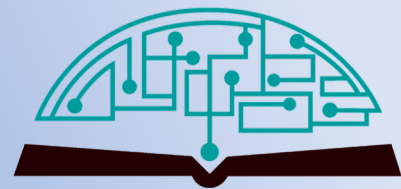


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Increasing the Beta-Carotene Content of Rice, Maize, and Potatoes Through Genetic Modification

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ABSTRACT: Vitamin A deficiency is a global health problem that disproportionately affects people in developing nations. It can cause serious health issues, such as immune system weakness and eyesight impairment. Transgenic technology has emerged as a possible way to address this issue by increasing the beta-carotene content of staple crops like rice, maize, and potatoes. Rice, maize, and potatoes are significant staple crops globally but are deficient in essential nutrients like vitamin A. Because of this, scientists have successfully inserted the genes needed to enhance beta-carotene into these crops using various genetic engineering techniques like CRISPR-Cas gene editing, biolistic transformation, and Agrobacterium-mediated transformation, thereby providing a solution to vitamin A deficiency and malnutrition.

KEYWORDS: Biomedical and Health Sciences; Nutrition and Natural Products; Biofortification; Beta-carotene; Vitamin A.

■ Introduction

Although often dismissed as a way to coax children into eating vegetables, the idea that eating carrots can improve your vision is grounded in fact. Vitamin A, a nutrient gained by eating carrots and other vibrant fruits and vegetables, is essential for maintaining excellent eye health. The advantages of vitamin A, however, go beyond only improving vision. The body uses this crucial nutrient for other vital processes, including strengthening the immune system, fostering good skin, and promoting growth and development. This way, vitamin A should be included in a balanced diet.

However, vitamin A is the final product of a series of enzymatic reactions. The chemical consumed is the crucial precursor beta-carotene, metabolized in the liver by the enzyme beta-carotene dioxygenase to make retinaldehyde.¹ Retinol, the active form of vitamin A, is produced from retinaldehyde by the retinaldehyde reductase. This conversion is regulated by the body's feedback mechanisms, which ensure that the body maintains healthy amounts of vitamin A while preventing dangerous levels from rising. How efficiently beta-carotene is converted to vitamin A depends on factors like age, sex, and other dietary components like fat and protein.²

Nevertheless, consuming too much vitamin A is also detrimental due to the negative feedback systems in the body that control vitamin A levels.³ Therefore, taking excessive vitamin A supplements does not simply treat vitamin A insufficiency. The liver and adipose tissue both store vitamin A since it is fat-soluble. When vitamin A levels fall, the body increases dietary vitamin A absorption and releases stored vitamin A from the liver to maintain proper levels. On the other hand, when vitamin A levels are high, the body inhibits absorption and decreases mobilization of vitamin A, instead storing the surplus in the liver and adipose tissue. It is important to remember that it can take weeks or months for the systems that regulate the body's vitamin A levels to return to normal. Consequent-

ly, it is advised to refrain from consuming too many vitamin A supplements to prevent toxicity, which can result from the liver becoming saturated. This happens due to the liver's limited ability to process and eliminate toxins when saturated with vitamin A; once it reaches its limit, these substances can build up and harm liver cells, resulting in liver failure.⁴ Bile, which aids in the digestion of lipids and removes waste from the body, is also produced by the liver. When the liver is overwhelmed with toxins, it can fail to produce bile, which causes a buildup of waste products in the body. This can lead to toxicity and even death.⁵

Beta-carotene is a carotenoid, a class of naturally occurring pigments found in plants, algae, and bacteria. These organic compounds are responsible for many fruits and vegetables' bright red, orange, and yellow pigments. Beta-carotene is a tetraterpenoid with 40 carbon atoms in its core structure, made up of conjugated double bonds flanked by two beta-ionone rings. Beta-carotene has a symmetrical structure, so each molecule of beta-carotene produces two molecules of vitamin A. This occurs when the central 15,15' carbon-carbon bond is oxidatively cleaved, catalyzed by the enzyme beta-carotene monoxygenase.⁶

Once it is produced, vitamin A aids in the formation of rhodopsin, a light-sensitive pigment found in the retina of the eye. Rhodopsin's functions include light detection and the initiation of the visual response. A lack of sufficient vitamin A hinders the formation of rhodopsin, which can cause night blindness. Vitamin A is also necessary for the health of the cornea, conjunctiva, and other eye tissues, and it might aid in preventing age-related macular degeneration, one of the primary causes of vision loss in older adults.⁷

In addition, vitamin A is essential for the formation of strong lungs. The alveoli, which comprise the lung's gas exchange area and develop throughout pregnancy and several years after birth, require vitamin A to grow. Furthermore, functional

abnormalities and disease states have been connected to long-term vitamin A deficiency (VAD). Chronic dietary VAD alters the respiratory epithelium significantly, decreases alveolar septation, and encourages the growth of new interalveolar walls.⁸ To build the retinyl reserves in the developing lung needed for retinoic acid (RA) production during lung maturation and postnatal life, it is imperative to maintain adequate intake throughout the final month of pregnancy. RA controls normal organogenesis, cell differentiation, and proliferation throughout embryonic development.⁹

Vitamin A also helps prevent infection by directly boosting the immune system and supporting the daily renewal of skin cells and the production of oil and mucus, which serve as barriers to infection.¹⁰ T cells, B cells, and natural killer cells—all significant contributors to both innate and adaptive immune responses—need vitamin A to mature. The skin, the body's biggest organ, regularly renews itself by shedding old cells and producing new ones; vitamin A plays a crucial role in this process by promoting the differentiation of keratinocytes, the major cells that make up the skin's outer layer, and by regulating the production of sebum. This oily substance helps to keep the skin hydrated and protected.¹¹ Another crucial part of the body's defense against infection is the mucous membranes, which create mucus, a viscous fluid that coats the inside of the mouth, nose, throat, lungs, and other organs and traps and eliminates pathogens, including bacteria and viruses. As vitamin A encourages the growth and differentiation of the cells that create mucus, it is essential for establishing and maintaining mucous membranes. Dry, scaly, and rough skin and mucous membranes that are more vulnerable to damage and infection can result from a vitamin A deficiency.^{12,13}

Vitamin A's importance can be seen when supplements are given to populations experiencing VAD.¹⁴ The incidence of diarrhea in newborn children was reduced by 30% in the treated group of a randomized controlled trial conducted in Nepal, and in a second experiment, young children had a 25% lower likelihood of severe malaria after vitamin A supplementation.¹⁵

Childhood morbidity and mortality that are brought on by VAD primarily occur in the developing world, particularly in Southeast Asia and Africa. VAD is a significant cause of newborn death since it can lead to a vicious cycle of disease and weakness in children. Because only animal products provide preformed vitamin A, carotenoids fill most of the vitamin A needs in countries with limited animal product consumption.¹⁶

VAD is a severe public health problem in many developing nations, but directly supplementing vitamin A is costly and risks toxicity. Recently, genetic engineering of staple crops to improve their beta-carotene content has been proposed as a potential remedy. Researchers have identified and cloned the genes that produce beta-carotene in various organisms. This knowledge has enabled several approaches that are effective at increasing beta-carotene content.¹⁷ This process, called "biofortification," provides a practical and affordable way to raise the nutrient intake of vulnerable populations while avoiding the risk of overconsumption.¹⁸ Biofortification reduces the burden of the diseases brought on by VAD and makes strides

toward eradicating vitamin deficiencies globally. The following sections will expand on different methods of biofortifying various staple crops.

■ Methods

Employing a systematic literature review methodology, this study extensively investigated various biofortification methods for three staple crops, specifically focusing on enhancing beta-carotene content. Articles were identified through targeted keyword searches related to the crops and relevant biofortification techniques, primarily relying on the crop name paired with "beta-carotene biofortification." The review relies primarily on recent literature, and articles were meticulously examined to extract insights into the efficacy of biofortification techniques and their impact on beta-carotene enhancement. The review provides a well-rounded perspective by combining modern advancements with foundational approaches.

Insights and Observations:

With the aid of transgenic technology, the beta-carotene content of corn, rice, and potatoes can be increased. Rice, maize, and potatoes are widely consumed and account for billions of people's daily calorie consumption. Rice, the primary staple for more than half of the world's population, is essential, particularly in Asia.¹⁹ Potatoes are a versatile and nutritious crop that is a mainstay in many countries. In contrast, maize, commonly known as corn, is a staple in many parts of the world, including sections of Africa and the Americas. These crops have been widely studied and investigated because of their importance to global food security and the need to improve productivity, nutritional content, disease resistance, and sustainability.¹⁸ Rice, in particular, has garnered significant attention in research efforts because of its high demand and the need to improve its productivity, which has paved the way for further research on improving the beta-carotene of staple crops, along with the aid of transgenic technology.

Gene Editing Techniques:

The recent development of CRISPR-Cas9 technology makes it possible to make precise and effective alterations to a plant's DNA, completely changing the area of biofortification. The CRISPR system comprises the Cas9 protein and a guide RNA (gRNA). The gRNA is intended to complement the target DNA sequence. While the CRISPR-Cas9 complex is initially introduced through transfections, it subsequently enters the cells. Once inside the cells, the complex guides Cas9 to bind with the target DNA. Cas9 severs the DNA, resulting in a double-stranded break. Non-homologous end joining (NHEJ) and homology-directed repair (HDR) systems in the cell can then repair the break. Using a template DNA molecule, scientists can use the CRISPR system to guide precise gene alterations.²⁰ Gene editing enables targeted modifications without introducing foreign DNA, in contrast to conventional genetic modification techniques like transgenesis.²¹ Transgenesis is the process where a foreign gene is inserted into the genome of an organism, or biolistic transformation,²² where typically gold or tungsten microprojectiles coated with genetic material are launched into target cells. While gene editing is more efficient and precise, conventional methods of genetic modification make crops more readily accepted by regulatory

bodies and consumers. Additionally, the simultaneous modification of numerous genes through gene editing has made introducing more complex features into crops possible.

Another method of genetic modification commonly used is agrobacterium-mediated transformation, where the random integration of DNA into the genome is routinely employed to introduce foreign genes into plants. This can have unanticipated consequences,²³ such as undesired mutations or perturbations of native genes. CRISPR's focused editing reduces these unexpected consequences and gives one more control over the genomic changes.

Another commonly used technology is RNAi silencing, where small RNA molecules prevent gene expression. Although a valuable tool for gene knockdown, this method is limited to post-transcriptional regulation. Furthermore, RNAi silencing might not completely silence the target gene, and it might also have unwanted side effects that silence other genes.²³

Concerns:

Despite the efficacy of the aforementioned techniques differing, all of the techniques have shown consistent results that prove that the genetic modification of these staple crops can significantly improve the beta-carotene content of these plants, which, if globalized, can effectively fight VAD worldwide. But for this to occur, many countries must pass comprehensive laws that motivate deficient populations to try genetically modified foods. Since its conception, the development of genetically modified foods (GMOs) has sparked debate. Proponents of GMOs claim that they can help with VAD, a serious public health problem in developing nations. Meanwhile, detractors cite several concerns that could reduce the impact of GMOs. The foremost concern is whether GMOs are safe to eat. Still, a study conducted by the National Academies of Sciences, Engineering, and Medicine found no evidence that foods grown with GMOs are any more harmful to human health than crops grown conventionally.²⁴

Another concern regarding genetically modified crops, in general, is that the widespread adoption of these modified crops could lead to the loss of traditional varieties of these crops that have been cultivated for centuries and are adapted to local growing conditions. Additionally, the common practice of patenting bio-enriched varieties may lead to small-scale farmers needing the proper technology to create modified crops to compete with larger agricultural businesses. Developing genetically modified crops could be driven by profit motives rather than the need to address nutritional deficiencies in developing countries, leading to additional issues and a reduced impact.²⁵

In addition to the ethical concerns, some people may have trouble absorbing and converting beta-carotene to vitamin A if they have certain genetic variations or medical conditions known as malabsorption disorders. People who suffer from intestinal conditions like Crohn's disease, ulcerative colitis, or celiac disease may have irritation and damage in the intestinal lining, leading to trouble absorbing some nutrients from meals, including beta-carotene and vitamin A. This can result in various micronutrient deficiencies, including a VAD, which can

harm health. This means that people with absorption issues will need more than biofortified foods and must seek other means of supplementation as well, so biofortification is more than just a one-size-fits-all solution.²⁶

Despite these problems, many countries, most notably the Philippines, one of the world's largest rice producers, have approved the consumption of golden rice. The Philippines' decision to allow the commercial production of golden rice in 2019 marked a significant turning point in technology development. It aimed to deliver around 50% of the daily recommended Vitamin A for young children.²⁷

Rice:

Rice is an everyday food source for over half of the world's population,²⁸ and VAD is prevalent in many underdeveloped countries. To counter this problem, researchers have been looking for ways to biofortify rice to increase its vitamin A content.

Golden rice is a genetically engineered species created to combat VAD in impoverished nations. The rice was genetically modified to create beta-carotene, a precursor of vitamin A, in the rice grains' endosperm, the edible part of the rice grain. Golden rice technology entails inserting two genes, phytoene synthase (*psy*) and beta-carotene desaturase (*crtI*), found in the bacterium *Erwinia uredovora* into the rice genome via Agrobacterium-mediated transformation,^{18, 29} which involves adding foreign genes into the rice genome by Agrobacterium tumefaciens. This bacterium is a naturally occurring vector, and it inserts a Ti-plasmid (tumor-inducing) into the plant cell's nucleus.³⁰ This approach was developed before the discovery of CRISPR-Cas9 and has been used to produce a range of genetically modified crops, including herbicide-resistant Bt cotton and soybeans. The resulting rice lines' endosperm produces beta-carotene, which the body converts to vitamin A. The ability to insert larger DNA segments into the genome via Agrobacterium-mediated transformation is one benefit that can be useful for adding complex traits into plants. One disadvantage of this method is the possibility of foreign DNA insertion into unwanted locations in the genome, which could have unforeseen impacts on plant growth and development. On the contrary, CRISPR-Cas9 allows for precise and large alterations to the plant genome targeted to a specific locus.³¹

The development of golden rice has spurred research into various methods of biofortifying rice to improve its vitamin A concentration. One method includes utilizing CRISPR-Cas9 technology to modify the rice genome and insert genes necessary for beta-carotene production. One study employed CRISPR-Cas9 to modify the rice genome and add the aforementioned *psy* and *crtI* genes responsible for beta-carotene synthesis. The study discovered that the generated rice lines contained up to 21 times more beta-carotene than the control lines.³² This contains more beta-carotene than golden rice, which contains roughly 1.6 µg/g of rice.¹⁸

A highly effective method for genetically modifying crops, such as rice, to improve their nutritional content is called "biolistic transformation." Biolistic transformation,²⁹ also known as gene gun technology, has introduced three genes from the beta-carotene biosynthetic pathway into the rice endosperm. The result was rice grains with high levels of beta-carotene,

giving them a distinctive golden color. Results have shown carotenoid amounts ranging from 0.297 µg/g to 1.05 µg/g.³³

Overall, various methods for biofortifying rice with increased vitamin A content exist, including CRISPR-Cas9 technology, biolistic transformation, and bacterium-mediated transformation. These approaches can address VAD in many developing countries and improve the health of millions of people. Yet, it is important to carefully consider the potential environmental and social impacts of these technologies and any potential risks to human health.

Maize:

Maize, commonly known as corn, is a significant staple crop around the globe but is deficient in important nutrients like vitamin A, which causes malnutrition in people who rely heavily on maize as their primary food supply. The genetic modification of maize to produce extra vitamin A is one approach to solving this issue. CRISPR-Cas9 is one of the techniques used in genetic engineering to do this. To improve maize's nutritional value, scientists have employed CRISPR-Cas9 to introduce or change genes that promote the production of beta-carotene.

In one study, one standard corn line with favorable *lycE* alleles was the donor parental line, whereas four sweet corn lines were the recipient lines.³⁴ The favorable *lycE* allele was introduced into four sweet corn lines using marker-assisted selection (MAS). MAS is a breeding strategy used in agriculture and genetics to aid in selecting desirable features in plants and animals. It uses molecular markers, which are distinct areas of DNA linked to specific traits or genes of interest.³⁵ According to this study, the favorable *lycE* allele increases beta-carotene, proA, and total carotenoid content while decreasing alpha-carotene levels. The average increase in beta-carotene, proA, and total carotenoid concentration was 1.54, 1.85, and 6.77 µg/g, respectively. The beta-carotene/total carotenoid ratio increased across the three converted lines, except the K185-converted lines. According to the findings of this study, MAS effectively increases proA carotenoid content in sweet corn by introducing the favorable *lycE* allele.³⁴

In a different study, *PSY1*, the phytoene synthase gene found in maize, was introduced into maize plants using CRISPR-Cas9 technology. In maize, *PSY1* is a limiting factor in beta-carotene synthesis and is implicated in its biosynthesis. According to the study, maize plants' beta-carotene and vitamin A levels significantly increased due to CRISPR-Cas-mediated gene editing. The researchers verified that the *PSY1* gene was successfully introduced into the maize plants by PCR amplification and sequencing of the targeted genomic region. They found that the gene was integrated into the maize genome in all edited plants. The resulting maize plants had significantly higher levels of beta-carotene than non-edited plants. The study demonstrated that CRISPR-Cas9 technology could precisely and efficiently introduce beneficial genes into crops to enhance their nutritional content and improve their yield and resistance to pests and diseases.³⁶

Overall, the biofortification of maize with higher levels of beta-carotene using various technologies has the potential to benefit millions of people worldwide. Researchers can improve

the nutritional value of maize, a common food source in developing nations, by inserting genes that boost beta-carotene production. The aforementioned findings show that different genetic editing techniques effectively achieve this objective and emphasize their potential as a weapon for combating hunger worldwide.

Potatoes:

More than 1.3 billion people worldwide rely on potatoes as an integral source of nourishment.³⁷ However, they do not contain much beta-carotene. Even though some potato varieties contain trace amounts of carotenoids, their concentrations are frequently much lower than those of other vegetables. Because of this, people who rely on potatoes as their primary food source may be susceptible to VAD. Numerous studies have explored the possibility of using various transgenic technologies to biofortify potatoes with more beta-carotene.

In one study, researchers used a bacterial-derived mini-pathway to induce the synthesis of beta-carotene (Provitamin A) from geranylgeranyl diphosphate.³⁸ Three *Erwinia* genes encoding phytoene synthase (*CrtB*), phytoene desaturase (*CrtI*), and lycopene beta-cyclase (*CrtY*) were used under tuber-specific or constitutive promoter control. Eighty-six unique transgenic lines were produced and tested, each with a different promoter/gene combination. In these tubers, carotenoids increase approximately 20-fold to 114 µg/g dry weight, while beta-carotene increases 3600-fold to 47 µg/g dry weight. This is not only the highest carotenoid and beta-carotene content reported for biofortified potatoes, as well as any of the four primary staple foods, but it is also enough to provide 50% of the recommended daily allowance (RDA) of Vitamin A with 250 gms (fresh weight) of these modified potatoes, assuming a beta-carotene to retinol conversion of 6:1.³⁸

Another study silenced the beta-carotene hydroxylase gene (*bch*), which transforms beta-carotene into zeaxanthin, using RNA interference (RNAi). Three distinct potato lines were transformed using *Agrobacterium tumefaciens* to introduce two RNAi constructs.³⁹ The GBSS (tuber-specific granule bound starch synthase) promoter was present in one construct, whereas the CaMV 35S (strong constitutive) promoter was present in the other. The amount of beta-carotene in the wild-type tubers was enhanced from minimal quantities to 331 µg per 100 g of fresh weight. In general, GBSS-derived transformants had higher beta-carotene levels than CaMV 35S-derived transformants.

Future Directions:

As people in different places have different dietary preferences and cultural practices, having three different biofortified crops high in vitamin A is critical. Furthermore, because different crops have different growth requirements, it is essential to have various crops suitable for different environments. Rice, for example, is frequently grown in flooded areas, but maize and potatoes are typically planted on drier soil. Farmers in different places can plant biofortified crops suitable for their particular growing conditions if there is a diversity of biofortified crops.⁴⁰

Modifying staple crops to increase beta-carotene content is headed towards further research and development to im

prove the nutritional content to address malnutrition and related health problems in vulnerable populations. One of the key challenges facing the field is ensuring that the modified crops are safe for human consumption and have no adverse environmental impacts. This requires continued research to understand the potential long-term effects of consuming these crops and to establish regulatory frameworks to ensure their safety. In addition, the development and distribution of these crops must be done equitably and sustainably, which benefits small-scale farmers and communities in need. For the advantages of these improvements to be available to those who need them most, there needs to be a greater investment in infrastructure, research, and education. Collaboration between governments, researchers, and stakeholders from other sectors will also be crucial to advance and handle the complicated issues related to changing staple crops. Overall, the potential benefits of modified staple crops are substantial, even though there are still many challenges to be solved, and ongoing efforts to develop and implement them responsibly and equitably will be vital to enhancing world health and lowering malnutrition.

Biofortification has achieved increased nutrient content in crops, including maize, rice, and potatoes, offering a long-lasting and affordable remedy for micronutrient deficiencies. Biofortified crops are not a panacea and might only be effective for some. Regional differences in soil quality, climate, and agricultural methods can affect biofortification projects' effectiveness. Moreover, while biofortified crops can increase the availability of essential micronutrients, relying solely on them may not address the underlying causes of poverty and food insecurity, which can also contribute to malnutrition. Therefore, a comprehensive strategy considering various elements is required to enhance nutrition and decrease malnutrition, including education and awareness campaigns, access to healthcare, and social services. Implementing such a strategy would support biofortification initiatives and address the root causes of malnutrition, helping to provide a long-lasting solution.

Future research should concentrate on conducting comprehensive studies that delve into the specific impacts of consuming these genetically modified crops. To ensure the safety and efficacy of these crops, it is imperative to investigate various aspects. Firstly, bioavailability studies should be undertaken to ascertain how much the human body can absorb the enhanced beta-carotene. For instance, clinical trials could be designed to compare the bioavailability of beta-carotene from genetically modified crops with that from traditional sources, measuring blood levels of beta-carotene and its conversion to vitamin A. Turning to the environmental perspective, investigating the potential consequences of gene flow from genetically modified crops to their wild relatives is of utmost importance. This could be addressed through experimental plantings in controlled environments, observing the rates of gene transfer and potential impacts on the wild populations. Non-target organism studies should also be conducted to assess whether the modified crops have unintended effects on beneficial insects, soil microorganisms, and other components of local ecosystems. For instance, studying the impact of genetically modified crops on pollina-

tors like bees could provide insights into potential ecological disruptions.

Moreover, a comprehensive evaluation of the overall environmental sustainability is vital. This involves assessing the water usage patterns of these crops in comparison to traditional crops, monitoring soil health parameters, and quantifying potential shifts in biodiversity in fields where the crops are cultivated. For example, analyzing soil microbial diversity and nutrient levels before and after cultivating genetically modified crops can offer insights into their impact on soil health.

Ultimately, finding the balance between the benefits of improved nutrition and the potential risks to human health and the environment is pivotal. This requires a multi-faceted approach, integrating findings from health and environmental research. Policymakers and stakeholders must consider these findings when deciding about the cultivation and consumption of biofortified crops. Responsible integration involves not only understanding the scientific nuances but also engaging in open dialogues with the public to address concerns and build trust in the technology.

This review significantly advances our knowledge of enhancing beta-carotene content in rice, maize, and potatoes through genetic modification. The review offers a comprehensive view of biofortification approaches by covering diverse strategies like CRISPR-Cas9, biolistic transformation, and bacterium-mediated methods. It discusses specific genetic modifications, aiding our understanding of the underlying mechanisms. The comparison between techniques, such as the beta-carotene variations in golden rice and CRISPR-modified rice, informs decision-making in future research.

The review's real-world impact lies in its emphasis on combating vitamin A deficiency in regions reliant on these crops. It quantifies the increase in beta-carotene content, underscoring the potential to address a crucial public health issue. Implicitly, the review suggests directions for future exploration: refining precision in genetic modifications, considering trait stacking for multifaceted benefits, exploring long-term environmental and ethical implications, and addressing consumer acceptance and regulatory aspects. This multifaceted perspective positions the review as a valuable roadmap for advancing crop biofortification strategies responsibly and sustainably.

In summary, the review's comprehensive coverage of diverse biofortification strategies, comparative analysis, emphasis on real-world impact, and implicit directions for future research collectively contribute to an enriched understanding of enhancing beta-carotene content in staple crops.

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■ References

1. Institute of Medicine (US) Panel on Micronutrients. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*; National Academies Press (US): Washington (DC), 2001.

2. Johnson, E. J. Role of Lutein and Zeaxanthin in Visual and Cognitive Function throughout the Lifespan. *Nutrition Reviews* **2014**, 72 (9), 605–612. <https://doi.org/10.1111/nure.12133>.
3. National Institutes of Health. *Office of Dietary Supplements - Vitamin A*. Nih.gov. <https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/>.
4. MedlinePlus. *Vitamin A: MedlinePlus Medical Encyclopedia*. Medlineplus.gov. <https://medlineplus.gov/ency/article/002400.htm>.
5. Gupta, N. K.; Lewis, J. H. Review Article: The Use of Potentially Hepatotoxic Drugs in Patients with Liver Disease. *Alimentary Pharmacology & Therapeutics* **2008**, 28 (9), 1021–1041. <https://doi.org/10.1111/j.1365-2036.2008.03822.x>.
6. Grune, T.; Lietz, G.; Palou, A.; Ross, A. C.; Stahl, W.; Tang, G.; Thurnham, D.; Yin, S.; Biesalski, H. K. β -Carotene Is an Important Vitamin A Source for Humans. *The Journal of Nutrition* **2010**, 140 (12), 2268S–2285S. <https://doi.org/10.3945/jn.109.119024>.
7. Miyazono, S.; Isayama, T.; Delori, F. C.; Makino, C. L. Vitamin A Activates Rhodopsin and Sensitizes It to Ultraviolet Light. *Visual Neuroscience* **2011**, 28 (6), 485–497. <https://doi.org/10.1017/S0952523811000423>.
8. Biesalski, H. K.; Nohr, D. Importance of Vitamin-A for Lung Function and Development. *Molecular Aspects of Medicine* **2003**, 24 (6), 431–440. [https://doi.org/10.1016/s0098-2997\(03\)00039-6](https://doi.org/10.1016/s0098-2997(03)00039-6).
9. Ross, A. C.; Gardner, E. M. The Function of Vitamin A in Cellular Growth and Differentiation, and Its Roles during Pregnancy and Lactation. *Advances in experimental medicine and biology* **1994**, 352, 187–200. https://doi.org/10.1007/978-1-4899-2575-6_15.
10. Ross, A. C.; Manson, J. E.; Abrams, S. A.; Aloia, J. F.; Brannon, P. M.; Clinton, S. K.; Durazo-Arvizu, R. A.; Gallagher, J. C.; Gallo, R. L.; Jones, G.; Kovacs, C. S.; Mayne, S. T.; Rosen, C. J.; Shapses, S. A. The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know. *The Journal of Clinical Endocrinology & Metabolism* **2011**, 96 (1), 53–58. <https://doi.org/10.1210/jc.2010-2704>.
11. Stephensen, C. B. Vitamin A, Infection, and Immune Function. *Annual Review of Nutrition* **2001**, 21, 167–192. <https://doi.org/10.1146/annurev.nutr.21.1.167>.
12. Semba, R. D. Vitamin A and Immunity to Viral, Bacterial and Protozoan Infections. *Proceedings of the Nutrition Society* **1999**, 58 (3), 719–727. <https://doi.org/10.1017/s0029665199000944>.
13. West, K.; Katz, J.; Shrestha, S.; LeClerq, S.; Khatri, S.; Pradhan, E.; Adhikari, R.; Wu, L.; Pokhrel, R.; Sommer, A. Mortality of Infants < 6 Mo of Age Supplemented with Vitamin A: A Randomized, Double-Masked Trial in Nepal. *The American Journal of Clinical Nutrition* **1995**, 62 (1), 143–148. <https://doi.org/10.1093/ajcn/62.1.143>.
14. Villamor, E.; Fawzi, Wafaie W. Vitamin A Supplementation: Implications for Morbidity and Mortality in Children. *The Journal of Infectious Diseases* **2000**, 182 (s1), S122–S133. <https://doi.org/10.1086/315921>.
15. West, K. P. Vitamin Deficiency Disorders in Children and Women. *Food and Nutrition Bulletin* **2003**, 24 (4_suppl2), S78–S90. <https://doi.org/10.1177/15648265030244s204>.
16. Gilbert, C. What Is Vitamin A and Why Do We Need It? *Community eye health* **2013**, 26 (84), 65.
17. de Pee, S.; Bloem, M. W. Current and Potential Role of Specially Formulated Foods and Food Supplements for Preventing Malnutrition among 6- to 23-Month-Old Children and for Treating Moderate Malnutrition among 6- to 59-Month-Old Children. *Food and Nutrition Bulletin* **2009**, 30 (3_suppl3), S434–S463. <https://doi.org/10.1177/15648265090303s305>.
18. Paine, J. A.; Shipton, C. A.; Chaggar, S.; Howells, R. M.; Kennedy, M. J.; Vernon, G.; Wright, S. Y.; Hinchliffe, E.; Adams, J. L.; Silvestro, A. L.; Drake, R. Improving the Nutritional Value of Golden Rice through Increased Pro-Vitamin A Content. *Nature Biotechnology* **2005**, 23 (4), 482–487. <https://doi.org/10.1038/nbt1082>.
19. Potrykus, I. Golden Rice and Beyond. *PLANT PHYSIOLOGY* **2001**, 125 (3), 1157–1161. <https://doi.org/10.1104/pp.125.3.1157>.
20. Liu, X.; Xie, C.; Si, H.; Yang, J. CRISPR/Cas9-Mediated Genome Editing in Plants. *Methods* **2017**, 121–122, 94–102. <https://doi.org/10.1016/j.ymeth.2017.03.009>.
21. Niemann, H.; Carnwath, J. W. *Transgenesis - an overview | ScienceDirect Topics*. www.sciencedirect.com/topics/medicine-and-dentistry/transgenesis.
22. Kikkert, J. R.; Vidal, J. R.; Reisch, B. I. Stable Transformation of Plant Cells by Particle Bombardment/Biostics. *Transgenic Plants* **2019**, 061–078. <https://doi.org/10.1385/1-59259-827-7:061>.
23. Boettcher, M.; McManus, Michael T. Choosing the Right Tool for the Job: RNAi, TALEN, or CRISPR. *Molecular Cell* **2015**, 58 (4), 575–585. <https://doi.org/10.1016/j.molcel.2015.04.028>.
24. National Academies of Sciences, E. *Genetically Engineered Crops: Experiences and Prospects*; National Academies Press, 2016.
25. Council, I. of M. and N. R. *Safety of Genetically Engineered Foods: Approaches to Assessing Unintended Health Effects*; National Academies Press, 2004.
26. Cao, G.; Lungaro, L.; Caputo, F.; Zoli, E.; Giancola, F.; Chiarioni, G.; De Giorgio, R.; Zoli, G. Nutritional Treatment in Crohn's Disease. *Nutrients* **2021**, 13 (5), 1628. <https://doi.org/10.3390/nu13051628>.
27. Stone, G. D.; Glover, D. Disembedding Grain: Golden Rice, the Green Revolution, and Heirloom Seeds in the Philippines. *Agriculture and Human Values* **2016**, 34 (1), 87–102. <https://doi.org/10.1007/s10460-016-9696-1>.
28. Rutledge, K.; McDaniel, M.; Teng, S.; Hall, H.; Ramroop, T.; Sproul, E.; Hunt, J.; Boudreau, D.; Costa, H. *Food Staple | National Geographic Society*. education.nationalgeographic.org/education.nationalgeographic.org/resource/food-staple/.
29. Ye, X. Engineering the Provitamin A (β -Carotene) Biosynthetic Pathway into (Carotenoid-Free) Rice Endosperm. *Science* **2000**, 287 (5451), 303–305. <https://doi.org/10.1126/science.287.5451.303>.
30. Rao, C. K. *Golden Rice - A Transgenic Variety of Rice, with Genes for the Synthesis of β -carotene*. www.fbae.org. http://www.fbae.org/2009/FBAE/website/special-topics_golden-rice.html.
31. Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J. A.; Charpentier, E. A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity. *Science* **2012**, 337 (6096), 816–821. <https://doi.org/10.1126/science.1225829>.
32. Shan, Q.; Wang, Y.; Li, J.; Gao, C. Genome Editing in Rice and Wheat Using the CRISPR/Cas System. *Nature Protocols* **2014**, 9 (10), 2395–2410. <https://doi.org/10.1038/nprot.2014.157>.
33. Datta, K.; Baisakh, N.; Oliva, N.; Torrizo, L.; Abrigo, E.; Tan, J.; Rai, M.; Rehana, S.; Al-Babili, S.; Beyer, P.; Potrykus, I.; Datta, S. K. Bioengineered “Golden” Indica Rice Cultivars with β -Carotene Metabolism in the Endosperm with Hygromycin and Mannose Selection Systems. *Plant Biotechnology Journal* **2003**, 1 (2), 81–90. <https://doi.org/10.1046/j.1467-7652.2003.00015.x>.
34. Yang, R.; Yan, Z.; Wang, Q. *Marker-assisted backcrossing of *lcyE* for enhancement of *proA* in sweet corn*. *Euphytica*. <https://doi.org/10.1007/s10681-018-2212-5>.
35. Ashraf, M.; Akram, N. A.; Mehboob-ur-Rahman; Foolad, M. R. Marker-Assisted Selection in Plant Breeding for Salinity Tolerance. *Plant Salt Tolerance* **2012**, 305–333. https://doi.org/10.1007/978-1-61779-986-0_21.
36. Tang, X.; Liu, G.; Zhou, J.; Ren, Q.; You, Q.; Tian, L.; Xin, X.; Zhang, Z.; Liu, B.; Zheng, X.; Zhang, D.; Malzahn, A.; Gong, Z.; Qi, Y.; Zhang, T.; Zhang, Y. A Large-Scale Whole-Genome Sequencing Analysis Reveals Highly Specific Genome Editing by Both Cas9 and Cpf1 (Cas12a) Nucleases in Rice. *Genome Biology*

- 2018, 19 (1). <https://doi.org/10.1186/s13059-018-1458-5>.
37. Devaux, A.; Goffart, J.-P.; Kromann, P.; Andrade-Piedra, J.; Polarr, V.; Hareau, G. The Potato of the Future: Opportunities and Challenges in Sustainable Agri-Food Systems. *Potato Research* **2021**. <https://doi.org/10.1007/s11540-021-09501-4>.
38. Diretto, G.; Al-Babili, S.; Tavazza, R.; Papacchioli, V.; Beyer, P.; Giuliano, G. Metabolic Engineering of Potato Carotenoid Content through Tuber-Specific Overexpression of a Bacterial Mini-Pathway. *PLoS ONE* **2007**, 2 (4), e350. <https://doi.org/10.1371/journal.pone.0000350>.
39. Van Eck, J.; Conlin, B.; Garvin, D. F.; Mason, H.; Navarre, D. A.; Brown, C. R. Enhancing Beta-Carotene Content in Potato by RNAi-Mediated Silencing of the Beta-Carotene Hydroxylase Gene. *American Journal of Potato Research* **2007**, 84 (4), 331–342. <https://doi.org/10.1007/bf02986245>.
40. Baker, N. T.; Capel, P. D. *Environmental Factors That Influence the Location of Crop Agriculture in the Conterminous United States*. pubs.usgs.gov. <https://pubs.usgs.gov/sir/2011/5108/>.

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The Influence of Figure Skating on the Central Nervous System, Social Wellbeing, and Mental Health

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ABSTRACT: Figure skating athletes experience repetitive training in intricate movements needed for their jumps, spins, and precise footwork on ice. Increased evidence shows that these trainings are associated with anatomic and functional changes in their central nervous systems. Specifically, long training induces the habituation of the vestibular system to the unusual movements in figure skating. Figure skaters are also more adaptive to vestibular-otolithic stimuli. On the cerebral level, figure skaters have larger cortical representations responsive to lower extremity activities on motor maps. In addition, skaters have higher gray matter volume, changes in white matter fractional anisotropy, and increased volume of their right cerebellum hemisphere and vermian lobules VI-VII. These adaptive changes benefit the athletes' overall physical wellness and impact their long-term behavior, learning, and cognitive landscape. The following review discusses studies that demonstrate the impact of figure skating on structures within the central nervous system. The social and mental benefits of figure skating, as well as possible future directions in this area of research, are also addressed.

KEYWORDS: Neuroscience; Behavioral and Social Wellbeing; Figure Skating; Central Nervous System; Mental Health.

■ Introduction

Figure skating is a sport that requires individuals to execute intricate movements, including jumps, spins, and precise footwork along the ice. It demands that athletes showcase their artistic abilities and perform a wide range of freestyle moves. The sport originated as a mode of transportation in northern European countries¹ before evolving over time into a popular recreational activity. As figure skating quickly popularized, its required physical routines became increasingly challenging, transitioning it into a demanding sport that now requires high levels of endurance, strength, and flexibility. The combination of artistic and technical abilities demands a high degree of physical exertion. Thus, many figure skating athletes develop enhanced abilities and responses throughout their organ systems. Because of the sport's demanding physical requirements, figure skaters develop adaptations in their central nervous systems, some of which are unique to individuals participating in figure skating. These include adaptations in the vestibular system and the motor map and changes in the volume of cerebral white matter and cerebellum.²⁻⁵

This review will summarize specific habituation to coordination and the mechanisms behind developing specialized motor skills in figure skaters. In addition, the social and mental health benefits of figure skating will also be discussed. We hypothesize that figure skating training improves the adaptive plasticity of the central nervous system, including the cerebrum, cerebellum, and vestibular system.

■ Discussion

The long-term roles of figure skating in brain development:

- The influence of figure skating on the anatomy of the brain

Extensive training in sports such as figure skating is increasingly shown to lead to adaptive changes or plasticity of the central nervous system.^{6, 7} Motor activities in body muscles are reflected in a region of the brain cortex in the frontal lobe, termed the motor cortex. The motor cortex is composed of primary and secondary motor cortices. The primary motor cortex is responsible for the motor control of different body parts, referred to as the cortical representation of muscles or motor map (Figure 1). The motor map is disproportionate to the mass of the corresponding muscles but related to the number of cortical neurons needed to control body muscles precisely. In this respect, regions controlling the movement of hands and face are much larger than those responsible for the trunk and legs (Figure 1).

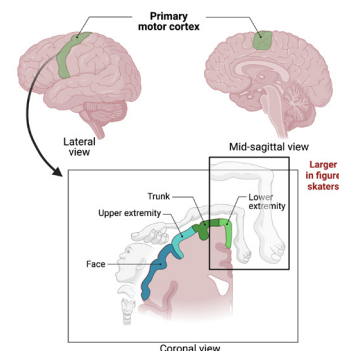


Figure 1: Figure skaters have a larger cortical representation responsive to lower extremity activities. The motor map is proportional to the number of cortical neurons needed to control body muscles. In skaters, the motor cortex responsible for the control of lower extremities is larger than in non-skaters. The figure was created with BioRender.

The long-term practice of figure skating, typically in professional figure skaters, is reported to affect the landscape of motor maps.⁸ This is consistent with many studies showing that motor training induces plasticity across the motor cortex in elite athletes.⁹ However, not all sports activities will lead to changes in the representations within the motor cortex. For example, animal experiments using rats demonstrated that strength training does not induce the reorganization of movement representations.¹⁰ Studies in rats also showed that endurance training was sufficient to induce angiogenesis but failed to alter the motor map.¹¹ Unlike strength and endurance training, sports training involving skilled activities, such as figure skating, has different impacts on the landscape of motor maps. For example, in animal experiments, reach-trained rats showed an expansion of distal, but not shoulder and elbow, representation of the motor cortex.¹² Squirrel monkeys trained on skilled digit manipulation exhibited an expansion of areas representing digit motor activity.¹³ In human studies, Vaalto *et al.* revealed that the cortical representation area of the tibialis anterior, the muscle critical for jumps, was larger in figure skaters when compared with the controls (Figure 1).⁸ An increase in the corresponding cortical representations in the frontal lobe was also observed in humans trained for digit, ankle, and tongue movements.¹⁴⁻¹⁶ Similarly, cortical representations of medial deltoid and carpi radialis muscles, which are critical for upper arm motor activities, have been shown to be enlarged in volleyball players.¹⁷

These studies support the hypothesis that repeated and long-term training in skill motor activities causes the enlargement of the areas of corresponding cortical representations. However, exceptions exist in specific skilled training. Vaalto *et al.* also found that the representation area of the left abductor digiti minimi, muscles critical for reaching right tones, was smaller in the nondominant hemisphere in string players compared to the controls.⁸ The authors concluded that motor map reorganization may differ depending on the skill and the roles of the muscles in the skill.⁸

The influence of cortical motor representation by extensive figure skating training persists even when the skaters are aged. The motor representation areas in the brain are believed to be shared with action execution and observation.¹⁸⁻²⁰ Studies have shown that retired and experienced figure skaters had better skills in predicting figure skating elements and other movements than age-matched individuals. This indicates that figure skating training could prevent an aging-associated decline in motor representations.²¹

Another aspect of the central nervous system that long-term figure skating may influence is the action observation network (AON).²² AON consists of ventral premotor cortex, inferior parietal cortex, and superior occipitotemporal cortex. The AON cortices are reciprocally connected and responsible for action interpretation to a common code. In elite sports athletes, the connections often extend beyond these regions of the canonical AON network. Diersch *et al.* reported that the caudate nucleus plays a critical function in connecting AON cortices to predict less familiar figure skating actions in figure

tice could modulate the distribution of AON networks.²³ In other sports, action anticipation has been observed beyond the AON system in expert basketball players.¹⁹

- **Plasticity in the brain white and gray matters in figure skaters**

Figure skating is reported to influence other aspects of brain anatomy as well. Broadly, the main components of the brain include gray matter and white matter based on their relative light appearance. Gray matter comprises neurons, whereas white matter encompasses bundles of nerve fibers (axons) derived from neurons. The connectivity of axons to different brain regions, which could be detected by diffusion tensor imaging (DTI), dictates the changes in white matter due to diseases or other conditions. In this respect, Hummel *et al.* reported that figure skaters showed similar cerebral white matter diffusivity changes compared to patients with bilateral vestibular loss and balance deficits.² Similar findings were also observed in athletes involved in other balance training, such as ballet dancing and slacklining.^{2, 24, 25} These studies indicate that repeated balance training, experienced in both athletes and balance deficit patients, shares similar mechanisms in shaping the white matter fractional anisotropy (Figure 2).

Long-term figure skating training can also induce structural plasticity in the gray matter. Compared with age-matched non-athletes, elite figure skaters had higher gray matter volume in different brain regions, including the posterior cerebellum, frontal lobe, temporal lobe, posterior cingulate, caudate, and thalamus. The change is specifically prominent in the cerebellum. The connection between the posterior cerebellum and fusiform gyrus is also stronger in figure skaters.⁷

- **Influence on cerebellum in figure skating**

Similar to what has been found in other sports, intensive figure skating training can induce brain structural changes, particularly in the cerebellum.^{4, 26} These findings are not surprising, given that the cerebellum is pivotal in coordinating various movements and controlling motor and balance. Like the cerebrum, the cerebellum comprises two hemispheres divided by an array of large folds called the vermis. The vermis is further divided by lobules. Using three-dimensional magnetic resonance imaging, Park *et al.* examined the volumes of the cerebellum in short-track speed skaters and compared them with the matched control individuals. They found that the volumes of the right cerebellar hemisphere and vermician lobules VI-VII are bigger in skaters.⁴ The authors suggest that long-term training in skating increases the volume of the aforementioned regions of the cerebellum that are most critical for balance and coordination during skating.

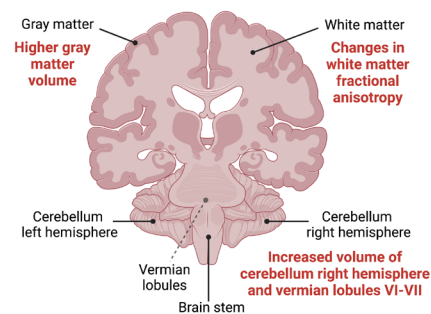


Figure 2: Skaters experience changes in the cerebrum and cerebellum. The figure shows a coronal view of the cerebrum and cerebellum in the normal brain. The skaters have higher gray matter volume, changes in white matter fractional anisotropy, and increased volume of cerebellum right hemisphere and vermian lobules VI-VII as depicted in red. The dotted line indicates that vermian lobules are located behind the illustrated regions.

Other studies investigating female short-track speed skaters also identified an increased volume of vermian lobules VI-VII. Compared with the control individuals and male skaters, the increased volume of vermian lobules VI-VII was shown to be correlated with more enhanced balance in female skaters.⁵

In summary, these reports demonstrate the positive influence of figure skating on the plasticity and functions of the brain. Nevertheless, most of these studies include only a limited number of athletes and control individuals. Future studies with an increased enrollment of study subjects would solidify these findings.

The relationship between figure skating and the vestibular system:

In humans, the vestibular system is an essential sensory structure that detects motion, head position, and spatial orientation. More specifically, this biological system is critical for rotation, movement, body position, and tilt. Like other human organ systems, the vestibular system is complex and composed of an array of delicately regulated anatomic and neurologic elements. The main component of the vestibular system is the vestibular labyrinth, which is continuous with the cochlea for hearing detection. The cochlea and vestibular apparatus are together covered by a bony labyrinth, which additionally has a membranous labyrinth underneath.

The vestibular labyrinth contains semicircular canals, which are critical to detect nodding, shaking, and tilting. A body fluid known as endolymph circulates throughout the canals and serves to travel according to body movements. Under circumstances where the head experiences rotation, for example, the endolymph flows into the ampulla. Within it, the sensory portion, known as crista ampullaris containing the cupula, hair cells, and vestibular nerves, will act analogously with rotations.²⁷ The vestibular system is also composed of the otolith organs to detect acceleration, tilting, and other gravitational forces. The two otolith organs are specifically named the utricle and saccule, which serve to detect movement on the horizontal or vertical plane. Within both the crista ampullaris and otolith organs, movement of the endolymph causes hair cells to act where neurotransmitters will be released to signal information regarding the plane of movements to the brain (Figure 3).²⁷

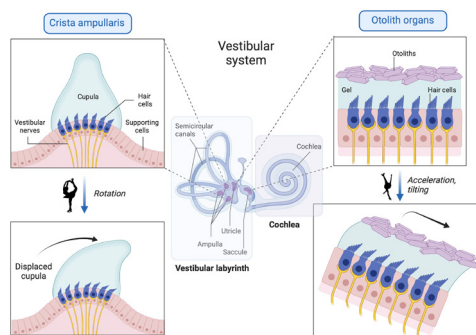


Figure 3: The anatomy and functions of the vestibular system related to figure skating. The vestibular system is composed of a vestibular labyrinth and cochlea. The vestibular labyrinth contains semicircular canals, which connect to the ampulla. Within the ampulla, crista ampullaris senses body rotations through the displaced cupula. The vestibular labyrinth also contains otolith organs, named utricle and saccule, to detect acceleration and tilting. The microscopic composition of crista ampullaris and otolith organs includes hair cells connected to vestibular nerves, which transmit the signal to the brain. The figure was created with BioRender.

The vestibular system is one of the most important organ systems for successfully performing the essential components of figure skating, such as rotation, acceleration, and tilting (Figure 3).²⁷ Conversely, practice in figure skating can also influence the functional plasticity of the vestibular system. In this respect, Tanguy *et al.* compared the vestibulo-ocular reflex (VOR) and motion sickness intensity in female figure skaters and matched control individuals.³ Through the measurement of sickness symptoms after vestibular and nauseogenic stimulations evoked by sinusoidal rotation or off-vertical axis rotation, the authors found that figure skaters exhibited a lower gain of VOR and less motion sickness compared to the control individuals. The VOR gain is calculated by the amount of eye rotation divided by the amount of head rotation, which is usually close to 1 in a healthy individual. However, in sports such as figure skating, the athlete needs to follow the objects during rapid rotations, which often is reflected by a lower gain of VOR. The lower VOR gain in figure skaters suggests adaptation of the vestibular-ocular system to the specialty of the skaters. The authors conclude that the repeated stimulations of VOR during figure skating practice could induce the habituation of the vestibular system to these unusual movements.³ The same conclusion was also made in a study by Alpini *et al.*,²⁸ which similarly studied VOR gain in figure skaters.

Additionally, these authors also found that vestibular habituation depends on the specific tasks of the skaters. For example, their study showed that VOR gain was reduced only in dancer skaters but not in single or paired skaters in their study.²⁸ Notably, the number of skaters enrolled in these studies is low, which may bias their findings.

These findings of vestibular habituation in figure skaters were also identified in animals such as monkeys^{29, 30} and cats.³¹ Both species experience frequent vestibular stimuli in their daily life. Similarly, athletes in other sports, such as dancing and gymnastics, are reported to have reduced gain of VOR in experimental conditions with vestibular ocular stimuli.^{32, 33}

The vestibular system also includes otolith organs comprising the utricle and saccule, which detect linear head acceleration and tilt, respectively. Otolithic inputs are reported to be interpreted better in figure skaters.³⁴ Tanguy *et al.* assessed otolith-ocular reflex in figure skaters and control individuals using vertical axis rotation (OVAR), a stimulus in which an individual is rotated along a tilted axis. The authors found that compared to the non-skaters, figure skaters interpret otolith signals more as rotation but less as inclination, again suggesting that figure skaters are more adaptive to the vestibular-otolith stimuli.³⁴

The Impact of figure skating on Social and mental health:

The influence of figure skating training extends beyond the physical changes and adaptations in the central nervous system.

Many reports show evidence demonstrating the benefits of figure skating in the mental and social health of the skaters. Lonescu and Badau recruited 143 students who performed recreational figure skating and evaluated their perceptions of the benefits of figure skating through questionnaires. The results showed a strong correlation between figure skating and improved physical development and fitness. The practice also contributes to the improvement of mental and social abilities.³⁵ While the study lacks a control group of students, the research provides substantial evidence that figure skating benefits mental and social health, even for recreational purposes.

Figure skating can also be therapeutic for mental diseases. A study by Casey *et al.* designed to test whether therapeutic skating could benefit patients with autism spectrum disorder recruited two autistic boys into various skating sessions.³⁶ These boys participated in three 1-hour skating sessions every week for 12 weeks. The study found that skating can produce physical benefits for these children, including balance, motor behavior, and functional capacity, and serve as an alternative approach to other activities.³⁶ Fragala-Pinkham *et al.* conducted a study with a much larger participant group, enrolling 22 children aged 5-12 years with developmental disabilities in figure skating once per week over six weeks. Using a questionnaire, the authors found that the parents of participating children were satisfied with the program and indicated overall improvements in their children's balance, endurance, self-esteem, and confidence.³⁷ The study supports Casey *et al.*'s conclusion that figure skating benefits the mental and physical health of neurodivergent children.

Conversely, mental training can also be influential to the performance of figure skating. An older study by Mumford *et al.* revealed that mental imagery training, a mental practice that could improve the performance of sports, significantly impacted figure skating in senior skaters. However, the effects on younger athletes were not apparent.³⁸

Figure skating-related medical problems pertinent to the neurologic system:

While practicing figure skating is undeniably beneficial, as with any sports activity, the sport has issues concerning injuries or other medical problems. The most common injuries in figure skating are in the muscular motor system,³⁹ which is not the focus of this review. Eating disorders and other medical problems are commonly observed in athletes across various disciplines, including figure skating.

Data from the 1980s and 90s showed that over half of the female skaters were dieting and took far less caloric needs than required.^{40, 41} Figure skaters also tend to show concerns about their weight and body dissatisfaction, well-known risk factors for eating disorders.⁴² A more recent study revealed that 13% of participating female skaters reported a range of problematic eating habits, which were irrelevant to their skating skills.⁴³ While eating disorders are a risk factor for many potential medical problems, especially the injuries associated with frequent jumps and landing, figure skaters do not appear to have an increased incidence of stress fractures. This could be because forces produced by these activities protect the skaters' skeletons.⁴⁴

Other injuries stemming from figure skating often directly impact the head. Knox *et al.* found that figure skaters had a greater percentage of head injuries (13.3%) than roller skaters (4.4%). It was also found that concussions occurred more often among figure skaters resulting from falls. The authors also found from each skating activity that children six years of age or younger participating in each skating activity experienced more head and facial injuries than older skaters.⁴⁵

Head injuries were also found common in synchronized skating. For example, in a study conducted before and during the World Synchronized Skating Championships in 2004, 19.8% of acute injuries were head injuries.⁴⁶ Generally, head injuries occurred more often in pairs and synchronized skating primarily due to lifting elements.

■ Conclusion

The field of figure skating continues to serve as a growing discipline in each category. This review discusses several neurological effects on activities such as speed skating, singles, pair skating, and synchronized skating. We conclude from the literature review that participating in these skating activities leads to changes in the physical adaptations in athletes, including vestibular habituation, the plasticity of the motor cortex, and white and gray matter in the central nervous system.²⁻⁵ Understanding the neurological benefits behind figure skating is significant as it can assist in improving athletic performance among experienced skaters and coaches while preventing injuries. In addition, analyzing the correlations between brain and bodily functions is crucial in future enhancements of varying skills and elements.

In addition to physical changes, figure skating offers mental and social benefits. Thus, it serves as an alternative physical and mental therapeutic approach for individuals with special needs, ultimately assisting them with their physical, social, and emotional well-being.³⁵⁻³⁷ Figure skating is commonly regarded as a physically demanding sport. However, uncovering social benefits can promote further social interactions within and outside of the sport. Additionally, understanding mental factors may be critical in helping athletes learn to manage stress and overcome obstacles during practice and competition.

As figure skating continues to gain popularity, a corresponding increase in athlete participation is likely. Involving more skaters in future studies is critical to increasing the power of analyses since many of the discussed studies have limited enrollments. Additional studies are needed to demonstrate the long-term impact of figure skating on the mental health of the general population and neurodivergent individuals. Furthermore, studies involving the comparative analyses of figure skating with other sports would provide important novel insights into how balance training shapes neuroplasticity. Society should encourage the support of figure skating programs to be used as a way to improve motor skills and balance among diverse individuals as well as increase awareness of the benefits of balance training such as figure skating.

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■ References

1. Cheng, D. Breaking the Ice: Figure Skating. *Handbook of the Mathematics of the Arts and Sciences* **2019**, 1-45.
2. Hummel, N.; Hufner, K.; Stephan, T.; Linn, J.; Kremmyda, O.; Brandt, T.; Flanagan, V. L. Vestibular loss and balance training cause similar changes in human cerebral white matter fractional anisotropy. *PLoS One* **2014**, 9 (4), e95666. DOI: 10.1371/journal.pone.0095666 From NLM Medline.
3. Tanguy, S.; Quarck, G.; Etard, O.; Gauthier, A.; Denise, P. Vestibulo-ocular reflex and motion sickness in figure skaters. *Eur J Appl Physiol* **2008**, 104 (6), 1031-1037. DOI: 10.1007/s00421-008-0859-7 From NLM Medline.
4. Park, I. S.; Lee, N. J.; Kim, T. Y.; Park, J. H.; Won, Y. M.; Jung, Y. J.; Yoon, J. H.; Rhyu, I. J. Volumetric analysis of cerebellum in short-track speed skating players. *Cerebellum* **2012**, 11 (4), 925-930. DOI: 10.1007/s12311-012-0366-6.
5. Park, I. S.; Yoon, J. H.; Kim, N.; Rhyu, I. J. Regional cerebellar volume reflects static balance in elite female short-track speed skaters. *Int J Sports Med* **2013**, 34 (5), 465-470. DOI: 10.1055/s-0032-1327649.
6. Nakata, H.; Yoshie, M.; Miura, A.; Kudo, K. Characteristics of the athletes' brain: evidence from neurophysiology and neuroimaging. *Brain Res Rev* **2010**, 62 (2), 197-211. DOI: 10.1016/j.brainresrev.2009.11.006 From NLM Medline.
7. Zhang, K.; Liu, Y.; Liu, J.; Liu, R.; Cao, C. Detecting structural and functional neuroplasticity in elite ice-skating athletes. *Hum Mov Sci* **2021**, 78, 102795. DOI: 10.1016/j.humov.2021.102795 From NLM Medline.
8. Vaalto, S.; Julkunen, P.; Saisanen, L.; Kononen, M.; Maatta, S.; Karhu, J. Long-term plasticity may be manifested as reduction or expansion of cortical representations of actively used muscles in motor skill specialists. *Neuroreport* **2013**, 24 (11), 596-600. DOI: 10.1097/WNR.0b013e3283628636 From NLM Medline.
9. Adkins, D. L.; Boychuk, J.; Remple, M. S.; Kleim, J. A. Motor training induces experience-specific patterns of plasticity across motor cortex and spinal cord. *J Appl Physiol (1985)* **2006**, 101 (6), 1776-1782. DOI: 10.1152/jappphysiol.00515.2006 From NLM Medline.
10. Remple, M. S.; Bruneau, R. M.; VandenBerg, P. M.; Goertzen, C.; Kleim, J. A. Sensitivity of cortical movement representations to motor experience: evidence that skill learning but not strength training induces cortical reorganization. *Behav Brain Res* **2001**, 123 (2), 133-141. DOI: 10.1016/s0166-4328(01)00199-1.
11. Kleim, J. A.; Cooper, N. R.; VandenBerg, P. M. Exercise induces angiogenesis but does not alter movement representations with in rat motor cortex. *Brain Res* **2002**, 934 (1), 1-6. DOI: 10.1016/s0006-8993(02)02239-4.
12. Kleim, J. A.; Barbay, S.; Nudo, R. J. Functional reorganization of the rat motor cortex following motor skill learning. *J Neurophysiol* **1998**, 80 (6), 3321-3325. DOI: 10.1152/jn.1998.80.6.3321.
13. Nudo, R. J.; Milliken, G. W.; Jenkins, W. M.; Merzenich, M. M. Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *J Neurosci* **1996**, 16 (2), 785-807.
14. Pascual-Leone, A.; Nguyet, D.; Cohen, L. G.; Brasil-Neto, J. P.; Cammarota, A.; Hallett, M. Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. *J Neurophysiol* **1995**, 74 (3), 1037-1045. DOI: 10.1152/jn.1995.74.3.1037.
15. Perez, M. A.; Lugholt, B. K.; Nyborg, K.; Nielsen, J. B. Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. *Exp Brain Res* **2004**, 159 (2), 197-205. DOI: 10.1007/s00221-004-1947-5.
16. Svensson, P.; Romaniello, A.; Arendt-Nielsen, L.; Sessle, B. J. Plasticity in corticomotor control of the human tongue musculature induced by tongue-task training. *Exp Brain Res* **2003**, 152 (1), 42-51. DOI: 10.1007/s00221-003-1517-2.
17. Tyc, F.; Boyadjian, A.; Devanne, H. Motor cortex plasticity induced by extensive training revealed by transcranial magnetic stimulation in human. *Eur J Neurosci* **2005**, 21 (1), 259-266. DOI: 10.1111/j.1460-9568.2004.03835.x.
18. Hommel, B.; Musseler, J.; Aschersleben, G.; Prinz, W. The Theory of Event Coding (TEC): a framework for perception and action planning. *Behav Brain Sci* **2001**, 24 (5), 849-878; discussion 878-937. DOI: 10.1017/s0140525x01000103.
19. Abreu, A. M.; Macaluso, E.; Azevedo, R. T.; Cesari, P.; Urgesi, C.; Aglioti, S. M. Action anticipation beyond the action observation network: a functional magnetic resonance imaging study in expert basketball players. *Eur J Neurosci* **2012**, 35 (10), 1646-1654. DOI: 10.1111/j.1460-9568.2012.08104.x.
20. Sunderland, A. Action, perception and postural planning when reaching for tools. *Exp Brain Res* **2013**, 227 (2), 211-222. DOI: 10.1007/s00221-013-3501-9.
21. Diersch, N.; Cross, E. S.; Stadler, W.; Schutz-Bosbach, S.; Rieger, M. Representing others' actions: the role of expertise in the aging mind. *Psychol Res* **2012**, 76 (4), 525-541. DOI: 10.1007/s00426-011-0404-x From NLM Medline.
22. Kilroy, E.; Cermak, S. A.; Aziz-Zadeh, L. A Review of Functional and Structural Neurobiology of the Action Observation Network in Autism Spectrum Disorder and Developmental Coordination Disorder. *Brain Sci* **2019**, 9 (4). DOI: 10.3390/brainsci9040075 From NLM PubMed-not-MEDLINE.
23. Diersch, N.; Mueller, K.; Cross, E. S.; Stadler, W.; Rieger, M.; Schutz-Bosbach, S. Action prediction in younger versus older adults: neural correlates of motor familiarity. *PLoS One* **2013**, 8 (5), e64195. DOI: 10.1371/journal.pone.0064195 From NLM Medline.
24. Hufner, K.; Binetti, C.; Hamilton, D. A.; Stephan, T.; Flanagan, V. L.; Linn, J.; Labudda, K.; Markowitsch, H.; Glasauer, S.; Jahn, K.; et al. Structural and functional plasticity of the hippocampal formation in professional dancers and slackliners. *Hippocampus* **2011**, 21 (8), 855-865. DOI: 10.1002/hipo.20801 From NLM Medline.
25. Hanggi, J.; Koeneke, S.; Bezzola, L.; Jancke, L. Structural neuroplasticity in the sensorimotor network of professional female ballet dancers. *Hum Brain Mapp* **2010**, 31 (8), 1196-1206. DOI: 10.1002/hbm.20928 From NLM Medline.
26. Di Paola, M.; Caltagirone, C.; Petrosini, L. Prolonged rock climbing activity induces structural changes in cerebellum and parietal lobe. *Hum Brain Mapp* **2013**, 34 (10), 2707-2714. DOI: 10.1002/hbm.22095.
27. Khan, S.; Chang, R. Anatomy of the vestibular system: a review. *NeuroRehabilitation* **2013**, 32 (3), 437-443. DOI: 10.3233/NRE-130866 From NLM Medline.
28. Alpini, D.; Botta, M.; Mattei, V.; Tornese, D. Figure ice skating induces vestibulo-ocular adaptation specific to required athletic skills. *Sport Sciences for Health* **2010**, 5, 129-134.
29. Blair, S.; Gavin, M. Response of the vestibulo-ocular reflex to differing programs of acceleration. *Invest Ophthalmol Vis Sci* **1979**, 18 (10), 1086-1090.
30. Jager, J.; Henn, V. Habituation of the vestibulo-ocular reflex (VOR) in the monkey during sinusoidal rotation in the dark. *Exp Brain Res* **1981**, 41 (2), 108-114. DOI: 10.1007/BF00236599.
31. G, C.; JM, E.; JH, C. Comparison between habituation of the cat vestibulo-ocular reflex by velocity steps and sinusoidal vestibular stimulation in the dark. *Experimental Brain Research* **2002**, 142, 259-267.
32. Ionescu, A.; Badau, D. Student Perceptions of Motor, Mental and Social Benefits and the Impact of Practicing Recreational Figure

- e Skating. *Behav Sci (Basel)* **2018**, 8 (12). DOI: 10.3390/bs8120110 From NLM PubMed-not-MEDLINE.
36. Casey, A. F.; Quenneville-Himbeault, G.; Normore, A.; Davis, H.; Martell, S. G. A therapeutic skating intervention for children with autism spectrum disorder. *Pediatr Phys Ther* **2015**, 27 (2), 170-177. DOI: 10.1097/PEP.000000000000139 From NLM Medline.
 37. Fragala-Pinkham, M. A.; Dumas, H. M.; Boyce, M.; Peters, C. Y.; Haley, S. M. Evaluation of an adaptive ice skating programme for children with disabilities. *Dev Neurorehabil* **2009**, 12 (4), 215-223. DOI: 10.1080/17518420902980100 From NLM Medline.
 38. Mumford, B.; Hall, C. The effects of internal and external imagery on performing figures in figure skating. *Can J Appl Sport Sci* **1985**, 10 (4), 171-177.
 39. Han, J. S.; Geminiani, E. T.; Micheli, L. J. Epidemiology of Figure Skating Injuries: A Review of the Literature. *Sports Health* **2018**, 10 (6), 532-537. DOI: 10.1177/1941738118774769 From NLM Medline.
 40. Brock, R. M.; Striowski, C. C. Injuries in Elite Figure Skaters. *Phys Sportsmed* **1986**, 14 (1), 111-115. DOI: 10.1080/00913847.1986.11708969.
 41. Kjaer, M.; Larsson, B. Physiological profile and incidence of injuries among elite figure skaters. *J Sports Sci* **1992**, 10 (1), 29-36. DOI: 10.1080/02640419208729904.
 42. Porter, E. B.; Young, C. C.; Niedfeldt, M. W.; Gottschlich, L. M. Sport-specific injuries and medical problems of figure skaters. *WJ* **2007**, 106 (6), 330-334.
 43. Dana K. Voelker, D., Justine J. Reel. Prevalence and correlates of disordered eating in female figure skaters. *Psychology of Sport and Exercise* **2014**, 15 (6), 14.
 44. Oleson, C. V.; Busconi, B. D.; Baran, D. T. Bone density in competitive figure skaters. *Arch Phys Med Rehabil* **2002**, 83 (1), 122-128. DOI: 10.1053/apmr.2002.26246.
 45. Knox, C. L.; Comstock, R. D.; McGeehan, J.; Smith, G. A. Differences in the risk associated with head injury for pediatric ice skaters, roller skaters, and in-line skaters. *Pediatrics* **2006**, 118 (2), 549-554. DOI: 10.1542/peds.2005-2913 From NLM Medline.
 46. Dubravcic-Simunjak, S.; Kuipers, H.; Moran, J.; Simunjak, B.; Pecina, M. Injuries in synchronized skating. *Int J Sports Med* **2006**, 27 (6), 493-499. DOI: 10.1055/s-2005-865816 From NLM Medline.

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Analysis of the Application of Chlorophylls and Anthocyanins in Food Coloring

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ABSTRACT: The solubility of pigments is of significant importance for the successful coloring of various products in the food industry. In this work, the solubility of anthocyanins and chlorophylls was analyzed by thin-layer chromatography. Plant pigments were applied to filter paper and placed in mixtures of hydrophilic, ethanol, methanol, hydrophobic solvents, heptane, chloroform, and petroleum ether. In addition, solutions of different pH were used to examine how pH changes the color of the plant pigments. By adding small amounts of a strong acid (hydrochloric acid), a strong base (sodium hydroxide), weak acids (citric acid and acetic acid), and salts (sodium hydrogen carbonate and sodium hypochlorite) to the pigment extracts, a palette of various colors in the pigments was observed. Based on the solubility data and the results of the color change in the pigments, anthocyanins were successfully used as a food color in a food product with acidic pH, lemonade, and a food product with basic pH, cookies.

KEYWORDS: Biochemistry, analytical biochemistry, anthocyanins, chlorophylls, food coloring, thin-layer chromatography.

Introduction

In the last decade, the use of food colors in the food industry has become increasingly prevalent. More and more artificial food colors have been included on the ingredient lists of food products in the interest of marketability and consumer attraction.¹ Moreover, the presence of such food colors negatively impacts human health, causing learning disabilities, kidney and liver damage, skin rashes, asthma, allergies, sleep disturbance, and tumors.² That is why alternatives to artificial food colorants should be sought.

Natural plant pigments, like anthocyanins and chlorophylls, may be used as a substitute for harmful food colors.³ Anthocyanins are water-soluble plant pigments in many plants, such as berries, beetroot, red cabbage, and purple potato.⁴ They are widely known for their health benefits: lowering chances of obesity, cardiovascular diseases, and inflammation.³ Anthocyanins' molecular structure consists of two benzene rings connected to a pyrylium ring.⁴ According to the position of the methoxy and hydroxyl group, anthocyanins appear in different colors and may be divided into six groups: pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin (Figure 1).

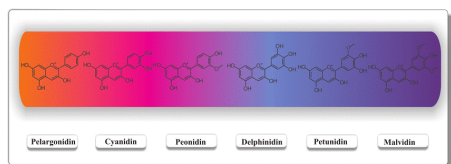


Figure 1: Structure of Anthocyanins.

In the presence of an alkaline solution, the polar covalent bond between the hydrogen and the oxygen atom may be easily broken, i.e., the anthocyanins may be deprotonated.⁵ In the presence of an acidic solution with a high proton concentration, however, anthocyanins may be protonated due to the positive

charge of the oxygen atom in the pyrylium ring, which is unstable and seeks an electron donor.⁵ As a result, anthocyanins' color changes to red, blue, or yellow-greenish, based on the pH of the environment they are placed in.⁴

Another vital plant pigment is chlorophyll, which, however, is insoluble in water. It enables the absorption of light by the plants and, eventually, helps transform the light energy into chemical energy, i.e., ATP synthesis, during photosynthesis.⁶ Chlorophyll is the most abundant green plant pigment, which consists of a porphyrin ring bound to magnesium.⁷ According to the side chain of the seventh carbon atom in the second pyrrole, whether it is a formyl group or a methyl group, two types of chlorophyll are distinguished: *chlorophyll A* and *chlorophyll B*.² (Figure 2)

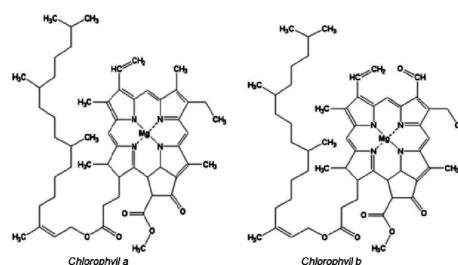


Figure 2: Structure of *Chlorophyll A* and *Chlorophyll B*.

Chemicals induce changes in the structure of the chlorophyll molecule due to the initiation of pheophytinization, epimerization, pyrolysis, hydroxylation, or oxidation.⁸ When placed in acidic conditions, chlorophyll loses its green color and turns greenish-brown due to its transformation into pheophytin.⁹ In contrast, when chlorophyll is mixed with alkaline solutions, its color changes to bright green due to chlorophyll's transformation to chlorophyllin, a water-soluble compound.¹⁰ (Figure 3)

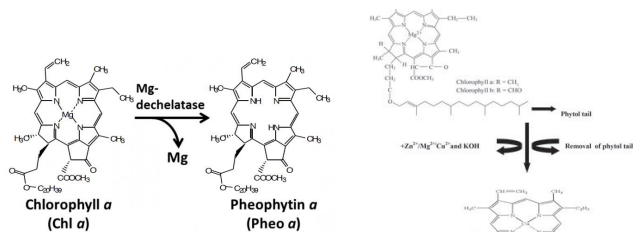


Figure 3: Chlorophylls structural change in different pH.

One of the purposes of this report is to explore chlorophylls and anthocyanins' applicability for food coloring based on their solubility. Chlorophylls and anthocyanins are isolated through thin-layer chromatography (TLC). This method is used to separate and identify different biomolecules with low molecular weight.¹¹ Two phases are used in TLC: stationary and mobile phase.¹¹ The stationary phase in this experiment is the strip of filter paper, on which a mixture of anthocyanins and chlorophylls is applied. It is dipped in the so-called mobile phase, a mixture of two organic solvents. The mobile phase carries the pigments up the filter paper. Depending on the solubility of the pigments in the individual components of the organic mixture, they travel at different rates up the filter paper and reach different endpoints.¹² Thus, they separate from each other. The higher point a pigment reaches, the more soluble in the most mobile component of the solvent it is.¹² The ratio between the distance traveled by the pigment and the distance traveled by the solvent is called a retention factor, R_f .¹² (Figure 4)

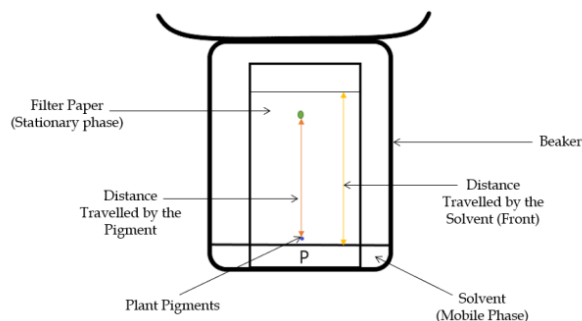


Figure 4: A Chromatography Plate.

Once the solubility of anthocyanins and chlorophyll in organic mixtures was determined, solvents of different pH were used to examine how pH changes the color of the plant pigments. Anthocyanins are anticipated to turn yellow in stronger alkaline solutions, blue in weaker alkaline solutions, and red in acidic solutions, while chlorophyll is expected to turn bright green in alkaline solutions and greenish-brown in acidic solutions.⁹

The report is organized as follows. In section III, the solubility of chlorophyll and anthocyanins in different organic mixtures is determined. In section IV, the change in the color of anthocyanins and chlorophyll is examined. In Section V, the results obtained in Sections III and IV are used to test the viability of anthocyanins in food coloring. In Section VI, all the data are discussed, and the initial hypothesis is confirmed. In section VII, an interdisciplinary connection between Chemistry and Biology is made. Section VIII presents a conclusion

about the entire experiment. In Appendix A, a chromatogram of a different stationary phase, silica gel, is presented.

Research Question: How may the plant pigments (anthocyanins and chlorophylls) be used as food colors based on their solubility in hydrophilic and hydrophobic solvents, determined by thin-layer chromatography, and their color change in solutions of different pH [pH = 0, 2, 3, 10, 11, 14]?

Measurement of the Solubility of Chlorophylls and Anthocyanins in Mixtures of Organic Solvents:

Materials used:

A number of materials were used for the determination of chlorophylls and anthocyanins' solubility (Table 1).

Table 1: Materials necessary for the conduction of the first part of the experiment and their purposes.

Material	Purpose
Beaker	A place to submerge the filter paper into organic solvents
Porcelain mortars with pestles	A place to crush the plants
Ethanol, heptane, chloroform, methanol, petroleum ether	Organic solvents used to determine the solubility of chlorophyll and anthocyanins
Filter paper	A place to lay the plant pigments
Quartz sand	Used for the extraction of chlorophyll from the plants
Spinach, kale, celery, lettuce, red cabbage, and beetroot	Plants used for extraction of chlorophyll and anthocyanins

Experimental Procedure:

Step 1: Preparation of plant pigment extracts

To obtain a chlorophyll extract, 10 g spinach leaves, 10 g kale, 10 g celery, and 10 g lettuce leaves were crushed in a porcelain mortar. For an easier breakdown of the plants, 15 g quartz sand was used. The obtained blend was mixed with 100 mL of 70% ethanol.

To extract anthocyanins, 20 g of red cabbage and 20 g of beetroot were mixed with 100 mL of water in two separate beakers.

Step 2: Preparation of a mixture of the plant pigments

In a beaker, 4 mL of the chlorophyll extract and 4 mL of each anthocyanins extract were placed. The mixture was stirred for a homogenate to be obtained. The purpose is to separate the chlorophylls from the anthocyanins using TLC.

Step 3: Applying the plant extracts to strips of filter paper

On six strips of filter paper, at the center, 0.5 cm from the bottom of the strip, eight drops of the pigment mixture from Step 2 were placed using a capillary tube.

Step 4: Separation of pigments in organic mixtures by TLC

In five beakers, different combinations of organic solvents were poured. Each strip of paper was placed in a beaker. A separation of the pigments is observed for 10 minutes (Figure 5).

Organic Solvents					
Ethanol	Ethanol: petroleum ether = 1:1	Ethanol: methanol = 1:1	Ethanol: heptane = 1:1	Ethanol: Chloroform = 1:1	Heptane: chloroform: petroleum ether: ethanol = 1:1:1:1

Figure 5: Separation of chlorophylls from anthocyanins in organic mixtures.

Step 5: Determination of retention factor of anthocyanins and chlorophyll

Finally, the distance traveled by each pigment on the filter paper was noted. The data is given in Table 2.

Table 2: Determination of retention factor for the chlorophyll and the anthocyanins.

Organic solvent	Initial point of the solvent s_i	Final point of the solvent s_f	Distance traveled by the solvent Δs	Initial point of the pigments c_i	Final point of chl. c_f	Distance traveled by chl. Δc	Final point of anth. a_f	Distance traveled by anth. Δa	Retention factor of chl. R_{fc}	Retention factor of anth. R_{fa}
Ethanol	0.3	4.5	4.2	0.5	1.7	1.2	3.7	3.2	0.29	0.8
Ethanol & petroleum ether	0.4	4	3.6	0.5	3.4	2.9	1.7	1.2	0.81	0.3
Ethanol & methanol	0.3	4.6	4.3	0.5	4	3.5	4.4	3.9	0.81	0.9
Ethanol & heptane	0.6	5	4.4	0.5	4.4	3.9	1.5	1	0.89	0.2
Ethanol & Chloroform	0.2	5	4.8	0.5	2.6	2.1	3.9	3.4	0.44	0.7
Heptane, chloroform, petroleum ether & ethanol	0.4	5.4	5	0.5	5	4.5	1.1	0.6	0.9	0.1

First, the distance traveled by the solvent Δs is determined by the formula:

$$\Delta s = s_f - s_i, \quad (1)$$

where s_i is the initial point of the solvent measured at the level the filter paper was dipped in the solvent ($t = 0$ sec), and s_f is the final point the solvent reached on the filter paper ($t = 10$ min).

Second, the distance traveled by chlorophyll Δc was determined by the formula:

$$\Delta c = c_f - c_i, \quad (2)$$

where c_i is the initial point at which the pigments' mixture was applied to the filter paper, and c_f is the final point the chlorophyll pigment reached ($t = 10$ min).

Third, the distance traveled by anthocyanins was determined by the formula:

$$\Delta a = a_f - c_i, \quad (3)$$

where a_f is the final point anthocyanins reached ($t = 10$ min).

In order for the solubility of each pigment in each organic mixture to be determined, the retention factor of chlorophylls is found by the formula:

$$R_{fc} = \frac{\Delta c}{\Delta s}, \quad (4)$$

where Δc and Δs are given by Eq. (2) and (1), while the retention factor of anthocyanins is:

$$R_{fa} = \frac{\Delta a}{\Delta s}, \quad (5)$$

Where Δa and Δs are given in Eq. (3) and (1).

The greater the value of the retention factor of the plant pigment is (i.e., the higher the pigment traveled up the strip of filter paper), the more soluble the pigment is in the particular component of the mixture (Table 2).

Measurement of the Change of the Color of Chlorophyll and Anthocyanins in Solutions of Different pH:

Materials used:

For the conduction of the second part of the experiment, a number of materials were used (Table 3).

Table 3: Materials necessary for the conduction of the second part of the experiment and their purposes.

Materials	Methods
Distilled water	Used for the extraction of anthocyanins
Test tubes	A place to mix plant pigments with solutions of different pH
Hydrochloric acid, citric acid, acetic acid, sodium hydrogen carbonate, sodium hypochlorite, sodium hydroxide	Solutions used to measure the change in the color of chlorophyll and anthocyanins in different pH

Step 1: Preparation of pigment extract

To obtain a chlorophyll extract, 10 g spinach leaves, 10 g kale, 10 g celery, and 10 g lettuce leaves were crushed in a porcelain mortar. For an easier breakdown of the plants, 15 g quartz sand was used. The obtained mixture was combined with 100 mL ethanol.

To extract anthocyanins, 20 g of red cabbage and 20 g of beetroot were mixed with 100 mL of water in two separate beakers.

Step 2: Preparation of solutions in a different range of pH

1 M hydrochloric acid, citric acid, acetic acid, sodium hydrogen carbonate, sodium hypochlorite, and sodium hydroxide were prepared (Table 4).

Table 4: pH of solutions used to test the color change of the pigments.

	HCl	CH ₃ COOH	Citric Acid	NaHCO ₃	Bleach - NaClO	NaOH
Concentration [M]	1.00	1.00	1.00	1.00	1.00	1.00
Molar Mass [g/mol]	36.46	60.05	192.12	84.01	74.44	40.00
Mass [grams]			9.61	4.20		2.00
Moles [mol]	0.99	1.00	0.05	0.05	0.08	0.05
Final Volume [L]	0.05	0.05	0.05	0.05	0.05	0.05
Volume of solution [mL]	83.33	94.34			90.09	
Initial molar concentration [M]	11.85	10.59			0.89	
Mass percentage	0.36	0.60			0.06	
Mass solution [g]	100.00	100.00			100.00	
Density [g/ml]	1.20	1.06			1.11	
Ka		1.8×10^{-5}	4.1×10^{-7}			
Kb				2.3×10^{-8}	8.5×10^{-7}	
pH	0.00	2.38	2.70	10.18	10.97	14.00

2.1.: Determination of the volume of water necessary to obtain 1 M solution using a solid sample of acid, base, or salt

Since citric acid, sodium hydrogen carbonate, and sodium hydroxide were solids, the mass of each compound necessary to obtain a 50 mL, 1 M solution should be determined. First, the necessary number of moles of each compound is given by:

$$n = cV \quad (6)$$

where V is the volume of the solution ($V = 50$ mL) and c is the required concentration ($c = 1$ M). Then, the number of moles is converted to mass by

$$m = nM \quad (7)$$

where M is the molar mass of the compound. Therefore, (combining both Eq. (6) and (7)) the required mass is given by:

$$m = cMV \quad (8)$$

Different values of mass m are obtained for the different solutions (Table 4).

2.2.: Determination of the mass of the acid necessary to obtain 1 M solution using a sample of aqueous acids.

Sine the hydrochloric acid, acetic acid, and bleach were in an aqueous form, the volume of water needed to be added to each solution to obtain a 50 mL, 1 M solution was found. For this purpose, the percentage concentration of each solution is

converted to molar concentration. From the known percentage concentration, the mass of the pure compound was found

$$m_{\text{compound}} = \frac{w_{\text{solution}} m_{\text{solution}}}{100} \quad (9)$$

where w_{solution} is the percentage concentration of each solution and $m_{\text{solution}}=100\text{g}$. Next, the mass of the compound was converted to moles:

$$n_{\text{compound}} = \frac{m_{\text{compound}}}{M_{\text{compound}}} \quad (10)$$

where M is the molar mass of the acid. Therefore, (combining both Eq. (9) and (10)) the required number of moles is given by:

$$n_{\text{compound}} = \frac{w_{\text{solution}} m_{\text{solution}}}{M_{\text{compound}} \times 100}, \quad (11)$$

The volume of the solution is found by the formula:

$$V_{\text{solution}} = \frac{m_{\text{solution}}}{\rho_{\text{solution}}} \quad (12)$$

where ρ_{solution} is the density of the solution. After the volume of the solution is obtained, the molar concentration of the solution is found by the formula:

$$c = \frac{n_{\text{compound}}}{V_{\text{solution}}} \quad (13)$$

Since the molar concentration of the initial solution is found, the law of solubility may be applied:

$$c_1 V_1 = c_2 V_2 \quad (14)$$

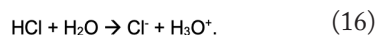
where c_1 is the initial concentration of the solution, V_1 is the initial volume of the solution, c_2 is the final concentration of the solution ($c=1\text{M}$), and V_2 is the final volume of the newly obtained solution ($V_2=50\text{mL}$). To find the necessary amount of volume needed to obtain a 1M solution, equation (14) was transformed into:

$$V_1 = \frac{c_2 V_2}{c_1} \quad (15)$$

Different values of volume V_1 are obtained for the other solutions (Table 4).

2.4.: Determination of pH of the strong acid

The pH of hydrochloric acid was found by determining the concentration of hydroxonium ions in the solution:



Since the ratio between the hydrochloric acid and hydroxonium ions in the balanced chemical equation is 1:1, then the concentration of HCl determines the pH of the solution. More precisely, the pH of the solution is determined by the formula:

$$\text{pH} = -\lg [\text{H}_3\text{O}^+], \quad (17)$$

where $[\text{H}_3\text{O}^+]$ is the concentration of H_3O^+ in the solution.

2.4.: Determination of pH of NaOH

NaOH dissociates irreversibly in water:



Using the concentration of OH^- , which is equal to the concentration NaOH as seen in Eq. 15, the pOH is determined:

$$\text{pOH} = -\lg [\text{OH}^-], \quad (19)$$

where $[\text{OH}^-]$ is the concentration of OH^- . The pH of NaOH is found using the relationship:

$$\text{pOH} + \text{pH} = 14. \quad (20)$$

2.5.: Determination of pH of weak acids

Since citric and acetic acids are weak acids, their dissociation is reversible, and the above formulas for determining pH are not applicable. That is why their pH is determined by the formula:

$$\text{pH} = \sqrt{K_a [\text{initial concentration}]}, \quad (21)$$

where the coefficient K_a is given in Table 4.

2.6.: Determination of pH of salts

Sodium hydrogen carbonate and sodium hypochlorite are basic salt products of reactions between the strong base NaOH and, respectively, the weak acids, H_2CO_3 and HClO . Their pOH is determined by using the formula:

$$\text{pOH} = \sqrt{K_b [\text{initial concentration}]}, \quad (22)$$

where the coefficient K_b is given in Table 4.

The pH of each of the salts is determined by using Eq. (17).

Step 3: Mixture of plant pigment extracts with solutions of different pH

The prepared solutions in step 2 were distributed into 18 test tubes, six containing 5 mL of anthocyanins extract from red cabbage, another six containing 4 mL of anthocyanins extract from beetroot, and the last six, 4 mL of chlorophyll extract. 5 mL of 1M acid, base, or salt solution was added to each test tube. The changes in the color were recorded (Figure 6).

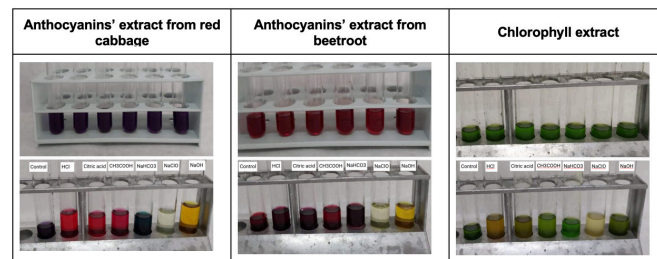


Figure 6: Changes in the color of plants' pigments as a result of changes in the pH.

Effect of Plant Pigments on the Food Industry:

To test the viability of anthocyanins in food coloring, pink lemonade was prepared with anthocyanin extract from red cabbage. 100 mL of lemon juice was mixed with 100 mL of sparkling water and 50 mL of still water. The mixture was stirred. Anthocyanin extract from 30 g red cabbage was prepared in 100 mL of still water. 50 mL of the anthocyanin extract was added to the mixture. 20 g of honey was added to the lemonade for a sweeter taste.

Moreover, the coloring effect of the red cabbage's anthocyanins was tested on cookies. Two egg whites were mixed with 2 mL of sweetener. Then 20 mL of anthocyanins extract was added. Cookies were made from the dough and baked for 10 min at 150 °C. The mixture turned greenish-blue due to the alkaline environment provided by the egg white (Figure 7). In

the interest of consumer attraction, the cookies were filled with whipping cream.

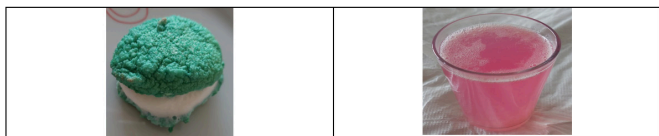


Figure 7: Cookies and Lemonade

■ Discussion

When the R_f values of anthocyanins and chlorophylls were compared, it was concluded that chlorophylls have the highest R_{fc} in the mixture of the solvents: heptane, chloroform, and petroleum ether ($R_{fc} = 0.90$), while anthocyanins have the highest R_{fa} in the mixture of ethanol and methanol ($R_{fa} = 0.9$) (Table 2). The results show that chlorophylls have traveled longer distances in hydrophobic solvents, while the anthocyanins have traveled longer distances in hydrophilic solvents. The data can be explained by one of the solubility rules that “like dissolves like,” since chlorophylls are hydrophilic molecules, while anthocyanins are hydrophobic molecules. The obtained results from the first part of the experiment show that chlorophylls are to be added to hydrophobic solvents since their solubility is greater. In contrast, anthocyanins are added to hydrophilic solvents.

Moreover, different colors are produced depending on whether the pigments are placed in alkaline or acidic solutions (Figure 6). When hydrochloric acid, citric acid, and acetic acid are added to chlorophyll, the expected results of greenish-brown are produced (Figure 6). The higher the pH of the acid, the more saturated the color is. The initial green color becomes brighter in the basic salt, NaHCO_3 , and even brighter in the strong base, NaOH (Figure 6).

A greater variety of colors is produced in the anthocyanin extracts when mixed with solutions of different pH. In acidic solutions, the extracts from red cabbage and beetroot show different nuances of red due to the presence of different types of anthocyanins in them. The red cabbage appears purple due to the predominant amount of cyanidin and peonidin, while the beetroot appears red due to a higher amount of pelargonidin.¹³

As the pH increases, the color of the red cabbage extract becomes lighter. In weak basic solutions (pH=10), the extract becomes bluish, and in highly alkaline solutions (pH =14), the color becomes yellowish (Figure 6). However, due to the different types of anthocyanins, beetroot contains, a change from red to yellowish is observed when NaOH is added (pH =14).

The results from the experiment's second part show that chlorophylls are appropriate to be used as food colors in basic solutions for a pleasant bright green color to be obtained. Anthocyanin extract from beetroot is to be used in both acidic and basic foods for red food coloring to be observed. The red cabbage's anthocyanins may be used for a wider variety of colors since different colors are obtained in acidic and basic pH solutions. Yellow food coloring could not be achieved using anthocyanins since a strong alkaline solution (pH =14) is needed; such pH does not exist in food products.

The addition of NaClO turned not only the chlorophyll solution colorless but also the two extracts of anthocyanins. The induced may be explained by the bleaching action of the atomic oxygen released during the decomposition of NaClO (Eq. 20).



Due to the hydrophilic properties of the red cabbage anthocyanins and their wide color change, an extract from red cabbage was used as a food color in the preparation of lemonade and cookies. The acidity of the lemonade led to a pink color change, which confirmed the results obtained in the second part of the experiment that anthocyanins turn red in an acidic environment (Figure 6). The basic environment of the cookies turned the anthocyanins greenish-blue, which also was in excellent agreement with the obtained results in the second part of the experiment (Figure 6). Thus, the viability of plant pigments as food colors was confirmed.

The great variety of colors and nuances the pigment produces when placed in different pH may find multiple applications in the food industry as a replacement for artificial colors, which will be both environmentally friendly and a healthier option for the people.

■ Conclusion

The rule of solubility (“like dissolves like”) is confirmed quite well in the measurements, as seen in the values of R_{fc} and R_{fa} . In hydrophilic solvents, the polar molecules of anthocyanins travel at a higher rate than the less polar molecules of chlorophyll and reach a higher endpoint on the filter paper. In hydrophobic solvents, the less polar molecules of chlorophyll travel faster and reach a higher point. The greater the solubility of the pigments in the solvent component, the higher the values of R_f are. It was proven that hydrophilic molecules dissolve in hydrophilic solvents and vice versa. The obtained results in Table 2 are essential for the future use of plant pigments in food coloring since the viability of the pigments as food colors depends on the solubility of the pigment in the food.

As expected, various colors are produced when bases and acids of different pH are used (Figure 6). This allows a greater variety of colors to be obtained and the plant pigments to be used as food colors. The production of pink lemonade and greenish-blue cookies confirmed the hypothesis that plant pigments may be used to dye food.

The future application of plant pigments in the food industry would be essential for environmental protection and would open new opportunities for using biomolecules in food.

■ References

1. Potera, C. The artificial food Dye Blues <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2957945/>.
2. Grumezescu, A. M.; Holban, A. M. Natural and Artificial Flavoring Agents and Food Dyes; Academic Press, 2017.
3. Alappat, B.; Alappat, J. Anthocyanin pigments: Beyond aesthetics <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7727665/>.
4. Khoo, H. E.; Azlan, A.; Tang, S. T.; Lim, S. M. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5613902/#:~:text=Anthocyanins%20are%20colored%20water%2Dsoluble,fruits%20have%20high%20ant>

- hocyans%20content.
5. McMurry, J. Organic Chemistry; 9th ed.; Brooks/Cole Pub, 2016.
 6. Reece, J.; Urry, L. Campbell Biology; 11th ed.; Pearson, 2017.
 7. Grimm, B. Chlorophyll: Structure and Function. Dissertation, ENCYCLOPEDIA OF LIFE SCIENCES, 2001.
 8. Mangos, T. J.; Berger, R. G. Determination of Major Chlorophyll Degradation Products. Zeitschrift für Lebensmitteluntersuchung und -Forschung A 1997, 204, 345–350.
 9. Koca, N.; Karadeniz, F. Effect of PH on Chlorophyll Degradation and Colour Loss in Blanched Green Peas. Dissertation, 2010.
 10. Del Pozo, M.; Guerrero, L. Lab report # 1_ Fruits and Vegetables .pdf - shem Cheng 920768313 HTM 301 lab report # 1: Fruits and vegetables 9/14/2021 in this lab report, the effects: Course hero <https://www.coursehero.com/file/p2skvhfg/Influence-of-alkaline-treatment-on-structural-modifications-of-chlorophyll/>.
 11. Bele, A. A. An overview on thin layer chromatography: International Journal of Pharmaceutical Sciences and Research <http://ijpsr.com/bft-article/an-overview-on-thin-layer-chromatography/?view=fulltext#:~:text=The%20principle%20of%20TLC%20is,moving%20over%20the%20solid%20phase.>
 12. Coskun, O. Separation techniques: Chromatography <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5206469/>.
 13. Fang, S.; Lin, F.; Qu, D.; Liang, X.; Wang, L. Characterization of purified red cabbage anthocyanins: Improvement in HPLC separation and protective effect against h₂O₂-induced oxidative stress in hepg2 cells <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6337153/>.
 14. Liu, Y.; Feng, X.; Zhang, Y.; Zhou, F.; Zhu, P. Simultaneous changes in anthocyanin, chlorophyll, and carotenoid contents produce green variegation in pink-leaved ornamental kale <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8212504/#CR3>.

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Distributed Glaucoma Detection Method Using Privacy-Preserving Federated Learning

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ABSTRACT: With over 200,000 US cases per year, glaucoma is the second most common cause of blindness worldwide. Showing no symptoms till the later stages, glaucoma is one of the most challenging conditions to diagnose. Currently, glaucoma is diagnosed through a dilated eye exam, which is both inefficient and prone to error. Therefore, this paper proposes a new, efficient method to diagnose glaucoma through machine learning. However, machine learning generally requires access to users' biomedical data being stored in the centralized server for training purposes, which creates many concerns regarding security and privacy for users. Since the security and privacy of users are essential, especially in a medical environment, the proposed approach is a distributed learning method for glaucoma detection in federated learning where data is decentralized. With a dataset of nearly 3000 retinal images, the federated learning system displays a detection accuracy of around 88% with an optic disc/optic cup segmentation dice score of around 0.987. This system should help to improve the detection accuracy of glaucoma while also maintaining user privacy.

KEYWORDS: Computational Biology and Bioinformatics, Glaucoma detection, Federated learning, U-Net, Machine Learning.

■ Introduction

Glaucoma is a progressive and chronic eye condition that damages the optic nerve, which sends visual information from the eye to the brain. This damage is usually associated with elevated intraocular pressure (IOP). Due to this, when the optic nerve is damaged, this can result in irreversible blindness. This eye disease advances very slowly, typically without noticeable symptoms in the early stages, making it one of the most challenging conditions to diagnose. As shown in Figure 1, vision loss by glaucoma usually starts with the peripheral vision (side views) and then later the central view.¹ Each year, the number of patients diagnosed with glaucoma increases daily, becoming more of an issue worldwide. As shown in Figure 2, more than 2.7 million Americans over age 40 have glaucoma, and this number is estimated to more than double by 2050.²

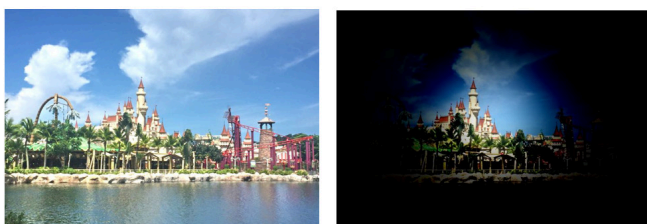


Figure 1: Vision loss affected by Glaucoma, (a) normal vision, (b) vision loss (glaucoma).¹

Projections for Glaucoma in 2030 and 2050 (in millions)

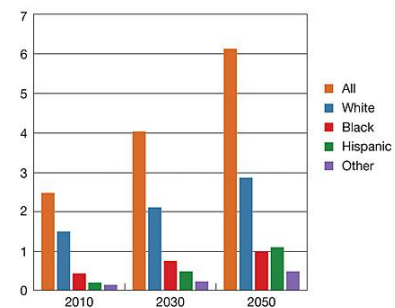


Figure 2: Increasing trend of glaucoma patients.²

Currently, the most common way to identify and diagnose glaucoma is through a dilated eye exam.³ A dilated eye exam is a widespread procedure performed by eye care professionals to examine the parts inside the eye. Using eye drops to increase the size of a patient's pupils, the eye doctor can thoroughly examine the health of the optic nerve and retina. By observing the optic nerve's shape, color, vessels, and size, the eye doctors will make assessments and a final diagnosis. However, this method is prone to errors due to the false readings and interpretations that can be made through the exam process.

Recently, image-based detection through machine learning has been popularized as a new efficient way to detect conditions like glaucoma with improved accuracy. For example, fundus images are used to look beyond the optic disc and im-

age regions outside the optic nerve head to detect glaucoma.⁴ An ensemble-based deep learning model for glaucoma diagnosis was the method used, which is the first of its kind ever developed.⁵ Fundus images were applied not only for the early identification of glaucoma but to determine the classification of its severity as well. Furthermore, an offline Computer-Aided Diagnosis (CAD) system is proposed for glaucoma detection using Le-Net architecture for input image validation.⁶ Additionally, an algorithm is suggested using a convolutional neural network (CNN) to diagnose glaucoma through fundus images.^{1,7}

With machine learning, advancements, and improvements, new problems like privacy issues also appear. Machine learning generally requires access to users' private and confidential data stored in the centralized server for training, creating many security concerns. To maintain the privacy of patients' records, this study proposes a federated learning approach where data is decentralized and instead performs training in a distributed manner. A federated learning system consists of a server and several clients. Each client can train the local network using its own local data,⁸ not through a server network, with the risk of exposing private data. The clients then send the local networks, not the local data, to the server. The server can then update the global network by aggregating the local networks. The federated learning approach often lets clients use low-cost devices like cell phones and sensors.⁹ The main contributions of this paper are summarized as follows:

- A federated learning model is proposed to detect glaucoma accurately while preserving data privacy.
- UNet model is implemented for the segmentation of optic disc and optic cup, by which the optic cup to disc ratio is calculated.
- Numerous simulations are performed with multiple fundus images for training the UNet-based federated learning, which results in a detection accuracy of 88% and a segmentation of 98.7%.

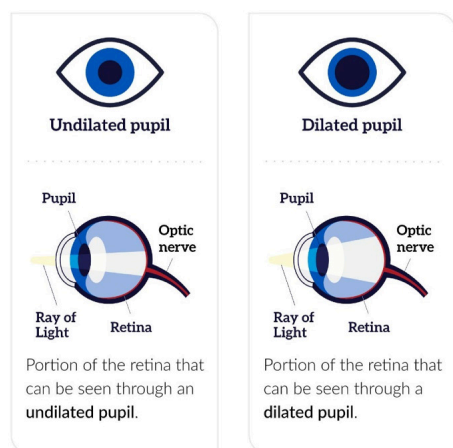


Figure 3: Dilated eye exam test.³

■ Clinical Diagnosis Method of Glaucoma

One method to diagnose a patient with glaucoma is to look at the optic cup-to-disc ratio in a fundus image. To date, glaucoma is clinically diagnosed using slit-lamp biomicroscopy, where an ophthalmologist often dilates the patient's pupils. A

dilated eye exam is the best and only method to check for eye diseases in the early stages. But, since this method totally relies on clinical expertise, there is quite a high possibility of misdiagnosis and high inter-examiner variability.⁶



Figure 4: Retinal images with and without glaucoma, where the arrows show optic cup, (a) normal optic nerve head, and (b) glaucomatous cupping. The increased optic cup is due to glaucoma.¹²

As shown in Figure 3, pupil dilation is performed to purposefully increase the size of the pupils during an eye exam so that the eye doctor can thoroughly examine the health of the optic nerve and retina. The exam is critical to preventing and treating eye conditions that could lead to vision loss. With a dilated pupil, more light is being passed through your eyes, allowing medical professionals to see much more of the retina, which helps diagnose glaucoma through a dilated eye exam.

The exam includes:

- A visual acuity test
- A visual field test
- An eye muscle function test
- A pupil response test
- A tonometry test
- Dilation

Technologies such as automated perimetry, tonometry, and optical coherence tomography (OCT) come with a few drawbacks, the most important of which is the risk of misinterpreting the data due to false positives and false negatives. It is reported that false positive reading for glaucoma patients ranges from 29% to 68%.¹⁰ Differentiating glaucomatous from non-glaucomatous neuropathy can be a difficult task in clinical practice, even for experienced professionals. Although glaucoma is the leading cause of disc cupping, 20% of the patients can be misdiagnosed.¹¹

Figure 4 illustrates the retinal image of a normal patient and that of a patient with glaucoma.¹² Detection of glaucoma is estimated based on CDR, which is defined as

$$\text{CDR} = \frac{\text{Optic cup size}}{\text{Optic disc size}}$$

Note that the glaucomatous eye generally shows a large value of CDR (≥ 0.5). The image with glaucoma shows an increased optic cup (OC) compared to the optic disc (OD), which results in a large optic cup-to-disc ratio (CDR). The optic cup is increased due to glaucoma; thus, calculating the optic cup and disc ratio from retinal images can provide a simple and easy detection method, which machine learning can perform exceptionally well with high accuracy.

Existing Diagnosis Methods Using Machine Learning Diagnosis using Machine Learning Methods

Over the past years, machine learning has been widely applied to various aspects of ophthalmology, in which glaucoma is one of the most popular. Using fundus images, many researchers have developed algorithms to distinguish glaucoma from normal eyes.¹³

A machine learning framework is presented in which three convolutional network architectures are used for glaucoma detection.⁴ VGGNet-16, ResNet-50, and GoogLeNet trained on the ImageNet dataset are tested with fundus images for glaucoma detection. The U-Net model is used for the machine learning algorithm to perform segmentation of optic disc and optic cup in retinal images, from which the cup-to-disc ratio is calculated and used for the detection.⁵

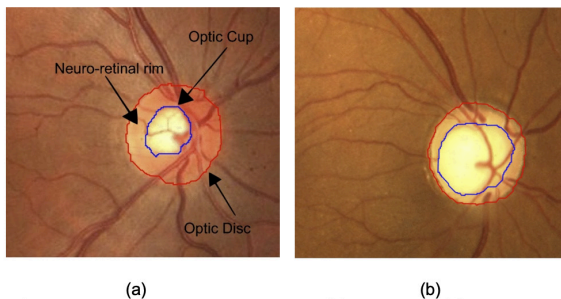


Figure 5: Segmentation of optic cup and optic disc. (a) normal eye, (b) glaucomatous eye.¹⁴

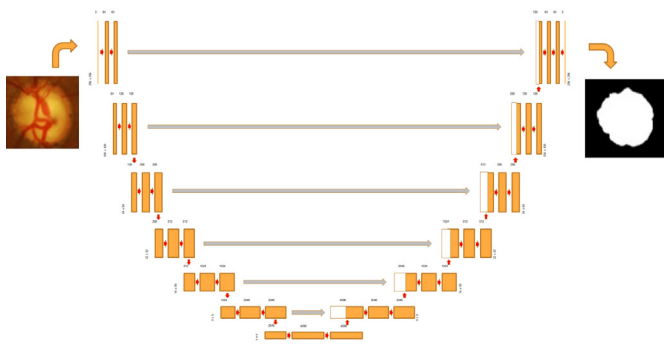


Figure 6: U-Net machine learning network for segmentation.

Segmentation:

The optic cup and disc must be identified and segmented from fundus retinal images to calculate CDR.⁷ Figure 5 illustrates retinal images of the normal eye and glaucomatous eye.¹⁴ The optic cup is the bright central portion inside the optic disk. The optic disk is the region where blood vessels and nerve fibers enter the retina surrounding the optic cup. It also depicts the segmented optic cup and the optic disc, where the glaucomatous eye shows a more considerable CDR value than the normal eye. U-Net, shown in Figure 6, is a popular machine-learning network model for segmentation.

U-Net:

U-Net is an architecture for semantic segmentation. As shown in Figure 6, it consists of a contracting path and an expansive path. The contracting path follows the typical architecture with repeated 3x3 convolutions and max pooling operations with stride 2 for downsampling. Each downsampling

operation with stride 2 for downsampling. Each downsampling step doubles the number of feature channels. Every step in the expansive path performs an upsampling of the feature map followed by a convolution that halves the number of feature channels.

Issues of Conventional Machine Learning-based Detection Methods:

Conventional machine learning methods, regardless of network models used, are centralized computational models, which means that the datasets are stored at the central machine learning server where training and evaluation are done. This centralized model provides excellent performance due to a large volume of datasets directly available at the server for training.

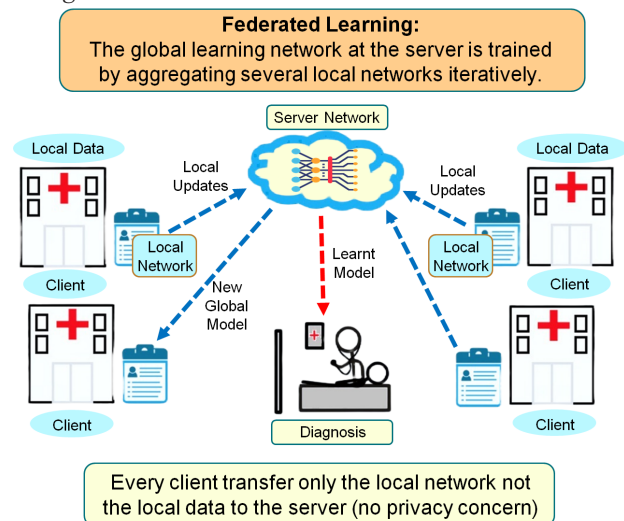


Figure 7: Federated learning architecture

However, this centralized model may not be possible for medical applications since medical images possess personal information, so privacy concerns exist. Due to this privacy issue, it may need help to collect many datasets for training machine learning networks easily. In addition, the number of medical images available at a single clinical facility may be limited since the datasets are only obtained by patients. Due to these reasons, a distributed learning method is desirable for medical applications, such as glaucoma detection.

Proposed Diagnosis Methods Using Federated Learning Federated Learning:

Google introduced federated learning in 2016 when the misuse of personal data was gaining global attention. Figure 7 depicts federated learning architecture.¹⁵ Federated learning is a distributed learning that consists of a server and several clients. Each client has its own dataset (local data), so the server does not need the dataset for training.^{10,16,17} Aledhari *et al.* demonstrate using a federated learning system within the healthcare sector.¹⁸ Another use for federated learning is an anomaly detection system to detect various IoT devices.¹⁹

The server first sends a deep-learning network model to all the clients. The network at the server is called a global network, and the network at each client is a local network. Each client (i.e., a clinic) uses its local data (medical images) to train its local network. Once all the clients have trained using their

own local data, the local networks are sent back to the server, where all the local networks are aggregated to update the global network. Then, the updated global network is again sent to the clients and copied to the local network. The clients perform further training to update their local network using their own dataset. This collaborative process using iteration continues as long as it goes until the model is fully trained. The more iterations, the better performance it may achieve.

This paper’s model assumes that the communication between the server and clients is perfect, with no errors. In the real world, however, a transmission between a client and the server can be expensive and unstable, making it prone to errors. The impact of such erroneous communications on performance is difficult to analyze and is an active ongoing research area. The dataset is distributed randomly over the clients, each with similar amounts of samples.

Procedure of Glaucoma Detection Using Federated Learning:

Figure 8 shows the process of the proposed glaucoma detection method using federated learning. First, fundus retinal images are downloaded, and pre-processing of those images is done. Pre-processing aims to resize or crop the images to focus on the region of interest. The next step is to implement a federated learning model, in which the U-Net network model for a server and the clients are built. We used ten clients in our simulation. Once the network model is implemented, training is performed, where 300 iterations (epochs) are executed. Then, we analyze the detection accuracy as well as segmentation performance.

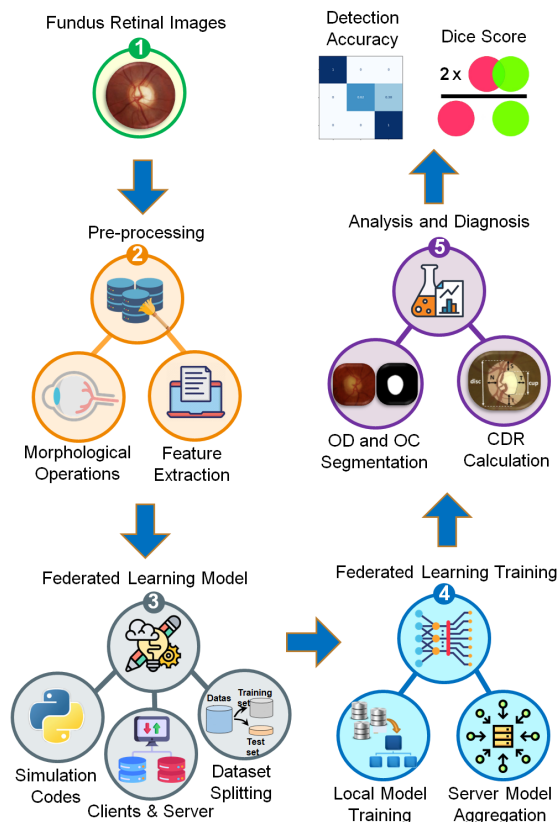


Figure 8: The procedure of glaucoma detection using federated learning.

Fundus Image Dataset:

This study uses publicly available fundus image datasets at Kaggle.com.²⁰ Fundus retinal images and OD/OC masks from ORIGA, REFUGE, and G1020 datasets, with 2,870 high-resolution retinal images available.¹¹ These images are split into 90% of the dataset for training and 10% for testing.

Simulations and Implementation:

An extensive number of simulations are performed to evaluate the performance of the federated learning-based classifier. For a development environment, Jupyter Lab and Anaconda tools are used on an Intel Core (TM) i7-1165G7 CPU @ 2.80GHz accelerated by GPU Nvidia RTX 3090. A cloud GPU server, Google Colab, was also utilized. Training the model with a large dataset during implementation requires considerable time for an ordinary CPU to execute. Therefore, a GPU is commonly used to build and run the model to save a significant amount of time.

A federated learning simulation is implemented using Tensorflow 2.0 and Python 3. U-Net is built under the Jupyter lab environment for local and server networks. The dataset is split into 90% of the dataset as a training dataset and the remaining 10% as the test dataset. A loss function is used in the network to calculate the loss, where the network is always trained to minimize the loss. There was also a validation procedure to avoid overfitting while training. In the federated learning system considered in this paper, ten clients are created where the images are divided equally among each client, and a local network is trained using its own local dataset.

Performance Analysis

Detection Accuracy:

The performance of the proposed detection method is evaluated by two metrics. Detection accuracy is calculated by the proposed federated learning using the test datasets. Figure 9 illustrates a confusion matrix with a detection accuracy of 88%. The accuracy is calculated by the number of true positives and true negatives out of all the test datasets.

Actual	Pos	158	31
	Neg	3	95
		Pos	Neg
		Predicted	

Figure 9: Glaucoma detection accuracy.

Table 1: Segmentation performance is shown by dice score.

Segmentation	Dice score
Optic cup	0.9873
Optic disc	0.9873

Since glaucoma detection is calculated using CDR, optic cup and disc segmentation performance is even more important. Generally, segmentation performance is evaluated by either the intersection of union (IoU) or Dice score. In this study, we use dice scores as the performance metric for segmentation. As shown in Table 1, after 300 epochs of training,

U-Net-based federated learning gives a dice score of 0.9873 for both optic cup and optic disc segmentation, which means the optic cup and optic disc are segmented with 98.7% accuracy from retinal images on average.

■ Conclusion

Glaucoma detection using federated learning is implemented and simulated in this paper. Compared to the clinical glaucoma detection method, this method is simple, easy, and more accurate. In addition, unlike the other machine learning methods for glaucoma detection, this method uses federated learning to circumvent privacy concerns of medical images shared at the central server for training. With a dataset of 2,870 fundus images, the system gives an overall detection accuracy of 88% and a dice score of 0.987 for segmentation performance. This new method to detect glaucoma will hopefully help doctors in their diagnosis while also helping patients by making it more accessible and less prone to errors.

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■ References

1. H. Fu, J. Cheng, Y. Xu, and J. Liu, "Glaucoma Detection Based on Deep Learning Network in Fundus Image," *Deep Learning and Convolutional Neural Networks for Medical Imaging and Clinical Informatics*, vol. 10, pp. 119-137, Sep. 2021.
2. National Eye Institute, "10 things you should know about glaucoma," U.S. Department of Health and Human Services, <https://www.nei.nih.gov/about/news-and-events/news/10-things-you-should-know-about-glaucoma>, Jan. 2017
3. National Eye Institute, "Get a dilated eye exam," U.S. Department of Health and Human Services, <https://www.nei.nih.gov/learn-about-eye-health/healthy-vision/get-dilated-eye-exam>, May.20 21
4. R. Hemelings, B. Elen, J. Barbosa-Breda, M. B. Blaschko, P. D. Boever, and I. Stalmans, "Deep learning on fundus images detects glaucoma beyond the optic disc." *Scientific Reports*, vol. 11, 20313 (2021), Oct. 2021
5. S. Joshi, B. Partibane, W. A. Hatamleh, H. Tarazi, C. S. Yadav, and D. Kraha, "Glaucoma detection using image processing and supervised learning for classification," *Journal of Healthcare Engineering*, vol. 2022, article ID 2988262, Mar. 2022
6. R. Shinde, "Glaucoma detection in retinal fundus images using U-Net and supervised machine learning algorithms," *Intelligence-based Medicine*, vol. 5, 2021, 100038, Jul. 2021
7. S. Ajitha, J.D. Akkara, and M.V. Judy, "Identification of glaucoma from fundus images using deep learning techniques," *Indian Journal of Ophthalmology*, vol. 69, no. 10, pp. 2702-2709, 2021
8. T. Li, M. Sanjabi, A. Beirami, and V. Smith, "Fair resource allocation in federated learning," 2019, arXiv:1905.10497. [Online]. Available: <http://arxiv.org/abs/1905.10497>
9. R. Doku, D. B. Rawat, and C. Liu, "Towards federated learning approach to determine data relevance in big data," *Proceedings of IEEE 20th International Conference Information Reuse Integration for Data Science (IRI)*. Los Alamitos, CA, USA, Jul. 2019, pp.184-192, doi: 10.1109/iri.2019.00039
10. W. B. Potter and J. L. Fanelli, "Red Disease: Is it Haunting Your Glaucoma Diagnoses?," *Review of Optometry*, Mar. 2017, <https://www.reviewofoptometry.com/article/red-disease-is-it-haunting-your-glaucoma-diagnoses>

11. D. T. Dias, M. Ushida, R. Battistella, S. Dorairaj, and T. S. Prata, "Neurophthalmological conditions mimicking glaucomatous optic neuropathy: analysis of the most common causes of misdiagnoses," *BMC Ophthalmology*, vol. 17, Jan. 2017, <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5223567/>
12. L. K. Singh, Pooja, H. Garg, M. Khanna, and R. S. Bhadoria, "An enhanced deep image model for glaucoma diagnosis using feature-based detection in retinal fundus," *Medical & Biological Engineering & Computing*, vol. 59, pp. 333-353, Jan. 2021
13. Mayo Clinic "Glaucoma." Mayo Foundation for Medical Education and Research, 30 Sept. 2022
14. A. Diaz-Pinto, S. Morales, V. Naranjo, T. Köhler, J. M. Mossi and A. Navea, "CNNs for automatic glaucoma assessment using fundus images: an extensive validation," *BioMedical Engineering*, vol. 1 8, Article number: 29, Mar. 2019
15. Q. Yang, Y. Liu, and Y. Cheng, *Federated Learning*. Morgan & Claypool Publishers, 2020.
16. H. Zhu, Z. Li, M. Cheah, and R. Siow Mong Goh, "Privacy-preserving weighted federated learning within oracle-aided MPC framework," 2020, arXiv:2003.07630. [Online]. Available: <http://arxiv.org/abs/2003.07630>
17. R. Shao, H. He, H. Liu, and D. Liu, "Stochastic channel-based federated learning for medical data privacy preserving," 2019, arXiv: 1910.11160. [Online]. Available: <http://arxiv.org/abs/1910.11160>
18. M. Aledhari, R. Razzak, R. M. Parizi and F. Saeed, "Federated Learning: A Survey on Enabling Technologies, Protocols, and Applications," *IEEE Access*, vol. 8, pp. 140699-140725, 2020.
19. T. D. Nguyen, S. Marchal, M. Miettinen, H. Fereidooni, N. Asokan, and A.-R. Sadeghi, "DfIoT: A federated self-learning anomaly detection system for IoT," *Proc. IEEE 39th Int. Conf. Distrib. Comput. Syst. (ICDCS)*, May 2019, pp. 756-767.
20. A. Jain, "Glaucoma Fundus Imaging Datasets." Kaggle, 26 June 2022

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Spectrophotometric Investigation of the Effect of the Type of Adsorbent on the Removal of Methylene Blue Dye

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ABSTRACT: This paper investigates the effects of different types of adsorbents on the removal of methylene blue dye. Three adsorbent types that will be utilized in this research are black tea, rice husk, and eggshells. Adsorption is the process of removing a substance called adsorbate in any physical state by adhering to the surface of another material called an adsorbent. In this paper, the adsorbate is a methylene blue dye stock solution with a concentration of 0.030 g L^{-1} . To evaluate the effectiveness of the adsorbents their adsorption capacities and removal efficiencies will be computed using Beer-Lambert Law and the absorbance data obtained from a UV-Vis spectrophotometer as the analytical method. It has been concluded that the most effective adsorbent among the three was rice husk, and the least efficient was eggshell powders. Ultimately, possible extensions to enlarge the spectrum of the research were proposed.

KEYWORDS: Chemistry; Environmental Chemistry; Adsorption; Removal Efficiency; Methylene Blue Dye.

■ Introduction

In alignment with the rapid development of technology and society, industrial wastes have been considerably increasing in size and type. There are numerous kinds of industrial waste, such as concrete, depending on the processes and raw materials utilized in the industry. One of them, which affects the environment exceedingly, is synthetic dyes. Synthetic dyes have been commonly used in the textile industry since 1856, after their discovery by William Henry Perkin.¹ Although synthetic dyes contribute immensely to the success of sales in the fashion, textile, and cosmetics industries, waste synthetic dyes root for over 20% of the pollution of water resources globally.² Each year, over 140,000 tons of non-treated dyes have been discharged into water.³ Methylene blue is one of these synthetic dyes, which is preponderantly applied in textile and many other industries.

Methylene blue (see Figure 1) is a cationic dye that can be dissociated into positively charged ions in aqueous environments.⁴ Its chemical formula is $\text{C}_{16}\text{H}_{18}\text{ClN}_3\text{S}$ and its scientific name is 7-(Dimethylamino) phenothiazin-3-ylidene-dimethylazanium chloride.⁴ Because it contains the N group as an azo group, it is also considered an azo dye. It has carcinogenic properties. Even though its name suggests otherwise, methylene blue has a dark green appearance. Methylene blue is usually utilized in paper coloring as biological stains and dyeing cotton.⁵

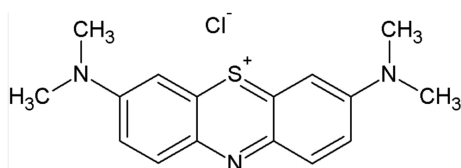


Figure 1: Chemical structure of methylene blue.⁶

To decrease the detrimental effects of waste synthetic dyes, specific methods are used for water treatment: coagulation, chemical oxidation, flocculation, and precipitation. However, these procedures are costly, or their adsorbents create problems such as regeneration, which is why sometimes they are not favored. An economical and efficient alternative practice for water purification is adsorption. Adsorption is the adhesion of ions or molecules from a substance, an adsorbate, which could be a solid, liquid, or gas onto the surface of another material, which is called an adsorbent.⁷ Adsorption can be chemical or physical. In chemical adsorption, a chemical bond between the adsorbate and the adsorbent is formed whereas, in physical adsorption, which will be the case in this research, there are weak Van der Waal forces between those two.⁸ Synthetic and natural adsorbents could be utilized in adsorption to remove particles from water. However, natural adsorbents are more beneficial because they are biodegradable wastes and can be recycled. Therefore, their utilization is safe for the environment. Some examples of low-cost adsorbents are tea wastes, eggshells, rice husks, moringa seeds, lemon seeds, orange peels, palm seeds, and papaya seeds.

This study will utilize powdered black tea waste, eggshell powder, and rice husk because of their prevalent consumption in Turkey. Their efficiency will be compared using spectrophotometry. A spectrophotometer is an instrument that measures the quantity and intensity of UV light absorbed by a substance at different wavelengths of visible light. "This instrument consists of a source that emits all wavelengths of light in the visible region (wavelengths of ~400 to 700 nm)."⁹ This tool is designed based on a principle, Beer's Lambert Law, which states that the concentration of a solute is proportional to the absorbance as mentioned in Encyclopedia of Spectroscopy and Spectrometry by Alison Gilchrist and Jim Nobbs.¹⁰ In this ex-

periment, this law will be applied to the measurements of the UV-VIS spectrophotometer.

Although there are recent studies on removing dyes from water, primarily the impact of time, pH, adsorbent dosage, and initial methylene blue dye concentration are measured, meaning that the comparison of different adsorbent types is rarely found. Thus, in this study three adsorbents, black tea, rice husk, and eggshells, that were not studied together were chosen. Additionally, most of the research papers only present the results. Few research papers explain the chemistry behind the adsorption of materials, specifically referring to the structure of the adsorbents. The explanations, if there are any, are generally superficial. Therefore, this research paper aims to determine the influence of the nature of adsorbents on the removal of methylene blue dye from the water. Primarily, the research question will be given in this research paper. After that, materials and methods will be presented. In this section, materials and chemicals will be respectively listed. Afterward, how the adsorbents and adsorbate were prepared will be described. Finally, the analytical method will be explained, and the molar absorptivity value, which is essential for calculations in the results section, will be computed. Following materials and methods, results will be provided through step-by-step mathematical calculations and explanations. Tables will also be displayed to give a clearer understanding of the processes. Then, the results will be discussed. The chemistry behind removing methylene blue dye, specifically black tea, rice husk, and eggshells will be elucidated while endeavoring to explain the difference between their removal capabilities. The paper will be concluded with the limitations and possible extensions of this research.

Research Question:

How does the type of adsorbent (black tea, rice husk, and eggshell) affect the removal of solid methylene blue dye (0.030 g L⁻¹) from 100.0 mL of distilled water measured by spectrophotometry under constant pH (7.00), room temperature (25.0°C), and adsorbent dosage (1.000 g) for 120 minutes?

Methods

Chemicals:

- 0.030 g Methylene Blue Dye (C₁₆H₁₈ClN₃S) (s) (± 0.001 g)

Preparation of Adsorbents:

Eggshells and black tea bags were collected in the household for three months. Rice husks (1 kg) were bought from a nearby shop. After the collection was complete, the tea bags were cut, and the bags were separated from black tea particles. Black tea particles were put in a 250.0 mL Erlenmeyer flask. Later, 200.0 mL of distilled water was added to the flask. The flask was swirled for several minutes. Rice husks were prepared using the same process after being put in a 250.0 mL Erlenmeyer flask. In addition, a 500 mL bowl was filled with distilled water to prepare the eggshells. After that, the eggshells were submerged in the bowl for several minutes and then taken outside of the bowl. After all the materials (black tea, rice husk, and eggshells) were washed, they were left to dry at 95°C for 16 h in a laboratory oven. When they were all dry, particles were collected in a 50 mL Florence flask.

Preparation of Adsorbate:

This experiment selected Methylene Blue as an adsorbate due to its strong adsorption ability onto adsorbents. Using a volumetric flask, the stock solution was prepared by dissolving 0.030 g of methylene blue dye in 1 liter of distilled water. Thus, 0.030 g L⁻¹ of the methylene blue stock solution was obtained.

Analytical Method:

The investigation was conducted by using the UV-Vis spectrophotometer. The adsorption capacity, which is the amount of adsorbate taken up by the adsorbent per unit mass or volume of the adsorbent, and removal efficiency, which is the percentage the adsorbent removes an adsorbate, will be computed to measure the removal of methylene blue dye.¹¹ While performing these calculations, the number of decimal places will be written without rounding to increase the results' accuracy and precision, which will be rounded at the very end. To be able to utilize the data obtained from the spectrophotometer in the equations for adsorption capacity and removal efficiency, first, the molar absorptivity must be determined using the Beer-Lambert Law, which states:⁹

$$A = \epsilon \cdot l \cdot c \text{ (Eq. 1)}$$

where A = absorbance (no units), ϵ = Molar Absorptivity (L mol⁻¹ cm⁻¹), l = Path Length (cm),

c = concentration (mol L⁻¹).

The molar absorptivity will be found by substituting A , l , and c values into Eq. 1. The sample solution that was used to calculate the molar absorptivity had a concentration of 0.030 g L⁻¹. Since the units are not compatible with Eq. 1, the equivalence of the value was found in mol L⁻¹. The molar mass of methylene blue (C₁₆H₁₈ClN₃S) was calculated:

$$(16 \cdot 12.011) + (18 \cdot 1.00797) + 35.453 + (3 \cdot 14.0067) + 32.07 = 319.85256 \text{ g mol}^{-1}$$

Using the formula for calculating the number of moles, which is $n = \frac{\text{Mass (g)}}{\text{Molar mass (g mol}^{-1}\text{)}}$,

$$0.030 \text{ g L}^{-1} = 9.379321522 \cdot 10^{-5} \text{ mol L}^{-1}$$

After that, the path length, which is the width of a cuvette, was measured as 1.00 cm using a ruler with an uncertainty of ± 0.05 cm. Subsequently, the highest absorbance of the sample was measured as 0.887 at 642.40 nm with the UV-Vis spectrophotometer. Although there were absorbance values higher than this value, they were all greater than 1.000, which decreases the precision and accuracy of the absorbance since it indicates that the sample was highly concentrated. Using all these values,

$$0.887 = \epsilon \cdot 1.00 \text{ cm} \cdot 9.379321522 \cdot 10^{-5} \text{ mol L}^{-1}$$

Thus,

$$\epsilon = 9456.974024 \text{ L mol}^{-1} \text{ cm}^{-1}$$

Because this is a constant value for the dye at this (642.40 nm) wavelength, it will be utilized in every adsorbent capacity equation in the Results section of this research paper.

Results and Discussion

Effect of Powdered Black Tea on Dye Adsorption:

The absorbance values determined using the UV-Vis spectrophotometer can be seen in Table 1. Normally, for each adsorbent, five trials were carried out. However, when the ab-

sorbance values obtained were evaluated, the absorbance value found in Trial 1 was considerably distinguishable from those obtained in other trials. Therefore, an additional trial was performed. Since the value found was close to the values of other trials, Trial 1 will be considered an outlier in this experiment.

Table 1: The absorbance of powdered black tea for each trial.

Number of trials	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
Absorbance value for powdered black tea at 642.40 nm (± 0.001)	0.019	0.147	0.132	0.145	0.158	0.164

An average of 5 trials was taken and the average absorbance was found to be

$$(0.147 + 0.132 + 0.145 + 0.158 + 0.164) \div 5 = 0.1492$$

This value is substituted in **Eq. 1:**

$$0.1492 = 9456.974024 \text{ L mol}^{-1} \text{ cm}^{-1} \cdot 1.00 \text{ cm} \cdot c_{\text{final}}$$

Hence, the final concentration of methylene blue in 100.0 mL sample is computed as

$$c_{\text{final}} = 1.57767167 \cdot 10^{-5} \text{ mol L}^{-1} \text{ (Eq. 2)}$$

The final concentration will be used in the calculation of adsorption capacity of powdered black tea waste. The equation for adsorption capacity (q) is

$$q = \frac{(c_{\text{initial}} - c_{\text{final}})}{m_{\text{adsorbent}}} \cdot V \quad \text{(Eq. 3)}$$

where the unit for c_{final} and c_{initial} is mg L^{-1} , $m_{\text{adsorbent}}$ = mass of adsorbent (g), and

$$V = \text{volume (L)}$$

In this equation, q is found in terms of mg g^{-1} . Therefore, the final concentration computed in **Eq. 2** should be converted into mg L^{-1} :

$$1.57767167 \cdot 10^{-5} \text{ mol L}^{-1} \cdot 319.85256 \text{ g mol}^{-1} \cdot 1000 = 5.0546223225 \text{ mg L}^{-1}$$

The initial concentration was 0.030 g L^{-1} , which is equal to 30 mg L^{-1} . Substituting related values into **Eq. 3**,

$$q = \frac{(30 - 5.0546223225) \text{ mg L}^{-1}}{1.000 \text{ g}} \cdot 0.100 \text{ g L}^{-1} = 2.495377678 \text{ mg g}^{-1} \approx 2.5 \text{ mg g}^{-1}$$

This signifies that for unit mass (1 g) of adsorbent (powdered black tea), approximately 2.5 mg of the adsorbate (solid methylene blue dye) is adsorbed. For this to be more understandable removal efficiency is preferred to be computed. The equation is

$$\text{Removal efficiency (\%)} = \frac{(c_{\text{initial}} - c_{\text{final}})}{c_{\text{initial}}} \cdot 100 \quad \text{(Eq. 4)}$$

Where the unit for c_{final} and c_{initial} is mg L^{-1}

Thus, the removal efficiency for powdered black tea waste is calculated as

$$\text{Removal efficiency (\%)} = \frac{(30 - 5.05)}{30} \cdot 100 = 83.17925592 \% \approx 83\%$$

Effect of Rice Husk on Dye Adsorption:

The absorbance values obtained from the UV-Vis spectrophotometer can be seen in Table 2.

Table 2: The absorbance of rice husk for each trial.

Number of trials	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Absorbance value for rice husk at 642.40 nm (± 0.001)	0.020	0.023	0.025	0.028	0.027

An average of 5 trials was calculated, and the average absorbance was found to be

$$(0.020 + 0.023 + 0.025 + 0.028 + 0.027) \div 5 \approx 0.0246$$

Then, to find the final concentration, this value is substituted in **Eq. 1:**

$$0.0246 = 9456.974024 \text{ L mol}^{-1} \text{ cm}^{-1} \cdot 1.00 \text{ cm} \cdot c_{\text{final}}$$

Thus, the final concentration of methylene blue in 100.0 mL sample is calculated as

$$c_{\text{final}} \approx 2.601254898 \cdot 10^{-6} \text{ mol L}^{-1}$$

Subsequently, the adsorption capacity of rice husk will be determined. Firstly, the value found in the previous step is converted to mg L^{-1} :

$$2.601254898 \cdot 10^{-6} \text{ mol L}^{-1} \cdot 319.85256 \text{ g mol}^{-1} \cdot 1000 \approx 8.32018038 \cdot 10^{-1} \text{ mg L}^{-1}$$

Utilizing the initial concentration as 30 mg L^{-1} and **Eq. 3**,

$$q = \frac{(30 - 8.32018038 \cdot 10^{-1}) \text{ mg L}^{-1}}{1.000 \text{ g}} \cdot 0.100 \text{ g L}^{-1} = 2.495377678 \text{ mg g}^{-1} \approx 2.5 \text{ mg g}^{-1}$$

This indicates that for a unit mass of adsorbent (rice husk), approximately 2.9 mg of the adsorbate (solid methylene blue dye) is adsorbed.

After the adsorption capacity was identified, the removal efficiency of rice husk is calculated using **Eq. 4** as

$$\frac{(30 - 8.32018038 \cdot 10^{-1}) \text{ mg L}^{-1}}{30 \text{ mg L}^{-1}} \cdot 100 = 97.22660654 \% \approx 97\%$$

Effect of Eggshell Powders on Dye Adsorption:

The absorbance values obtained from the UV-Vis spectrophotometer can be seen in Table 3.

Table 3: The absorbance of powdered black tea for each trial.

Number of trials	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Absorbance value for eggshell powders at 642.40 nm (± 0.001)	0.199	0.196	0.197	0.196	0.197

An average of 5 trials was taken and the average absorbance was found to be

$$(0.199 + 0.196 + 0.197 + 0.196 + 0.197) \div 5 = 0.197$$

Now, this value is substituted in **Eq. 1:**

$$0.197 = 9456.974024 \text{ L mol}^{-1} \text{ cm}^{-1} \cdot 1.00 \text{ cm} \cdot c_{\text{final}}$$

The final concentration of methylene blue in 100.0 mL sample is therefore computed as

$$c_{\text{final}} = 2.08311876 \cdot 10^{-5} \text{ mol L}^{-1}$$

Following this, the adsorption capacity of eggshell powders will be computed. The value found in the previous step is converted to mg L^{-1} :

$$2.08311876 \cdot 10^{-5} \text{ mol L}^{-1} \cdot 319.85256 \text{ g mol}^{-1} \cdot 1000 \approx 6.662908682 \text{ mg L}^{-1}$$

Making use of **Eq. 3** and considering the initial concentration as 30 mg L^{-1} ,

$$q = \frac{(30 - 6.66) \text{ mg L}^{-1}}{1.000 \text{ g}} \cdot 0.100 \text{ g L}^{-1} = 2.333709132 \text{ mg g}^{-1} \approx 2.3 \text{ mg g}^{-1}$$

This suggests that for a unit mass of adsorbent (eggshell powders), approximately 2.3 mg of the adsorbate (solid methylene blue dye) is adsorbed. As a final step, the removal efficiency of the adsorbent is computed utilizing **Eq. 4:**

$$\frac{(30 - 6.66) \text{ mg L}^{-1}}{30 \text{ mg L}^{-1}} \cdot 100 = 77.79030439 \% \approx 78\%$$

Discussion of the Results

From the adsorption capacity results and the percentages for removal efficiency, it can be concluded that the most effective adsorbent is rice husk, and the least efficient adsorbent is powdered eggshells. Although the distinction between the efficiency is more subtle in the adsorption capacity values, the removal efficiency was calculated to interpret this difference that might seem insignificant.

Both rice husk and black tea have cellulose in their composition. Since cellulose contains a vast number of hydroxyl groups in its structure, materials composed of cellulose as well as cellulose itself can be potentially utilized to adsorb materials.³ The hydroxyl groups in cellulose can form strong Hydrogen bonds with the amine groups or other nitrogen atoms present in the structure of methylene blue as can be seen in Figure 2.

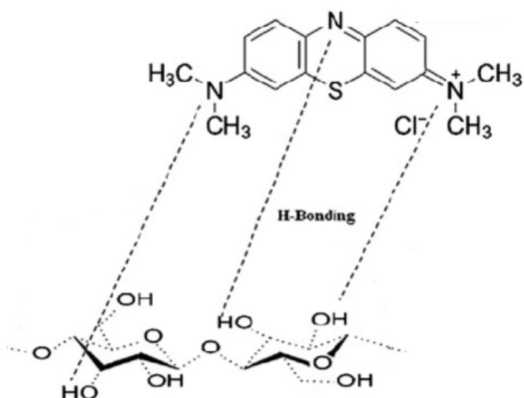


Figure 2: Hydrogen bonding between hydroxyl groups of cellulose and nitrogen atoms in methylene blue.¹²

Nonetheless, the percentage of cellulose is not equal in rice husk and black tea: nearly 50% of rice husk comprises cellulose whereas cellulose makes up only 20% of black tea.^{13,14} Moreover, rice husk contains 25% to 30% lignin and 15% to 20% hemicellulose.¹³ Lignin and hemicellulose contain hydroxyl groups, as shown in Figures 3 and 4, which also assist the formation of hydrogen bonds with the nitrogen atoms in methylene blue. This leads to the conclusion that there is almost four times more possibility that methylene blue particles can adhere to the hydroxyl groups of rice husk when compared to those of black tea, supporting the difference in the absorbance values depicted in Tables 1 and 2 as well as the conclusion that rice husk is more effective than black tea.

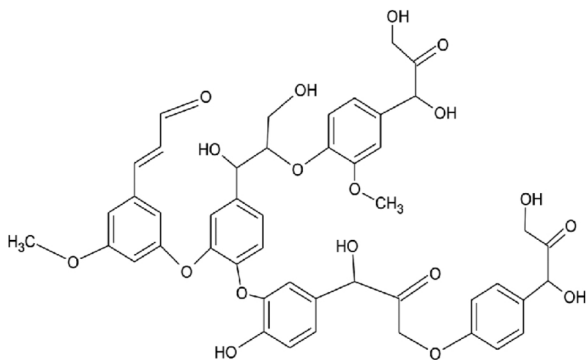


Figure 3: Chemical structure of lignin.¹⁵

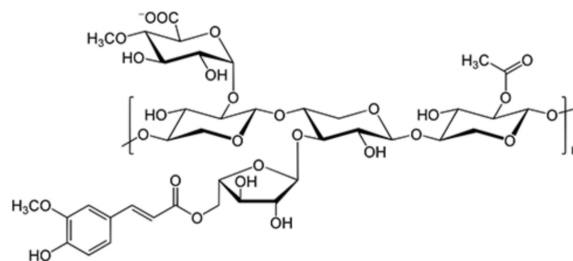


Figure 4: Chemical structure of hemicellulose.¹⁶

On the other hand, eggshells contain CaCO_3 as a crucial component in their structure.¹⁷ Methylene blue is a cationic dye that contains tertiary amine groups, meaning that three radical groups are bound to the nitrogen atom in the amine group of the dye.¹⁷ The tertiary amine group binds the Ca^{2+} ion of the CaCO_3 in the structure of eggshell powders.¹⁷ However, quaternary amine groups that always have a positive charge are included in the chemical structure of methylene blue dye.¹⁷ The positively charged amine group repels the Ca^{2+} ion of CaCO_3 .¹⁷ The repulsion decreases the strength of the electrostatic attraction between Ca^{2+} and methylene blue. This results in a decline in dye adsorption, as demonstrated in Table 3, smaller adsorption capacity, and removal efficiency of eggshell powders, parallel to the results obtained in this research paper.

Another possible explanation for the difference in adsorption capacity and removal efficiency between rice husk, black tea powders, and eggshell powders could be related to a major limitation of this experiment conducted, which is the surface area of the three adsorbents. Even though adsorbents were ground with mortar and pestle or even cut by scissors to minimize the large difference between the surface areas of each adsorbent, it was inevitable to have some differentiation. The difference in the surface areas of powdered black tea and eggshell powders was very subtle; meanwhile, the rice husk and the other two adsorbents were larger. Since the surface area of the rice husk was wider, the possibility of rice husk and methylene blue particles colliding was somewhat higher. This might explain the larger difference between the removal efficiency percentage of rice husk and black tea powders when compared to the difference in the removal efficiency percentage of black tea and eggshell powders.

Conclusion

This research aimed to investigate the effects of powdered black tea, rice husk, and eggshell powders on the removal of methylene blue dye from water. With this regard, the preparation of the adsorbents and the adsorbates were delineated; the analytical method, which was the usage of UV-Vis Spectrophotometer data in Beer-Lambert Law, was explained; the calculations for finding the adsorption capacity and removal efficiency for each adsorbent were performed, and the results were discussed and compared. Accordingly, a conclusion was reached: the most efficient adsorbent is rice husk, and the least effective adsorbent is eggshells among the three adsorbents.

In conclusion, this research could be further developed by enlarging its scope. In the future, more types of adsorbents

could be investigated; the effect of pressure – apart from the variables measured in many scientific studies, such as time, pH, temperature, adsorbent or adsorbate dosage – could be measured, or even the behavior of adsorbents, when put in filthy seawater, could be searched with the new advancements in technology and artificial intelligence.

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■ References

1. William Henry Perkin. <https://www.sciencehistory.org/historical-profile/william-henry-perkin#:~:text=With%20the%20accidental%20discovery%20in,the%20first%20commercialized%20synthetic%20dye.> (accessed Feb 2, 2023).
2. Dyeing for fashion: Why our clothes are so bad for the environment. <https://www.euronews.com/green/2022/02/26/dyeing-for-fashion-why-the-fashion-industry-is-causing-20-of-water-pollution#:~:text=Dyeing%20and%20finishing%20are%20responsible,cent%20of%20global%20water%20pollution.> (accessed Feb 12, 2023).
3. authors, A.; Lv, D.; Additional information Funding This work was supported by Key R & D and promotion projects in Henan Province (CN) [Grant Numbers 192102310220. Ultrafast removal of methylene blue from water by Fenton-like pretreated peanut hull as biosorbent. <https://www.tandfonline.com/doi/full/10.1080/17518253.2021.2024277.> (accessed Feb 9, 2023).
4. Methylene Blue. [https://pubchem.ncbi.nlm.nih.gov/compound/ethylene-blue#:~:text=Methylthioninium%20chloride%20\(INN%2C%20or%20methylene,inhibitor%20of%20Tau%20protein%20aggregation.](https://pubchem.ncbi.nlm.nih.gov/compound/ethylene-blue#:~:text=Methylthioninium%20chloride%20(INN%2C%20or%20methylene,inhibitor%20of%20Tau%20protein%20aggregation.) (accessed Feb 17, 2023).
5. Mulushewa, Z.; Dinbore, W. T.; Ayele, Y. Removal of methylene blue from textile waste water using kaolin and zeolite-X synthesized from Ethiopian kaolin. [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8207001/#:~:text=Methylene%20blue%20\(MB\)%20is%20a,and%20coating%20for%20paper%20stock.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8207001/#:~:text=Methylene%20blue%20(MB)%20is%20a,and%20coating%20for%20paper%20stock.) (accessed Feb 23, 2023).
6. Hui, T. S.; Zaini, M. A. A. Isotherm studies of methylene blue adsorption onto potassium salts. https://www.researchgate.net/publication/281738079_Isotherm_Studies_of_Methylene_Blue_Adsorption_onto_Potassium_Salts-Modified_Textile_Sludge. (accessed Feb 17, 2023).
7. Absorption vs adsorption. https://www.diffen.com/difference/Absorption_vs_Adsorption#:~:text=Adsorption,-Differ%20%E2%80%BA%20Science%20%E2%80%BA%20Chemistry&text=Absorption%20is%20the%20process%20in,a%20surface%20of%20the%20adsorbent. (accessed Feb 24, 2023).
8. Topics, C. Physical adsorption and chemical adsorption. <https://pandakajal42.medium.com/physical-adsorption-and-chemical-adsorption-8f4d67434ca9#:~:text=Physical%20adsorption%20occurs%20when%20the,by%20the%20sharing%20of%20electrons.> (accessed Feb 25, 2023).
9. Zumdahl, S. S. In *Chemistry*; D.C. Heath: Lexington, 1989; ppA 18–A21.
10. Colorimetry. <https://www.sciencedirect.com/topics/chemical-engineering/colorimetry#:~:text=Colorimetry%20is%20a%20scientific%20technique,is%20proportional%20to%20the%20absorbance.> (accessed Feb 18, 2023).
11. Adsorption Capacity. [https://www.sciencedirect.com/topics/engineering/adsorption-capacity#:~:text=1%20Adsorption%20Capacity-,Adsorption%20capacity%20\(or%20loading\)%20is%20the%20amount%20of%20adsorbate%20taken,adsorbed%20per%20mass%20of%20desiccant.](https://www.sciencedirect.com/topics/engineering/adsorption-capacity#:~:text=1%20Adsorption%20Capacity-,Adsorption%20capacity%20(or%20loading)%20is%20the%20amount%20of%20adsorbate%20taken,adsorbed%20per%20mass%20of%20desiccant.) (accessed Feb 20, 2023).
12. Ahmad, T.; Danish, M.; Rafatullah, M.; Mohamad, M. N. The use of date palm as a potential adsorbent for wastewater treatment: A review. https://www.researchgate.net/figure/Schematic-representation-of-hydrogen-bonding-between-nitrogen-atoms-of-Methylene-Blue-and_fig1_51970876. (accessed Feb 21, 2023).
13. Rice Husk. <https://www.sciencedirect.com/topics/engineering/rice-husk#:~:text=Rice%20husk%20constitutes%20about%20,%E2%80%93150%20kg%2Fm3.> (accessed Feb 14, 2023).
14. Tea (beverage). [https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/tea-beverage#:~:text=Polysaccharides%20and%20cellulose%20\(approximately%2020,5%25%20of%20dry%20weight%20each.](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/tea-beverage#:~:text=Polysaccharides%20and%20cellulose%20(approximately%2020,5%25%20of%20dry%20weight%20each.) (accessed Jan 23, 2023).
15. Jahangeer, M.; Mahmood, Z.; Yameen, M.; Riaz, M. Chemical structure of lignin [15] - researchgate. https://www.researchgate.net/figure/Chemical-structure-of-lignin-15_fig1_346503389. (accessed Jan 24, 2023).
16. 6.2C Hemicellulose. <https://www.e-education.psu.edu/egee439/node/664.> (accessed Feb Jan 27, 2023).
17. Preparation of core-shell structured caco - royal society. <https://royalsocietypublishing.org/doi/10.1098/rsos.170697.> (accessed Feb 24, 2023).

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A Comparison of Post-Traumatic Stress Disorder Rates and Determining Factors in New Jersey Female and Male Emergency Medical Technicians

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ABSTRACT: Post-traumatic stress disorder, or PTSD, is a severe mental condition a person can develop after experiencing or witnessing a traumatic event. It may have long-term psychological effects as well as reduced work performance. Compared to the general population, emergency medical technicians (EMTs) responsible for arriving on the scene to treat and transport medical patients in emergencies experience a higher prevalence of PTSD. However, in existing publications on PTSD among EMTs, there is a lack of research on PTSD among specifically female EMTs. To fill the gap, this study examined the prevalence of PTSD and its determining factors in female EMTs compared to their male co-workers working in New Jersey. Overall, this study found that 27% of respondents met PTSD criteria, while the prevalence of PTSD among male and female EMTs was similar. However, among the determining factors analyzed, the married women EMTs who took on statistically more family duty responsibilities also showed higher PTSD severities than married men. The discovery of this clear correlation between family duties and PTSD offers enlightenment for relevant agencies in providing support for EMTs.

KEYWORDS: Behavioral and Social Sciences, Post-traumatic stress disorder (PTSD); emergency medical technician (EMT); determining factors; comparison; gender.

■ Introduction

Post-traumatic stress disorder, or PTSD, is a mental condition that an individual could develop after experiencing or witnessing a disturbing event.¹ Such events are common encounters for emergency medical technicians (EMTs) who treat and transport medical patients. Their experiences range from superficial lacerations and bone fractures to horrific car crashes and homicides. Other triggering factors for PTSD in EMTs include stressful conditions like low pay and long working hours; the average income for EMTs is 30% less than that of other working Americans.² PTSD can lead to severe anxiety, trouble sleeping, sudden changes in character, and even suicidal thoughts,¹ significantly affecting an individual's social and occupational life.³ In a professional setting, PTSD has been linked to reduced work performance and impaired decision-making,⁴ with displayed symptoms including high levels of acute stress, intense and unpleasant reactions when faced with high acuity situations, "performance deficits on complex cognitive tasks, verbal memory impairment and heightened assessment of risk".⁵ This means that EMTs impacted by PTSD may not be able to fully concentrate on their work, putting themselves and their patients at risk. This risk is even more alarming with the knowledge that the rate of PTSD among EMTs ranges from 11% to 35%,³ much higher than 6% for the general population developing PTSD at some point in their life.⁶ All these studies and statistics have demonstrated the profound need for investigating, understanding, and mitigating PTSD among EMTs.

To study PTSD among EMTs, a comprehensive evaluation of PTSD-determining factors is important for a non-biased

and in-depth understanding. However, in existing literature, there is an evident lack of research on female EMTs and how they differ from their male counterparts. One such illustration is a study by Khazaei *et al.*, in which 259 participants surveyed were all male, leading the authors to admit in their limitations section that the study may have been gender biased.³ This exclusion of female EMTs in research is not an isolated case but a general trend. After analyzing twelve articles, Alghamdi recognized this gap in his literature review.⁴

In a limited number of studies that included female respondents, it was disappointing that no attempts had been made to determine the correlation between gender and PTSD severity. For instance, Russ surveyed an EMT population with a male-to-female ratio of 224:79 but failed to analyze if there was any difference in PTSD prevalence between them.⁷ Ntatamala & Adams determined that 35% of females and 27% of males included in their study had PTSD. Still, similarly, they did not attempt to investigate the reason for the gender contrast.⁸ Another example is Olf's study, where women were determined to have a two to three times higher risk of developing PTSD than men.⁹ Among all the studies, none of the authors separated the genders to examine them individually or to evaluate what PTSD-determining factors are most significant for each. This gap exists even though 39.1% of over 175,000 EMTs currently employed in the United States are women.¹⁰

The presumption that men and women react similarly to various situations is not a legitimate reason to ignore these current research gaps. In reality, women biologically respond differently to stress and exhibit different PTSD symptoms

than men.¹¹ This is illustrated when male and female rats were exposed to single prolonged stress (SPS) in a study by Pooley *et al.*¹¹ SPS is a model of PTSD that involves exposing the rats to several successive stressful situations, including a forced swim experience and a brief loss of consciousness after exposure to a chemical compound of ether. Males showed higher acoustic startle response (ASR) or enhanced muscular activity produced reflexively in response to the stimulus, replicating a well-established effect of SPS. On the contrary, female rats showed no changes in ASR. A similar trend has been observed in humans as well. “More than half of women with PTSD do not show the male-typical increase in negative feedback control of the HPA axis.

Similarly, women with PTSD are less likely to show enhanced ASR, and in some cases, show diminished startle”.¹¹ These findings indicate that female EMTs may not show as many visible PTSD signs as men. However, this silence does not translate to more resilience in women to PTSD. Instead, this silence may exacerbate the problem, resulting in women being overlooked and not receiving the necessary care.

In addition to physical differences, all the articles in this literature review have ignored the fact that, albeit the roles of men and women have become more equitable over the last few decades, they are still fundamentally dissimilar even today. For example, women’s roles often include more family obligations than men, such as caregiving for children and elderly parents, all on top of their regular jobs.¹² According to a Gallup poll conducted in 2019, women are more likely to do the laundry, prepare meals, and care for children daily in a household compared to men.¹³ Considering that these added responsibilities could induce more stress in women, they are included in this study to help identify the determining factors responsible for the gender difference in PTSD of EMTs.

Due to the lack of analysis regarding PTSD-determining factors for female EMTs specifically, this study aims to tackle this research gap by answering the question: *How does the prevalence of PTSD and its determining factors in female EMTs compare to their male co-workers working in New Jersey?*

By generating conclusions about how gender impacts PTSD rates differently, this study hopes to help inform legislators and healthcare providers about how to assist EMTs best and if gender should play a factor in their decisions. Such assistance may include government support to address the issues arising from gender variations, especially considering donations fully fund some stations. In general, resources for EMTs are not readily available. In some states, emergency medical services (EMS) are not considered essential, so taxpayer dollars do not necessarily support them.¹⁴ This lack of resources may explain why Spitzer found that “only 55% of respondents had ever received any information or education about PTSD, and only 13% of respondents sought treatment for their symptoms”.¹⁴ There are many ongoing legislative debates about these mental health concerns which have led to disparities between states, with some states offering significant, while others offering little or no worker compensation for traumatic experiences.¹⁵ Should more gender-specific resources be needed, this general lack of funding for EMS would present additional challenges

to diagnosing and treating PTSD in female EMTs, emphasizing the need for further research on this gender gap.

New Jersey is an ideal context for conducting such research since it can act as a microcosm of the United States. For instance, the national median age is 38.8 years as of 2021,¹⁶ close to the median age of 40 in New Jersey.¹⁷ This type of similarity can be seen across multiple other variables such as educational attainment, employment rate, and ethnicity, making New Jersey one of the top three states most representative of the US.¹⁸ Such research in a representative setting is important due to the lack of extensive national studies on PTSD among EMTs.¹⁴ Thus, conducting this study in New Jersey is a suitable alternative while generating a significant impact.

■ Methods

The survey research method was the most optimal for this study. The survey research method refers to “collecting information about a group of people by asking them questions and analyzing the results.”¹⁹ It is one of the best tools to gain insights into topics of interest from a predefined group of respondents. This aligns well with the purpose of this study --- to identify the prevalence and possible determining factors of PTSD among EMTs and assess the differences in males and females.

The survey used in this study consisted of three major parts. The first consisted of only one question asking respondents’ consent to participate in the survey. The second part of the survey collected demographic information as potential PTSD-determining factors, and the third part contained questions used to determine the presence of PTSD among respondents.

The questions in the second part were chosen based on studies by Khazaei *et al.* and Ntatalama & Adams, who studied PTSD in the general EMT population.^{3,8} They included questions in their surveys relating to gender, years of work experience, marital status, employment status, time worked, and previous training with stress management. Gender defined in this study is self-identified by the respondents. In the survey for this study, the question concerning marital status was sourced directly from the National Institutes of Health PhenX Toolkit. This platform provides various materials for survey research studies. This version included more comprehensive marital status selections, such as “widowed,” which was not part of the selections in the studies by Khazaei *et al.* or Ntatalama since being “widowed” could be very different from simply being “single.” The survey in this study asked respondents the number of children they have, which was necessary for uncovering its effect on PTSD for men and women, respectively, as the roles of women often include more family obligations than men, such as caring for children.¹² The survey also included questions relating to the respondent’s lifestyle on a scale of agreement ranging from “strongly disagree” to “strongly agree.” These questions were formulated according to a Gallup poll conducted in 2019, in which respondents recognized that compared to men, women are more likely to do the laundry, prepare meals, and care for children daily in a household.¹³

The third and last part of the survey took inspiration from a study by Khazaei *et al.* to determine the prevalence of PTSD among the participating EMTs. They used the PCL-5 survey, a PTSD Checklist based on the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5). The PCL-5 survey contains a total of twenty questions, which is three more than the older PCL-4 version. These additional items were added to align with the updated DSM-5 edition, with updated DSM-5 symptoms, including persistent trauma-related negative emotions, persistent blame, and reckless or self-destructive behavior. Of the remaining seventeen items, the wording of thirteen was revised to better align with the DSM-5 modifications to clarify symptom expression.²⁰ These changes make the PCL-5 survey more advantageous than PCL-4.

To further support the use of the PCL-5 survey, in a study examining the psychometric properties of the PCL-5 survey in firefighters, EMTs, and police officers, the results “indicated that PCL-5 scores showed strong internal consistency and convergent and discriminant validity.”²¹ Internal consistency is a method of determining if all the questions on a test or survey are measuring the same thing.²² For instance, if a test is about algebra, all the questions on the test should be relevant to algebra. Convergent validity refers to whether the results of a test designed to assess a specific topic or construct agree with the results of other tests that assess the same topic.²³ Discriminant validity is the other side of the coin and determines whether a test designed to measure a particular element does not correlate with other tests that measure different elements.²³ In the study by Morrison *et al.*, convergent validity was determined by correlating the PCL-5 survey results with those from other tools measuring PTSD.²¹ Discriminant validity was determined by correlating the PCL-5 survey results with measures of other constructs such as anxiety, depression symptoms, and alcohol abuse.²¹ In all the correlations, the PCL-5 survey displayed expected and appropriate end results, validating its adoption by the author in this study.

The PCL-5 survey also has two optional portions: the Life Events Checklist and Criterion A, which gather information about different events participants have experienced, including earthquakes, etc. Since the two components were not directly related to this study's scope or demographic nature, they were excluded to keep the survey at a manageable length. Only the basic version of the PCL-5 survey was used to determine the severity of PTSD in the participants.

The survey was developed on the online Google Forms platform. The settings were selected so that no Google email accounts were collected from the participants, thus keeping the survey anonymous. Also, participants were given the ability to edit their answers immediately after submitting the form in case inaccurate information was accidentally submitted. The author of this paper is an EMT cadet at a local ambulance station. The Google Forms link was shared on various personal Facebook accounts of the employees at the ambulance station. It was also shared on a Facebook group of EMTs around New Jersey and through the New Jersey EMS Council. Paper copies were also distributed at the author's ambulance station to en-

sure the survey was accessible to those with difficulties using electronic technology.

Surveys provide a significant first step by offering the researcher concrete numbers to run statistical analyses.²⁴ The data were analyzed via independent t-tests using the JASP analytical program. T-tests determine whether there is a statistical difference between the averages of the two groups.²⁵ This aligns with the purpose of this study --- to analyze how different factors contribute to PTSD severity among men vs. women. T-tests were also used by Iranmanesh *et al.* to analyze PTSD among paramedic and hospital emergency personnel.²⁶ A t-value of the absolute value of 1.96 or more indicates statistical significance, and this can be conveniently cross-checked with the p-value simultaneously generated by JASP, with 0.05 or below indicating statistical significance. P-values measure the probability that an observed difference between groups is due to chance and if the difference would present itself again if the study were repeated. The correlational analysis did not fit well with this research study because there could be a correlation between various factors for men and women, respectively. Still, it would be challenging to compare how these correlations are different for men and women, thus defeating the purpose of this study. The data collected from the PCL-5 survey can all be quantified by associating a numerical value with the extent of severity, namely, 0 = Not at all, 1 = A little bit, 2 = Moderately, 3 = Quite a bit, and 4 = Extremely. The total PTSD severity score equals the sum of all twenty items on the survey, resulting in the highest possible score of 80. A cutoff score between 31-33 indicates meeting PTSD criteria.²⁷ The higher end of the range (33) was chosen as the cutoff score for this study to replicate the study by Khazaei *et al.*³ For analysis, the data was first split based on categories like “married” and then further isolated into sub-groups of “married men” and “married women” for t-test analyses. The averages of each sub-group were plotted in bar charts or line graphs to illustrate comparisons.

To mathematically analyze the questions relating to lifestyle in the survey, the answers were also quantified so that 0 = Strongly disagree, 1 = Disagree, 2 = Neither agree nor disagree, 3 = Agree, and 4 = Strongly agree.

■ Results

There were 103 responses in total to the distributed questionnaire. Among them, 33% of respondents (34 out of 103) identified as women, 66% (68 out of 103) as men, and less than 1% (1 out of 103) as nonbinary. Nearly a third, or 27% of respondents (28 out of 103), met PTSD criteria with a score of 33 or more. The PTSD for different genders was compared regarding various potential determining factors, and t-test results were tabulated to determine the statistical significance of the factors evaluated. The average PTSD severity among men, women, non-binary, and all respondents was compared and displayed in Figure 1. Table 1 shows the t-test results comparing PTSD between men and women. Unfortunately, a t-test could not be performed for the one non-binary response as a minimum of two data points were required to run a t-test.

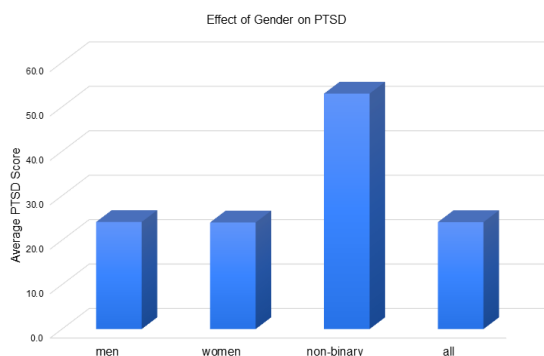


Figure 1: Effect of Gender on PTSD. There was no obvious difference in PTSD between men and women. However, the one non-binary submission appeared significantly higher than most other participants. Even though this one respondent may not be a comprehensive representation of the non-binary population, it could present areas for further research.

Table 1: Results of t-test Examining PTSD in Men vs. Women. There was no statistically significant difference in PTSD between male and female EMTs.

	t	df	p
PTSD men vs women	0.03	100	0.976

A t-value of absolute value 1.96 or more indicates statistical significance, and this can be conveniently cross-checked with the p-value, where a number of 0.05 or below indicates statistical significance. The value of df in the t-test, or degrees of freedom, indicates the number of independent values that can vary in a statistical analysis without breaking any constraints. In Table 1, neither the t-value of 0.03 nor the p-value of 0.976 met statistical significance.

Figure 2 displays the average PTSD in respondents of various marital statuses for men and women, while Table 2 contains the results of the t-tests.

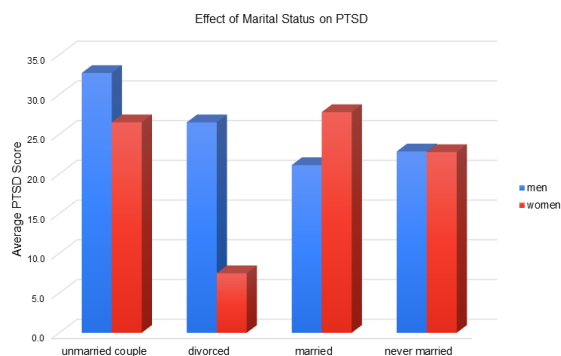


Figure 2: Effect of Marital Status on PTSD. For unmarried couples or divorcees, men tended to have higher PTSD severity. However, for those in a marriage, women tended to have higher PTSD severity, though these differences did not meet statistical significance.

Table 2: Results of t-test Examining Effect of Marital Status on PTSD. The PTSD differences in different marital statuses were insignificant.

	t	df	p
Member of an unmarried couple	0.617	20	0.544
Divorced	0.924	2	0.453
Married	-1.116	38	0.272
Never married	0.012	30	0.991

Family duty combines responsibilities, including cleaning, doing the laundry, caring for children and elderly relatives, and cooking. These responsibilities were analyzed for men, women, and all genders in association with the average PTSD in the respective groups, as displayed in Figure 3.

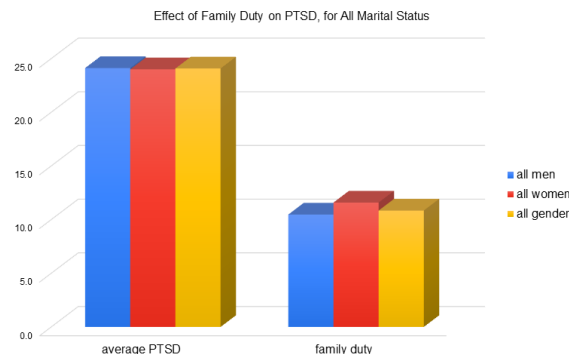


Figure 3: Effect of Family Duty on PTSD for All Marital Statuses. There was no difference in the amount of family duty held by men or women, resulting in no discernible difference in PTSD between genders.

However, encouraged by the differences observed in PTSD in different marital statuses (Figure 2), the researcher examined married men and married women and their relationship with family duties, as summarized in Figure 4. Table 3 contains the t-test results used to determine statistical significance between these groups.

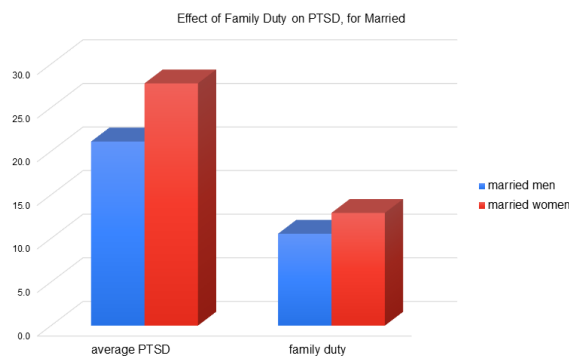


Figure 4: Effect of Family Duty on PTSD for Married EMTs. There was a discernible difference between the family duties performed by married men and women, with females responsible for more family duties. A similar trend was also displayed for PTSD, with married women experiencing higher severity than married men.

Table 3: Results of t-test Examining Effect of Family Duty on PTSD for Married Men and Women. The difference in family duties between men and women was statistically significant.

	t	df	p
Married men vs women PTSD	-1.116	38	0.272
Family duties	-2.120	38	0.041

The more severe PTSD in married women than married men, though statistically insignificant, positively correlated with more family duties for women, which showed statistical significance ($t > |1.96|$, $p < 0.05$, Table 3).

Having a backup plan for childcare in the event of a call and its correlation with PTSD were compared for men and women in Figure 5.

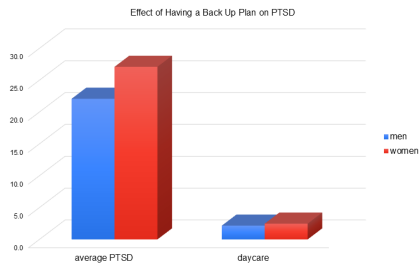


Figure 5: Effect of Having a Backup Plan on PTSD. There was no difference in the accessibility of childcare between men and women if they got an ambulance call, based on responses to the prompt “When I get a call, I have a backup plan for childcare.” Both groups had ratings slightly higher than two, translating to “neither agree nor disagree.”

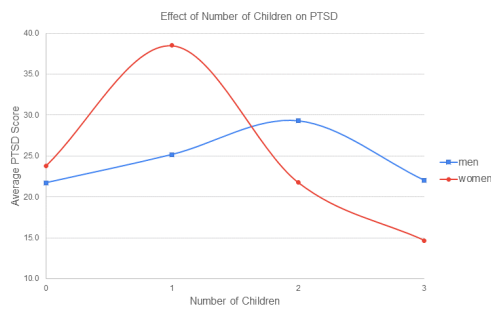


Figure 6: Effect of Number of Children on PTSD. Men and women with no children had similar PTSD scores, whereas women with one child have noticeably higher PTSD than men with one child. For those with two or three children, men have more PTSD than women, indicating that the number of children affects genders differently.

Figure 7 demonstrates the effect of having previous stress management training on PTSD, and Table 4 represents the results from the t-tests.

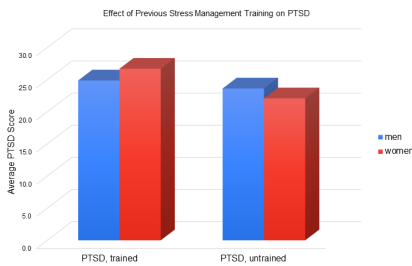


Figure 7: Effect of Previous Stress Management Training on PTSD. The PTSD among trained women was slightly higher than that among trained men, while the opposite was true for untrained EMTs.

Table 4: Results of t-test Examining Effect of Previous Stress Management Training on PTSD. No statistical differences were found between men and female EMTs, with and without previous stress management training.

	t	df	p
Does not have previous training	0.302	60	0.764
Has previous training	-0.286	38	0.777

Years of working experience were compared in relation to PTSD visually and statistically in Figure 8 and Table 5, respectively.



Figure 8: Effect of Years of Working Experience on PTSD. Among EMTs with less than twenty years of working experience, men experienced higher PTSD, whereas, for EMTs with more than twenty years of work experience, women experienced higher PTSD.

Table 5: Results of t-test Examining Effect of Years of Working Experience on PTSD. The differences in PTSD levels displayed by men and women showed no statistical significance for any work experience groups.

	t	df	p
<10	0.533	52	0.596
10-20	0.204	24	0.840
>20	-0.558	20	0.583

The effect of the number of hours worked per week by EMTs on PTSD was compared in Figure 9 and statistically analyzed in Table 6.

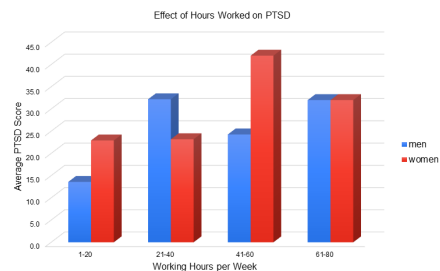


Figure 9: Effect of Hours Worked per Week on PTSD. Women working 1-20 hours or 41-60 hours per week had higher PTSD than their male counterparts. Conversely, men working 21-40 hours had higher PTSD than females with the same number of hours.

Table 6: Results of t-test Examining the Effect of Working Hours per Week on PTSD. There were no statistically significant differences between PTSD in men and women with various numbers of working hours per week.

	t	df	p
<20	-1.696	27	0.101
21-40	1.419	30	0.166
41-60	-1.515	23	0.143
61-80	Not enough responses	Not enough responses	Not enough responses

Figure 10 and Table 7 analyze the effect of employment status on PTSD between men and women, including paid, volunteer, and retired EMTs.

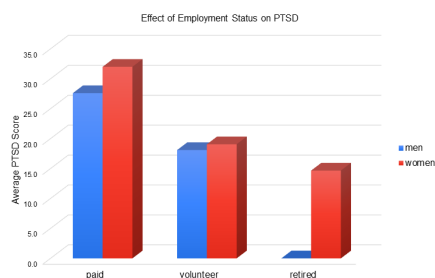


Figure 10: Effect of Employment Status on PTSD. In each case, female EMTs had higher PTSD than men with the same employment status.

Table 7: Results from t-test Examining Effect of Employment Status on PTSD. No statistically significant differences were found between men and women EMTs for any of the groups examined based on employment status.

	t	df	p
Paid	-0.711	57	0.480
Volunteer	-0.198	37	0.844

■ Discussions

An overall analysis of the data in this study demonstrates that nearly a third, or 27%, of respondents meet PTSD criteria. This aligns with the findings of previous studies, including one by Khazaei *et al.*, who determined a 22% prevalence within their EMT population,³ and Ntatamala & Adams, who found a 30% prevalence.⁸ Thus, the PTSD among EMTs in New Jersey is significantly higher than PTSD among the general population, which is only around 6%, a phenomenon attributed to the nature of the job and the type of traumatic experiences associated with the field of emergency medicine.⁶

The prevalence of PTSD in female and male EMTs working in New Jersey is quite similar. However, evaluation of PTSD resulting from various PTSD-determining factors reveals moderate to significant differences between men and women EMTs, providing a more in-depth understanding of the PTSD prevalence among EMTs in New Jersey.

Marital status, one of the determining factors evaluated in this study, has also been studied previously. Khazaei *et al.* found that EMTs who are married have higher levels of PTSD.³ Even though they did not analyze females and males individually, their conclusions about the general effect of marriage on PTSD support the findings of this study. Garcia & Umberson and Cleary & Mechanic directly support the finding that married women experience more stress than married men, though their study did not target PTSD.^{28,29}

An explanation for higher stress in married women could be that women are responsible for more household duties, which showed a statistically significant difference from men based on the data in this study. This corroborates the findings of many previous studies as well.³⁰ As recognized by Cleary & Mechanic, housewives experience more stress than women who do not have children due to more significant amounts of housework.²⁹ The uniqueness of this research is that these greater responsibilities were tied to higher levels of PTSD among married female EMTs than their male counterparts.

However, in Cleary & Mechanic's study, compared to housewives, employed women with children had more stress.²⁹ A similar finding is illustrated by this study in that women EMTs without any children had similar levels of PTSD as

men. In contrast, an apparent disparity appeared when female EMTs had higher PTSD than men, with both groups having one child. The combination of stress from work and childcare can push a female EMT to experience more PTSD than men. However, it is unclear why women in this study with two or three children experience less PTSD than men, an avenue that can be explored in future research.

With such stress, EMTs must learn how to recognize PTSD, cope with it, and stay healthy. Khazaei *et al.* determined that EMTs with no previous stress management training have higher levels of PTSD.³ However, this study contradicts their finding, namely, both men and women EMTs without previous training exhibit lower levels of PTSD. Those who have received training may be more aware of the symptoms of PTSD and, therefore, better at identifying them. Having experienced PTSD could also be why they received the training in the first place, for them to better prepare against and know how to cope with future traumatic experiences.

Another contradiction between this study and Khazaei *et al.*'s study is in terms of EMT work experience. Khazaei *et al.* found that EMTs with ten or fewer years of work experience tend to have more PTSD,³ while this study finds that women with more than twenty years of experience have higher levels of PTSD, with men maintaining relatively the same between different groups. This difference could be explained by the fact that this study purposely included female participants, a sample group that Khazaei *et al.* lacked.³ The inclusion of women in this study makes the conclusion unbiased and, therefore, more convincing, especially since the female gender is a factor that has been linked to greater levels of physical and emotional exhaustion.³¹ The positive correlation between work experience and PTSD in women observed by this study could be explained by accumulated traumatic experiences resulting from greater years of work experience.

Similar to the findings about years of work experience, this study also finds that women who worked long hours (41-60 hours per week) experience the greatest PTSD. Though analyzed slightly differently, this finding is similar to that of Khazaei *et al.* They found that EMTs who had worked more than eight shifts per month had the greatest PTSD, defining a shift as 24 hours. For this study, 50 hours per week can be translated into approximately 200 hours per month, which equals 8.33 shifts, corroborating the findings of Khazaei *et al.*³

A positive correlation was observed between being formally employed as a paid EMT and higher levels of PTSD. This may be attributed to the fact that paid EMTs, on average, work more than double the hours of volunteer EMTs and thus have higher chances of encountering traumatic experiences.

Building off existing literature, the new understanding from this study is that although the prevalence of PTSD among female and male EMTs working in New Jersey is similar, various determining factors cause higher rates of PTSD in female EMTs than their male counterparts. Among these factors, responsibilities relating to family duties show statistically higher amounts in married women than men.

The results of this study were not unexpected, as previous studies have shown that there is a biological difference between

how men and women react to stress¹¹. These findings can act as guidelines in emergency medicine for healthcare providers and policymakers as they attempt to provide wellness support for EMTs. Various PTSD-determining factors affect men and women differently and should be considered when developing preventative methods to help EMTs most effectively. For example, it would be recommended that programs targeting female EMTs should include a component relating to handling family duties. This component may not be as effective for lowering levels of PTSD in male EMTs. Help given to EMTs will also have broad-ranging effects on people around them, including their family members, friends, and patients.

In many of the comparisons (bar graphs) in this study, a visually discernible difference in PTSD can be noted in the groups investigated. Still, most of the t-tests did not yield statistically significant results. This is most likely due to the limited number of responses collected. When the responses were divided into various groups for analyses, each group needed more data to run effective t-tests. This also reveals a limitation of this study: the limited number of responses collected. However, it is still important to visually determine how various characteristics affect PTSD among male and female EMTs for a deeper understanding. With approximately 26,000 EMTs currently in New Jersey³² and a 95% confidence interval, the optimal size for this study to ensure accurate results should be 379 respondents.³³ The ultimate sample size of a little over 100 respondents in this study makes it difficult to extrapolate the results to a broader population. The results are probably most representative of the region immediate to where the author lives, as the author's local ambulance station sent out the survey and thus did not accurately represent the entire state of New Jersey or the United States. Additionally, with a larger sample size, there could be more determining factors in addition to family duties that could yield statistically significant results. This would enrich the overall new understanding of this research. Another limitation is that respondents can be inaccurate in their responses, intentionally or not, so a larger sample size could offset these issues. Therefore, future research with a greater sample size is recommended to resolve previously described issues.

Another area of future research involves studies with more gender identifications. There was one nonbinary participant in this study, and their PTSD severity was significantly higher than that of the rest of the participants. Though they cannot represent the entire nonbinary EMT population, this data presents a promising avenue for further research regarding how the nonbinary gender status contributes to PTSD severity and the PTSD-determining factors among EMTs.

■ Conclusion

In this study, we examined the prevalence of PTSD and its determining factors in female EMTs working in New Jersey and compared them to their male co-workers. Among all the respondents, 27% met PTSD criteria, higher than the general population. Although the prevalence of PTSD among female and male EMTs was similar, an apparent disparity was displayed between some demographic groups broken down by PTSD-determining factors such as marital status, household

responsibilities, and age. Among these factors investigated, obligations relating to family duties showed statistically higher levels in married women, directly correlating with the higher levels of PTSD observed in this group.

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■ References

1. Mayo Clinic Staff. Post-Traumatic Stress Disorder (PTSD). <https://www.mayoclinic.org/diseases-conditions/post-traumatic-stress-disorder/symptoms-causes/syc-20355967> (accessed 2023-05-21).
2. Kernstine, K.; Buchman, C. EMTs Nationwide Leaving Profession Because of Low Pay, High Stress. <https://www.newsnationnow.com/health/low-pay-high-stress-lead-to-emt-shortage-across-country/> (accessed 2023-05-21).
3. Khazaei, A.; Navab, E.; Esmaili, M.; Masoumi, H., Prevalence and Related Factors of Post-Traumatic Stress Disorder in Emergency Medical Technicians; a Cross-Sectional Study. *Archives of Academic Emergency Medicine* **2021**, *9* (1), 7.
4. Alghamdi, A. A., The Psychological Challenges of Emergency Medical Service Providers During Disasters: A Mini-Review February 2022. *Front. Psychiatry* **2022**, *13*, 8.
5. Regehr, C.; LeBlanc, V. R., PTSD, Acute Stress, Performance and Decision-Making in Emergency Service Workers. *J. Am. Acad. Psychiatry Law* **2017**, *45* (2), 184-192.
6. Staff at VA's National Center for PTSD. How Common Is PTSD in Adults? . https://www.ptsd.va.gov/understand/common/common_adults.asp (accessed 2023-05-21).
7. Russ, T., Post-traumatic stress disorder comparison between fire and EMS Personnel. *Journal of Emergency Medical Services*. **2022**
8. Ntatamala, I.; Adams, S., The Correlates of Post-Traumatic Stress Disorder in Ambulance Personnel and Barriers Faced in Accessing Care for Work-Related Stress. *Int. J. Environ. Res. Public Health* **2022**, *19* (4), 14.
9. Olf, M., Sex and gender differences in post-traumatic stress disorder: an update. *Eur. J. Psychotraumatol.* **2017**, *8*, 2.
10. Zippia. Emergency Medical Technician Demographics and Statistics in the US. <https://www.zippia.com/emergency-medical-technician-jobs/demographics/> (accessed 2023-05-21).
11. Pooley, A. E.; Benjamin, R. C.; Sreedhar, S.; Eagle, A. L.; Robison, A. J.; Mazei-Robison, M. S.; Breedlove, S. M.; Jordan, C. L., Sex differences in the traumatic stress response: PTSD symptoms in women recapitulated in female rats. *Biol. Sex Differ.* **2018**, *9*, 11.
12. Cleveland Clinic. Women and Stress. <https://my.clevelandclinic.org/health/articles/5545-women-and-stress> (accessed 2023-05-21).
13. Brenan, M. Women Still Handle Main Household Tasks in U.S. <https://news.gallup.com/poll/283979/women-handle-main-household-tasks.aspx> (accessed 2023-05-21).
14. Spitzer, A., First Responders and PTSD: A Literature Review.

Journal of Emergency Medical Services. 2020

15. Patterson, J. Workers' Compensation for PTSD: Which States Offer Benefits? <https://www.gerberholderlaw.com/workers-comp-ptsd-by-state/> (accessed 2023-05-21).
16. United States Census Bureau. Nation Continues to Age as It Becomes More Diverse. <https://www.census.gov/newsroom/press-releases/2022/population-estimates-characteristics.html> (accessed 2023-05-21).
17. Data USA. New Jersey. <https://datausa.io/profile/geo/new-jersey> (accessed 2023-05-21).
18. Kolko, J. "Normal America" Is Not a Small Town of White People. <https://fivethirtyeight.com/features/normal-america-is-not-a-small-town-of-white-people/> (accessed 2023-05-21).
19. McCombes, S. Survey Research: Definition, Examples & Methods. <https://www.scribbr.com/methodology/survey-research/> (accessed 2023-05-21).
20. LeardMann, C. A.; McMaster, H. S.; Warner, S.; Esquivel, A. P.; Porter, B.; Powell, T. M.; Tu, X. M.; Lee, W. W.; Rull, R. P.; Hoge, C. W.; Millennium Cohort Study, T., Comparison of Posttraumatic Stress Disorder Checklist Instruments From Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition vs Fifth Edition in a Large Cohort of US Military Service Members and Veterans. *JAMA Netw. Open* **2021**, *4* (4), 11.
21. Morrison, K.; Su, S. Y.; Keck, M.; Beidel, D. C., Psychometric properties of the PCL-5 in a sample of first responders. *J. Anxiety Disorders*. **2021**, *77*, 9.
22. Cornell, D. 15 Internal Consistency Reliability Examples. <https://helpfulprofessor.com/internal-consistency-reliability-examples/> (accessed 2023-05-21).
23. Questionmark. Understanding Convergent & Discriminant Validity. <https://www.questionmark.com/resources/blog/understanding-convergent-discriminant-validity/> (accessed 2023-05-21).
24. Survey Monkey. Why Survey Research and Survey Methodology Matter. <https://www.surveymonkey.com/mp/why-survey-understanding-survey-methodology/> (accessed 2023-05-21).
25. Staff at Laerd Statistics. Independent T-Test for Two Samples. <https://statistics.laerd.com/statistical-guides/independent-t-test-statistical-guide.php> (accessed 2023-05-21).
26. Iranmanesh, S.; Tirgari, B.; Bardsiri, H. S., Post-traumatic stress disorder among paramedic and hospital emergency personnel in south-east Iran. *World J. Emerg. Med.* **2013**, *4* (1), 26-31.
27. Staff at VA's National Center for PTSD. PTSD Checklist for DSM-5 (PCL-5). <https://www.ptsd.va.gov/professional/assessment/adult-sr/ptsd-checklist.asp> (accessed 2023-05-21).
28. Garcia, M. A.; Umberson, D., Marital Strain and Psychological Distress in Same-Sex and Different-Sex Couples. *J. Marriage Fam.* **2019**, *81* (5), 1253-1268.
29. Cleary, P. D.; Mechanic, D., Sex-Differences in Psychological Distress Among Married People. *J. Health Soc. Behav.* **1983**, *24* (2), 111-121.
30. Cerrato, J.; Cifre, E., Gender Inequality in Household Chores and Work-Family Conflict. *Front. Psychol.* **2018**, *9*, 11.
31. Hsu, H. C., Age Differences in Work Stress, Exhaustion, Well-Being, and Related Factors From an Ecological Perspective. *Int. J. Environ. Res. Public Health* **2019**, *16* (1), 15.
32. State of New Jersey Department of Health. Emergency Medical Services. <https://www.nj.gov/health/ems/> (accessed 2023-05-21).
33. Qualtrics. Sample Size Calculator. <https://www.qualtrics.com/blog/calculating-sample-size/> (accessed 2023-05-21).

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Discovery of Novel Genetic Alteration Using Meta-analysis of Colorectal Cancer

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ABSTRACT: More than 5.25 million people worldwide are diagnosed with colorectal cancer (CRC), representing 10% of the global cancer incidence and 9.4% of all cancer-caused deaths. It is common to find genetic and epigenetic alterations in CRC, which are the driving force of tumorigenesis. Therefore, discovering a novel genetic alteration in colorectal cancer can support early diagnosis and finding novel targets for cancer treatment. However, genetic alterations found in colorectal cancer are not fully elucidated. A meta-analysis of colorectal cancer genomics data sets was performed using 12 different studies provided by cBioPortal to identify the novel genetic alteration in colorectal cancer patients. Through cBioPortal analysis, it was hypothesized that chromosome 17q21 amplification is associated with tumor progression. Patient survival analysis was performed through cBioPortal analysis. Also, nine genes located in 17q21 were further analyzed with GeneMania, a web-based program that predicts the function of gene sets. Using cBioPortal analysis, it was discovered that chromosome 17q21 amplification was enriched in deceased patients. Furthermore, through patient survival analysis, amplification of each of the nine genes located in chromosome 17q21 was significantly associated with decreased patient survival rate. Hence, using GeneMania analysis, it was discovered that the gene network of the nine genes was significantly associated with DNA integrity checkpoint function. Through this study, chromosome 17q21 amplification, which may alter the part of DNA integrity in cancer cells, can be used as a biomarker that predicts poor patient survival.

KEYWORDS: Human genetics; Colorectal cancer; Patient survival rate; Chromosome 17; DNA integrity checkpoint.

■ Introduction

Colorectal cancer (CRC) mostly begins as a polyp, a non-cancerous growth that develops in the colon's inner lining.¹ Polyps are classified as either adenomatous or serrated.² Similar to adenomas, serrated polyps, traditional serrated adenomas, and large hyperplastic polyps are associated with an increased risk for CRC.³ Because sessile serrated polyps (SSPs) are difficult to detect during colonoscopy as they are usually flat, these features make them the precursors for a large proportion of cancers.⁴

Gene mutation found in colorectal cancer affects overall patient survival.⁵ For example, approximately half of all colorectal cancers show *TP53*, otherwise known as P53, gene mutations, with higher frequencies observed in the distal colon and rectal tumors.⁶ The role of the *TP53* gene is to regulate the cell cycle and apoptosis. Specifically, the P53 protein induces G1 cell-cycle arrest and controls the repairing of the DNA before the cell goes into DNA replication.⁷ If the DNA repair is unsuccessful, P53 causes cell death. *TP53* mutation occurs at the time of transition from adenoma to cancer. Several studies attempted to explain the significance of *TP53* mutation in colorectal cancer, with conflicting results. A study concluded that the survival rate for P53 positive patients was far greater than that for P53 negative patients. However, overexpression of P53 in stage three CRC carried a better overall survival in CRC patients.⁸

Gene deletion in colorectal cancer also affects colorectal patients' survival. In chromosome 18, loss of heterozygosity

(LOH) in the region of 18q21 is often seen in advanced colorectal cancer. LOH is defined as the loss of one allele at a specific locus.¹⁰ Often, the remaining allele is affected by a deletion mutation or a loss of chromosome from a chromosome pair. Some studies found an inverse relationship between CRC patient survival and 18q LOH. A previous study evaluated the effect of 18q LOH on 532 non-MSI-high, stage I-IV CRC tumors; in patients with non-MSI-high CRC, 18q LOH were not significantly associated with a difference in survival.¹¹

The cBio Cancer Genomics Portal (cBioPortal) contains numerous multidimensional cancer genomics data sets.¹² The cBioPortal minimizes the tasks needed to collect data by summarizing complex genomic data from large-scale cancer genomics projects.¹³ Through cBioPortal, it is possible to better understand biology and clinical applications.

GeneMania provides hypotheses about gene function by showing a list of genes and categorizing genes based on their functions.¹⁴ After receiving comprehensive gene lists, GeneMania groups the genes based on their function, followed by genomics and proteomics data. GeneMania determines whether the functional genomic dataset follows its predictive value during this process. GeneMania also predicts gene function. Using a single gene, GeneMania searches for several genes with the same function based on its interactions with other genes.¹⁴ Overall, GeneMania allows researchers to analyze genes more efficiently and intuitively.

■ Methods

Analyzing genomic alteration on colorectal patients' genomic data using cBioPortal:

Through cBioPortal, 4341 colorectal patients (4488 samples) were analyzed to find a novel genetic alteration associated with patients' survival. After the patient samples were divided into two groups: living group (n = 1275) and deceased group (n = 273), amplified genes enriched in the deceased group were found. The gene location, percentage of alteration in each group, log-ratio between living and deceased group, p-value, and q-value was analyzed using cBioPortal. The log-rank statistical test was used to calculate the p-value.

Gene network analysis using the GeneMania program:

Gene network analysis was performed with eleven genes found in the cBioPortal (*GJD3*, *CCR7*, *TOP2A*, *CDC6*, *IGFBP4*, *WIPF2*, *KRT222*, *SMARCE1*, *RARA*). GeneMania predicts the function of listed genes and their genetic network, including co-expression, physical interaction, shared domains, and biological pathways. GeneMania analyzes the gene lists and prioritizes the genes for functional assays. It finds functionally similar genes within the genomics and proteomics data that have been previously published. Since hundreds of millions of interactions had been collected by the database from GEO, BioGRID, IRefIndex, and I2D, the interaction databases were used to predict the gene function.

Patient survival analysis using cBioPortal:

cBioPortal for cancer genomics provides visualization analysis of overall patient survival status. The overall survival of patient groups between the gene amplified group (*TOP2A* and *CDC6*) and the non-amplified group was analyzed. Kaplan-Mier analysis and log-rank test were performed to calculate the p-value. The median survival month in each group was also investigated.

■ Results and Discussion

Table 1: The amplified genes located on 17q21.2 enriched in the deceased colorectal cancer patient's group.

Gene	Cytoband	LIVING (n = 1275)	DECEASED (n = 273)	Log Ratio	p-Value	q-Value	Enriched in
<i>GJD3</i>	17q21.2	19 (1.49%)	15 (5.49%)	-1.88	2.57E-04	5.97E-03	DECEASED
<i>CCR7</i>	17q21.2	17 (1.33%)	12 (4.82%)	-1.85	1.06E-03	0.0149	DECEASED
<i>TOP2A</i>	17q21.2	19 (1.49%)	13 (4.76%)	-1.68	1.72E-03	0.0209	DECEASED
<i>CDC6</i>	17q21.2	19 (1.49%)	12 (4.82%)	-1.69	2.13E-03	0.0239	DECEASED
<i>IGFBP4</i>	17q21.2	19 (1.49%)	12 (4.82%)	-1.69	2.13E-03	0.0239	DECEASED
<i>WIPF2</i>	17q21.2	19 (1.49%)	12 (4.82%)	-1.69	2.13E-03	0.0239	DECEASED
<i>KRT222</i>	17q21.2	14 (1.10%)	10 (4.02%)	-1.87	2.65E-03	0.0289	DECEASED
<i>SMARCE1</i>	17q21.2	15 (1.18%)	10 (4.02%)	-1.77	3.79E-03	0.0358	DECEASED
<i>RARA</i>	17q21.2	28 (1.27%)	26 (2.73%)	-1.1	3.84E-03	0.0359	DECEASED

Gene amplification is when there is an increase in the copy number of DNA present in a specific region of the chromosome or an increase in the RNA and protein made from that gene. Cancer cells often produce multiple copies of genes, and some of the amplified genes can cause cancer cells to grow faster or become resistant to anticancer drugs. 4341 colorectal patients (4488 samples) were analyzed to find a novel genetic alteration associated with patients' survival through the cBioPortal database. First, the patient samples were divided into two groups: living group (n = 1275) and deceased group (n = 273). It was found that chromosome position 17q21.2 amplification is enriched in the deceased patient group (Ta

ble 1). In total, nine amplified genes are located in 17q21.2: *GJD3*, *CCR7*, *TOP2A*, *CDC6*, *IGFBP4*, *WIPF2*, *KRT222*, *SMARCE1*, and *RARA*. Overall, it was found that the novel amplification of nine genes in colorectal cancer patient samples enriched in decreased patient groups.

Previous studies showed that 17q21 amplification was detected in gastric cancer and breast cancers.^{15,16} The comparative analysis of DNA copy number and microarray in gastric cancer shows that the 17q12-q21 region is amplified and many genes in this region are overexpressed. A breast cancer study indicated that *HER2/NEU* amplification (both positioned on 17q21) is responsible for the development of Trastuzumab, one of the first immunotherapeutic drugs for the successful treatment of breast cancers.¹⁷ In conclusion, the amplification in region 17q21 not only caused colorectal cancer but also gastric cancer and breast cancer. This shows how 17q21 plays a critical role in cancer progression.

Table 2: The result of functional prediction of nine amplified genes with extended genes that are functionally similar using GeneMANIA.

Function	P-value	Coverage
DNA integrity checkpoint	3.33e-1	4/147
Negative regulation of epithelial cell proliferation	3.33e-1	3/54
Hormone receptor binding	3.33e-1	4/148
Mitotic cell cycle checkpoint	3.33e-1	4/136
DNA replication checkpoint	3.68e-1	2/10
Insulin-like growth factor binding	5.30e-1	2/13
Cell cycle checkpoint	5.75e-1	4/201
Regulation of chromosome organization	6.13e-1	4/239
Positive regulation of DNA-templated transcription, initiation	6.13e-1	2/23
DNA recombination	6.13e-1	4/229

GeneMANIA analysis was performed to find a novel function of nine amplified genes on colorectal cancer progression. It was found that DNA integrity checkpoint, negative regulation of epithelial cell proliferation, hormone receptor binding, and mitotic cell cycle checkpoint were determined to be the most significant functions of the nine amplified genes (p-value = 3.33e-1) (Table 2). Since the maintenance of genomic integrity is important in normal cell growth and development, gene alteration on DNA integrity checkpoints is found in many cancer cells. In addition, DNA integrity checkpoints provide cells with time to repair damaged DNA, but cancer-initiating cells have lost DNA repair or cell-cycle checkpoints. In conclusion, four genes among nine amplified genes were significantly associated with the function of the DNA integrity checkpoint meaning alteration in this function may be linked to the poor survival rate of colorectal patients.

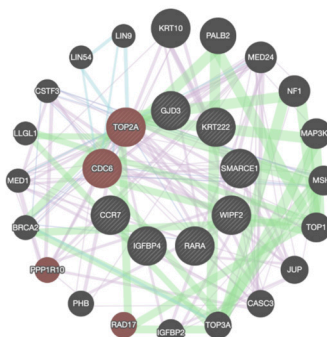


Figure 1: Gene network analysis of ten DEGs, located in the center as slash patterned. The gene sets associated with ten DEGs are located around the ten DEGs. The four red genes are associated with the DNA integrity checkpoint. The green line indicates the physical interaction between the genes. The purple line indicates the co-expressed genes. The blue line indicates the co-localized genes.

Cancer-associated necrosis produces more abnormal DNA fragments than apoptosis in human cells. Therefore, this study focused on the DNA integrity checkpoints because DNA integrity, which relates to the uneven copy number of DNA fragments, is highly related to cancer. According to the model, *TOP2A* and *CDC6* are among the nine amplified genes, and *PPP1R10* and *RAD17* are functionally related to the nine amplified genes. In conclusion, amplification of *TOP2A* and *CDC6* and the alteration of relative genes (*PPP1R10* and *RAD17*) may affect DNA integrity checkpoints, which shortens the survival rate of colorectal cancer patients (Figure 1).

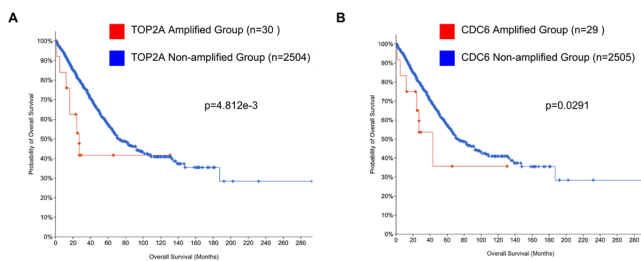


Figure 2: The analysis of overall survival of *TOP2A* and *CDC6* amplified patient group compared to *TOP2A* and *CDC6* non-amplified patient group. (A) *TOP2A* amplified group (n=30) showed a lower survival rate compared to *TOP2A* non-amplified group (n=2504) ($p = 4.812e-3$). (B) *CDC6* amplified group (n=29) showed lower survival rate compared to *CDC6* non-amplified group (n=2505). ($p = 0.0291$) Log-rank test was used to calculate the p-value.

To validate the effect of *TOP2A* and *CDC6* amplification on colorectal cancer patients' survival, the overall patient survival rate was further analyzed with the cBioPortal database. The patients were divided into two groups: the groups with amplified genes and the groups without amplified genes. A graph in Figure 2 shows the probability of overall survival (Y-axis) and overall survival months (X-axis). It was found that the median survival months of *TOP2A* amplified patients (27 months) was significantly lower than those of *TOP2A* non-amplified patients (71.93 months). Similarly, the median survival months of *CDC6* amplified patients (43.17 months) was significantly lower than the median survival months of *CDC6* non-amplified patients (72.47 months). In conclusion, it was speculated that since *TOP2A* and *CDC6* are associated with the function of DNA integrity checkpoint, the lower survival rate in amplified groups may have abnormal DNA structure with severe DNA damage.

Conclusion

To reiterate the findings shown in Figure 2, two genetic alterations *TOP2A* and *CDC6* both significantly decreased the patient survival rate. Using the given information, when analyzing genomics for colorectal cancer patients in the future, it is possible to more accurately diagnose and predict the survival rate of the patients. Furthermore, it was found that 17q21 amplification affected DNA integrity checkpoints the most. With a better understanding of the impact of amplified genes,

ways to recover the affected DNA integrity checkpoint can be found, possibly supporting the development of a novel treatment for colorectal cancer. Lastly, a considerably large sample size of 4448 patients was analyzed. Therefore, the results from this study provide a more accurate interpretation of the impact of 17q21 amplification, further reducing the margin of error when treating colorectal patients in general.

However, the study is limited in the methods used, as only data analysis was performed. The media used to perform meta-analysis has the potential for publication bias, skewed data, and difficulties in combining studies that may have differences in population, interventions, etc. Furthermore, the study is limited in scope, as only gene copy alterations, disregarding other genetic alterations, such as mutation and fusion genes, were focused on. Therefore, cancer cell experiments to not only validate the real implications of 17q21 amplification on cancer cell development can be performed but it is also possible to find potential treatments for colorectal cancer. In addition, cBioPortal can be used to further analyze other genetic variations which could have played a significant role in tumor progression.

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References

- Bond, J. H. Colon Polyps and Cancer. *Endoscopy* **2003**, *35* (1), 27–35.
- Alec, M.; Simion, L.; Straja, N.; Brătu, E. Multiple Polyps and Colorectal Cancer. *Chirurgia (Bucur)* **2014**, *109* (3), 342–346.
- Demetriades, H.; Kanellos, I.; Blouhos, K.; Tsachalis, T.; Vasiliadis, K.; Pramateftakis, M. G.; Betsis, D. Synchronous Polyps in Patients with Colorectal Cancer. *Tech. Coloproctol.* **2004**, *8* Suppl 1, s72–5.
- Farris, A. B.; Misdraji, J.; Srivastava, A.; Muzikansky, A.; Deshpande, V.; Lauwers, G. Y.; Mino-Kenudson, M. Sessile Serrated Adenoma: Challenging Discrimination from Other Serrated Colonic Polyps. *Am. J. Surg. Pathol.* **2008**, *32* (1), 30–35.
- Cavagnari, M. A. V.; Silva, T. D.; Pereira, M. A. H.; Sauer, L. J.; Shigueoka, D.; Saad, S. S.; Barão, K.; Ribeiro, C. C. D.; Forones, N. M. Impact of Genetic Mutations and Nutritional Status on the Survival of Patients with Colorectal Cancer. *BMC Cancer* **2019**, *19* (1), 644.
- Iacopetta, B. TP53 Mutation in Colorectal Cancer. *Hum. Mutat.* **2003**, *21* (3), 271–276.
- Guimaraes, D. P.; Hainaut, P. TP53: A Key Gene in Human Cancer. *Biochimie* **2002**, *84* (1), 83–93.
- Williams, D. S.; Mouradov, D.; Browne, C.; Palmieri, M.; Elliott, M. J.; Nightingale, R.; Fang, C. G.; Li, R.; Mariadason, J. M.; Fargher, I.; et al. Overexpression of TP53 Protein Is Associated with the Lack of Adjuvant Chemotherapy Benefit in Patients with Stage III Colorectal Cancer. *Mod. Pathol.* **2020**, *33* (3), 483–495.
- Armaghany, T.; Wilson, J. D.; Chu, Q.; Mills, G. Genetic Alterations in Colorectal Cancer. *Gastrointest. Cancer Res.* **2012**, *5* (1), 19–27.

10. Chang, S.-C.; Lin, J.-K.; Lin, T.-C.; Liang, W.-Y. Loss of Heterozygosity: An Independent Prognostic Factor of Colorectal Cancer. *World J. Gastroenterol.* **2005**, *11* (6), 778–784.
11. Ogino, S.; Nosho, K.; Irahara, N.; Shima, K.; Baba, Y.; Kirkner, G. J.; Meyerhardt, J. A.; Fuchs, C. S. Prognostic Significance and Molecular Associations of 18q Loss of Heterozygosity: A Cohort Study of Microsatellite Stable Colorectal Cancers. *J. Clin. Oncol.* **2009**, *27* (27), 4591–4598. □
12. Cerami, E.; Gao, J.; Dogrusoz, U.; Gross, B. E.; Sumer, S. O.; Aksoy, B. A.; Jacobsen, A.; Byrne, C. J.; Heuer, M. L.; Larsson, E.; *et al.* The CBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data. *Cancer Discov.* **2012**, *2* (5), 401–404.
13. Gao, J.; Aksoy, B. A.; Dogrusoz, U.; Dresdner, G.; Gross, B.; Sumer, S. O.; Sun, Y.; Jacobsen, A.; Sinha, R.; Larsson, E.; *et al.* Integrative Analysis of Complex Cancer Genomics and Clinical Profiles Using the CBioPortal. *Sci. Signal.* **2013**, *6* (269), p11.
14. Warde-Farley, D.; Donaldson, S. L.; Comes, O.; Zuberi, K.; Badrawi, R.; Chao, P.; Franz, M.; Grouios, C.; Kazi, F.; Lopes, C. T.; *et al.* The GeneMANIA Prediction Server: Biological Network Integration for Gene Prioritization and Predicting Gene Function. *Nucleic Acids Res.* **2010**, *38* (Web Server issue), W214–20.
15. Tanner, M.; Hollmén, M.; Junttila, T. T.; Kapanen, A. I.; Tommila, S.; Soini, Y.; Helin, H.; Salo, J.; Joensuu, H.; Sihvo, E.; *et al.* Amplification of HER-2 in Gastric Carcinoma: Association with Topoisomerase IIalpha Gene Amplification, Intestinal Type, Poor Prognosis and Sensitivity to Trastuzumab. *Ann. Oncol.* **2005**, *16* (2), 273–278.
16. McDonald, S. L.; Stevenson, D. A. J.; Moir, S. E.; Hutcheon, A. W.; Haites, N. E.; Heys, S. D.; Schofield, A. C. Genomic Changes Identified by Comparative Genomic Hybridisation in Docetaxel-Resistant Breast Cancer Cell Lines. *Eur. J. Cancer* **2005**, *41* (7), 1086–1094.
17. Negri, T.; Tarantino, E.; Orsenigo, M.; Reid, J. F.; Gariboldi, M.; Zambetti, M.; Pierotti, M. A.; Piloti, S. Chromosome Band17q21 in Breast Cancer: Significant Association between Beclin1 Loss and HER2/NEU Amplification. *Genes Chromosomes Cancer* **2010**, *49* (10), 901–909.

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Violence Against Women: Addressing the Effectiveness of the Due Diligence Framework in Pakistan

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ABSTRACT: Violence against women is a global problem that has affected the lives of millions of women physically, mentally, socially, and economically. Within this paper, violence against women will be defined from a radical feminist approach as any act of violence rooted in multiple and intersecting forms of discrimination and inequalities that result in or are likely to result in physical, sexual, or psychological harm or suffering to women. Under the pillars of due diligence outlined in the Declaration of Violence Against Women (DEVAV), namely, prevention, protection, prosecution, and remedy, a country must fulfill its obligation to residing women regarding all four aspects. However, upon theoretical and factual analysis of the geopolitical, cultural, and religious factors that influence the legal system in Pakistani society, it can be concluded that the State is unable to uphold the due diligence standard for violence against women effectively, and consequently, unable to provide effective redress to victims of self, interpersonal or communal violence.

KEYWORDS: Behavioral and Social Sciences; Psychology and Sociology; Violence Against Women; Due Diligence Standard; South Asian Perspectives.

■ Introduction

Violence against women (VAW) in Pakistan is a systemic problem that has prevailed since its inception. Today, up to 32% of women have experienced violence, and that is just the reported statistic; more than half of these women do not report the domestic abuse, acid attacks, and honor killings that plague their lives, making the depth of factual and legal analysis of VAW difficult to conduct.¹ Although legislation such as the banning of the two-finger rape test has been passed, the extent to which facilities for victims of violence against women or cultural stigma in prosecution systems have been dealt with remains inadequate, and thus, the violence prevails.²

This paper will assess how the due diligence framework regarding violence against women effectively protects women and girls from violence in Pakistan. In the first section, the definition of violence against women will be established, considering the number of different interpretations and different terminologies of VAW that exist: VAW as a public health issue and as a legal issue, with the feminist critiques of radical feminism and liberal feminism being assessed in both. After defining violence against women, the second section of the paper will break down what the due diligence standard means, first looking at it holistically as a matter of international law and then specifically in the context of violence against women. Defining the requirements of States in upholding the four pillars of prevention, protection, prosecution, and remedy will also be developed under this section. The paper will discuss the manifestations of VAW in Pakistan:

■ Discussion

Definition and Manifestation of VAW:

It is important to acknowledge that there is no clear definition of violence against women, and differences often arise in how various stakeholders define it. These differences lead to VAW and gender-based violence being used interchangeably. In her Special Rapporteur Report, 'The Continuum of Violence against Women and the Challenges of Effective Redress,' Rashida Manjoo defines violence against women from the perspective of a radical feminist as follows:

'Rooted in multiple and intersecting forms of discrimination and inequalities, and strongly linked to the social and economic situation of women, violence against women constitutes a continuum of exploitation and abuse.'³

The link between violence against women and a patriarchal gender hierarchy is made clear in her definition, and this link is again reinforced under the definition of gender-based violence (GBV) presented in the UN Declaration on the Elimination of Violence Against Women: 'any act of gender-based violence that results in or is likely to result in, physical, sexual or psychological harm or suffering to women.'⁴

Yet, in the context of the modern world, many of the more recent definitions of violence against women have begun to shift towards a constructive, feminist approach, focusing less on patriarchy as a structure that enables gender-based violence and more on an inclusive understanding of all forms of violence. These definitions mirror a gradual but significant shift away from the emphasis on gender discrimination as it is manifested in VAW. This is evidenced in how the European Union defines gender-based violence as 'violence directed

person because of that person's gender or as violence that affects persons of a particular gender disproportionately.' The need to be inclusive of violence against all genders in their definition overshadows the impact of violence on women and girls.⁵

However, such an approach can be problematic considering how recognizing the disproportionate impact of violence against women and girls within these definitions is necessary to truly encapsulate the degree of harm from such violence. When looking at the manifestations of VAW broadly, the World Report on Violence and Health categorized them into self-directed, interpersonal, and collective, as seen in the diagram below extracted from 'Violence Against Women.'⁶

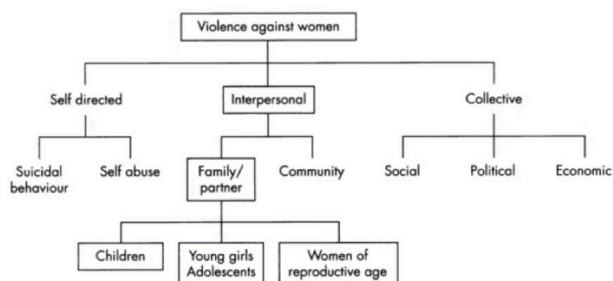


Figure 1: A typology of violence against women, modified after the World Report on Violence and Health, WHO

Self-directed manifestations of VAW can look like suicidal tendencies, particularly in cases where unmarried women become pregnant and do not see a way out of the financial or societal ostracisation that may result from it. Similarly, self-abuse in the form of eating disorders such as bulimia or anorexia becomes common due to increasing stress levels and self-loathing.⁷ Interpersonal manifestations exist within the family of these women where many are subjected to rape or molestation by their husbands, fathers, brothers, and other members of their family: in other cases, they are publicly honor killed for bringing 'dishonor' to the family name. The last manifestation of violence against women encompasses the violence inflicted by larger groups such as 'states, organized political groups, militia groups, and terrorist organizations.'⁸ Within all of these manifestations, the nature of violent acts can be physical, sexual, psychological or involving deprivation or neglect.

As a result of the variety of manifestations, many conceptual debates about the classification of VAW have arisen in recent years on whether to classify VAW as a violation of human rights, as discrimination, or as a public health issue. Previously, violence against women was understood as a social and legal problem. The Convention on the Elimination of All Forms of Discrimination against Women (CEDAW) General Recommendation No.19 states that gender-based violence against women does impair or nullify women's enjoyment of human rights and fundamental freedoms.⁹ The Committee also affirmed that violence against women constitutes discrimination within the meaning of Article 1 of the Convention: 'discrimination against women shall mean any distinction, exclusion or restriction made based on sex which has the effect or purpose of impairing or nullifying the recognition, enjoyment or exer-

cise by women, irrespective of their marital status, based on equality of men and women, of human rights and fundamental freedoms in the political, economic, social, cultural, civil or any other field.'¹⁰

All violence directed against a woman because she is a woman, or violence that disproportionately affects women is thus classified as discriminatory. Alternatively, a variety of feminist lawyers tend to view VAW as a legal issue because of the general belief in law as a catalyst for social change – the desire to use the law as a mechanism for structural change is the foundation for this constructive feminist approach. However, in 1996, the World Health Assembly reframed VAW as a public health issue by adopting a resolution that declared that 'violence is a leading public health problem worldwide'¹¹ due to the physical and mental costs a woman has to endure upon being inflicted with violence through rape, battery, assault, harassment and so on. In arguing for this perspective, those in favor of the public health model stipulate that the only way to make VAW a palpable problem for States is to make it a public health disaster, something they already have the responsibility to protect people from: the classification makes alleviating VAW a priority for States by making it a more palatable problem.¹²

This perspective is critiqued by many radical feminists for its lack of functionality. Viewing VAW as a public health issue forces society to view it as a disease or condition that needs to be cured without analyzing individual, communal, or structural barriers. Consequently, this approach tunnels in on a disease conceptualization of VAW and, in doing so, prioritizes 'treating' manifestations of VAW above the root problem of patriarchy. Addressing violence against women as a public health issue requires a comprehensive approach that includes prevention, intervention, and support for survivors, as well as efforts to address the underlying social and cultural norms that perpetuate violence and inequality, hence making it difficult to have effective intervention.¹³

For this paper, then, VAW will be defined from a radical feminist approach as any act of violence rooted in multiple and intersecting forms of discrimination and inequalities that results in or is likely to result in physical, sexual, or psychological harm or suffering to women. This definition clarifies the disproportionate impact of violence on women and the gendered perspective required in addressing such violence.

Due Diligence Framework: An Overview:

In the context of international human rights law, due diligence refers to the degree of care that is reasonably expected or legally required to solve world problems.¹⁴ Specifically, in the context of VAW, due diligence measures the extent to which States uphold their responsibility to protect and respond to such acts of violence perpetrated against women.

The General Recommendation, 19 of the Committee on Elimination of Discrimination against Women, was the first to introduce the due diligence standard for addressing violence against women in 1992, placing responsibility on the State for failing to prosecute acts of violence in both a private and public setting.¹⁵ The Declaration on the Elimination of Violence Against Women expanded upon it in 1993 to bring about the current due diligence for a State's responsibility in tack

ling VAW, which consists of four key principles: prevention, protection, prosecution and punishment, and remedy.¹⁶ In addition to these pillars, there is an overarching understanding that States should be non-discriminatory and consider all forms of violence women suffer when applying the due diligence standard.¹⁷

When analyzing a State's responsibility to follow the due diligence framework, two legal cases come to mind as being pioneers in establishing this standard: the 1988 Inter-American Court of Human Rights *Velásquez Rodríguez v Honduras* case, and the European Court of Human Rights *M.C. v Bulgaria* case.¹⁸ When Angel Manfredo Velásquez was abducted, the court passed a landmark judgment in holding the State of Honduras accountable for failing to prevent and protect its civilians from violence, even setting out the required compensation to be provided to the victim by the government.¹⁹ The second landmark ruling came in *M.C. v Bulgaria*, where the State of Bulgaria was held accountable by the European Court of Human Rights for failing to properly investigate or charge the crime of rape of a 14-year-old girl. In doing so, the Court stated they violated Articles 3 and 8 of the European Convention on Human Rights (ECHR), again making them compensate the victim as they did in *Velásquez Rodríguez v Honduras*.²⁰ In both cases, forcing the States to compensate the victims was proof of a State's responsibility to uphold a due diligence standard and thus protect women from violence within their borders.

The first pillar under the due diligence standard is the prevention pillar. This aims to tackle the structural problems that enable violence against women and girls, most pertinently, that of institutional patriarchy. From a radical feminist perspective, this means reforming patriarchal structures entirely by introducing more female-lead parliamentarians or passing legislation transforming rape and advocacy laws. For example, legislation guaranteeing gender equality has become intrinsic in national constitutions worldwide, dealing directly with what Whaley and Messner have referred to as the ameliorative hypothesis (a study that predicts increased gender equality will decrease levels of rape victimization through financial independence for women).^{21,22} By contrast, the constructionist feminist theory argues for policies such as campaigns, education, and training of men to increase public safety and awareness under this pillar. Both theories, however, agree on the baseline concept that prevention of VAGW needs to be incorporated into all State structures, whether legal, judicial, administrative, or public, for States to be considered compliant with the standard of due diligence.

The second pillar of protection obligates the State to provide services for victims of violence against women. Rashida Manjoo explains how fulfilling obligations under this pillar consists 'mainly of the provision of services such as telephone hotlines, health care, counseling centers, legal assistance, shelters, protection/restraining orders, and financial aid to victims of violence.'²³ Yet, one of the major drawbacks of this pillar is that these protective measures often suffer from failures in implementation. In extreme cases, they can even result in greater difficulty for the women who choose to report their

perpetrators. In third-world countries where the provision of such services outside of urbanized city centers is scarce to none, a lack of available resources such as shelters can force victims to continue living in homes where they experience domestic violence.²⁴ Furthermore, the process of effective investigation into the perpetration of violence against women comes under this pillar as well, with the State being obligated to ensure that investigations by State law enforcement (such as the police) are carried out as quickly as possible and with as little harm to the victim's privacy or general life standard.

Under the third pillar of prosecuting and punishing VAW perpetrators, States are responsible for responding to violent crimes in all three manifestations: violence against the self, interpersonal violence, and communal violence. As a result, even those crimes occurring within private spaces (including intimate partner violence and domestic violence) must be prosecuted by the State, and it may be held liable for failing to fulfill this duty. Under this pillar, states must ensure that their courts provide adequate sentencing for perpetrators and try them according to the bare minimum requirement of international law (which states that traditions or religious practices may not hamper the effective prosecution of VAW).²⁵ However, in many instances, the lack of special investigative agencies and cultural traditions interfere with the proper enactment of prosecution. According to the Human Rights Commission of Pakistan, over 470 cases of honor killings were reported in Pakistan in 2021, but estimated the real statistic to be closer to 1,000 due to the prevalence of underreporting and lack of effective prosecution.²⁶ As a result, these inefficiencies are barriers to effective redress of VAW under the due diligence standard.

The last pillar of remedying acts of violence is highlighted in both CEDAW and the DEVAW, which obligate the State to compensate victims of violence.^{27,28} Compensation may include adequate reparations, monetary or otherwise, to compensate for the severity of the damage faced by women who are victims of violence. Yet, due to the variegated nature of violent acts against women, such reparations must be specific to the victims. However, there is rarely an attempt by States to take a gendered and specialized approach to providing for them. Where reparations should focus on subverting the pre-existing social hierarchies and gendered law bodies that exist to support women who are victims of violence, they often are not provided at all, with women receiving no reparations beyond watching their attacker (and even this occurs only in rare cases) go to prison. Overall, the State's responsibility to fulfill the due diligence standard regarding VAWG can only be upheld when both prevention at a grass-root level and redress measures are simultaneously carried out, providing compensation and protection to victims of these crimes.

VAW in Pakistan: A Factual Context:

Detailing violence against women and girls in Pakistan is a difficult task to undertake. In a society where lack of education, cultural and religious values, and, as a result, underreporting is rife, it becomes hard to qualify the extent to which VAWG prevails and obtain accurate statistics on the number of women subjected to it. In the CEDAW's Concluding Observations on the 5th Periodic Report of Pakistan, it is estimated that '34

percent of ever-married women have experienced spousal physical, sexual, or emotional violence, and 56 percent of ever-married women who reported experiencing physical or sexual violence have neither sought help to stop the violence nor told anyone.²⁹ That notwithstanding, these statistics do not even begin to cover the other, uglier manifestations of VAWG in Pakistan. This may be due to cultural stigma, which results in very little reporting on manifestations of self-violence – as a result, more readily available information exists on the manifestations of interpersonal and community violence.

Domestic or intimate partner violence (IPV) is the most commonly reported manifestation of violence against women.³⁰ Defined by the United Nations as ‘a pattern of behavior in any relationship that is used to gain or maintain power and control over an intimate partner,’ many women, particularly in rural areas like villages in Balochistan, face physical and emotional abuse at the hands of their husbands and other family members.³¹ The abuse they experience is not just physical beatings but also emotional abuse through verbal insults and intimidation. In specific households, this can even look like threatening financially dependent women with the revocation of shelter or money if they refuse to comply with their husbands’ or families’ demands. Also, sex-selective infanticide and abortion have emerged as a traditional practice that is on the rise in Pakistan. Between January 2017 and April 2018, 345 newborn babies were found dumped in the garbage in Karachi alone – and 99 percent were girls.³²

Community-level VAWG in Pakistan is fundamentally characterized by stripping away their agency. Forced child marriages, prostitution, and trafficking for sexual exploitation are all too common, with women in many cases being honor killed by their family members for being ‘beghairat’ and trying to retain some of that agency. The word ‘ghairat’ here roughly translates to honor and ‘beghairat’ to dishonorable. Still, the term itself goes deeper – as the Aurat Foundation states, it “is considered a chivalrous adherence to tradition and culture in the context of social relations,” the breaking of which is akin to losing all respect for the family.³³ This can include anything from choosing a partner against the family’s wishes to being accused of adultery or other impropriety. The murder of Qandeel Baloch is a horrifying example of this – after supposedly posting what her family considered ‘shameful’ pictures online, she was strangled to death by her brother. Despite the heinous nature of the act, such VAWG has become so prevalent that all her brother (Wazeem Azam) had to say in the aftermath was: “Yes of course, I strangled her,” he said. “[I’m] not embarrassed at all over what I did.”³⁴

In extreme cases, branding women with the mark of ‘beghairat’ subjects them to acid attacks, disfiguring their bodies and leading to lifelong physical and psychological trauma. These attacks are not limited to within the family but also extend to men whose marriage proposals are rejected, such as the case of a 27-year-old female domestic helper who was attacked with acid in Lahore by a man for this exact reason. Upon refusal of his proposal, it is reported that he threatened her, saying, “I will not leave you worth anything.”³⁵

When tracing back the root factors that contribute to such VAWG, many analysts turn to the socio-cultural fabric of the society itself. The belief that women are inferior to men is instilled from childhood: a woman is raised to depend on others for her daily needs, losing her sense of self and relying on her relationships for support.³⁶ The traditional association of men’s honor with women’s freedom and liberty places the responsibility in the hands of men to control how women lead their lives – by extension, VAW becomes a means to exert this control and exercise a man’s right to ‘discipline’ or ‘use’ what is considered ‘his woman’ as he chooses. What is more pressing is how these women are force-fed this narrative, trapping them in a constant state of fear of speaking up or reporting what they consider normal behavior by the men in their families.

Nabeela Falak highlights poverty as another factor that goes “hand in hand” with domestic abuse in her article, ‘Causes and Effects of Domestic Violence against Women in Pakistan: An Analytical Study.’ Tied to this poverty is a lack of education and unemployment, all of which make men far more likely to take their frustrations out on their wives and children and women far less likely to understand their rights enough to seek legal recourse.³⁷ However, the economic underpinnings of VAWG are not as black and white as this. In fact, women attaining literacy and financial independence can sometimes trigger increased rates of domestic violence as their husbands lash out in response to the new power hierarchy in their relationship, making it harder for a woman to seek refuge in any economic circumstance.³⁸

Another factor that influenced the potential for VAWG was the onslaught of the COVID-19 pandemic – the UN Women’s 2020 report established that when the COVID-19 pandemic struck, violence against women and girls in Pakistan rose exponentially.³⁹ Women who had previously been unaffected by IPV suddenly found themselves being subjected to it at the hands of their husbands with whom they were near: “My husband has lost his job as he was working in a cement-producing factory. He is in stress and depression due to this loss. And I rarely had this issue of physical violence from him only since he has lost his job; he brings away all his frustration on me by tormenting me.”⁴⁰ Ultimately, patriarchal narratives form the baseline for the prevalence of VAWG in Pakistan: the institutionalized notion that women are inferior to men sets a precedent for these men to inflict violence upon women (both in an interpersonal and communal capacity).

VAW in Pakistan: A Due Diligence Analysis :

In general, due diligence has been used to hold States accountable despite not actually being binding. When looking at due diligence for Pakistan specifically, although there have been no Special Rapporteur visits to assess the state of VAW in the country, the degree to which a general framework of due diligence has been followed can be inferred through analysis of research published by NGOs and news agencies.

To comply with the due diligence standard, a State must aim to prevent VAW from occurring as a whole. Former Special Rapporteur Yakin Ertürk provided the general rule that by ‘adopting specific legislation, developing public awareness campaigns and training professionals,’ States can fulfill their

prevention obligations under the due diligence standard.⁴¹ Regarding national legislation, certain acts of violence such as acid attacks, honor killings, and forced marriage have been labeled a crime under Pakistani criminal law, with some even being punishable by the death penalty. An example is the Zahir Jaffer case. When 27-year-old Noor Mukadam was brutally beheaded and raped by Zahir Jaffer, the Islamabad High Court (IHC) ruled for the death sentence and overturned the trial court's 25-year jail term into the death penalty.⁴²

The existing legal system only deals with these major and specific violence cases without defining violence or violence against women under their Penal Code.⁴³ Even acts such as the 'Protection of Women against Violence Act, 2016' are only passed in large, urbanized provinces like Punjab, leaving those most vulnerable in the rural provinces of Balochistan or northern provinces of Khyber Pakhtunkhwa without any legal avenues for protection.^{44,45} In the context of international legislation, although Pakistan has ratified CEDAW, it is one of many countries to have not ratified the Optional Protocol to CEDAW, and as a result, the complaint and inquiry mechanisms into VAW are non-existent in the country.⁴⁶

On the point of public awareness campaigns, the government has made itself clear in its efforts to eliminate the continuance of VAW in Pakistan – it collaborated with UN Women in 2011 to launch a 'One Million Signature Campaign' to 'demand and say no to violence.'⁴⁷ Similarly, to reduce institutional barriers for rape victims and set a standard in training medical examiners working with rape survivors, the 'two-finger rape test' was banned from determining virginity and consent in rape trials. As the newspaper, 'Courting the Law' stated:

"Until recently, rape survivors had to go to a medico-legal officer (MLO) for a physical examination after an incident of rape, where they had to go through the two-finger test to determine their consent, virginity and moral standing, which was no less traumatic than the rape itself."⁴⁸ The Anti-Rape (Investigation and Trial) Ordinance, 2020 was also established to set up a national sex-offender registry as well as special courts to expedite the trial of violent sex crimes.⁴⁹

Moving further, when examining the country's efforts on prosecution and punishment, there are differing perspectives of feminism that can be used to assess the effectiveness of prosecution redress in Pakistan. Liberal feminists argue from a solution-based approach, where prosecution and protection methods look like state-designed public campaigns or sensitizing the police. In reality, this kind of approach is ineffective. In Andrew Byrnes and Eleanor Bath's paper, 'Violence against Women, the Obligation of Due Diligence, and the Optional Protocol to the Convention on the Elimination of All Forms of Discrimination against Women: Recent Developments,' they highlight the case of *Goecke v Austria*, stating:

"[There was] a failure by public prosecutors to take domestic violence seriously as a threat to life, and their failure to request detention as a matter of principle in such cases; and a failure to collect data and maintain statistics on domestic violence."⁵⁰

What the case showcases is not just the failure of the Austrian Government to provide adequate bodies that respond to

threats of sexual violence but also that the problem does not lie in the creation of avenues for help. It instead lies in the inherent problem that Mackinnon points out in her 'Theory of a Feminist State' that to stop rape; women need to be made less rapeable. In other words, the solution lies in altering the structure of the State entirely rather than working within the structure.⁵¹ Pakistan operates under the Liberal feminist approach to solving VAW, and consequently, prosecution and punishment for rapists or other perpetrators of VAW is slim to none, with the conviction rate of rapists estimated by War Against Rape to be less than 3%.⁵²

Lastly, when providing a remedy, even if a State is not directly responsible in a case of VAW, it is still required to compensate the victim of VAW. *Opuz v. Turkey* set a precedent for this when the ECHR concluded that the State must compensate the victim because of the violence imposed by her husband and the State's inability to prevent the violence.⁵³ In Pakistan, there are no compensation programs in place, so even though compensation is a necessary part of the national laws against rape, the burden is placed on the perpetrator to pay the compensation fee rather than the State. As a result, not only does the State not fulfill its obligation to victims of VAW, but sometimes it even hampers the administration of justice. In the case of Safdar Ali, who had been sentenced to 25 years in prison for rape, the payment of 750,000 PKR reduced his sentence from 25 to just 10 years, an unnecessary reduction given that compensation should be a requirement unrelated to the sentencing of the perpetrator. In most cases, the Lahore High Court noted that trial courts provided victims no compensation at all, which means that the victim of violence has to shoulder the burden of relocation or medical fees.⁵⁴ Often, the stress of supporting themselves adds to the physical and emotional trauma they have been subjected to.⁵⁵

Ultimately, the State of Pakistan is hindered in its path to properly observing the due diligence standard. On a first level, the geopolitical climate of Pakistan is such that long-term policies for VAW are unlikely to be implemented as political parties come into and out of power (an indication of this is how no Prime Minister has ever completed their full term in Pakistan, making it difficult for competing parties to carry on the projects of the previous party in power).⁵⁶ Similarly, the cultural climate, as discussed in the Factual Analysis section of this paper, prevents actual legislation from coming into place and hinders the ability of trained officers (both men and women) to sympathize with victims of sexual violence. Even the ability to set up domestic abuse shelters or hotlines is limited in places such as Balochistan, where rugged valleys and mountains do not allow for infrastructural development.

■ Conclusion

While numerous papers analyze the extent and manifestations of violence against women across the globe, barely any of them focus on the link between due diligence and VAW. Even fewer examine the specificity of the nature of violence in Pakistan, and as a result, much of the information non-governmental organizations rely upon to help on-ground victims is derived from testimonials or news articles. Hence, it is recommended that future research focus on looking at the

intersecting relationships between factors such as culture and economic circumstances to understand the root enabler of VAW in Pakistan. Moreover, not enough literature exists examining the legal or political aspect of the government's role in enabling perpetrators of VAW to go free, which would be useful in understanding how to reinforce existing due diligence measures and create new policies to keep officials in check. Given the prevalent and severe nature of violence against women in Pakistan, this review concludes that on-ground victims would benefit from further research into the unique factors from which VAW stems, as well as an analysis of how the government could be held accountable under the due diligence standard to ensure these factors do not persist.

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■ References

1. Time to Vow to End Violence against Women." UNFPA Pakistan, November 26, 2022. [https://pakistan.unfpa.org/en/news/time-to-vow-to-end-violence-against-women#:~:text=32 per cent of women, the stories of survivors do.](https://pakistan.unfpa.org/en/news/time-to-vow-to-end-violence-against-women#:~:text=32%20per%20cent%20of%20women,stories%20of%20survivors%20do.)
2. Ali, Umed; K, Miyon. "Sigh of Relief: Two-Finger Test Abolished, Anti-Rape Ordinance Promulgated." *Courting The Law*, May 25, 2021. [https://courtingthelaw.com/2021/03/01/commentary/sigh-of-relief-two-finger-test-abolished-anti-rape-ordinance-promulgated/.](https://courtingthelaw.com/2021/03/01/commentary/sigh-of-relief-two-finger-test-abolished-anti-rape-ordinance-promulgated/)
3. Manjoo, R. "The continuum of violence against women and the challenges of effective redress," *International Human Rights Law Review*, 1(1), pp. 1–29. 2012. Available at: <https://doi.org/10.1163/22131035-00101008>.
4. DEVAW "Declaration on the Elimination of Violence Against Women." OHCHR. United Nations, December 20, 1993. <https://www.ohchr.org/en/instruments-mechanisms/instruments/declaration-elimination-violence-against-women>.
5. "What Is Gender-Based Violence?" European Commission. European Union. [https://commission.europa.eu/strategy-and-policy/policies/justice-and-fundamental-rights/gender-equality/gender-based-violence/what-gender-based-violence_en#:~:text=gender%2Dbased%20violence-,Gender%2Dbased%20violence%20\(GBV\)%20by%20definition,of%20a%20particular%20gender%20disproportionately.](https://commission.europa.eu/strategy-and-policy/policies/justice-and-fundamental-rights/gender-equality/gender-based-violence/what-gender-based-violence_en#:~:text=gender%2Dbased%20violence-,Gender%2Dbased%20violence%20(GBV)%20by%20definition,of%20a%20particular%20gender%20disproportionately.)
6. Krug, Etienne; Dahlberg, Linda; Mercy, James; Zwi, Anthony; Lozano, Rafael. "World Report on Violence and Health." Geneva. World Health Organisation, 2002. https://apps.who.int/iris/bitstream/handle/10665/42495/9241545615_eng.pdf?sequence=1.
7. Krantz, Gunilla; Claudia Garcia-Moreno. "Violence against Women." *Journal of Epidemiology & Community Health* 59, no. 10 (October 1, 2005): 818–20. <https://doi.org/10.1136/jech.2004.022756>.
8. Krantz, Gunilla; Claudia Garcia-Moreno (Note 7)
9. CEDAW, General recommendations made by the Committee on the Elimination of Discrimination Against Women. 1992. United Nations. United Nations. Available at: <https://www.un.org/womenwatch/daw/cedaw/recommendations/recomm.htm>
10. CEDAW (Note 9)
11. Manjoo, R (Note 3)
12. Öhman, Ann; Burman, Monica; Carbon, Maria; Edin, Kerstin. "The Public Health Turn On Violence Against Women': Analysing Swedish Healthcare Law, Public Health and Gender-Equality Policies - BMC Public Health." *BMC Public Health*, May 24, 2020. <https://bmcpublihealth.biomedcentral.com/articles/10.1186/s12889-020-08766-7>.
13. COFEM, Coalition of Feminists for Social Change. "Reframing language of 'gender-based violence' away from feminist underpinnings," *Feminist Perspectives on Addressing Violence Against Women and Girls Series*, Paper No. 2. 2017.
14. Manjoo, R (Note 3)
15. CEDAW (Note 9)
16. DEVAW (Note 4)
17. DEVAW (Note 4)
18. Witten, Samuel M. "Velásquez Rodríguez Case: American Journal of International Law." Cambridge Core. Cambridge University Press, February 27, 2017. <https://www.cambridge.org/core/journals/american-journal-of-international-law/article/abs/velasquez-rodriguez-case/6A85F6193511E1BAA894E4CB50611AF4>.
19. Zada, Sebghatullah Qazi. "Breach of Afghanistan's International Obligations Using the Due Diligence Standard to Combat Violence against Women." *The International Journal of Human Rights*, 2021. <https://www.tandfonline.com/doi/full/10.1080/13642987.2021.1895764>.
20. "M.C. v. Bulgaria." Legal Information Institute. Cornell Law School, 2003. https://www.law.cornell.edu/women-and-justice/resource/mc_v_bulgaria#:~:text=v-,Bulgaria,therefore%20rape%20had%20not%20occurred.
21. UN Women, *Progress of the World's Women: In Pursuit of Justice* (UN Women, 2011) available at: <http://progress.unwomen.org/pdfs/EN-Report-Progress.pdf>.
22. Whaley, R. B.; Messner, S. F. (2002). Gender equality and gendered homicides. *Homicide Studies*, 6, 188–210.
23. Manjoo, R (Note 3)
24. Manjoo, R (Note 3)
25. Manjoo, R (Note 3)
26. Anees, M.S. (2022) 'honor killings' continue unabated in Pakistan, – *The Diplomat*. for *The Diplomat*. Available at: <https://thediplomat.com/2022/07/honor-killings-continue-unabated-in-pakistan/>
27. CEDAW (Note 9)
28. DEVAW (Note 4)
29. UN official documents (no date) CEDAW 5th Review. United Nations. Available at: <https://www.un.org/en/delegate/page/un-official-documents>.
30. "Frequently Asked Questions: Types of Violence against Women and Girls." UN Women. <https://www.unwomen.org/en/what-we-do/ending-violence-against-women/faqs/types-of-violence#:~:text=Domestic%20violence-,Domestic%20violence%2C%20also&text=It%20encompasses%20all%20physical%2C%20sexual,violen%20experienced%20by%20women%20globally.>
31. What is domestic abuse? (no date) United Nations. United Nations. Available at: <https://www.un.org/en/coronavirus/what-is-domestic-abuse>.
32. Durrani, F. (2018) "Karachi becoming a killing field for newborn girls," *thenews*. The News International. Available at: <https://www.thenews.com.pk/print/309162-karachi-becoming-a-killing-field-for-newborn-girls>.
33. Hadi, R. (2014). *Violence Against Women in Pakistan - A Qualitative Review of Reported Incidents*. Karachi: Aurat Foundation.
34. "Qandeel Baloch's brother admits killing her, say Pakistani police" (2016) *The Guardian*. Guardian News and Media. Available at: <https://www.theguardian.com/world/2016/jul/17/qandeel-baloch-brother-admits-killing-her-say-pakistani-police>.
35. "Another acid attack," (2021) *The Express Tribune*. Available at:

- <https://tribune.com.pk/story/2304350/another-acid-attack>.
36. Babur, Z. U. (2007). *Violence Against Women in Pakistan: Current Realities and Strategies for Change*. Burg, Austria: European University Center for Peace Studies.
 37. Falak, N. (2022). *Causes and Effects of Domestic Violence against Women in Pakistan: An Analytical Study*. *Sustainable Business and Society in Emerging Economies*, 4 (2), 619- 626.
 38. LaBore, Kathryn; Ahmed, Tooba; Ahmed, Rashid. (2019) "Prevalence and predictors of violence against women in Pakistan," *Journal of Interpersonal Violence*, 36(13-14), pp. 4-5. Available at: <https://doi.org/10.1177/0886260518824652>.
 39. *Violence against women and girls data collection during COVID-19* (no date) UN Women – Headquarters. Available at: <https://www.unwomen.org/en/digital-library/publications/2020/04/issue-brief-violence-against-women-and-girls-data-collection-during-covid-19>.
 40. Munir, M.M.; Munir, M.H.; Rubaca, U. (no date) *The Shadow Pandemic: Violence against Women in Pakistan during COVID-19 Lockdown*, *Journal of International Women's Studies*. Bridge Water State University. Available at: <https://vc.bridgew.edu/cgi/viewcontent.cgi?article=2450&context=jiws>.
 41. Zada, Sebghatullah Qazi (Note 19)
 42. "Pakistan: Zahir Jaffer Gets Double Death Sentence in Noor Mukadam Murder Case." Web Desk. *Khaleej Times*. March 13, 2023. <https://www.khaleejtimes.com/world/asia/pakistan-zahir-jaffer-gets-double-death-sentence-in-noor-mukadam-murder-case>.
 43. Ali, Rafia Naz. "National Legislation on Violence against Women in Pakistan" *Journal of Peace, Development and Communication*, 2022. <https://journalseeker.researchbib.com/view/issn/2663-7901>.
 44. "The Punjab Protection of Women Against Violence Act 2016." Act XVI of 2016. Punjab, February 29, 2016. <http://punjablaws.gov.pk/laws/2634.html>.
 45. Ali, Rafia Naz (Note 43)
 46. "Optional Protocol to the Convention on the Elimination of All Forms of Discrimination against Women." United Nations Treaty Collection. United Nations, 2023. https://treaties.un.org/pages/ViewDetails.aspx?src=IND&mtdsg_no=IV-8-b&chapter=4&clan_g=en.
 47. "Events and Campaigns." Asia. <https://asiapacific.unwomen.org/en/countries/pakistan/evaw-pakistan/events-and-campaigns>.
 48. Ali, Umed, and K Miyon (Note 2)
 49. Siddiqui, Naveed. "President Approves Anti-Rape Ordinance to Ensure Speedy Trial, Strict Punishments." *DAWN.COM*, December 15, 2020. <https://www.dawn.com/news/1595907>.
 50. Byrnes, A.; E. Bath. "Violence against Women, the Obligation of Due Diligence, and the Optional Protocol to the Convention on the Elimination of All Forms of Discrimination against Women-Recent Developments." *Human Rights Law Review* 8, no. 3 (2008): 517-33. <https://doi.org/10.1093/hrlr/ngn022>.
 51. MacKinnon, Catharine A. "Feminism, Marxism, Method, and the State: Toward Feminist Jurisprudence [1983]." *Feminist Legal Theory*, 2018, 181-200. <https://doi.org/10.4324/9780429500480-11>.
 52. "Conviction Rate in Rape Cases under 3% in Pakistan: Report." *Global Village Space*, September 15, 2020. <https://www.globalvillagespace.com/conviction-rate-in-rape-cases-under-3-in-pakistan-report/>.
 53. Echr. "European Court of Human Rights." HUDOC. European Court of Human Rights. <https://hudoc.echr.coe.int/fre#%7B%22itemid%22:%5B%22001-92945%22%5D%7D>.
 54. "Heinous Crime: LHC Directs Lower Courts to Order Compensation for Rape Victims." *The Express Tribune*, August 9, 2015. <https://tribune.com.pk/story/935195/heinous-crime-lhc-directs-lower-courts-to-order-compensation-for-rape-victims>.
 55. "The Need for Victim Compensation Programmes ." Pakistan and Globally. <https://www.asafeworldforwomen.org/global-news/asia/pakistan/5017-need-for-victim-compensation.html>.
 56. Hassan, Syed Raza. "Factbox: No Pakistani Prime Minister Has Completed a Full Tenure." *Reuters*. April 9, 2022. <https://www.reuters.com/world/asia-pacific/no-pakistani-prime-minister-has-completed-full-tenure-2022-04-09/>.

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The Evidence of Vitamins on the Treatment and Prevention of COVID-19

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ABSTRACT: Coronavirus disease 2019 (COVID-19) is an infectious disease caused by a novel coronavirus called SARS-CoV-2. The disease first emerged in Wuhan, China, in December 2019 and quickly spread to become a global pandemic. Through this pandemic, one of the lessons learned is the importance of nutrition, which can substantially impact the prevention and severity of infections. Supplementing vitamins and minerals into a balanced diet may be beneficial in decreasing the risk of contracting COVID-19. This study intends to review published human clinical trials on the role of vitamins in preventing and treating COVID-19. Only randomized, double-blind, placebo-controlled clinical trials between 1 January 2020 and 1 June 2023 are included, and 18 publications indexed in PubMed qualified. Through this study, it was determined that there is strong evidence to support the beneficial effects of Vitamin D3 in the treatment and prevention of COVID-19 if the vitamin is taken over multiple doses rather than a single high dosage and if the vitamin is taken during the early stages of COVID-19 infection. There is weaker evidence to support the beneficial effect of Vitamin C in the prevention and treatment of COVID-19.

KEYWORDS: Biomedical and Health Sciences; Nutrition and Natural Products; COVID-19; Nutraceuticals; Vitamins; Vitamin D3.

■ Introduction

Since it was first reported in Wuhan, China, in December 2019, COVID-19 has quickly spread worldwide in a few months. On 31 December 2019, the World Health Organization (WHO) announced that SARS-CoV-2 is responsible for COVID-19, and on 11 March 2020, COVID-19 was declared a global pandemic.¹ SARS-CoV-2 is a positive-sense, single-stranded RNA virus.^{2,3} The primary site of infection of COVID-19 is the respiratory system,⁴ although other organs can also be affected. The infection of COVID-19 typically progresses through up to five stages: (a) Incubation period, which can range from 2 to 14 days; (b) Early stage, the days when symptoms first appear, including fever, cough, fatigue, body aches, and loss of taste or smell; (c) Pulmonary phase, when the disease progresses to the lungs, leading to shortness of breath, difficulty breathing, and in severe cases, pneumonia, and acute respiratory distress syndrome (ARDS); (d) Hyperinflammatory phase, where the immune system overreacts to the virus and causes damage to organs and can be fatal if it is not controlled well; (e) Recovery phase when the disease resolves within 2-3 weeks with symptoms gradually improving over time. Not all infected individuals experience all five stages: people may experience mild symptoms and recover quickly. In contrast, other people are entirely asymptomatic and unknowingly transmit the disease to others.

Inadequate innate immune response in the first stages of COVID infection and immune-mediated damage due to the dysregulated hyperinflammatory phase are considered to be the significant determinants of poor outcomes in COVID-19 patients.⁵ Some vitamins regulate gene expression in immune

cells and support the maturation and differentiation of immune cells. Vitamins, including Vitamins A, B, C, D, and E, are well-known for their role in modulating the adaptive and innate immune systems. Vitamin A has been shown to act as a T-cell effector, facilitating adaptive and innate immunity.⁶ The Vitamin B family comprises a total of 8 vitamins, thiamine [B1], riboflavin [B2], niacin [B3], pantothenic acid [B5], pyridoxine [B6], biotin [B7], folate or folic acid [B9], and cyanocobalamin [B12]. They are essential to immune regulation and contribute to intestinal barrier function.⁷ Vitamin C is known for its antiviral properties, such as increasing interferon-alpha production, modulating cytokines, reducing inflammation, and restoring mitochondrial function.⁸ These properties make it a common nutraceutical therapy for respiratory sickness. Vitamin D3 is a secosteroid hormone that regulates many cellular mechanisms.^{9,10} The effects of Vitamin D3 on skeletal and bone metabolism have been well-recognized for decades. Only in the past 20 years has Vitamin D3 been demonstrated to have significant roles in regulating the immune response, oxidative stress, cancer biology, and the nervous system.¹¹⁻¹⁵ Vitamin E has also been demonstrated to be crucial in regulating and supporting immune system function.¹⁶

There has been much interest in the potential role of vitamins in COVID-19. For instance, multiple studies have shown that there is a correlation between Vitamin D deficiency and increased risk for infectivity, morbidity, and mortality associated with COVID-19.¹⁷ In addition, when Vitamin C was administered to COVID-19 patients, it increased the survival rate of COVID-19 patients by attenuating excessive activation of the immune response.¹⁸ Given the low cost and easy access to these products, it would be a great benefit to consumers if

treatment through vitamins can supplement frequent hospital visits.

The objective of the current study is to review high-quality published human clinical studies, specifically the randomized, double-blind, placebo-controlled trials, or the “gold standard” of clinical trials, to investigate the evidence for vitamin supplementations (e.g., Vitamins A, B, C, and D) in the treatment and prevention of COVID-19.

Methods

Since the start of the COVID-19 pandemic, the publications related to “COVID-19” indexed in Pubmed mushroomed from 0 publications before the year 2020 to 93,703 publications in year 2020, 140,378 publications in year 2021, 129,276 publications in year 2022, and 45,677 publications from 1 January 2023 to 1 June 2023. To support global efforts to combat the COVID-19 pandemic, many publishers made their COVID-19-related publications available for free. This includes many articles indexed in PubMed, a free biomedical and life sciences literature search engine. This policy, which made these publications available and accessible for all, has made this review possible.

A PubMed search using keywords “COVID-19” and “Vitamin” generated 2762 hits on 1 June 2023. From this list, using PubMed’s filter choice “Randomized Controlled Trial,” the list was narrowed down to 67 publications. These 67 studies were used for initial screening. Each article was downloaded and examined for its study design. Only randomized, double-blind, placebo-controlled studies were included; ClinicalTrials.gov was used to verify when the descriptions of the published trials were unclear.

Furthermore, only studies related to vitamins/supplements/nutrients were included. Trials with commercial combinations of ingredients that are not clearly listed or with over-the-counter medications were excluded. Dietary survey studies were also excluded. As a result, a total of 18 studies satisfied the inclusion criteria and were included in our analysis (Figure 1).

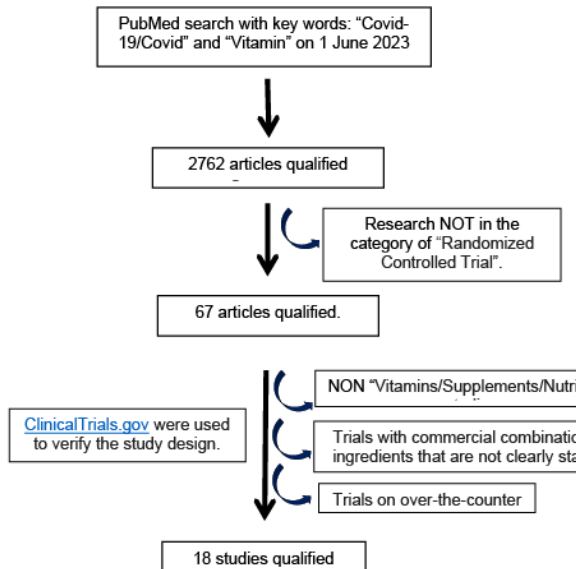


Figure 1: Research studies selection process.

Results and Discussion

Each of these 18 randomized, double-blind, placebo-controlled studies is summarized in Table 1¹⁹⁻³⁶, with details on the date of the publication, “Number of Subjects” in each group, the dosage of vitamin “Interventions” that each group took, and the duration of the intervention, the major finding “Results” from the study, and the Registration “ID” of the study at ClinicalTrials.gov.

Table 1: Randomized, double-blind, placebo-controlled clinical studies on Vitamin interventions for Covid-19.

Ref #	Publication Date	Publication Title	No. of Subjects	Interventions	Results	Clinical trial.Gov ID
19	30 December 2022	Vitamin D3 Supplementation at 5000 IU Daily for the Prevention of Influenza-like Illness in Healthcare Workers: A Pragmatic Randomized Clinical Trial	Vitamin D: 299 Control: 578	5000 IU daily vitamin D3 or Placebo for nine months	5000 IU daily reduces influenza-like illness (ILI), including COVID-19, in healthcare workers	NCT045 96567
20	14 September 2022	Efficiency of nicotinamide-based supportive therapy in lymphopenia for patients with ordinary or severe COVID-19: A randomized controlled trial	12 Vitamin B3 12 Placebo	100mg nicotinamide (vitamin B3) 5 times a day for 2 days	no statistically significant difference on the effect of nicotinamide on lymphopenia in COVID-19 patients	NCT049 10230
21	19 July 2022	Prevention of covid-19 and other acute respiratory infections with cod liver oil supplementation, a low dose vitamin D supplement: quadruple blinded randomized placebo-controlled trial	vitamin D, n=17278 Placebo, n=17323	5 mL/day of cod liver oil (10 µg of vitamin D), for up to six months.	Daily supplementation with cod liver oil, a low dose vitamin D, for six months during the SARS-CoV-2 pandemic in Norwegian adults, did not reduce the incidence of SARS-CoV-2 infection, serious covid-19, or other acute respiratory infections	NCT046 39086
22	29 May 2022	Evaluation and comparison of vitamin A supplementation with standard therapies in the treatment of patients with COVID-19	182 COVID-19 outpatients Experimental (n = 91) control (n = 91)	25 000 IU oral vitamin A or placebo for 10 days with standard Covid treatments for both groups	Vitamin A supplementation showed significantly greater decreases in the experimental group 10 days post-intervention in symptoms such as fever, body aches, weakness and fatigue, paracetamol symptoms, white blood cell count, and reactive protein, as compared with the standard treatment alone (p < 0.05)	Iran, no registrati on
23	25 March 2022	A Pilot of a Randomized Control Trial of Melatonin and Vitamin C for Mild-to-Moderate COVID-19	placebo (n = 34), vitamin C (n = 32) melatonin (n = 32)	COVID-19 Patients took placebo, vitamin C 1000 mg, or melatonin 10 mg orally for 14 days	Vitamin C 1000 mg once daily had no effect on disease progression. Melatonin 10 mg daily may have a statistically significant effect, but it is unclear if this represents a clinically significant benefit to those with mild-to-moderate symptoms of COVID-19 infection	NCT045 30339
24	26 July 2022	Positive Effects of Vitamin D Supplementation in Patients Hospitalized for COVID-19: A Randomized, Double-Blind, Placebo-Controlled Trial	50 COVID-19 patients	vitamin D (25,000 IU per day over 4 consecutive days, followed by 25,000 IU per week (up to 5 weeks)) or placebo	At Day 7, a significantly lower percentage of patients were still hospitalized in the vitamin D group compared to the placebo group (19% vs. 54%; p = 0.015). In none of the patients treated with vitamin D were hospitalized after 21 days compared to 44% of the placebo. Vitamin D significantly reduced the duration of supplemental oxygen among the patients who needed it (4 days vs. 7 days in the placebo group; p = 0.012) and significantly improved the clinical recovery of the patients, as assessed by the WHO scale (n = 0.0048)	NCT046 36968
25	27 May 2022	High-dose vitamin D versus placebo to prevent complications in COVID-19 patients: Multicenter randomized controlled clinical trial	218 Covid patients 115 participants were assigned to vitamin D3 and 105 to placebo	Participants were randomized to a single oral dose of 500 000 IU of vitamin D3 or matching placebo, did not prevent the respiratory worsening	Among hospitalized patients with mild-to-moderate COVID-19 and risk factors, a single high oral dose of vitamin D3 as compared with placebo, did not prevent the respiratory worsening	NCT044 11446
26	5 April 2022	Effect of Antemic in patients with COVID-19: A Phase I prospective study	50 hospitalized COVID-19 patients C or placebo oral supplement: Atemic: n=33 Placebo: n=17	Antemic (a formula with arbutin, curcumin, frankincense and vitamin C) or placebo oral supplement, twice daily on days 1 and 2, beside standard care	Antemic improved NEWS2 in 97% of patients and shortened the duration of abnormal oxygen levels, oxygen supplementation and fever.	NCT043 82040
27	11 April 2022	Efficacy and Safety of Vitamin D Supplementation to Prevent COVID-19 in Frontline Healthcare Workers: A Randomized Clinical Trial	Vitamin D: n=94 Placebo: n=98	Participants were randomly assigned to receive 4,000 IU (Vitamin D3) or placebo (P) daily for 30 d.	SARS-CoV-2 infection rate was lower in Vitamin D than in Placebo (8.4 vs. 24.5%, p < 0.001). Vitamin D supplementation in exposed individuals prevents SARS-CoV-2 infection without serious AEs and regardless of Vitamin D status.	NCT045 35791
28	15 December 2021	The Effect of Vitamin C on the Pathological Parameters and Survival Duration of Critical Ill Coronavirus Disease 2019 Patients: A Randomized Clinical Trial	The intervention group (n = 31) The control group (n = 69)	Participant received one capsule of 500 mg of vitamin C or placebo daily for 14 days	Vitamin C supplementation resulted in a higher mean survival duration compared with that of the control group (8 vs. 4 days, p = 0.01).	IRCT201 5124202 569985
29	14 November 2021	The effect of supplementation with vitamins A, B, C, D, and E on disease severity and inflammatory response in patients with COVID-19: a randomized clinical trial	Intervention group: n=30 Placebo: n=30	25,000 IU daily of vitamins A, B, C, D, and E once during the study (D, 200 IU twice daily of E, 500 mg four times daily of C, and one amp daily of B compared with placebo)	Significant changes were detected in serum levels of vitamins (p = 0.001 for vitamin E, p = 0.001 for vitamin C, p = 0.001 for vitamin A, p = 0.003 for vitamin B), TNF-α (p = 0.001), and SOFA score (p < 0.001) after intervention compared with the control group. The effect of vitamins on the mortality rate was not statistically significant (p = 0.112). The prolonged hospitalization rate to more than 7 days was significantly lower in the intervention group than the control group (p < 0.001).	IRCT202 0031984 681981
30	9 October 2021	Effect of Vitamin D Supplementation on Muscle Status in Old Patients Recovering from COVID-19 Infection	Vitamin D: n=15 Placebo: n=15	vitamin D (cholecalciferol: 2000 IU/day) and placebo was carried out for 6 weeks	Vitamin D supplementation produces decreases in indicators of muscle damage, which may ultimately contribute to improving the health status and quality of life of patients who have suffered from COVID-19, during the recovery process	Spain, no registrati on
31	15 September 2021	Treatment With 25-Hydroxyvitamin D3 (Calcifediol) Is Associated With a Reduction in the Blood Neutrophil-to-Lymphocyte Ratio Marker of Disease Severity in Hospitalized Patients With COVID-19: A Pilot Multicenter, Randomized, Placebo-Controlled, Double-Blinded Clinical Trial	Vitamin D: n=53 Placebo: n=53	a dose of 25µg of D3 that was equivalent to approximately 3000 to 6000 IU per day of vitamin D3 for 30 days and 60 days	We observed an overall lower trend for hospitalization, intensive care unit duration, need for ventilator assistance, and mortality in the 25(OH)D3 group compared with that in the placebo group, but differences were not statistically significant.	Iran, no registrati on
32	16 March 2021	Effect of a Single High Dose of Vitamin D3 on Hospital Length of Stay in Patients with Moderate to Severe COVID-19	Vitamin D: n=120 Placebo: n=120	Patients were randomly assigned to receive a single oral dose of 200,000 IU of vitamin D3 or placebo	Among hospitalized patients with COVID-19, a single high dose of vitamin D3, compared with placebo, did not significantly reduce hospital length of stay.	NCT044 49718
33	12 November 2020	Short term, high-dose vitamin D supplementation for COVID-19 controlled study (SHADE study)	40 SARS-CoV-2 positive individuals: Vitamin D: n=18 Placebo: (n=24)	Receive daily 60,000 IU of cholecalciferol (oral nano-liquid drops) or Placebo for 7 days.	10 (82.5%) participants in the intervention group and 5 (20.8%) in the control arm (p=0.018) became SARS-CoV-2 RNA negative. Greater proportion of vitamin D-deficient individuals with SARS-CoV-2 infection turned SARS-CoV-2 RNA negative with a significant decrease in fibrinogen on high-dose cholecalciferol supplementation.	NCT044 50247
34	24 August 2020	Effect of calcifediol treatment and best available therapy versus best available therapy on intensive care unit admission and mortality among patients hospitalized for COVID-19: A pilot randomized clinical study	76 Covid hospitalized patients receiving standard care Vitamin D: n=30 Placebo: (n=26)	On the day of admission take oral calcifediol (0.532 mg) or placebo. Day 5-7: oral calcifediol (0.265 mg) or placebo, and then weekly until discharge or ICU admission	Of 50 patients treated with calcifediol, one required admission to the ICU (2%), while of 25 untreated patients, 13 required admission (50%) p value X2 Fisher test p < 0.001	NCT043 69098
35	28 February 2023	Vitamin D Outcomes in Severe COVID-19 Patients—Randomized Controlled Trial	155 patients with COVID-19 disease in need of respiratory support admitted to the ICU. Vitamin D: (n=77) Placebo: (n=78)	Patients with low vitamin D levels were randomized to receive either the intervention group received daily supplementation of vitamin D (10,000 IU) of cholecalciferol daily for 14 days and the control group did not receive vitamin D supplementation.	There was no statistically significant difference in the survival rates between the two groups. Patients in the intervention group had delayed increases in CRP and PCT plasma levels and also in neutrophil/lymphocyte ratio compared to the control group. However, there were no significant differences between the two groups. However, the study findings should be interpreted with caution due to inadequate study power for primary outcome. With this caveat, the trial should be viewed as a contribution to a still under-researched body of evidence about the effect of vitamin D supplementation in critically ill patients with COVID-19.	NCT053 84574
36	27 October 2022	REaCue trial: Randomized controlled clinical trial with extended-release calcifediol (ERC) in symptomatic COVID-19 outpatients	patients with mild to moderate COVID-19. Vitamin D: n=65 Placebo: (n=66)	The dosing protocol consisted of 300 mcg (10 ERC capsules) on each of days 1, 2, and 3 followed by 60 mcg (2 ERC capsules) on days 4 through 27, administered at bedtime after fasting for 3 h.	Vitamin D levels were significantly raised in ERC treated group as compared to Placebo group. Symptom respiratory status was unchanged in the full analysis set for ERC. In the PP population, respiratory status was resolved 4 faster when 25D was elevated above baseline level at both days 7 and 14 (median 6.5 versus 10.5 d; HR, 1.372; 95% CI, 0.9451-991; P = 0.0962; Wilcoxon P = 0.0386).	NCT045 51911

Vitamin D3's Role in COVID-19:

The role of Vitamin D3 in COVID-19 has been of great interest to the scientific community. Multiple studies have shown a correlation between positive COVID-19 cases and Vitamin D deficiency. For example, one 2020 study evaluating the National Clinical Laboratory Database of the United States observed that out of 191,779 patients who tested positive for SARS-CoV-2 showed lower circulating Vitamin D concentrations.³⁷ Another 2020 study showed that hospitalized Korean patients with COVID-19 were three times more likely to be Vitamin D deficient than the control group.³⁸ A 2020 Israeli study showed that patients with Vitamin D deficiency had approximately 1.5 times higher odds of getting COVID-19 than patients with normal Vitamin D levels.³⁹ A 2021 Spanish study on COVID-19 patients showed that Vitamin D deficiency was approximately 1.7 times more prevalent in patients with COVID-19 than those without the disease.⁴⁰ These studies suggest a link between low Vitamin D levels and increased COVID-19 susceptibility in different populations.

Vitamin D3 is an essential nutrient that plays a crucial role in supporting a healthy immune system. Research has shown that Vitamin D3 helps boost the immune system's ability to fight infections. It has been suggested that Vitamin D3 may reduce the risk of respiratory tract infections, including COVID-19, by enhancing immune function. In addition, Vitamin D3 has been shown to have anti-inflammatory effects by inhibiting the overexpression of inflammatory cytokines such as IL-1 α , IL-1 β , and tumor necrosis factor- α —which may be particularly important in COVID-19 since severe cases of the disease are often characterized by an overactive immune response and inflammation.¹⁸ These mechanisms may have provided the basis for the observed benefits in the human clinical studies reviewed in this study and supported the use of Vitamin D3 to prevent and treat COVID-19.

Among the 18 randomized, double-blind, placebo-controlled studies which qualified for review,¹⁹⁻³⁶ 12 of them used Vitamin D3 only,^{19,21,24,25,27,30-36} and 1 used a composition of Vitamins A, B, C, D, and E.²⁹ Among the 13 studies investigating the effect of Vitamin D3 on the infection rate, duration of the hospital stays, the severity of the symptoms, and the mortality resulting from COVID-19, a total of 8 studies reported statistically significant benefits of the vitamin conditions compared to the placebo group,^{19,24,27,29,30,33,34,36}. In comparison, four studies did not demonstrate a statistically significant difference compared to outcomes from the placebo group.^{21,25,32,35} The last one of the 13 studies showed an overall lower trend for hospitalization, intensive care unit duration, need for ventilator assistance, and mortality in the Vitamin D3 group compared to the placebo group. However, the differences were not statistically significant.³¹ Seven of the eight positive studies used Vitamin D3 as the single intervention, with doses ranging from 2000 to 60,000 IU and the duration of the treatment from 7 days to 9 months.^{19,24,27,30,33,34,36} Although the outlier of the eight studies, which used a single dosage of Vitamin D3, showed a significant benefit, its treatment contained a composition of Vitamins A, B, C, D, and E: 25,000 IU daily of vitamins A, 600,000 IU once during the study of D, 300 IU

twice daily of E, 500 mg four times daily of C, and one amp daily of B complex for seven days.²⁹ Interestingly, among the four studies that did not show a statistically significant difference, two of them used a single high dose of 200,000 IU and 500,000 IU.^{32,21} Evidently, single doses of Vitamin D3, even at extremely high doses such as 500,000 IU, do not produce a significant benefit. Among the other two studies, one used a daily dose of 400 IU (10 mcg) for six months, and the other used a daily dose of 10,000 IU for 14 days.^{24,35} Interestingly, the latter study was given to patients with a very severe level of COVID-19, who were admitted to the ICU and had to be on respiratory support.³⁵ The dose was 10,000 IU and given over 14 days, and it did not show any difference in the severity of the condition. Although the study was underpowered and had some limitations, it nonetheless suggests that Vitamin D3 might need to be taken at an early stage of COVID-19 infection rather than a later stage.

These studies suggest that taking Vitamin D3 at a dosage higher than 2000 IU consistently for an extended period, such as 1-2 months, may be beneficial in preventing infection and reducing hospital stays, the severity of symptoms, and fatality of people with COVID-19 if it is taken at early stages of COVID-19 infection. These studies also indicate that a single high dosage of Vitamin D3 is not adequate. It may also be beneficial to take Vitamin D3 with other vitamins to have a synergistic effect. Future research should investigate whether Vitamin D3 has a dose-dependent effect on COVID-19 progression and the mechanism for Vitamin D3's beneficial effects during COVID-19 infection. Additionally, as most of these studies have a small sample size (e.g., 15-299 participants in the Vitamin D group), future studies should also include larger numbers of patients to improve the statistical power and validity of the studies.

Vitamin C's Role in COVID-19:

Vitamin C is known to play a role in the immune system, as it is a powerful antioxidant and a cofactor for multiple biosynthetic and gene regulatory enzymes.⁴¹ Some studies have suggested that it may help reduce the severity and duration of respiratory infections, including those caused by coronaviruses. However, while Vitamin C is an important nutrient for overall health and immune function, our analysis shows that there is not enough evidence to support the beneficial role of Vitamin C in treating or preventing COVID-19.

Among the 18 studies included in the review, three studies tested the effect of Vitamin C on COVID-19. One of these studies used a formulation of Vitamin C and other drugs.²⁶ In contrast, the other two used only Vitamin C.^{23,28} The first study gave ArtemiC (a formulation of Vitamin C with artemisinin, curcumin, and frankincense) or a placebo oral spray to 50 random hospitalized symptomatic COVID-19 patients. Besides standard hospital care, the treatment was given twice daily on days 1 and 2. Patients were then monitored until they were discharged from the hospital on day 15. Results showed that the Vitamin C formula improved symptoms in 91% of patients and shortened durations of abnormal SpO2 levels, oxygen supplementation, and fever.²⁶ The second study examined the effect of daily supplementation of 500 mg of Vitamin C

for 14 days on the pathological parameters and survival duration of critically ill patients with COVID-19. This study found that Vitamin C supplementation resulted in a higher mean survival duration than the control group (8 vs. 4 days, $p < 0.01$).²⁸ Conversely, the third study—in which groups of patients with mild-to-moderate symptoms of COVID-19 infection were given either 1000mg of Vitamin C, 10mg of melatonin, or a placebo for 14 days—showed that 1000 mg of Vitamin C taken once daily had no effect on disease progression and quality-of-life in patients.²³

These studies indicate that consistent Vitamin C intake may provide limited benefits for people with COVID-19. However, further studies of Vitamin C for COVID-19 infection are needed to clarify whether Vitamin C can benefit COVID-19 prevention and treatment. It is also important to investigate if Vitamin C has a dose-dependent effect on COVID-19 progression since this analysis shows that a 500 mg/d supplementation of Vitamin C showed promising results. Still, a 1000 mg/d supplementation of Vitamin C showed no significant difference despite taking place with the same number of days as the former study (14 days). Since the studies were performed with a small group of participants (e.g., 32–33 participants in the Vitamin C group), a well-powered study with a larger sample size would be needed to have a more precise conclusion.

Vitamin A and B's Role in COVID-19:

Vitamin A is a fat-soluble vitamin important for growth and development, the immune system, vision, reproduction, and proper organ function. It is commonly found in many foods like dairy products and leafy vegetables, making it a common vitamin in an individual's everyday diet.⁴² Vitamin A deficiency can lead to an increased risk of severe infections because the infection increases the demand for Vitamin A.⁴³ This suggests that Vitamin A may be a factor in helping in the prevention of infections like COVID-19. Furthermore, it seems that Vitamin A may also have a role in decreasing some COVID-19 symptoms. One study (out of the 18 studies reviewed) evaluated the effect of Vitamin A supplementation in patients with COVID-19 and found that a daily oral dose of 25,000 IU of Vitamin A for ten days with standard COVID-19 treatments decreased symptoms—such as fever, body ache, weakness, and fatigue—significantly more as compared to the standard treatment alone ($P < 0.05$).²² Despite the promising news, only one study showed these results, and the condition of the patients was not directly supervised as the study used a sample from an outpatient health center. Hence, further research is needed to verify the beneficial role of Vitamin A in COVID-19 treatment.

Vitamin B consists of eight water-soluble vitamins and is also found in everyday food items like dairy products and green leafy vegetables. It promotes energy production in the body and acts as a critical cofactor for neurological and cellular metabolic pathways.⁴⁴ Vitamin B3, specifically, is used by the body to digest food into energy and helps keep an individual's nervous system, digestive function, and skin healthy.⁴⁵ Limited evidence has indicated that Vitamin B3 does not play a significant role in alleviating the effects of COVID-19. Out of

the 18 studies, one study tested the effects of Vitamin B3 on COVID-19 by using 100 mg of Vitamin B3 5 times a day for two days in patients with ordinary or severe COVID-19. This study found no statistically significant difference in the effect of Vitamin B3 in COVID-19 patients versus placebo.²⁰ Although this study reported no statistically significant observed effects, further research on Vitamin B3 is necessary to clarify its potential role in COVID-19 prevention and treatment.

Conclusion

Our study reviewed all qualified, double-blind, placebo-controlled publications indexed on Pubmed from 1 January 2020 to 1 June 2023 for the roles of vitamins in COVID-19 prevention and treatment. Our review suggests that high doses of Vitamin D (over 2000 IU) given over an extended period of time (at least six weeks) to patients at the early stages of COVID-19 infection were found to have a beneficial role in preventing infection, improving quality-of-life, and reducing symptoms, the duration of hospital stay, and mortality. A single high dose of Vitamin D does not significantly benefit these areas. Vitamins C and A appear to have a limited beneficial role in COVID-19 treatment, and we found little to no evidence for the role of Vitamin B3 or other vitamins in COVID-19 treatment. Although combinations of vitamins and other ingredients may provide some synergistic benefits, further “gold standard” research of specific combined formulas is needed to verify these results. One of the limitations of these studies involves small sample sizes, which limits the statistical power and significance of the conclusions. Thus, this needs to be addressed in future studies with larger sample sizes. In addition, mechanisms of action need to be hypothesized and further investigated. If sufficient clinical evidence can be generated to support the role of vitamins in COVID-19 prevention and treatment, a case could be made for incorporating vitamins into treatment guidelines for COVID-19. This could reduce or alleviate this infectious disease's global burden. Additionally, since many of these vitamins are easily accessible to consumers at a reasonable price, using vitamins in COVID-19 prevention and treatment can help reduce the number of hospital visits and the cost of treating COVID-19 infections.

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References

1. World Health Organization. WHO Director-General's Opening Remarks at the Media Briefing on COVID-19 - 11 March 2020. URL: <https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020> (Accessed June 9, 2023).
2. Chan, J. F.; Yuan, S.; Kok, K. H.; To, K. K.; Chu, H.; Yang, J.; Xing, F.; Liu, J.; Yip, C. C.; Poon, R. W.; Tsoi, H.; Lo, S. K.; Chan, K.; Poon, V. K.; Chan, W.; Ip, J. D.; Cai, J.; Cheng, V. C.; Chen, H.; Hu, C. K.; Yuen, K. A Familial Cluster of Pneumonia Associated with the 2019 Novel Coronavirus Indicating Person-to-Person Transmission: A Study of a Family Cluster. *Lancet* **2020**, *395* (10223), 514–523.
3. Machhi, J.; Herskovitz, J.; Senan, A. M.; Dutta, D.; Nath, B.; Oleynikov, M. D.; Blomberg, W. R.; Meigs, D. D.; Hasan, M.; Patel, M.; Kline, P.; Chang, R. C.; Chang, L.; Gendelman, H. E.; Kevadiy

- a, B. D. The Natural History, Pathobiology, and Clinical Manifestations of SARS-CoV-2 Infections. *J. Neuroimmune Pharmacol.* **2020**, *15* (3), 359–386.
4. Rothan, H. A.; Byrareddy, S. N. The Epidemiology and Pathogenesis of Coronavirus Disease (COVID-19) Outbreak. *J. Autoimmun.* **2020**, *109* (May), 102433.
 5. Cao, W.; Li, T. COVID-19: Towards Understanding of Pathogenesis. *Cell Res.* **2020**, *30* (May), 367–369.
 6. Raverdeau, M.; Mills, K. H. G. Modulation of T Cell and Innate Immune Responses by Retinoic Acid. *J. Immunol.* **2014**; *192*(7):2958.
 7. Lindschinger, M.; Tatzber, F.; Schimetta, W.; Schmid, I.; Lindschinger, B.; Cvirn, G.; Stanger, O.; Lamont, E.; Wonisch, W. A Randomized Pilot Trial to Evaluate the Bioavailability of Natural Versus Synthetic Vitamin B Complexes in Healthy Humans and Their Effects on Homocysteine, Oxidative Stress and Antioxidant Levels. *Oxid Med Cell Longev* 2019.
 8. Carr, A. C.; Maggini, S. *Vitamin C and Immune Function Nutrients* **2017**; *9*(11):1211.
 9. Dursun, E.; Alaylioglu, M.; Bilgic, B.; Hanagasi, H.; Lohmann, E.; Atasoy, I. L.; Candas, E.; Araz, O. S.; Onal, B.; Gurvit, H.; Yilmazer, S.; Gezen-Ak, D. Vitamin D Deficiency Might Pose a Greater Risk for Apo-E4 Non-Carrier Alzheimer's Disease Patients. *Neurol Sci* **2016**; *37*:1633–1643.
 10. Holick, M. F. Vitamin D: A Millennium Perspective. *J. Cell. Biochem.* **2003**, *88* (February), 296–307.
 11. Visweswaran, R. K.; Lekha, H. Extraskelatal Effects and Manifestations of Vitamin D Deficiency. *Indian J. Endocrinol. Metab.* **2013**, *17* (July), 602–610.
 12. Gezen-Ak, D.; Dursun, E. Molecular Basis of Vitamin D Action in Neurodegeneration: The Story of a Team Perspective. *Hormones* **2018**, *18* (January), 17–21.
 13. Gezen-Ak, D.; Yilmazer, S.; Dursun, E. Why Vitamin D in Alzheimer's Disease? The Hypothesis. *J. Alzheimers Dis.* **2014**, *40* (April), 257–269.
 14. Annweiler, C.; Dursun, E.; Feron, F.; Gezen-Ak, D.; Kalueff, A. V.; Littlejohns, T.; Llewellyn, D.; Millet, P.; Scott, T.; Tucker, K. L.; Yilmazer, S.; Beauchet, O. Vitamin D and Cognition in Older Adults: *International Consensus Guidelines Geriatr Psychol Neuropsychiatr Vieil* **2016**; *14*:265–273.
 15. Dursun, E.; Gezen-Ak, D. Vitamin D Basis of Alzheimer's Disease: *From Genetics to Biomarkers Hormones* **2019**; *18*:7–15.
 16. Lee, G. Y.; Han, S. N. The Role of Vitamin E in Immunity Nutrients. *MDPI AG* **2018**.
 17. Charoenngam, N.; Shirvani, A.; Holick, M. F. Vitamin D and Its Potential Benefit for the COVID-19 Pandemic. *Endocr Pract.* **2021** May; *27*(5):484–493.
 18. Holford, P.; Carr, A. C.; Jovic, T. H.; Ali, S. R.; Whitaker, I. S.; Marik, P. E.; Smith, A. D. Vitamin C—An Adjunctive Therapy for Respiratory Infection, Sepsis and COVID-19. *Nutrients.* **2020** Dec 7; *12*(12):3760.
 19. Helmond, N.; Brobyn, T. L.; LaRiccia, P. J.; Cafaro, T.; Hunter, K.; Roy, S.; Bandomer, B.; Ng, K. Q.; Goldstein, H.; Mitrev, L. V.; Tsai, A.; Thwing, D.; Maag, M. A.; Chung, M. K. Vitamin D3 Supplementation at 5000 IU Daily for the Prevention of Influenza-like Illness in Healthcare Workers: *A Pragmatic Randomized Clinical Trial Nutrients* **2022**; *15*(1):180.
 20. Hu, Q.; Zhang, Q. Y.; Peng, C. F.; Ma, Z.; Han, Y. L. Efficiency of Nicotinamide-Based Supportive Therapy in Lymphopenia for Patients with Ordinary or Severe COVID-19: *A Randomized Controlled Trial Medicine (Baltimore)* **2022**; *101*(43):e31138.
 21. Brunvoll, S. H.; Nygaard, A. B.; Ellingjord-Dale, M.; Holland, P.; Istre, M. S.; Kalleberg, K. T.; Soraas, C. L.; Holven, K. B.; Ulven, S. M.; Hjartaker, A.; Haiser, T.; Lund-Johansen, F.; Dahl, J. A.; Meyer, H. E.; Soraas, A. Prevention of COVID-19 and Other Acute Respiratory Infections with Cod Liver Oil Supplementation, a Low Dose Vitamin D Supplement: *Quadruple Blinded, Randomised Placebo Controlled Trial BMJ* **2022**; *378*:e071245.
 22. Rohani, M.; Mozaffar, H.; Mesri, M.; Shokri, M.; Delaney, D.; Karimi, M. Evaluation and Comparison of Vitamin A Supplementation with Standard Therapies in the Treatment of Patients with COVID-19. *East Mediterr Health J* **2022**; *28*(9):673–681.
 23. Fogleman, C.; Cohen, D.; Mercier, A.; Farrell, D.; Rutz, J.; Bresz, K.; Vernon, T. A Pilot of a Randomized Control Trial of Melatonin and Vitamin C for Mild-to-Moderate COVID-19. *J Am Board Fam Med* **2022**; *35*(4):695–707.
 24. De Niet, S.; Trémège, M.; Coffiner, M.; Rousseau, A.; Calmes, D.; Frix, A.; Gester, F.; Delvaux, M.; Dive, A.; Guglielmi, E.; Hentt, M.; Staderoli, A.; Maesen, D.; Louis, R.; Guiot, J.; Cavalier, E. Positive Effects of Vitamin D Supplementation in Patients Hospitalized for COVID-19: *A Randomized, Double-Blind, Placebo-Controlled Trial Nutrients* **2022**; *14*(15):3048.
 25. Mariani, J.; Antonietti, L.; Tajer, C.; Ferder, L.; Inserra, F.; Sanchez Cunto, M.; Brosio, D.; Ross, F.; Zylberman, M.; López, D. E.; Luna Hisano, C.; Maristany Batisda, S.; Pace, G.; Salvatore, A.; Högrefe, J. F.; Turela, M.; Gaido, A.; Roderer, B.; Banega, E.; Iglesias, M. E.; Rzepeski, M.; Gomez Portillo, J. M.; Bertelli, M.; Vilela, A.; Heffner, L.; Annetta, V. L.; Moracho, L.; Carmona, M.; Melito, G.; Martínez, M. J.; Luna, G.; Vensentini, N.; Manucha, W. High-Dose Vitamin D Versus Placebo to Prevent Complications in COVID-19 Patients: *Multicentre Randomized Controlled Clinical Trial PLoS One* **2022**; *17*(5):e0267918.
 26. Hellou, E.; Mohsin, J.; Elemy, A.; Hakim, F.; Mustafa-Hellou, M.; Hamoud, S. Effect of Artemis in Patients with COVID-19: *A Phase II Prospective Study J Cell Mol Med* **2022**; *26*(11):3281–3289.
 27. Villasis-Keever, M. A.; López-Alarcón, M. G.; Miranda-Novales, G.; Zurita-Cruz, J. N.; Barrada-Vázquez, A. S.; González-Ibarrá, J.; Martínez-Reyes, M.; Grajales-Muñoz, C.; Santacruz-Tinoco, C. E.; Martínez-Miguel, B.; Maldonado-Hernández, J.; Cifuentes-González, Y.; Klünder-Klünder, M.; Garduño-Espinosa, J.; Lopez-Martínez, B.; Parra-Ortega, I. Efficacy and Safety of Vitamin D Supplementation to Prevent COVID-19 in Frontline Healthcare Workers: A Randomized Clinical Trial. *Arch Med Res* **2022**; *53* (4):423–430.
 28. Majidi, N.; Rabbani, F.; Gholami, S.; Gholamalizadeh, M.; Bour Bour, F.; Rastgoo, S.; Hajipour, A.; Shadnoosh, M.; Akbari, M. E.; Bahar, B.; Ashoori, N.; Alizadeh, A.; Samipour, F.; Moslem, A.; Doaei, S.; Suzuki, K. The Effect of Vitamin C on Pathological Parameters and Survival Duration of Critically Ill Coronavirus Disease 2019 Patients: *A Randomized Clinical Trial Front Immunol* **2021**; *12*:717816.
 29. Beigmohammadi, M. T.; Bitarafan, S.; Hoseindokht, A.; Abdollahi, A.; Amoozadeh, L.; Soltani, D. The Effect of Supplementation with Vitamins A, B, C, D, and E on Disease Severity and Inflammatory Responses in Patients with COVID-19: *A Randomized Clinical Trial Trials* **2021**; *22*(1):802.
 30. Caballero-García, A.; Pérez-Valdecantos, D.; Guallar, P.; Caballero-Castillo, A.; Roche, E.; Noriega, D. C.; Córdova, A. Effect of Vitamin D Supplementation on Muscle Status in Old Patients Recovering from COVID-19 Infection. *Medicina (Kaunas)* **2021**; *57*(10):1079.
 31. Maghbooli, Z.; Sahraian, M. A.; Jamalimoghaddamsiahkali, S.; Asadi, A.; Zarei, A.; Zendehdel, A.; Varzandi, T.; Mohammadnabi, S.; Alijani, N.; Karimi, M.; Shirvani, A.; Holick, M. F. Treatment With 25-Hydroxyvitamin D3 (Calcifediol) Is Associated with a Reduction in the Blood Neutrophil-to-Lymphocyte Ratio Marker of Disease Severity in Hospitalized Patients With COVID-19:

- A Pilot Multicenter, Randomized, Placebo-Controlled, Double-Blinded Clinical Trial *Endocr Pract* **2021**;27(12):1242-1251.
32. Murai, I. H.; Fernandes, A. L.; Sales, L. P.; Pinto, A. J.; Goessler, K. F.; Duran, C. S. C.; Silva, C. B. R.; Franco, A. S.; Macedo, M. B.; Dalmolin, H. H. H.; Baggio, J.; Balbi, G. G. M.; Reis, B. Z.; Antonangelo, L.; Caparbo, V. F.; Gualano, B.; Pereira, R. M. R. Effect of a Single High Dose of Vitamin D3 on Hospital Length of Stay in Patients With Moderate to Severe COVID-19: A Randomized Clinical Trial *JAMA* **2021**;325(11):1053-1060.
33. Rastogi, A.; Bhansali, A.; Khare, N.; Suri, V.; Yaddanapudi, N.; Sachdeva, N.; Puri, G. D.; Malhotra, P. Short Term, High-Dose Vitamin D Supplementation for COVID-19 Disease: A Randomized, Placebo-Controlled, Study (SHADE Study) *Postgrad Med J* **2022**;98(1156):87-90.
34. Entrenas Castillo, M.; Entrenas Costa, L. M.; Vaquero Barrios, J. M.; Alcalá Díaz, J. F.; López Miranda, J.; Bouillon, R.; Quesada Gomez, J. M. Effect of Calcifediol Treatment and Best Available Therapy Versus Best Available Therapy on Intensive Care Unit Admission and Mortality Among Patients Hospitalized for COVID-19: A Pilot Randomized Clinical Study *J Steroid Biochem Mol Biol* **2020**;203:105751.
35. Domazet Bugarin, J.; Dosenovic, S.; Ilic, D.; Delic, N.; Saric, I.; Ugrina, I.; Stojanovic Stipic, S.; Duplancic, B.; Saric, L. Vitamin D Supplementation and Clinical Outcomes in Severe COVID-19 Patients-Randomized Controlled Trial. *Nutrients* **2023**, *15* (5), 1234. DOI: 10.3390/nu15051234.
36. Bishop, C. W.; Ashfaq, A.; Melnick, J. Z.; Vazquez-Escarpanter, E.; Fialkow, J. A.; Strugnell, S. A.; Choe, J.; Kalantar-Zadeh, K.; Federman, N. C.; Ng, D.; Adams, J. S. REsCue trial: Randomized controlled clinical trial with extended-release calcifediol in symptomatic COVID-19 outpatients. *Nutrition* **2023**, *107*, 111899. DOI: 10.1016/j.nut.2022.111899.
37. Kaufman, H. W.; Niles, J. K.; Kroll, M. H.; Bi, C.; Holick, M. F. (2020) SARS-CoV-2 positivity rates associated with circulating 25-hydroxyvitamin D levels. *PLoS ONE* *15*(9): e0239252.
38. Im, J. H.; Je, Y. S.; Baek, J.; Chung, M. H.; Kwon, H. Y.; Lee, J. S. Nutritional status of patients with COVID-19. *Int J Infect Dis.* **2020** Nov;100:390-393. doi: 10.1016/j.ijid.2020.08.018. Epub 2020 Aug 11.
39. Merzon, E.; Tworowski, D.; Gorohovski, A.; Vinker, S.; Golan Cohen, A.; Green, I.; Frenkel-Morgenstern, M. (2020). Low plasma 25(OH) vitamin D level is associated with increased risk of COVID-19 infection: an Israeli population-based study. *The FEBS journal*, *287*(17), 3693–3702.
40. Hernández, J. L.; Nan, D.; Fernandez-Ayala, M.; García-Unzueta, M.; Hernández-Hernández, M. A.; López-Hoyos, M.; Muñoz-Cacho, P.; Olmos, J. M.; Gutiérrez-Cuadra, M.; Ruiz-Cubillán, J. J.; Crespo, J.; & Martínez-Taboada, V. M. (2021). Vitamin D Status in Hospitalized Patients with SARS-CoV-2 Infection. *The Journal of clinical endocrinology and metabolism*, *106*(3), e1343–e1353.
41. Maggini, S. Vitamin C and Immune Function. *Nutrients* **2017**, *9* (11), 1211. DOI: 10.3390/nu9111211.
42. National Institutes of Health. Vitamin A Fact Sheet for Consumers. Office of Dietary Supplements. URL: <https://ods.od.nih.gov/factsheets/VitaminA-Consumer/#:~:text=A%20and%20Carotenoids,oids%20and%20what%20do%20they,and%20other%20organs%20work%20properly> (Accessed June 9, 2023).
43. Gilbert, C. What is vitamin A and why do we need it? *Community Eye Health* **2013**, *26* (84), 65.
44. Hanna, M.; Jaqua, E.; Nguyen, V.; Clay, J. B. Vitamins: Functions and Uses in Medicine. *Perm. J.* **2022**, *26* (2), 89–97. DOI: 10.7812/TPP/21.204.
45. Mayo Clinic. Niacin (Vitamin B3): Overview. Mayo Clinic. URL: <https://www.mayoclinic.org/drugs-supplements-niacin/art-20364984#:~:text=Overview,from%20the%20food%20they%20eat> (Accessed June 9, 2023).

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An Accessible and Convenient Method to Synthesize Metal-Organic Frameworks with Household Appliances

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ABSTRACT: Metal-organic frameworks (MOFs) are a rising family of compounds consisting of metal ions (nodes) linked with organic ligands (linkers) to form crystalline structures. Among various methods, an accessible and convenient method with household appliances was adopted to produce the HKUST-1, a type of MOF synthesized using copper (II) acetate monohydrate and 1,3,5-benzenetricarboxylic acid. The mechanochemical synthesis method, which involved a solvent-free ball-milling technique, was utilized in this study through a mortar and pestle. After the synthesis of HKUST-1, its adsorption capacity of CO₂ was obtained. Then, the HKUST-1 sample was regenerated by heating it in a toaster oven. Finally, the morphology of the synthesized HKUST-1 sample was characterized by scanning electron microscopy (SEM). Our experimental results confirmed that the HKUST-1 with CO₂ adsorption capacity could be synthesized through a convenient and mechanochemical method.

The significance of this research lies in the potential impact of an accessible and environmentally friendly synthesis method, which requires no solvents and avoids the need for high-energy laboratory equipment. Additionally, the study reveals the promising CO₂ adsorption capacity of the mechanochemically synthesized HKUST-1. Despite the observed minimal CO₂ adsorption, this study establishes the groundwork for further exploration of low-cost and high-productivity MOF synthesis using the accessible mechanochemical approach.

KEYWORDS: Chemistry; Metal-Organic Frameworks (MOFs); HKUST-1; Mechanochemical Synthesis; Carbon dioxide; Desorption; Regeneration; Global warming.

■ Introduction

As a family of compounds, metal-organic frameworks (MOFs) are made of metal ions (nodes) linked with organic ligands (linkers) to form crystalline structures. MOFs have rapidly emerged in the research field with a wide range of applications. Due to their high adsorption capacity, large surface area, tunable porosity, hierarchical structure, and recyclability, MOFs can be readily employed in various applications, including analyte capture and separation, catalysis, sensors, photo/electromagnetics, and biomedical areas.¹ As a popular and versatile MOF, the HKUST-1 has been used for gas storage, especially for hydrogen and methane, due to its porous structure. Furthermore, due to its strong affinity for CO₂ and NH₃, the HKUST-1 can remove the two toxic gases in polluted air or flue gases. Among various types of MOFs, the HKUST-1 is the focus of this study.

The chemical formula of HKUST-1 is Cu(OAc)₂·H₂O. Consisting of dimeric metal units, the HKUST-1 has a central pair of Cu²⁺ ions coordinated by benzene-1,3,5-tricarboxylate linker molecules.² The “paddlewheel” unit is commonly used to describe the coordination of the metal center, as shown in Figure 1.

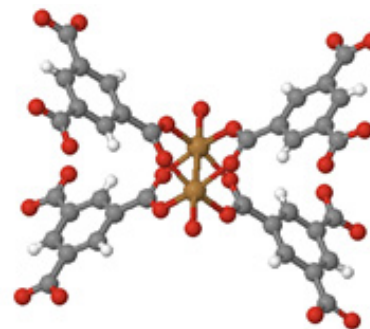


Figure 1: Molecular geometry of HKUST-1.³

The National Aeronautics and Space Administration (NASA) reported that the Earth's average surface temperature has risen by 1.11 °C (approximately 2 °F) since the late 19th century from global warming.⁴ The primary source of greenhouse gas emissions comes from CO₂ produced from burning fossil fuels, and there is an urgent need to find a solution.

The CO₂ removal process is often done by adsorption, which involves transferring fluid molecules from a fluid to the surface of a solid.⁵ Due to the three-dimensional structure, porosity, and high surface area per unit volume (specific surface area), the HKUST-1 is especially favorable for gas storage and removing toxic gases, including greenhouse gases.⁶

The HKUST-1 can be synthesized by various methods, including hydrothermal,⁷ sonochemical,⁸ electrochemical,⁹ and microwave-assisted¹⁰ techniques. However, most of the HKUST-1 has been exclusively synthesized on small scales by conventional electrical heating through hydrothermal or solvothermal techniques, which may involve a high-pressure and high-temperature chemical reaction that requires relatively high energy.

This study proposes a novel approach to synthesize HKUST-1 with desired adsorption capacity in less than 30 mins. As a mechanical technique that grinds powders into fine particles and blends different materials, mechanochemical synthesis can be considered a “green” method and can synthesize MOFs in a short time.¹¹ In addition, mechanochemical synthesis is also a highly accessible method, as it only uses rotational energy instead of requiring high-energy laboratory equipment and often does not require any solvent. In this study, the HKUST-1 was mechanochemically synthesized through a ball-milling method with household appliances, including a mortar and pestle. The adsorption and desorption capacities of the mechanochemically synthesized HKUST-1 were verified by heating it in a toaster oven at 140 °C.¹² This fast, simple, and convenient synthesis method described in the present work is solvent-free, environmentally friendly, and economically feasible, demonstrating its potential for further research and development.

■ Methods

The copper (II) acetate monohydrate (95%, Co, extra pure grade) was purchased from Samchun Chemical Co., and the trimesic acid (or 1,3,5-benzenetricarboxylic acid, 95%) was purchased from Sigma Aldrich Co. The CO₂ gas was purchased in ≥99.9% CO₂ gas cylinders from Sodastream.

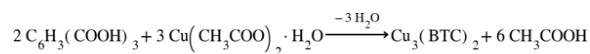
Synthesis:

The chemical reaction of the HKUST-1 synthesis can be described by Equation 1. In this study, 2.802 g of H3BTC and 3.993 g of copper (II) acetate (corresponding to a mole ratio of 2:3) were measured using a high-precision analytical lab scale (500 × 0.0001 g). All chemicals were measured with weighing papers before being placed in the mortar grinder. Table 1 in Results and Discussion summarizes that the reactants were ground with a pestle for 30 min with any changes recorded every 5 min. The same synthesis procedure was repeated to examine whether washing the product with an ethanol/water mixture (1:1 v/v) could improve the corresponding adsorption capability by removing any impurities trapped in the three-dimensional structure of the HKUST-1. After being washed with an ethanol/water mixture, the HKUST-1 was vacuum-filtered and dried in an oven at 100 °C under vacuum conditions, as shown in Figure 2.

The choice of mechanochemical synthesis through a mortar and pestle was motivated by the goal of developing an accessible and environmentally friendly method. Mechanochemical synthesis is known for its simplicity, efficiency, and reduced reliance on hazardous solvents, making it an ideal candidate for green synthesis. Additionally, using household appliances like a mortar and pestle made the process easily replicable, even in

non-laboratory settings, thereby democratizing the synthesis of MOFs.¹³

The decision to examine the effects of washing the HKUST-1 product with an ethanol/water mixture (1:1 v/v) was driven by the intention to improve the material's adsorption capabilities. The washing process was hypothesized to remove any impurities or residual reactants trapped within the three-dimensional structure of the HKUST-1, thereby enhancing the material's overall adsorption capacity.¹³



Equation 1: Balanced chemical equation of HKUST-1 synthesis reaction.



Figure 2: Experimental setup of the vacuum filtering procedure after HKUST-1 synthesis.

The third sample was produced using the traditional mechanochemical synthesis method with the ball-milling machine. The reactant chemicals were placed in a wide-mouth bottle (made of HDPE) with zirconia balls before being ball-milled at 300 rpm overnight without solvent using the WiseMix Ball Mill machine.

Adsorption:

Before testing the ability to adsorb CO₂, the HKUST-1 sample was initialized by heating it in a toaster oven for 30 min. This process can remove CO₂ or N₂ molecules that may have already been adsorbed onto the HKUST-1 surface from the laboratory environment. After initialization, 1 g of the HKUST-1 sample was placed into a 20 mL vial.

The adsorption process of CO₂ molecules by the HKUST-1 was carried out in a mason jar with a lid containing a straw hole. CO₂ was supplied into the mason jar using a Sodastream machine through the straw, shown in Figure 3. Specifically, CO₂ was supplied for 1 h to ensure that the HKUST-1 sample absorbed CO₂ in a closed environment. The mass of the sample was recorded after being placed in a CO₂-filled jar for 1 h.



Figure 3: Experimental setup of the CO₂ adsorption process.

Desorption:

After the adsorption process, the HKUST-1 was regenerated by heating the sample in the toaster oven again to remove the CO₂ molecules adsorbed on the surface of the HKUST-1. For the regeneration of HKUST-1, the sample was heated in the toaster oven at 150 °C for 30 min while recording the sample mass every 5 min.

Results and Discussion

Observations were recorded during the reaction, as summarized in Table 1. Before the reaction, the reactants were bright blue with no odor. After mortar grinding for 5 min, an unpleasant smell started to form, with the color changed to less bright blue. After 10 min, a weak acidic smell was found, although the color still remained light blue. After reacting for 25 min, a strong acidic smell started to become noticeable with the occurrence of smoke. The same situation remained till the end of the grinding period after 30 min. Figure 4 exhibits the pictures of the HKUST-1 during and after the synthesis process by mortar-grinding.

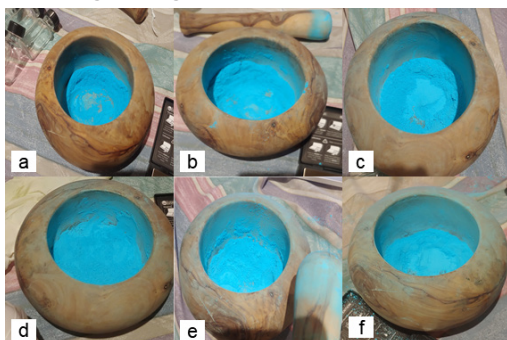


Figure 4: Photographs of the HKUST-1 after mortar-grinding for (a) 5 min, (b) 10 min, (c) 15 min, (d) 20 min, (e) 25 min, and (f) 30 min.

Table 1: Observations during the mechanochemical synthesis of HKUST-1.

Time	Observations
Before reaction	- Bright blue - No odor
5 min	- Color changed from cyan to light blue - Acidic smell formed
10 min	- Weak acidic smell - Still in light blue

15 min	- Same color - Odor remained constant
20 min	- Same color - Odor remained constant
25 min	- Strong acidic smell - Presence of smoke
30 min	- Same color - Odor remained constant - Smoke can be seen

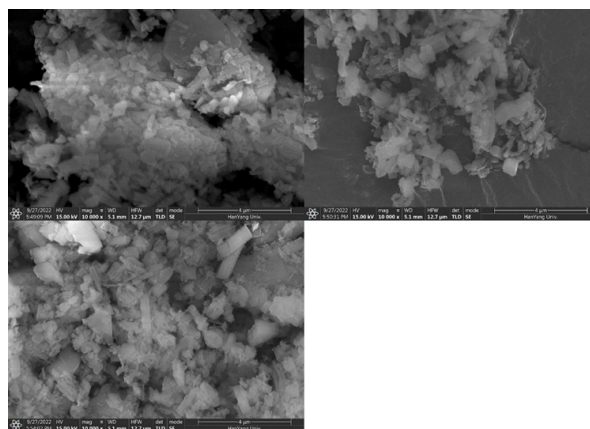


Figure 5: SEM images of the HKUST-1 samples.

The morphology of the prepared HKUST-1 samples was characterized using the SEM, Verios G4UC, and Thermo Fisher Scientific), as shown in Figure 5. During the mechanochemical synthesis process, some limitations and challenges were encountered, as evident from the SEM images showing inconsistent and uneven distributions of particle sizes and morphologies. These variations in crystal growth and morphology could be attributed to the mechanical energy input during the grinding process. As the reaction progressed, the mechanical forces applied to the particles might have led to differences in crystal nucleation and growth rates, resulting in the observed inconsistencies. Understanding and controlling these factors could be crucial for achieving more uniform particle sizes and improving the material's adsorption performance.

Another potential limitation is the use of a ball-milling machine for the third sample synthesis. While the ball-milling technique is effective for mechanochemical synthesis, the use of specialized equipment may not be as accessible or convenient as the mortar-and-pestle approach. Consequently, this could limit the scalability and practicality of the synthesis method for larger-scale production or home-based synthesis.

Three samples, including two mortar-ground samples and one ball-milled sample, adsorbed an average of 0.380 g of CO₂, as depicted in Figure 6 and summarized in Table 2. Figure 6 illustrates the mass of adsorbed CO₂ per unit mass of the HKUST-1 during the heating process. The HKUST-1 samples were regenerated after adsorption and can be reused for later adsorption. The ball-milled HKUST-1 sample washed with ethanol/water exhibited the highest CO₂ adsorption. At the beginning of heating, all three samples desorbed a significant amount of CO₂ to go through the regeneration process.

As time passed, the desorption rate decreased, as shown by the decrease in the mass of remaining CO₂ in the samples.

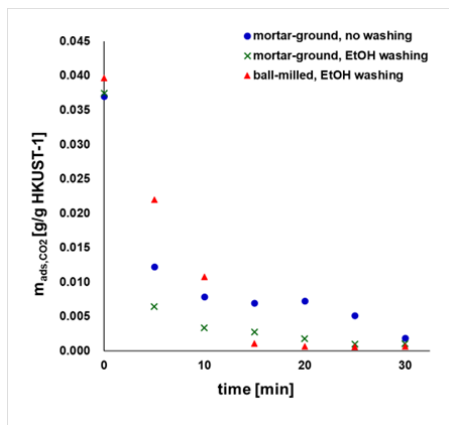


Figure 6: Mass of adsorbed CO₂ by unit mass of the HKUST-1 sample.

As summarized in Table 2, the ball-milled sample exhibited the least amount of remaining CO₂ at the end of the heating process. This indicates supreme “reversibility” for the adsorption/desorption process as the heated HKUST-1 can be reused to adsorb CO₂ again. However, the ball-milled sample was different from the other two HKUST-1 samples since it utilized the ball-milling machine. In addition, the ethanol washing process included vacuum-filtering and -drying in the oven overnight, which required an additional energy input compared to the mortar-ground samples without washing.

Table 2: Mass of adsorbed and desorbed CO₂ per unit mass of HKUST-1 sample.

	Mass of CO ₂ adsorbed	Mass of CO ₂ desorbed	CO ₂ removed
Mortar-ground, no washing	0.0371 g	0.0351 g	94.9 %
Mortar-ground with EtOH/water washing	0.0376 g	0.0364 g	97.1 %
Ball-milled with EtOH/water washing	0.0397 g	0.0390 g	98.2 %

Considering the amount of energy input in the washing process, the additional adsorbed CO₂ seems insignificant because all three samples resulted in a percent regeneration of over 90%. Although the washing and ball-milling process can result in different adsorbed CO₂ amounts and CO₂ removal rates, the two samples were not synthesized in an environmental-friendly way. From the results, the synthesis of other MOFs may be further explored through mechanochemical synthesis using a mortar grinder, as it can yield low-cost and high-productivity synthesis compared to traditional hydrothermal methods.

Furthermore, it should be noted that while the CO₂ adsorption capacity of the mechanochemically synthesized HKUST-1 was promising, the adsorption amount appeared relatively low compared to other reported MOFs. This could be attributed to the morphological inconsistencies observed in the SEM images, which might have affected the material's overall adsorption performance. Addressing these inconsistencies and optimizing the synthesis method could potentially lead to higher CO₂ adsorption capacities.

Despite these challenges, the mechanochemical synthesis method still holds great potential as an environmentally friendly and accessible approach for MOF synthesis. By further investigating the underlying mechanisms of crystal growth during mechanochemical synthesis and optimizing the reaction conditions, researchers can overcome these limitations and develop more efficient and reproducible synthesis procedures.

Conclusion

The successful synthesis of HKUST-1 through an accessible and environmentally friendly mechanochemical method holds significant implications for the field of metal-organic frameworks (MOFs) and gas capture technologies. The utilization of household appliances, such as a mortar and pestle, in the synthesis process, demonstrates the potential for low-cost and easily scalable production of MOFs with desirable properties. By avoiding the need for high-energy and high-pressure reactions, this green synthesis method presents an attractive alternative to traditional solvothermal approaches, which often involve energy-intensive processes and the use of hazardous solvents.

The observed CO₂ adsorption capacity of the mechanochemically synthesized HKUST-1 adds to its potential as a practical material for addressing greenhouse gas emissions. Despite the morphological inconsistencies observed in the SEM images, the high percentage of regeneration after desorption indicates the material's ability to be reused efficiently for gas capture applications. This suggests that the adsorption and desorption properties of the MOF can be effectively tuned and optimized through further research and development.

Furthermore, the exploration of other MOFs using the mechanochemical synthesis approach with a mortar grinder or other household equipment presents an avenue for future research. Different metal ions and organic ligands could be utilized to synthesize a wide range of MOFs with diverse properties and functionalities. By systematically studying the effects of various reaction conditions, grinding times, and precursor ratios, researchers can identify novel MOFs with enhanced adsorption capacities for different gases, making significant contributions to gas storage and separation technologies.

The accessible and straightforward nature of the mechanochemical synthesis method makes it a feasible option for home-based synthesis of MOFs, democratizing the process and encouraging more widespread interest in the field. This could foster a community-driven approach to MOF research, allowing individuals to contribute to solving real-world challenges related to greenhouse gas emissions and environmental pollution. The accessible synthesis method presented in this study could pave the way for innovative research and advancements in the field of MOFs, making significant contributions to the ongoing efforts to combat climate change and improve air quality.

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■ References

1. Adegoke, K. A.; Agboola, O. S.; Ogunmodede J.; Araoye, A. O.; Bello, O. S. Metal-Organic Frameworks as Adsorbents for Sequestering Organic Pollutants from Wastewater. *Mater. Chem. Phys.* **2020**, *253*, 123246.
2. Šimėnas, M.; Kobalz, M.; Mendt, M.; Eckold, P.; Krautscheid, H.; Banys, J.; Pöpl, A. Synthesis, Structure, and Electron Paramagnetic Resonance Study of a Mixed Valent Framework Containing Cu₂ Paddle-Wheel Units. *J. Phys. Chem. C* **2015**, *119*(9), 4898–4907.
3. University of Liverpool. ChemTube3D. <https://www.chemtube3d.com/mof-hkust-1-2/> (accessed Oct 31, 2022).
4. Gaeta, K. NASA Says 2022 Fifth Warmest Year on Record, Warming Trend Continues. *National Aeronautics and Space Administration (NASA)* [Online], **2023**, <https://www.nasa.gov/press-release/nasa-says-2022-fifth-warmest-year-on-record-warming-trend-continues> (accessed Jan 28, 2023).
5. Vrtovec, N.; Mazaj, M.; Buscarino, G.; Terracina, A.; Agnello, S.; Arčon, I.; Kovač, J.; Logar, N. Z. Structural and CO₂ Capture Properties of Ethylenediamine-Modified HKUST-1 Metal-Organic Framework. *Cryst. Growth Des.* **2020**, *20*(8), 5455–5465.
6. Li, H.; Wang, K.; Sun, Y.; Lollar, C. T.; Li, J.; Zhou, H.-C. Recent Advances in Gas Storage and Separation Using Metal-Organic Frameworks. *Mater. Today* **2018**, *21*(2), 108–121.
7. Domán, A.; Madarász, J.; Sáfrán, G.; Wang, Y.; László, K. Copper Benzene-1,3,5-Tricarboxylate (HKUST-1)-Graphene Oxide Pellets for Methane Adsorption. *Microporous Mesoporous Mater.* **2021**, *316*, 110948.
8. Li, Z.-Q.; Qiu, L.-G.; Xu, T.; Wu, Y.; Wang, W.; Wu, Z.-Y.; Jiang, X. Ultrasonic Synthesis of the Microporous Metal-Organic Framework Cu₃(BTC)₂ at Ambient Temperature and Pressure: An Efficient and Environmentally Friendly Method. *Mater. Lett.* **2009**, *63*(1), 78–80.
9. Van Assche, T.R.C.; Desmet, G.; Ameloot, R.; De Vos, D.E.; Terryn, H.; Denayer, J.F.M. Electrochemical Synthesis of Thin HKUST-1 Layers on Copper Mesh. *Microporous Mesoporous Mater.* **2012**, *158*, 209–213.
10. Zou, F.; Yu, R.; Li, R.; Li, W. Microwave-Assisted Synthesis of HKUST-1 and Functionalized HKUST-1-@H₃PW₁₂O₄₀: Selective Adsorption of Heavy Metal Ions in Water Analyzed with Synchrotron Radiation. *Chemphyschem.* **2013**, *14*(12), 2825–2832.
11. Tanaka, S. *Mechanochemical Synthesis of MOFs*. In *Metal-Organic Frameworks for Biomedical Applications*, 13th ed.; Woodhead Publishing, 2020; pp 197–222.
12. Raganati, F.; Gargiulo, V.; Ammendola, P.; Alfe, M.; Chirone, R. CO₂ Capture Performance of HKUST-1 in a Sound Assisted Fluidized Bed. *Chem. Eng. J.* **2014**, *239*, 75–86.
13. Klimakow, M.; Klobes, P.; Thunemann, A. F.; Rademann, K.; Emmertling, F. Mechanochemical Synthesis of Metal-Organic Frameworks: A Fast and Facile Approach toward Quantitative Yields and High Specific Surface Areas. *Chem. Mater.* **2010**, *22*(18), 5216–5221.

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Kevin's research interests span a variety of fields and are still flourishing, from nanoparticles in materials chemistry to therapeutics in biomedicine and biochemistry. In the future, Kevin would like to study medicine and become a biomedical researcher focusing on molecular and cellular research and cancer research.

Quantum 4x4 Tic-Tac-Toe

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ABSTRACT: Game theory studies interactive decision-making, where the outcome for each participant or player depends on the actions of all. Quantum game theory introduces new strategies and outcomes that are impossible in classical game theory, hence it is generally advantageous over classical game theory. One of the games in which quantum features add advantages to strategies and winning possibilities is the 3x3 tic-tac-toe. Here, we study the quantum 4x4 tic-tac-toe, a new tic-tac-toe model that incorporates quantum strategies into the classical 4x4 tic-tac-toe. This paper reviews some winning strategies and properties of the quantum 4x4 tic-tac-toe.

KEYWORDS: Computer Science, Classical Game Theory; Quantum Game Theory; Superposition; Entanglement; Measurements, Tic-Tac-Toe.

■ Introduction

Game theory is a framework for understanding choice in situations among competing players. It can help players reach optimal decision-making when confronted by independent and competing actors in a strategic setting and apply to disparate disciplines such as mathematics, psychology, and philosophy.¹

The basic principle of game theory is the abstract and deductive model of policy-making. It does not describe how people actually make decisions but rather how they would make decisions in competitive situations if they were entirely rational. Game theory has a direct association with human interaction, and it aims at the maximization of profit or the rationalization of decisions.² This theory analyses a player's decision-making based on how they expect other players to make a decision. In other words, we can say that it helps in determining optimal rational choices given a set of circumstances.³

The fundamental tenet of traditional game theory is that players have consistent preferences and are rational. Participants can get an optimal scheme under the given circumstances. Standard game theory assumes that each actor is perfectly rational and always acts in their best interest.⁴

Classical game theory states that individuals involved in a game are interrelated, as one's decision affects others and is also affected by others. Therefore, no one in the game can control the result completely, and no one is completely isolated from others. In addition, individuals in the game are rational decision-makers, which means they should make decisions that will maximize their expected payoff. Because of the interdependency of players, a rational decision must be made based on anticipating other players' actions.⁵

With the development of quantum computing, David A. Meyer was the first to merge quantum computing with game theory in 1999. Meyer applied general quantum algorithms to find a better strategy that improves the expected payoff of individuals. He generated a quantum strategy that

proved to be always at least as good as the classical strategies. This discovery thus serves as a foundation for a new chapter of game theory – quantum game theory.⁶ Since then, many mathematicians, physicists, and economists have explored this area by constructing quantum versions of classical game theory models. Quantum game theory allows the study of interactive decision-making by players with access to quantum technology. This technology can be used both as a quantum communication protocol and to randomize players' strategies more efficiently than in classical games.⁷

In this paper, I will evaluate the concepts of game theory and further review the differences between classical game theory and quantum game theory based on strategies, concepts, rules, and properties. First, this comparison will be made with the help of the comparison between classical 3x3 tic-tac-toe and quantum 3x3 tic-tac-toe. Then, I will further evaluate classical 4x4 grid tic-tac-toe to propose a new model of a game of quantum 4x4 grid tic-tac-toe, which incorporates quantum strategies and rules into classical 4x4 grid tic-tac-toe.

■ Discussion

Game Theory:

Game theory concepts provide a mathematical framework to formulate, structure, analyze, and understand such game scenarios. It provides applicable mathematical models and tools to understand the possible strategies that agents may follow when competing or collaborating in games. In game theory, a strategic interaction is defined as a game, and those involved in the decision-making are called the players, who are assumed to act rationally.⁸

Classical Game Theory:

Classical game theory studies games where players simultaneously or sequentially move, bet, or strategize. As a result, players often need to learn more about the rules, properties, and strategies of the game. Players of these games are more

likely to depend on prediction and assumptions for their successive moves in the game.⁹

The prisoner's dilemma is one of the most popular examples of game theory. Suppose two people, Prisoner-A and Prisoner B, are arrested for committing a crime. However, the prosecutor still needs proper evidence to decide their conviction. To get the confession from the prisoners, they are questioned in two different rooms. Both the prisoners are separated, and there is no communication between them. The officials propose some deals to both the prisoners. According to the deals, if both prisoners confess the crime, they will receive a prison sentence of five years. If the prosecutor receives a confession from Prisoner-A, but a denial from Prisoner-B, then Prisoner-A will receive a three years prison sentence, while Prisoner-B will be freed. The same holds true in the case where Prisoner-B confesses to the crime, but Prisoner-A denies it. If both do not confess, they will receive two years of prison.⁹

From all of the given deals, the most favorable one is that neither of the prisoners confess, as they receive the lesser punishment (two years of imprisonment) in this case only. However, the problem is that the prisoners do not know each other's strategies; they are not certain whether the other prisoner will confess. The prisoners are likelier to choose the deal that is best for their own interest but worse for both of them collectively.⁹

The repeated prisoner's dilemma is a game in which trust and cooperative behavior is established if the game is played several times, instead of non-cooperation if it is played one time. The expression 'Tit for Tat' is considered the best solution for repeated prisoner's dilemma as per the game theory. The concept of Tit for Tat was presented by an American mathematical psychologist, Anatol Rapoport, who stated that the players would likely not cooperate if provoked. At the same time, they are likely to cooperate if unprovoked.⁹

One of the most popular strategies for this game is based on this concept, where the player starts by cooperating, then copying whatever the other player did in the last move. Tit for Tat is similar to the philosophy of "an eye for an eye," where the punishment matches the crime. If someone defects against you, then immediately defect against them. If they start cooperating again, then you start cooperating again too.¹⁰

As Tit for Tat starts by cooperation and then copies the other player's last move, it can give the illusion of trust to the opponent as it is always cooperating initially. However, it can immediately switch to working against the opponent if there is any suspicion of betrayal. This adaptability is a solid strategy. However, one of the cons of this strategy is that it plays to tie, not to win. Amongst Prisoner's Dilemma fans, Tit for Tat was considered the best strategy for a couple of decades, as it generally does better than other strategies in the long run.¹⁰

While discussing game theory, there are some important terms to keep in mind that are used recurrently. These include the N-person game theory, the zero-sum game, the optimal strategy, the minimax principle, and the Nash equilibrium.¹¹

The N-person game theory provides a logical framework for analyzing contests with more than two players or sets of con-

flicting interests-anything, from a hand of poker to the tangled web of international relations.¹¹

A zero-sum game is a term used in game theory to describe both real games and situations of all kinds, usually between two players or participants. The gain of one person is equal to the loss of the other, due to which the net sum is zero. For instance, if a person plays a single game of rock-paper-scissors with someone else, one person will lose, and one person will win. The win (+1) added to the loss (-1) equals zero.¹¹

A player's strategy set defines what strategies they can play. A player has a finite approach set if they have several discrete strategies. For instance, a game of rock-paper-scissors comprises a single move by each player, and each player's move is made without the knowledge of the others, not as a response, so each player has a finite strategy set (e.g., rock, paper, and scissors).¹¹

An optimal strategy maximizes a player's expected payoff. The two players of a matrix game carry out one of the pairs of mixed strategies. Each player adjusts their strategy to minimize the maximum loss that an opponent can inflict. In the finite case, the normal form for such a game is a matrix, where each row represents one of Player 1's strategies, and each column represents one of Player 2's strategies.¹¹

The minimum-maximum (min-max) theorem is a decision-making condition used to calculate the optimal move. The condition evaluates the minimum loss and maximum profit.¹¹

The Nash equilibrium is when each player is assumed to know the equilibrium strategies of the other players, and no player has anything to gain by changing only their own strategy. It is a concept that determines the optimal solution in a non-cooperative game in which each player lacks any incentive to change their initial strategy. Under the Nash equilibrium, a player gains nothing from deviating from the initially chosen, assuming the other players keep their strategies unchanged. A game may include multiple Nash equilibria or none.¹¹

Quantum Game Theory:

Quantum game theory differs from the classical version in three ways: superposed initial states, the quantum entanglement of initial states, and the superposition of strategies.

For a classical two-strategy case, one player's choice can be represented by the classical bit, i.e., 0 or 1. The quantum version replaces a bit with a qubit and is initially prepared in a superposition of 0 and 1. The player's choice corresponds to the operation on the qubit. This is the superposition of initial states.¹²

The quantum entanglement of initial states is when the set of qubits provided to each player can be initially entangled, so one player's operation on their qubit can affect others' qubits, which means one's choice can alter others' expected pay-offs of the game.¹²

In the quantum case, since a qubit represents the initial state, a player choosing a strategy is analogous to rotating the qubit to a new state or applying a unitary matrix to the state vector of the qubit. The new state may not be definite and can still be in the superposition of basis states with changed probability amplitudes. This is the superposition of strategies.¹²

A unitary operation is a transformation that preserves the structure and properties of the game. It is performed on the strategies of all the players simultaneously, without changing the relative proportion/probability distribution between strategies.

For several zero-sum games, it has been shown that if one of the players can use quantum tools and, thereby, quantum strategies, they can increase their chance of winning. Just as a classical mixed strategy can be thought of as a strategy conditioned on the realization of some classical random variable, a quantum strategy can be considered a strategy conditioned on the value of some quantum mechanical observable.¹³

Quantum computation has outperformed its classical counterpart in various fields. It has been shown that a quantum strategy dominates classical strategies in examples such as the prisoner's dilemma.¹⁴

In the Quantum Prisoner's Dilemma, both parties betraying each other is still an equilibrium. However, there can also exist multiple Nash equilibria that vary based on the entanglement of the initial states. When the states are only slightly entangled, a specific unitary operation exists for Player 1. If Player 2 chooses betrayal every turn, Player 1 will profit more than Player 2 and vice versa. Thus, a profitable equilibrium can be reached in two additional ways. The case where the initial state is most entangled shows the most change from the classical game. In this game version, Players 1 and 2 have an operator Q that allows for a pay-out equal to mutual cooperation with no risk of betrayal.¹³

Classical 3x3 Tic-Tac-Toe:

The tic-tac-toe game is a two-person, zero-sum game that involves two players. It is a nine-square grid where each player has only four plays to justify their winning strategies. The opponent then devises a strategy to either win or bring it to a tie.¹⁵

This game involves filling up a 3x3 grid with either crosses ('X') or noughts ('O'). The first player will get a maximum of five turns, and the second player will get a maximum of four turns, after which the victory/draw of the game is declared. The player who first encounters three crosses ('X') or three noughts ('O') in a particular row, column, or diagonal is declared the winner.¹⁶

There are $3^9=19,683$ possible states in the game. The game's goal is to fill the nine spaces with a maximum of three boxes in a row, column, or diagonal. Therefore, there are $9! = 362,880$ ways to fill the 9th position.¹⁶

The optimal strategy in the tic-tac-toe is the one that puts the player in the most preferred position, irrespective of the strategy of his opponents. Tic-tac-toe uses the strategy that puts the player in the most desired position, regardless of the strategy of his opponents.¹⁶

The tic-tac-toe game uses crosses ('X') to specify the first player's move and noughts ('O') to the second player's move. Initially, there were only three types of moves: corner, edge, and center.¹⁶

1	2	3
4	5	6
7	8	9

Figure 1: Classical 3x3 tic-tac-toe grid boxes labeled from 1 to 9.

In Figure 1, positions 0, 2, 6, and 8 are called 'corners,' 1, 3, 5, and 7 are called 'edges,' and position 4 is called the 'center' position.

The first player, i.e., player X, has an advantage as the player has access to a free board and can set the pace of the game. These are some of the winning strategies for player X.

1. Place the first mark in any corner, as in boxes 1,3,7, or 9 from Figure 1. For example, in Figure 2, X's move is marked in box 3. If their opponent does not place their O in the center, move on to the next step.

2. Now, place X in the opposite corner of the first one. In Figure 2, the X is played in box 7. The opponent's O mark should not be between the marks.

3. Place X in either one of the remaining corners. In Figure 2, X is played in box 1.

Optimally, the opponent will try to block this move by placing their O in boxes 2, 4, or 5. Two possible ways of winning have been secured by placing X in the last corner.

4. For example, if O is placed in box 5, player X can win horizontally or vertically by placing an X in box 2 or 4. This is also called the forking strategy, where the player tries to secure more than one winning path so that a win can still be secured if their path is blocked.¹⁷

X	X	X
X	O	
X		

Figure 2: Horizontal and vertical winning sequence for player X in the classical 3x3 tic-tac-toe.

Winning tic-tac-toe going second is more difficult due to the advantage going first can offer if the opponent places their X in a corner. If they do not, then player O can follow the strategy in the previous section to win; if not, player O can instead cause a draw (or possibly win). These are some of the strategies for player O to cause a draw.

1. Assuming that the opponent placed their X in the corner, place the O in the center.

2. The opponent will now attempt to secure a position in every corner, so player O can respond by placing O between their marks to block all possible winning paths.¹⁷

Depending on the course of the game, it is possible to win or secure a draw with this strategy. It is usually the latter, but if the opponent makes a mistake, a victory can be secured by getting three in a row in the middle of the board.¹⁷

Quantum 3x3 Tic-Tac-Toe:

The quantum version of tic-tac-toe adds the rule of superposition. On every move, two marks must be placed in separate squares. These two marks are subscripted with the number of the moves, so X gets the odd number moves $X_1, X_1, X_3, X_3, \dots$, and O gets the even number moves $O_2, O_2, O_4, O_4, \dots$. The two states of a singular move, such as X_1 and X_1 , are also spooky marks. Quantum moves are indicated with hyphens, 1-3, 6-9...¹⁸

Quantum tic-tac-toe provides superposition with an immediate and obvious interpretation. Figure 3 shows the first move of a game where X places his spooky marks in squares 1 and 2. A superposition in the quantum tic-tac-toe means that two games of classical tic-tac-toe are being played. In the first classical game, X has moved to square 1; in the second, X has moved to square 2. The two classical games are in simultaneous play; they are not independent.¹⁸

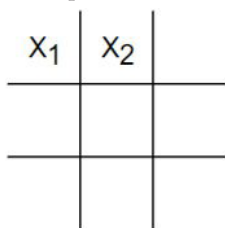


Figure 3: Superposition in quantum 3x3 tic-tac-toe with two games in a classical ensemble.

Figure 4 shows the situation if player O places their spooky marks in squares 4 and 5. The existing two classical games are duplicated, so there are two identical sets, each consisting of two games. In the first set, the move O in row 1 has moved to square 4; in the second set, the move O in row 2 has moved to square 5. Thus, there are now four games in the classical ensemble.¹⁸

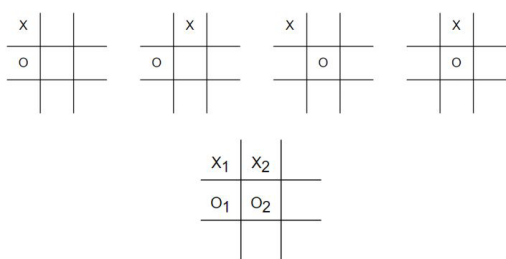


Figure 4: Superposition in quantum 3x3 tic-tac-toe with four games in a classical ensemble.

A slightly different move can occur for O in which player O plays in squares 5 and 2, as shown in Figure 5. X already has a spooky mark in square 2, so this move looks incorrect at first glance. However, such moves must be permitted to accommodate all the spooky marks of the incoming moves. As before, the classical games are duplicated into two sets; one of O's spooky marks is in the first set, and the other spooky mark is in the second set. The difference is that in one of the classical games, there is an X and an O in square 2.¹⁸

With both X and O in square 2, the first two moves of the game are no longer independent. When a measurement finally occurs, if X ends up in square 2, O will have to end up in square 5. Similarly, if O ends up in square 2, X must be in square 1.

These two moves have become entangled: the state of one affects the other. Any pair of quantum moves that share a square are necessarily entangled.¹⁸

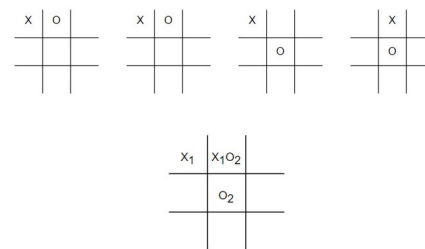


Figure 5: Superposition in quantum 3x3 tic-tac-toe where two quantum moves share one square.

Figure 6 shows a third move where X places his two spooky marks in squares 1 and 5, entangling with both moves one and two. The resulting entanglement has a new property: tracing a path around the entanglement back to the starting point is possible. The entanglement is cyclic.¹⁸

Starting in square 2, X_1 is entangled with O_2 because they share the same square. However, O_2 is also entangled with X_3 in the center square, and similarly, X_3 is entangled with X_1 in the corner square. If X_1 collapses to square 2, this collapse forces O_2 to collapse into square 5, forcing X_3 into square 1, which consistently collapses X_1 into square 2, where we started the analysis. Alternatively, if X_1 collapses to square 1, that forces X_3 into square 5, O_2 into square 2, and thus forces X_1 into square 1. Assuming a particular collapse for any of the moves creates a causal chain that forces the same collapse as was assumed.¹⁸

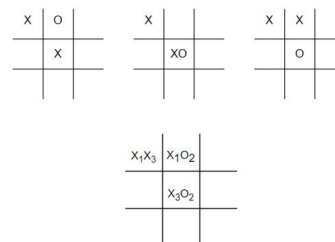


Figure 6: Superposition in quantum 3x3 tic-tac-toe where one quantum moves share one box in three positions on the 3x3 grid.

Cyclic entanglements will be indicated by underlining the spooky marks. In the quantum tic-tac-toe, either collapse leads to a classical game. No matter how complicated the cyclic entanglement is, there are always only two possible collapses. One must be chosen; the other will be eliminated from the classical ensemble, resulting in a single game of the classical tic-tac-toe.¹⁸

To balance the game strategically, the other player gets to choose the collapse. Because X made the move that created a cyclic entanglement, O gets to choose the collapse. Afterward, they will still get to make their regular quantum move of two spooky marks. Once a square has collapsed, it has a single real classical mark, and further play in that square is prohibited.¹⁸

Choosing a collapse is a strategic choice. In this case, O can choose their move to end up either in the center square or the side square; the center square is tactically stronger, so Player O chooses the collapse that puts their mark there. The other game in the classical ensemble is rejected as a matter of choice, not because of an entanglement-induced contradiction, X and

O now find themselves three moves into a classical game of tic-tac-toe, the only one left in the ensemble.¹⁸

Figure 7 shows the result after the collapse and a listing of the classical moves corresponding to the final reality.

$X_1 X_3$	$X_1 O_2$	
	$X_3 O_2$	

Figure 7: Results after the collapse in the 3x3 quantum tic-tac-toe, where the spooky mark that remains is bolded.

A successful conclusion of a game of quantum tic-tac-toe is where X or O has achieved a winning 3-row down the middle column. A win requires three real classical marks in a row; spooky marks do not count. Any game that goes to nine moves without generating a classical 3-row is a tie. If the board collapses on move eight without a win, the opponent is forced to place his spooky marks in the remaining square.¹⁸ Some winning strategies for Quantum 3x3 tic-tac-toe are listed below.

- Play at least one mark of a pair in an empty square. This increases the chances of having the lowest maximum subscript in a tiebreaker, as on each play, you add another square where you have the mark with the lowest subscript. X does this up until the last move.

- Play to increase the number of probable wins. For example, in Figure 8, after playing X_1 in opposite corners, play one X_3 in the middle and the other in a corner. When one X_1 collapses, the other X_1 that is now fixed will align with either X_3 . The same is true for X_3 in relation to X_1 .¹⁹

These are some moves to avoid.

- Do not play on your marks. X never does this until the final move, when there is no other option.

- Refrain from letting your last mark be part of your three in a row. Your previous move has the highest subscript and can make you lose the tie-breaker.¹⁹

X_1		
O_2	$X_3 O_2$	
X_3		X_1

Figure 8: Winning strategies for the quantum 3x3 tic-tac-toe.

Classical 4x4 Tic-Tac-Toe:

4x4 tic-tac-toe is a game that adheres to the same rules of 3x3 tic-tac-toe, played on a grid of 16 squares. It is a two-person, zero-sum game involving two people, a player, and a computer. However, this game is played in a grid of 16 squares instead of 9 squares as the board's width increases.²⁰

This variant of the tic-tac-toe game involves filling up a 4x4 grid with either crosses ('X') or noughts ('O'). The first player will get a maximum of eight turns, and the second player will get a maximum of eight turns, after which the victory/draw of the game is declared. The player who first encounters four crosses ('X') or four noughts ('O') in a particular row, column, or

diagonal is declared the winner. There are $4^{16}=4,294,967,296$ possible states in the game. The purpose of filling the sixteen spaces can be considered as filling the sequence of nine boxes which is a maximum of three in a row, column, or diagonal. Therefore, there are $16! = 20,922,789,888,000$ ways to fill the 16th position.²⁰

The optimal strategy is the strategy that puts the player in the most preferred position irrespective of the strategy of their opponents. This game uses a min-max algorithm to choose an optimal move for a player, assuming that the opponent is also playing optimally. The goal of the min-max in the game is to minimize the maximum loss.²⁰

The winning strategy in a game of classical 4x4 tic-tac-toe is similar to that of the classical 3x3 tic-tac-toe, with the only substantial difference being the increase in width of the grid and subsequent increase in choices to be made by the players. These are some of the common strategies.²⁰

1	2	3	4
5	6	7	8
9	10	11	12
13	14	15	16

Figure 9: Representation of the grid for the 4x4 tic-tac-toe, numbered from 1 to 16.

- Start in the center (6, 7, 8, 9 from Figure 9): this option provides more options for future moves and can also help to block the opponent from forming a winning sequence.

- Control the corners (1, 4, 13, 16 from Figure 9): corners are extremely powerful as they provide options for future moves; controlling two corners can force the opponent into a defensive position.

- Rather than creating a single winning line, attempt to create multiple threats on the board, forcing the opponent to make defensive moves and limit their options, thus giving more options for future moves.

- Remain mindful of the opponent's moves and look out for potential winning combinations; anticipating these moves will enable you to take steps to block them and gain an advantage.

- Use a fork, a move that creates two potential winning lines on the board. You can win on the other line if the opponent can only block one of them. The fork strategy is only possible if the players mark their move in one of the four corners.²⁰

In Figure 9, for example, obtaining control of corners 4, 13, and 16 would be an optimal move, such that if your opponent blocks the 4, 8, 12, and 16 lines, you still have an opportunity to create a diagonal sequence in the 4, 7, 10, 13 lines and win the game.²⁰

In an optimal game, the first player has an advantage as they set the pace. Hence, optimally, the first player would win. The best outcome for the second player is to either secure a win or block the first player from a win so that the game ends in a draw.²⁰

The Pairing Strategy can be used by the second player in a game of classical 4x4 Tic-Tac-Toe. This strategy enables the second player to block the first player from a winning move

and end the game in a draw. The second player mimics the first player's moves by placing their moves symmetric to the first player.²⁰

X	O	X	X
O	X	O	O
X	X	O	X
O	O	X	O

Figure 10: Pairing strategy in classical 4x4 tic-tac-toe, where the same color marks every pair.

As depicted in Figure 10, the colored line through the middle represents the line of symmetry. Every X move is mirrored through the line of symmetry by an O move. The same color marks each mirrored pair, and the game ends in a tie.²⁰

Quantum 4x4 Tic-Tac-Toe:

Quantum 4x4 tic-tac-toe is a model incorporating quantum rules and properties, including superposition, entanglement, and measurements, into a 4x4 grid. This game is played in various stages. First, this game will be explained through a specific example.

In Stage 1, once every move, one mark must be played in 2 separate squares to entangle those squares. However, all the boxes must be filled with one move each before filling each box with more than one move. Therefore, Stage 1 is complete only once all the boxes in the grid are filled with one move each. You can move on to Stage 2 only after Stage 1 is complete.

X ₁	X ₂	O ₄	O ₂
X ₇	O ₂	O ₈	O ₆
O ₈	X ₇	X ₁	O ₄
X ₂	O ₆	X ₃	X ₃

Figure 11: Stage 1 of the 4x4 tic-tac-toe.

In Stage 2, every box will begin to hold two or more moves. This stage is played similarly to Stage 1, with one difference—the employment of the pairing law. Again, a vertical symmetry line passes down the board's middle. Each player must play one mark in two boxes in every move. However, one of the two marks must mirror one of the marks that the opponent played in the previous turn.

For example, in Figure 12, the line in the board's middle represents the symmetry line. Every one out of two X moves is mirrored by one O move, and the second O move is played in any other box of the player's choosing. This O move is mirrored by an X move, so the pattern continues until all the boxes in the grid contain two moves each.

In the given figure, the move X₉ in box 8 is mirrored by one O₁₀ move in box 9. After that, the second O₁₀ move can be played in any box of the player's choosing. In this case, it is played in box 11, mirrored by one X₁₁ move in box 6, so the pattern continues. The same color marks every mirror pair. Eventually, the first move is mirrored by the last move, as is

seen by X₉, which is played in box two and is mirrored by O₁₀, placed in box 15.

If they wish, the players can begin to form measurements, that is, collapse the boxes to either of the two moves in this stage. In every turn, the players choose between forming a measurement and continuing with forming entanglements.

X ₁₃ X ₁	X ₉ X ₂	O ₁₂ O ₄	X ₁₅ O ₂
O ₁₄ X ₇	X ₁₁ O ₂	X ₁₅ O ₈	X ₉ O ₆
O ₁₀ O ₈	O ₁₄ X ₇	O ₁₀ X ₁	X ₁₃ O ₄
O ₁₆ X ₂	X ₁₁ O ₆	O ₁₆ X ₃	O ₁₂ X ₃

Figure 12: Stage 2 of the quantum tic-tac-toe, where the colored moves display the pairing strategy.

Assuming that the players continue to Stage 3, the game continues without the pairing law. The players are no longer required to mirror each other's moves through a line of symmetry. Instead, the players continue forming entanglements until one of the players chooses to form a measurement. The measurements can be formed in any of the boxes in the grid.

X ₁₃ X ₁	X ₉ X ₂	O ₁₂ O ₄	X ₁₅ O ₂ X ₁₇
O ₁₄ X ₇	X ₁₁ O ₂ O ₁₈	X ₁₅ O ₈ X ₁₇	X ₉ O ₆
O ₁₀ O ₈ O ₁₈	O ₁₄ X ₇	O ₁₀ X ₁ X ₁₉	X ₁₃ O ₄
O ₁₆ X ₂	X ₁₁ O ₆ X ₁₃	O ₁₆ X ₃	O ₁₂ X ₃

Figure 13: Stage 3 of the quantum 4x4 tic-tac-toe.

Figure 13 shows a game where a cyclic entanglement is formed. In this particular example, an optimal strategy is used where cyclic entanglements have been formed in Stage 3. This increases the chances of getting desirable results when measurements are formed.

Here, the two states of X₁₇ are in boxes 4 and 7. X₁₅ is also a move that previously existed in boxes 4 and 7, leading to an entanglement formed in both boxes. The next move, O₁₈, is played in box 9. At first glance, this may seem to be a random move; however, O₈ is a move that's common in boxes 7 and 9. Due to this, boxes 7 and 9 are entangled. Similarly, the second state of O₁₈ and the two states of the move X₁₉ are played in boxes 6, 14, and 11, respectively, so that pre-existing moves in these boxes lead to a case where all these boxes are entangled.

X ₁₃ X ₁	X ₉ X ₂	O ₁₂ O ₄	X ₁₅ O ₂ X ₁₇ O ₂
O ₁₄ X ₇	X ₁₁ O ₂ O ₁₈	X ₁₅ O ₈ X ₁₇ X ₁₇	X ₉ O ₆
O ₁₀ O ₈ O ₁₈ O ₁₈	O ₁₄ X ₇	O ₁₀ X ₁ X ₁₉ O ₁₀	X ₁₃ O ₄
O ₁₆ X ₂	X ₁₁ O ₆ X ₁₃	O ₁₆ X ₃	O ₁₂ X ₃

Figure 14: Measurements formed in Stage 3 of the quantum 4x4 tic-tac-toe, where the moves marked in red represent those that remained due to the measurement.

As the game progresses, the players can either continue forming entanglements or begin developing measurements. In the given example, as player X chooses to form an entanglement with the move X_{19} , player O can decide to continue forming entanglements or begin collapsing the states in either box. In this example, player O begins forming a measurement in box 4. The player decides to collapse that box to move O_2 , providing an advantage to player O. However, there are two other moves in that box- X_{17} and X_{15} . In any case, where there are 3 states in a box, once a measurement has been formed in one state, it is the second player's turn to decide which box will continue forming the measurements.

In this example, Player X chooses to continue collapsing box 7 to state X_{17} as this move benefits Player X. Now, it's Player O's turn to decide if the measurements should continue with move O_8 or X_{15} . However, it can be observed that move X_{15} exists in boxes 4 and 7. However, both boxes have already collapsed into one specific state. The move X_{15} gets canceled out as it can no longer be measured in a box where a measurement has already been made. Therefore, Player O has no other choice but to continue measuring with move O_8 .

As boxes 7 and 9 contain the two states of O_8 , box 7 has already been measured to state X_{17} . Therefore, box 9 is measured to O_8 . As the pattern continues, Player X can choose if the measurement continues with moves O_{18} or O_{10} . In the example, player X chooses to continue the measurement with the move O_{10} , thus forming a measurement in box 11. This is an optimal strategy as box 11 contains two X states and one O state, so the subsequent measurement formed will have to be with the collapse of an X state in box 1 or 14. Therefore, Player O continues a measurement with the move X_1 in box 1.

$X_{13}X_1$ X_1	X_9X_2 X_2	$O_{12}O_4$ O_4	$X_{15}O_2$ X_{17} O_2
$O_{14}X_7$ X_7	$X_{11}O_2$ O_{18} X_{11}	$X_{15}O_8$ X_{17} X_{17}	X_9O_6 X_9
$O_{10}O_8$ O_{18} O_{18}	$O_{14}X_7$ O_{14}	$O_{10}X_1X_{19}$ O_{10}	$X_{13}O_4$ X_{13}
$O_{16}X_2$ O_{16}	$X_{11}O_6$ X_{13} O_6	$O_{16}X_3$ X_3	$O_{12}X_3$ O_{12}

Figure 15: Consequent measurements formed as the game progresses in Stage 3 of the quantum 4x4 tic-tac-toe, where the red marks represent those that remain in the box due to the measurement.

As there are only two states in this box, this will lead to a consecutive measurement formed throughout the board as the boxes are entangled with each other. For example, as box 1 collapses to the state X_1 , X_{13} is the second state in box 1. Therefore, box 12, which contains the second state of X_{13} , will collapse to X_{13} . As this box also contains only two states, a similar cyclic collapse will occur with O_4 , the second state in box 12. Therefore, as the cycle progresses, box 3 is collapsed to the state O_4 .

Consequently, box 16 is collapsed to the state O_{12} , box 15 is collapsed to the state X_3 , box 13 is collapsed to the state O_{16} , box 2 is collapsed to the state X_5 , box 8 is collapsed to the state X_9 , and box 14 is collapsed to the state O_6 . This sequence of measurements took place as the respective boxes contained

only two entangled states. Therefore, there needed to be a choice as to which state must be measured.

$X_{13}X_1$ X_1	X_9X_2 X_2	$O_{12}O_4$ O_4	$X_{15}O_2$ X_{17} O_2
$O_{14}X_7$ X_7	$X_{11}O_2$ O_{18} X_{11}	$X_{15}O_8$ X_{17} X_{17}	X_9O_6 X_9
$O_{10}O_8$ O_{18} O_{18}	$O_{14}X_7$ O_{14}	$O_{10}X_1X_{19}$ O_{10}	$X_{13}O_4$ X_{13}
$O_{16}X_2$ O_{16}	$X_{11}O_6$ X_{13} O_6	$O_{16}X_3$ X_3	$O_{12}X_3$ O_{12}

Figure 16: Consequent measurements formed as the game progresses in stage 3 of the quantum 4x4 tic-tac-toe, where the red marks represent those that remain in the box due to the measurement.

However, box 14 contains three states; now, player X needs to choose whether to continue the measurements with move X_{11} or X_{19} . As can be seen in Figure 16, X_{19} is a state that exists in boxes 11 and 14; however, both these boxes have been measured to states O_{10} and O_6 respectively. Therefore, the move X_{19} cannot be collapsed in a box that has already been measured. Hence, it gets canceled out.

In such a case, player X has no choice but to continue measurements with the move X_{11} in box 6. In this box, the two other states are O_2 and O_{18} . O_2 has already been measured in box 4, and O_{18} must be canceled out as its other state is in box 9, which has already been measured to move O_8 . It is important to note that new entanglements cannot be formed in boxes that have already been measured. Therefore, if Player O wishes to continue the game, they can form more entanglements only in boxes 5 and 10, as these are the only boxes that have not been measured. However, as this option does not lead to any desirable outcome, player O must continue forming measurements with the aforementioned boxes. If player O chooses to measure box 5 to move X_7 , box 10 will be measured to the move O_{14} .

X_1	X_2	O_4	O_2
X_7	X_{11}	X_{17}	X_9
O_8	O_{14}	O_{10}	X_{13}
O_{16}	O_6	X_3	O_{12}

Figure 17: Return to the classical state in the quantum 4x4 tic-tac-toe.

The game will end here as the entire board has returned to the classical state, as shown in Figure 17, with only one move existing per box. As seen in this figure, the winner is Player X at the end of this game, as a successful horizontal sequence is formed in boxes 5, 6, 7, and 8.

The above example was one case of a game with moves that the average player is most likely to play. However, the outcome can very easily be altered at any stage by using specific strategies.

One optimal strategy is that players should begin to form measurements only after they reach Stage 3. However, forming measurements before that stage would not lead to beneficial

outcomes. On the contrary, it would be restricting, as further measurements cannot be included in boxes that have collapsed to a singular move.

When forming entanglements in Stage 3, players should form cyclic entanglements to increase their chances of forming a desirable measurement. For example, when forming entanglements in Stage 3, Player X should play their move in a box that contains two states of O, and Player O should play their move in a box that contains two states of X. For example, in Figure 13, X_{17} should be played in box 3 which contains two states of O. This increases the chances of forming a desirable measurement of X, as without this move, box 3 will be inevitably measured to a state of O as there is no X component.

Players should also note the possibility of certain moves getting canceled altogether, as is seen in the case of moves X_{15} , X_{19} , and O_{18} . This occurs in cases where these states cannot be measured in either of the boxes in which they exist, as those boxes have already been measured to another move. Players can take advantage of this property by forming entanglements in specific positions to ensure that their opponent's moves get canceled.

The above example examined a case where no further entanglements were formed after measurements; however, entanglements can be formed at any stage. Therefore, the game can be much longer and more complicated in cases where entanglements and measurements are formed simultaneously instead of in stages. Consequently, it is important to anticipate the consequences of every move and the consequent measures that can be formed. Anticipating the opponent's moves will give the player an advantage so that they can block the opponent from forming entanglements that will lead to beneficial measurements.

■ Conclusion

Observations were recorded during the reaction, as summarized. This paper discussed game theory and its concepts, terms such as the N-person game theory, zero-sum game, different winning strategies, optimal strategy, minimax principle, and Nash equilibrium. The differences between classical and quantum game theory have been explored in depth by analyzing the differences between their rules, strategies, and properties in the context of tic-tac-toe. The differences between the 3x3 classical tic-tac-toe and the 3x3 quantum tic-tac-toe were analyzed. We showed that quantum game theory significantly increases the number of solutions and leads to more optimal outcomes than classical game theory.

To extend this game, we have explored the 4x4 classical tic-tac-toe. First, we proposed the quantum version of the 4x4 tic-tac-toe, which incorporates quantum rules, properties, and strategies. We demonstrated the advantages of the quantum version over the classical one. Next, we suggested new rules and strategies by updating the 3x3 quantum tic-tac-toe. The 4x4 version is significantly complicated because players potentially have more options regarding the number of boxes, rounds of the games, and length of entanglement cycles. Finally, we presented some winning strategies for the 4x4 quantum tic-tac-toe through explicit examples and results.

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■ References

1. Picardo, E. How Game Theory Strategy Improves Decision Making <https://www.investopedia.com/articles/investing/111113/advanced-game-theory-strategies-decisionmaking.asp> (accessed 2023-03-30).
2. Barsha. Game theory: Meaning, importance, types, and examples. <https://www.sociologygroup.com/game-theory/> (accessed 2023-03-31).
3. Borad, S. B. Game Theory – Meaning, Example, Types, Importance and More <https://efinancemanagement.com/financial-management/game-theory> (accessed 2023-03-30).
4. Dong, J. TY - BOOK AU - Dong, Jingjing PY - 2020/01/01 SP - T1 - Comparison Between Classical Game Theory and Evolutionary Game Theory Focused on Prisoner's Dilemma https://www.researchgate.net/publication/347404089_Comparison_Between_Classical_Game_Theory_and_Evolutionary_Game_Theory_Focused_on_Prisoner's_Dilemma (accessed 2023-03-30).
5. Khan, F. S.; Solmeyer, N.; Balu, R.; Humble, T. S. Quantum games: a review of the history, current state, and interpretation <https://www.osti.gov/servlets/purl/1513429> (accessed 2023-03-30).
6. H, W. ADVANTAGES AND APPLICATIONS OF QUANTUM GAME THEORY. <https://math.uchicago.edu/~may/REU2022/> (accessed 2023-03-31).
7. Szopa, M. Efficiency of Classical and Quantum Games Equilibria <https://search.proquest.com/openview/3074be8f0f4e698424d25c0410079483/1.pdf?pq-origsite=gscholar&cbl=2032401> (accessed 2023-03-30).
8. Barguillo, J. C. Self-organizing Coalitions for Managing Complexity: Emergence, Complexity and Computation, vol 29. Springer, Cham... <https://link.springer.com/book/10.1007/978-3-319-69898-4> (accessed 2023-03-30).
9. Kaur, G. 8 game theory examples in real life. https://studiousguy.com/game-theory-examples-in-real-life/#1_Prisoners_Dilemma (accessed 2023-03-31).
10. Benson, B. A prisoner's dilemma cheat sheet <https://medium.com/thinking-is-hard/a-prisoners-dilemma-cheat-sheet-4d85fe289d87> (accessed 2023-03-30).
11. Budihal, R. M. GAME THEORY – NASH EQUILIBRIUM AND ITS APPLICATIONS <https://www.jetir.org/papers/JETIR1701220.pdf> (accessed 2023-03-30).
12. Alkaraz, S. H.; El-Seidy, E.; Morcos, N. S. Tic-Tac-Toe: Understanding the Minimax Algorithm <http://article.sapub.org/10.5923.j.jgt.20200901.01.html> (accessed 2023-03-31).
13. Quantum game theory - Wikipedia https://en.wikipedia.org/wiki/Quantum_game_theory (accessed 2023-03-31). Alkaraz, S. H.; El-Seidy, E.; Morcos, N. S. Tic-Tac-Toe: Understanding the Minimax Algorithm
14. Quantum game theory- Wikiwand https://www.wikiwand.com/en/Quantum_tic-tac-toe (accessed 2023-03-31).
15. <http://article.sapub.org/10.5923.j.jgt.20200901.01.html> (accessed 2023-03-31).
16. Samuel, S.; Mahawar, K.; França, I. J. Improved technique in Tic-Tac-Toe game to minimize the condition of draw using minimax over optimal strategy <https://www.citefactor.org/journal/pdf/Improved-technique-in-Tic-Tac-Toe-game-to-minimize-the-condition-of-draw-using-min-max-over-optimal-strategy.pdf> (accessed 2023-03-30).

17. Klinger-Meyers, B. How to Always Win at Tic Tac Toe | Best Tic Tac Toe Strategy <https://twinfinite.net/2022/08/how-to-always-win-at-tic-tac-toe-best-tic-tac-toe-strategy/> (accessed 2023-03-30).
18. Goff, A. Quantum tic-tac-toe: A teaching metaphor for superposition in quantum mechanics (accessed 2023-03-31).
19. Mosher, R. What is the optimal strategy in Quantum Tic Tac Toe? - Board & Card Games <https://boardgames.stackexchange.com/questions/7085/what-is-the-optimal-strategy-in-quantum-tic-tac-toe> (accessed 2023-03-31). <https://aapt.scitation.org/doi/abs/10.1119/1.2213635> (accessed 2023-03-30).
20. Alkaraz, S. H.; El-Seidy, E.; Morcos, N. S. Tic-Tac-Toe: Understanding the Minimax Algorithm <http://article.sapub.org/10.5923.j.jgt.20200901.01.html> (accessed 2023-03-31).
21. Choi, A. S. Alyssa Choi - Tic-Tac-Toe <https://momath.org/wp-content/uploads/2021/08/Alyssa-Choi-Tic-Tac-Toe.pdf> (accessed 2023-03-30).

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Evolutions of Vaccines and Recent Developments

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ABSTRACT: Despite significant advances in modern medicine that have led to an improved understanding of pathogenic diseases and proven success with vaccination approaches over several years, several hurdles exist to improving confidence or acceptance in the people. Improving awareness among the general population will significantly reduce infections, deaths, and financial toxicity to families and society. Over the years, numerous effective vaccines for various pathogenic diseases have been developed. This review describes the historical perspective of vaccines, types of vaccines, reasons for the failure of certain vaccinations, successes and failures, and religious misconceptions. The emergence of the recent COVID-19 pandemic, which led to several deaths worldwide in a short time, was an eye-opener that enlightened the general population, governments, and scientific communities in many ways. The robust technology development was made in anticipation of future pandemics, which means developing safe vaccines in record time while also at the same time facing challenges such as vaccine hesitancy among people.” Further, we focus on developing COVID-19 Vaccines and the technologies used to manufacture these vaccines. The scientific achievement in the successful COVID-19 vaccine development has proven that vaccines can prevent deaths from such lethal diseases and contain the spread of outbreaks.

KEYWORDS: Translational Medical Sciences; Disease Prevention; Vaccine; Vaccination; Infection; COVID-19; SARS-COV-2.

■ Introduction

Historical perspective of vaccines:

Human diseases have always been known throughout history. The human body is constantly exposed to and is fighting against pathogens. The invention and evolution of vaccines benefited humankind against several deadly diseases (Figure 1). A vaccine protects from infections by building defenses against pathogens when exposed. Over several years, vaccines significantly reduced the risk of getting ill, infections, or even death. Vaccines work by modulating the immune system, bolsters, and helping the immune system to develop preventive immunity against diseases safely. Sometimes, after receiving a vaccine, one experiences few symptoms of the disease the vaccine was made to fight off. Once those symptoms disappear, there remains a memory of those immune responses; the body will remember to fight the real disease better. However, some individuals can get the disease after vaccination because it takes time for the human body to build immunity.

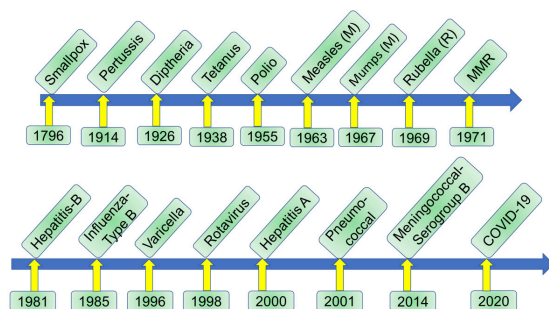


Figure 1: Journey of vaccines – In the last 225 years.

Many people died from diseases like smallpox before they were cured. Drawing on the discovery that serum taken from a recovered animal had protective powers, it began with the final realization that the disease could be cured by using the immune serum.¹ Many others reported similar instances, like Hippocrates in 400 BC when he described mumps and diphtheria; also, in the 19th century, the father of vaccination, Edward Jenner, published his work on smallpox.² There were many other pioneers and historical events essential in developing vaccines, which have eradicated or controlled many dangerous diseases. Edward Jenner was the first to give a scientific description of vaccination when he published his monograph “An Inquiry into the Causes and Effects of the Variolae Vaccine” in 1789. While he was not the first to discover vaccination, as said above, he was the first to confer scientific status on the procedure and to pursue its scientific investigation.³ While it was Louis Pasteur who developed the modern science of vaccination, he was the one who developed vaccines in the laboratory by using the same agent that caused the disease.⁴ Many other scientists took advantage of these techniques from the mile marks of the evolution of vaccines and developed vaccines for typhoid, cholera, and plague before the 19th century ended. Through the 20th century, many infectious diseases became defendable with the evolution of vaccines (Figure 1).

In human history, only two diseases were eradicated, according to the World Health Organization (WHO): smallpox and rinderpest. Smallpox is an ancient disease dating back to China in the 4th century CE (Common Era); it resulted in about 300–500 million deaths in the 20th century as eradication is hard to conceptualize; As an infectious disease anthropologist,

Thomas Aiden Cockburn defined disease eradication as “the extinction of the pathogen that causes disease”.⁵ There were some ways of controlling smallpox called variolation, a process named after the virus that causes smallpox (variola virus). The people who had never been exposed to smallpox were exposed to the material from smallpox sores by scratching it into their bodies or inhaling it. After variolation, individuals would develop symptoms of smallpox, but fewer people died from variolation than if they had it naturally. As stated above, Edward Jenner was the pioneer of the anti-smallpox vaccination. He noted that milkmaids who had already got and survived another cowpox did not get smallpox. In 1796, he inoculated an 8-year-old boy with material obtained from the lesion on the udder of a cow infected with cowpox and found that the boy did not get infected by smallpox.⁶

On the other hand, Rinderpest was a cattle plague primarily affecting cattle and buffalo. Infected animals suffered from symptoms such as fever, mouth wounds, diarrhea, nose, and eye discharge, and eventually death.⁷⁻⁹ Rinderpest never affected the humans; it did affect their lives as they used cows and buffalo primarily for milk and meat back then. The rinderpest outbreaks caused famines to break out, responsible for millions of deaths. The virus was spread through the water in the animal's body as animals got infected by inhaling the virus from sick animals' breath, secretion, or excretions. However, with the development of a potent vaccine, the Plowright tissue culture rinderpest vaccine (TCRV), in 1960, it was eradicated in 2011.

When most of the population in a country is immunized to a disease/virus, the likelihood of even those who are not immunized, getting the disease/virus is unlikely because the chances of it breaking out are small with most vaccinated. This is known as herd immunity. There are many stages of how a vaccine is developed and made, which start from initial research to distribution, then to hospitals and doctors' offices. To first test whether the vaccine can work safely on humans, it must be synthesized and undergo animal studies and clinical trials. As with other drugs or medicines, vaccines are also tested on animals such as rats or mice to ensure that if they show the intended effects on a mammal, they might show the intended efficacy on a human. When the possible vaccine is ineffective or has any serious side effects, it won't continue through the trials. But when the potential vaccine is effective, it succeeds the human trials. The developers must seek approval from other national/international agencies, such as the FDA or the WHO.

In this review, we discuss the types of vaccines, reasons for the failure of certain vaccinations, vaccination successes and failures, religious misconceptions, and emerging success with COVID-19 vaccination.

■ Discussion

Types of vaccines:

There are several types of vaccines, like live-attenuated vaccines, inactivated vaccines, subunit conjugates, toxoids, messenger mRNA vaccines, and viral vector vaccines. Live-attenuated vaccines are weakened forms of the virus that can no longer replicate in the body. Live-attenuated chicken cholera, inactivated anthrax, and live-attenuated rabies vaccines were

generated with attenuation by oxygen or heat at the turn of the 20th century by Louis Pasteur. Other alternative attenuation methods, such as using the serial passage of *Mycobacterium bovis*, led to the live Bacille Calmette-Guerin (BCG)¹⁰ vaccine, which is still used today to prevent tuberculosis. Yellow fever vaccines were developed using serial passage¹¹ which are grown in chicken embryo tissues.¹²

The Inactivated vaccine is created using heat, radiation, or certain chemicals that inactivate the pathogen. The pathogen will no longer cause illness but can still be recognized by the immune system. Successful inactivated vaccines are whole-cell typhoid, cholera, and pertussis vaccines that resulted at the end of the 19th Century. Some bacterial diseases are not caused by bacteria but by their toxin. Immunizations created using inactivated toxins are called toxoids.^{12,13} Toxoids can be grouped/considered as inactivated vaccines but are given a category to themselves because they contain an inactivated toxin, not an inactivated form of the bacteria. Inactivated bacterial toxins, diphtheria, and tetanus toxoid vaccines were developed using formaldehyde by Alexander Glenny and Barbara Hopkins in 1923. The live oral polio, measles, rubella, mumps, and varicella virus vaccines came into existence due to advances made in virus culturing *in vitro*, which allowed viral pathogens to be studied in greater detail and the development in attenuation methods where the viral particles were cultivated in artificial conditions.

Further advancement in vaccine development was seen by using capsid proteins/polysaccharides. In the 1960s, vaccines were developed using encapsulated polysaccharides from Meningococci and later pneumococci¹⁴⁻¹⁶ including *Haemophilus influenzae* type b (Hib)¹⁷ by the Walter Reed Army Institute of Research. A subunit vaccine contains only portions of the microbe that can be presented as antigens to the human immune system instead of the microbe as a whole. The microbe portions called antigens are usually selected to protect against multiple serotype variants (multi-valent vaccines) that best activate the immune response to induce memory B cells through T cells.¹⁸ Conjugate vaccines only contain a small portion of the microbe that is presented as antigens to the immune system in the human body; they are combined with a carrier protein chemically linked to the bacterial coat derivatives. Altogether, they generate a more powerful, combined immune response: typically, the “piece” of bacteria being presented would not generate a robust immune response on its own, while along with the carrier, protein would. The piece of bacteria can't cause illness, but combined with a carrier protein, it can generate immunity against future infection.^{12, 13} The whole-cell pertussis vaccine is replaced by the acellular vaccine, which consists of 5 different subunits of protein antigens and one or more bacterial components. This was developed to avoid the unwanted side effects of the whole-cell vaccine¹⁹.

A great deal of advancement was seen in microbiology and immunology with development in molecular biology, where a greater knowledge/understanding of pathogen epitopes and host immune responses to vaccination was allowed. Unique technologies in genetic sequencing and molecular genetics have driven the development of vaccines against RNA viruses

such as Influenza²⁰ and Rotavirus, covering multiple variants of the epitopes. Genetic engineering made possible the DNA manipulation and the use of surface antigens for hepatitis B viral vectors²². L1 antigen of the human papillomavirus (HPV) virus induces protective immune responses by the formation of virus-like particles.²³ L1 particles are the main component of the HPV vaccine. In-depth genomic sequencing analysis of meningococcal antigens identified four proteins for the vaccine development for the meningococcal B vaccine.²⁴

A recent pandemic caused by a novel coronavirus, SARS-CoV-2, which emerged from China causing a severe acute respiratory illness, has challenged vaccine development. As per WHO, globally, as of 2023, there have been 765,222,932 confirmed cases of COVID-19, including 6,921,614 reported deaths.²⁵ COVID-19 disease was effectively tackled in a relatively shorter time using novel mRNA vaccines. Messenger mRNA vaccines make proteins to trigger an immune response. They have several benefits over the other vaccines because of their short manufacturing time and that they do not contain a live virus, lowering the chances of anyone getting it from the vaccine. Viral vector vaccines use a modified version of a different virus to give protection to the person, which has been used to protect against COVID-19. Vaccination was possible because various pharma companies were able to harness the existing and novel technologies, including recombinant vector vaccines (NCT04313127, NCT04324606), DNA vaccine platforms (NCT04336410), RNA vaccine platforms (NCT04283461), and adjuvants. As a result, the regulatory approval, manufacturing, and distribution of COVID-19 vaccines were expedited for global health and to combat the SARS-CoV-2 disease. There were many challenges the world had to face with vaccine development and success with COVID-19 vaccines. Due to the constant mutations of the COVID-19 virus led to the emergence of new variants, and it was challenging to create a successful vaccine that works against all the variants. Due to this, the available COVID-19 vaccines don't offer long-term protection. A new vaccine dose is required periodically to be protected, unlike other vaccines such as the MMR vaccine (plus a booster dose), which is usually only administered to a person once in their life. At the same time, the flu vaccine must be given yearly. Although the COVID vaccination was highly successful in protecting against new infections, it was not the case every time a new vaccine was developed for any disease. Sometimes, there will be faded protection after some time, even with the protective vaccine. Many immunizations failed several times due to many reasons.

Reasons why immunizations failed:

Vaccines stimulate the immune system to produce a protective immune response that mimics the response to natural infection, a process known as active immunization.^{12, 13} Still, despite the long-term protection from the disease, the antibody levels produced from the vaccination decrease over time. Sometimes, the primary and secondary host factors can cause vaccine failure. The primary factor is when the patient fails to become immune after the vaccine is given. The secondary factor is where the patient develops immunity but is challenged

by a natural infection caused by the inadequate body's response to prevent the disease.

There are usually two reasons why the immunizations don't work: 1) there is a failure to provide the potent vaccines properly to individuals in need, i.e., the failure in the vaccine delivery system, and 2) there is no response towards the vaccine; there is no immunity built up from the vaccine because of either inadequacy of the vaccine or the unknown factors in the human body. "The major factor contributing to the failure of the delivery system is failure to vaccinate; in the developing world, this is commonly a result of the inadequacy of the vaccine supply"²⁶ Other issues of the immunizations failing when there are barriers against them, improper use of vaccines, and the ineffectiveness of the vaccines at the time of use. As said above, vaccine failures are usually because of the inadequacy of vaccination regimes or the administration; also because of something in the human body, for example, genetics, immune status, age, health, or nutritional status. Nevertheless, there are many examples of where vaccines have prevented some of the worst diseases in history (Figure 1).

Successes and failures of vaccines:

There were not just successes in the evolution of vaccines but failures as well. For example, there was a vast BCG vaccine failure in India against Tuberculosis (TB) caused by *Mycobacterium tuberculosis*. In the 1920s, a vaccine was proved to have worked when tested against children, which led around 60 other countries to use the vaccine. However, the vaccine was found to protect children against TB meningitis and military (disseminated) TB; however, its efficacy in adults against the pulmonary form ranged between 0% and 80%.²⁷ Meaning that the vaccine was ineffective in adults, still leading to deaths. It was unclear why the vaccine didn't work. It wasn't until many years later that an effective vaccine was invented that works for children and adults. The most common successes are usually when researchers succeed in creating vaccines that work and stop or prevent the disease/virus from infecting anyone again. For example, the Poliovirus was an infectious virus that spread through contact, particularly in impoverished places. The first vaccine for polio discovered tissue culture. The discovery of tissue culture allowed researchers to grow a live form of the virus that was attenuated (or weakened) to form the basis of vaccines that could be given orally.²⁸ Researchers still use these tissue culture techniques for vaccine development and research. The success of mass vaccination led to the global eradication of the poliovirus, except in Afghanistan and Pakistan. Similarly, measles was a highly contagious virus that spread through the air by sneezes or coughs. Before the vaccine for measles was developed, it was deadly, causing 2.6 million deaths a year. Still, now with the vaccine, there is a global call to try to eradicate the virus, and while there still may be some cases, there aren't as many as when it first came out.

Religion and misconceptions:

Most religions don't have any vast issues against vaccines or taking them. However, there are some concerns about why the vaccines are administered or what ingredients the vaccine contains. It is not just religion that is stopping people from taking a vaccine. The most popular reasons for people's wariness abo-

out vaccines are that too many vaccines overwhelm the immune system, MMR vaccines cause autism, HPV vaccines increase the risk of getting an autoimmune disease, and the influenza vaccine given in early pregnancy increases the risk of miscarriage. The data from epidemiology and biology has shown that taking multiple vaccines is harmless to the human body, other than ensuring they are protected from the disease they took the vaccine for. Numerous large-scale studies have shown that taking an MMR vaccine does not cause children autism. While autism may have been seen in a few children, there is not enough evidence to prove that MMR causes autism (Immunization Action Coalition). According to Sarah Geoghegan, a Pediatric Infectious Disease Specialist at The Children's Hospital of Philadelphia, "More than 270 million doses of HPV vaccine have been administered," and there have been no reports of autoimmune disease from taking an HPV vaccine. Also, there is no connection between the influenza vaccine affecting pregnancy as there have been no reports or data that the influenza vaccine had an impact on pregnancy.^{29,30} Vaccination has always been an enormous issue to criticize as there is always some distribution of false information and conspiracy theories on the internet and social media that influence the minds of the public causing them to be wary of vaccines. When the COVID pandemic started, many reports of cases have occurred. People then started theorizing about the virus, causing them to think wild and sometimes unbelievable theories about it, giving roots of whether or not the vaccine would work.

The WHO has named vaccine hesitancy as one of the threats to global health because if some people don't take the vaccine, they could get infected and start spreading it to others, causing another pandemic. The goal of a vaccine is to prevent a pandemic from happening again. There have been a few issues of some side effects from vaccines, leading to people distrusting immunization and the scientific method that created the vaccine in the first place. The public has seen situations where vaccines have failed before and have cemented the vaccine wariness, such as the incident where, during the first year following the vaccination campaign against the H1N1 infection in 2009 – 2010, the risk for narcolepsy increased up to 14-fold for children and adolescents, and up to 7-fold for adults in several countries where the vaccine Pandemrix was used. They have taken the same vaccine in other countries, and there were no reports of narcolepsy. Still, many people see that anything relating to the incident is to blame, causing them to blame the vaccine, which had nothing to induce narcolepsy within children.

The COVID-19 pandemic and the development of COVID-19 vaccines:

COVID Pandemic and steps for vaccine development: The COVID pandemic has killed over 1.13 million Americans, and at least 7 million reported deaths worldwide. The highest deaths were reported in the elderly and immunocompromised individuals. An urge became prominent to create a vaccine for COVID-19 as death and panic emerged from the deadly disease, destroying many lives. This virus was unprecedented in the history of vaccination, making this disease very hard to

combat. Nevertheless, scientists did not take long to develop an effective vaccine against COVID-19. Several prior developments were ongoing in developing vaccines using novel platforms for other diseases. Soon after the genetic sequence of the COVID-19 virus was published, scientists were quick to use it to develop the vaccine. What also helped develop the vaccine quickly was how, a decade before, the U.S. could utilize the highly adaptable vaccine platforms such as RNA (among others) and adapt structural biology tools to design agents (immunogens) that powerfully stimulate the immune system.³¹ Because of these previously developed reagents, the creators of the COVID-19 vaccine could develop a vaccine that worked quickly. SARS-CoV-2, the virus that causes COVID-19, was first identified in December 2019. By December 11, 2020, the Pfizer vaccine became the first to receive emergency use authorization from the Food and Drug Administration (FDA). This vaccine was created in a year, which took a lot of effort and resources. It became the new normal of wearing masks and distancing ourselves from others until and after the vaccine development. People are wary of the vaccine as some felt it took too long to make it and that too many died. At the same time, some thought that the vaccine development process was quicker than usual and questioned the early development process itself. Also, most people asked how they would react to the vaccine if there were any side effects or severe reactions.

Usually, creating a vaccine takes up to 10-15 years because scientists need to know what and how the virus is and see how it reacts to people so that they can counter it. But with the coronavirus affecting everyone in the world, the global leaders had to come together and have their scientists share everything they could to collaborate in the vaccine research. In 2018, a study in *The Lancet Global Health* Trusted Source estimated the cost of early development and initial clinical safety trials for a typical vaccine to be \$31–68 million. Large-scale trials to determine the efficacy of a vaccine candidate would add to these figures.³² Looking at the average cost of creating a vaccine that would take around ten years to make, the price for the coronavirus was huge because of the accelerated times. The US Operation Warp Speed (OWS) Trusted Source partnered with multiple institutions, including the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC), to develop, manufacture, and distribute 300 million doses by early 2021.³³ The European Union and the United Kingdom had also funded several vaccine candidates.

CDC recognized vaccine developing companies: CDC (Centers for Disease Control and Prevention) states that children five and above should get their primary series of COVID-19 vaccines, and everyone aged 12 and above should also receive a booster dose. The vaccines that CDC recognizes are Pfizer-BioNTech, Moderna, and Johnson & Johnson's Janssen (Table 1). So, with the development of the COVID-19 vaccine, there had to be test trials to see if the vaccine was not harmful. At first, when some companies were releasing some of the data from the human trials, the general response from the public was generally good.

The Johnson & Johnson COVID-19 single-dose vaccine is compatible with standard vaccine storage and distribution

channels, with easy delivery to remote areas³⁴ (Table 1). Some of these vaccines' known general side effects have been reported.³⁴ Such side effects are from the injection site reactions: pain, redness of the skin, and swelling. Other general side effects: headache, feeling very tired, muscle aches, nausea, and fever. There is also a chance of getting an allergic reaction. The Pfizer vaccine has proven to be very effective against the virus as it promises to have individuals be immune to the pathogen SARS-CoV2, reducing the spread of COVID. Some risks of the vaccine are that the vaccine will lose its efficiency if not stored at a specific temperature; with the public unknowing of that, might disregard their precaution and take the vaccine not knowing if it properly works if not stored at the right temperature but other than that there are no other significant risks of the vaccine.

Table 1: Vaccines, dosing schedules, and their effects^{***}.

Manufacturer / vaccine	Age	Vaccine type	Dosage	# of doses needed**	Need for a Booster	Side effects & Adverse reactions	Shelf life
* BNT16b2 - Bivalent Pfizer-BioNTech COVID-19 Vaccine	5 years and older	mRNA	5-11 yrs-0.2 mL/10 µg; 12-older yrs-0.2 mL/30 µg	2 doses required- Dose 1 now; Dose 2 at least 8 weeks after Dose 1; Dose 3/booster at least after 8 weeks after Dose 2	Yes; 4 months after first dose	fever, chills, tiredness, and headache; myocarditis and pericarditis	2°C and 8°C up to 10 Wks
* mRNA-1273 Bivalent Moderna COVID-19 Vaccine	6 years and older	mRNA	6-11 yrs-0.25 mL/25 µg; 12-older yrs-0.5 mL/50 µg	2 doses required- Dose 1 now; Dose 2 at least 8 weeks after Dose 1; Dose 3/booster at least after 8 weeks after Dose 2	Yes; 4 months after first dose	fever, chills, tiredness, and headache; myocarditis and pericarditis	2°C and 8°C up to 30 Wks
SARS-CoV-2 rS (NVX-CoV2373). NUVAXOVID-Novavax-Monovalent vaccine	12 years and older	Protein sub-unit	12 yrs and older-0.5 mL/50 µg	2 doses required- Dose 1 now; Dose 2 at least 4-8 weeks after Dose 1; Dose 3/booster at least after 8 weeks after Dose 2	Yes; 4 months after first dose	fever, chills, tiredness, and headache;	2°C and 8°C up to 9 months
Ad26.COV2.S-Johnson and Johnson	18 years and older	Replication incompetent adenovirus type 26 expressing the SARS-CoV-2 spike protein	18 years and older-0.5 mL/100µg	1 dose: Dose 1 now	Yes, 2 months after first dose	fever, chills, tiredness, and headache; thrombocytopenia syndrome, or TTS	2°C and 8°C up to 9 -11 months
AZD1222 ChAdOx1 nCoV-19 AstraZeneca	18 years and older	ChAdOx1-S [recombinant] vaccine	18 years and older-0.5 mL/ 5 × 10 ¹⁰ viral particles	2 doses required- Dose 1 now; Dose 2 at least 8-12 weeks after Dose 1	Yes, 4-6 months after first dose	fever, chills, tiredness, and headache; anaphylaxis, thrombocytopenia syndrome, or TTS, myocarditis and pericarditis	2°C and 8°C up to 9 months
VidPrevtyn Beta is a monovalent, recombinant-protein vaccine-Sanofi	18 years and older	Beta variant and older-spike antigen	18 years and older-0.5mL/5µg	1 Booster dose use only in previously immunized	Yes, 4-6 months after first dose	chills, tiredness, arthralgia and headache	2°C and 8°C up to 1 yr

*Monovalent vaccine is no longer recommended bivalent is in use.

**doses for people who did not receive prior vaccinations

***The information was adapted from the publicly available Center for Disease Control and Prevention (CDC) website

(<https://www.cdc.gov/coronavirus/2019-ncov/vaccines/stay-up-to-date.html>;<https://www.cdc.gov/coronavirus/2019-ncov/vaccines/expect/after.html>; <https://www.cdc.gov/coronavirus/2019-ncov/vaccines/safety/adverse-events.html>)

Pfizer/BioNTech Vaccine has been recommended to people 16 years and older, with a dose of 30 µg (0.3 m). Initially, it showed immunogenicity for at least 119 days after the first vaccination and is 95% effective in preventing the SARS-COV-2 infection.³⁵ The AstraZeneca vaccine incorporates the spike gene from SARS-CoV-2 into another non-pathogenic virus.³⁶

There have been reports of people who have taken the vaccine to get clotting issues, and some have died.³⁷⁻³⁹ The most common symptoms of widespread blood clots, usually shown in women younger than 65 and a low platelet count sometimes associated with bleeding, have continued to be there. The Moderna vaccine was very effective against the virus, easier to transport, and less sensitive to temperatures. Some of the mild side effects of the vaccine are pain or swelling at the vaccination shot area, fever, fatigue, headache, muscle pain, nausea, vomiting, itching, chills, and joint pain, and rarely cause an allergic reaction. With the vaccine targeting the respiratory system, it also seems to be affecting the cardiovascular systems of both children and adults. There have been reports of myocarditis and pericarditis occurring in recipients within a week after receiving the vaccine, mostly young male adults. Myocarditis is the inflammation of the middle layer of the heart wall. This is caused by a viral infection that could lead to death; the myocarditis would have been caused by the little part of the COVID-19 virus that was made into the vaccine.⁴⁰ Because the benefits outweighed the risks, there was no reason to stop giving out the vaccines since they did work as intended.

The Pfizer-BioNTech vaccine contains messenger RNA, a genetic material that instructs cells in the body to make the distinctive "spike" protein of the SARS-CoV-2 virus (Table 1). The age recommended for taking the Pfizer-BioNTech was 5 – 11 years, with two doses, three weeks apart. They are fully vaccinated after two weeks of the final dose. There is another version of the Pfizer-BioNTech where the ages recommended to take it is 12 years and older. They also become fully vaccinated after two weeks from taking the final dosage, but this one requires a booster dose ~5 months after the last dose.

The ages recommended for the Moderna vaccine are 18 years and older, taking two doses, three to eight weeks apart; they are also fully vaccinated after two weeks from the final dose. They must also take the booster dose ~5 months after the last dose. The J&J/Janssen COVID-19 vaccine was 66.3% effective in clinical trials at preventing trial-confirmed COVID-19 infection in people who received the vaccine and had no evidence of being previously infected.^{34, 41, 42} People had the most protection two weeks after vaccination.^{34, 41, 42} The age recommended to take the J&J vaccine is 18 years and older, and there is only one dose. After two weeks, they are vaccinated but must take the booster dose later. It was initially recommended that no one mixes the vaccines as they might trigger a reaction inside the body. Later, some studies were undertaken to test the efficacies of mixing and matching different vaccines.

According to the CDC, after the initial development of the vaccine, they go through three phases of clinical trials to ensure they are safe and effective. So, when COVID came, they oversped the process but didn't miss any of the phases so that the vaccines could be used as quickly as possible to lessen the disease's damage. The clinical trials for COVID-19 vaccines have involved volunteers of different ages, races, and ethnicities. Therefore, they needed to account for the different outcomes of everyone participating in the clinical trials to see what differed. Though the FDA must approve of the vaccines

going out to the public, they assessed that the three vaccines were usable and had met the FDA's standards, so they were granted Emergency Use Authorization. EUAs allowed for the vaccines to be quickly distributed to the public but also kept the standards of the original vaccine testing.

It was a global collaboration on the creation of the COVID-19 vaccine. China stands out from the rest as a survey was done in China, and 97% said they would get the vaccine once it was released. While the US has been actively trying to get other countries to use the vaccine, many people still hesitate and don't want to use it. A poll was given to American citizens, and around 30% of adults hesitated about taking the vaccine. Per Bolson and Palm 2022, America still has one of the highest hesitations regarding the COVID-19 vaccine, along with Russia, where around 47% are unwilling to take it, and Australia, where about 30% of the population is not for the vaccine. More than twenty vaccines have been approved for emergency use in countries such as China, Russia, the United Kingdom, India, and the United States. Some countries have made significant progress in immunizing their citizens, while others have only vaccinated a fraction of their population. In India, a devastating surge of random spikes of COVID was seen.⁴³ The country had eventually begun actively promoting its campaign about vaccines. As a result, they could immunize around five hundred million people.⁴⁴ But WHO noticed that the lack of vaccines in some African countries would cause the pandemic to go on even longer. To keep up the progress of immunization, many countries have or are considering vaccine mandates as they believe that having it there would speed up immunizing people against COVID.

Initially, countries have implemented the vaccine mandate for only healthcare workers because they are the most likely to get infected and then start infecting their patients and other people nearby. Soon after wider availability, vaccinations were recommended to everyone. Children's access to the vaccine has begun to expand across most countries, such as China, having their children get it as young as three years old, while America gets their children the vaccine at around five years old. Scientists are divided about how much of a population must have COVID-19 antibodies to prevent new outbreaks, with estimates ranging from less than half to over 80 percent.⁴⁵ It is improbable for COVID-19 to get herd immunized because of how recent the pandemic still is and how easy it is for others to get it, along with how uneven the vaccination handout was and how the number of variants that are arising for which the current vaccines may be less effective. Mixing various types of COVID-19 vaccines may enhance the immune response and increase flexibility when a person needs a booster dose. Still, doses of the vaccine first received are not available.

Funding sources: Vaccinations are frequently collaborative efforts across sectors of society, with private pharmaceutical firms teaming up with public health agencies or university labs.⁴⁵ The governments, international institutions, private sectors, research institutions, and nonprofits were the ones who had a large hand in the COVID-19 vaccine development. Public Health agencies played a critical role in supplying and deploying COVID-19 vaccine research funds to the public.

In America, the government launched a project called Operation Warp Speed, where they were developing a workable vaccine and manufacturing vaccines enough for all three hundred million Americans. The government pledged millions of dollars to various companies. It brought several agencies to work together, such as the Department of Health and Human Services—including the Centers for Disease Control and Prevention, the National Institutes of Health (NIH), the Food and Drug Administration (FDA)—and the Department of Defense.

The European Commission has funded several hundred million euros to the development of vaccines, and the Chinese government has state-owned firms that are two-fifths of the country's vaccine industry, implying that China wants the pandemic to be done with how much time and money they are spending on the vaccine and getting it to their citizens. International Institutions such as WHO and other organizations are working on financing and manufacturing enough vaccines for the world to use. In particular, having an equal number of vaccines get to the countries otherwise would cause political and health issues the world isn't ready to face. Another of the multilateral efforts is the Coalition for Epidemic Preparedness Innovations (CEPI). This global alliance was founded by Norway, India, the Bill & Melinda Gates Foundation, the UK-based Wellcome Trust, and the World Economic Forum.⁴⁵ This coalition focuses on improving vaccine access in lower-income countries like the ones listed above. In addition, the WHO and other international medical organizations launched a global initiative called CORVAX, where the goal was to have around two billion doses of the vaccine by the end of 2021. On the other hand, the pharmaceutical industry has been a big help in developing and manufacturing vaccines because they understand all the health risks of not taking them. Many companies began researching COVID-19 to see what vaccine candidates can be made. Usually, early research receives government funding, but most of the funds come from private sources. But with the current pandemic, the need for a vaccine overcame the risk of those private sources. Much vaccine candidate research has come from research institutions and nonprofits, colleges, and universities. For example, in the case of the University of Oxford's vaccine, the research team was already working on vaccines for an unknown disease that could cause a pandemic; in January 2020, the group zeroed in on COVID-19. The Gates Foundation has been the leading nonprofit funding COVID-19 vaccine efforts.⁴⁵ Most vaccines that various medical organizations approved have been made and manufactured at firms and research groups in China, Russia, and the United States.

Technologies and Companies that manufactured COVID-19 vaccines:

A number of technologies have evolved over the years for the development of vaccines against various pathogens (Table 2). The leading companies that created the COVID-19 vaccines were Johnson & Johnson, Sanofi, Moderna, Pfizer-BioNTech, and AstraZeneca. These companies were the ones who stood out during the crisis and provided the vaccines to the people. Johnson & Johnson used the traditional

vector-based technology to deliver the COVID-19 vaccine. They have used a replication-incompetent adenoviral vector (type 26), which delivers DNA encoding the COVID spike protein into the host cell upon vaccination. It is transcribed to mRNA and translated to spike protein fragments, provoking the immune system to react against the COVID-19 virus. COVID-19 Unlike other companies' COVID vaccines, the Johnson & Johnson vaccine is given as a single dose. Sanofi utilized a recombinant protein-based technology to manufacture the COVID-19 vaccine.⁴⁶ This recombinant protein subunit vaccine used a COVID spike protein produced in insect cells via the Baculovirus vector. This vaccine contains copies of the spike protein. This recombinant-protein technology was previously used for seasonal flu vaccines by Sanofi. Recently, a booster vaccine was approved by the European Commission.⁴⁷ Pfizer and Moderna are mRNA-based vaccines. There was no use of the vector; these vaccines consisted of only mRNA, which, upon delivery, instructs host cells to translate mRNA to a specific CORONA virus spike protein that triggers an immune response to COVID. AstraZeneca has used a double-stranded DNA inserted into a modified version of a chimpanzee adenovirus known as the ChAdOx1 vector. The vector could enter the cells but cannot replicate inside the cell. Once inside the cell, the DNA will be read to produce mRNA and translated to the spike protein recognized by the host's immune system. Immune responses triggered were effective against COVID-19 infection.

Table 2: Evolution of technologies.

Technology	Definition	Example	Ref
Attenuation	Consists of live whole bacterial cells or viral particles that are treated in such a way that they have reduced virulence within the host but retain their ability to provoke an immune response	The measles, mumps, rubella (MMR), varicella (chickenpox), and polio are examples of attenuated vaccines	10; 28
Inactivation	Usually made by exposure of virulent virus or toxins to chemical or physical agents in order to destroy infectivity while retaining immunogenicity	Diphtheria, and tetanus toxoid vaccines are examples of inactivated vaccines	12;13
Subunit vaccines	A vaccine that contains purified parts of the pathogen that are antigenic or necessary to elicit a protective immune response	Hepatitis B and Acellular pertussis vaccines are examples of subunit vaccines	19
mRNA vaccines	It will teach cells how to make a protein that triggers an immune response against an antigen	Pfizer and Moderna COVID-19 vaccines are example of mRNA vaccines	35
DNA Vaccines	It is a type of vaccine that transfects a specific antigen-coding DNA sequence into the cells of an organism as a mechanism to induce an immune response	Hepatitis B, Influenza B, Meningitis and AstraZeneca	22; 36

Other COVID vaccine types and therapies under development :

COVID-19 virus variants have emerged since the initial strain, and there is demand for next-generation COVID-19 vaccines. The intranasal vaccine attracted many due to its delivery location which could provide better mucosal immune

responses against this respiratory virus. Intra nasal vaccination method has shown potent mucosal and systemic immune responses. Many intranasal COVID vaccines are in clinical trials, and some have been approved in China, India, Iran, and Russia.⁴⁸ An inhaled vaccine called Convidecia was approved in China. Intra-nasal technology for the COVID-19 vaccine developed by Curiel was approved in India. In 2020, the FDA approved an anti-viral COVID pill developed by Pfizer for treating mild or moderate disease in patients. This pill, called Paxlovid (nirmatrelvir tablets and ritonavir tablets, which are co-packaged for oral use), is effective if taken within five days of COVID-19 virus symptoms. However, it is only authorized for up to five days continuously.

Conclusion

Since the invention of the first vaccine, vaccination has gained much importance and contributed to global health. As diseases have rampaged throughout centuries, scientists invented vaccines to ensure people don't die from pathogenic diseases. It was believed that several dangerous infectious diseases are tackled by vaccines and therapies. In contrast, disease-resistant infectious diseases are increasing in high numbers due to increased immigration, changes in human behavior and demographics, feasibility/speed of international travel, novel infections by a variety of pathogens, and also the recent pandemic that have shaken the world. The current COVID pandemic has taught the world the importance of preparedness to combat future pandemics by improving and continuously investing in developing novel technologies and increasing vaccine research efforts (Table 2). There is still much room for improvement in the existing vaccines regarding feasibility, immunogenicity, lowering side effects, increasing safety, and cost-effectiveness. Of late, many new vaccines are being developed directed towards non-infectious diseases. Various terminologies and definitions are listed in Table 3. As discussed, whether vaccines are affordable for all populations worldwide is debatable. The emergence of COVID-19 has proven once again that vaccines can prevent deaths from such lethal diseases and contain the spread of outbreaks that might have caused a threat to global health and security.

Table 3: Table for terminologies and definitions.

DNA Vaccines	A type of vaccine that transfers a specific antigen-coding DNA sequence into the cells of an organism as a mechanism to induce an immune response.
RNA Vaccines	A type of vaccine that uses a copy of a molecule called messenger RNA to produce an immune response.
Narcolepsy	A chronic sleep disorder that causes overwhelming daytime drowsiness.
Vaccine	A substance used to stimulate immunity to a particular infectious disease or pathogen, typically prepared from an inactivated or weakened form of the causative agent.
Disease	A disorder of structure or function in a human, animal, or plant, especially one that has a known cause and a distinctive group of symptoms, signs, or anatomical changes
Infections	Is the invasion of tissues by pathogens, their multiplication, and the reaction of host tissues to the infectious agent and the toxins they produce.

Symptoms	A physical or mental feature which is regarded as indicating a condition of disease, particularly such a feature that is apparent to the patient
Immunization	The action of making a person or animal resistant to a particular infectious disease or pathogen, typically by vaccination
Herd Immunity	Resistance to the spread of an infectious disease within a population that is based on pre-existing immunity of a high proportion of individuals as a result of previous infection or vaccination
Drug	A medicine or other substance which has a physiological effect when ingested or otherwise introduced into the body
Genetic Engineering	The deliberate modification of the characteristics of an organism by manipulating its genetic material
Invention	Something, typically a process or device, that has been invented
Immune System	A complex network organs, cells, and proteins that defends the body against infection, while protecting the body's own cells
Platelet	Small, colourless cell fragments in our blood that form clots and stop or prevent bleeding
Fatigue	Extreme tiredness resulting from mental or physical exertion or illness
Nausea	A feeling of sickness with an inclination to vomit
Myocarditis	The inflammation of the heart muscle which reduces the heart's ability to pump blood
Pericarditis	The swelling and irritation of the thin, sac-like tissue surrounding the heart
Race	The division of people based on physical characteristics
Ethnicities	The quality or fact of belonging to a population group or subgroup made up of people who share common cultural background or descent
Recombinant Protein	Proteins encoded by recombinant DNA that has been cloned in an expression vector that supports expression of the gene and translation of messenger RNA

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■ References

- Taylor, W. P.; Roeder, P. L.; Rweyemamu, M. M., 11 - History of vaccines and vaccination. In Rinderpest and Peste des Petits Ruminants, Barrett, T.; Pastoret, P.-P.; Taylor, W. P., Eds. *Academic Press: Oxford*, **2006**; pp 222-246.
- Hajj Hussein, I.; Chams, N.; Chams, S.; El Sayegh, S.; Badran, R.; Raad, M.; Gerges-Geagea, A.; Leone, A.; Jurjus, A., Vaccines Through Centuries: Major Cornerstones of Global Health. *Front Public Health* **2015**, *3*, 269.
- Riedel, S., Edward Jenner and the history of smallpox and vaccination. *Proc (Bayl Univ Med Cent)* **2005**, *18* (1), 21-5.
- Iwasaki, A.; Omer, S. B., Why and How Vaccines Work. *Cell* **2020**, *183* (2), 290-295.
- Corona, A., Disease Eradication: What Does It Take to Wipe out a Disease?. *American Society of Microbiology* **2020**, (March 6).
- Dov L. Boros, Snippets of Vaccine History: Success, Failure, and Controversy. *Consultant for Pediatricians* **2010**, *9* (10).
- Pankhurst, R., Economic History of Ethiopia (1800 - 1935). Hail Selassie I University Press: 1968.
- Nations., F. a. A. O. o. t. U., The Global Rinderpest Eradication Programme Progress report on rinderpest eradication: Success stories and actions leading to the June 2011 Global Declaration. http://www.fao.org/ag/againfo/resources/documents/AH/GREP_flyer.pdf, 2011.

- Roeder, P.; Mariner, J.; Kock, R., Rinderpest: the veterinary perspective on eradication. *Philos Trans R Soc Lond B Biol Sci* **2013**, *368* (1623), 20120139.
- Calmette, A.; Guérin, C.; Boquet, A.; Nègre, L., La vaccination préventive contre la tuberculose par le" BCG," . Masson et cie: 1927.
- Theiler, M.; Smith, H. H., The use of yellow fever virus modified by in vitro cultivation for human immunization. *The Journal of experimental medicine* **1937**, *65* (6), 787.
- Plotkin, E. A. M. S. A., Vaccines. W.B. Saunders; 2nd Edition (January 1, 1988): 1988.
- Plotkin, S. A., Orenstein, W.A., Offit, P.A., Vaccines. 6th. ed. Elsevier; Philadelphia: , 2013.
- Austrian, R., Pneumococcal polysaccharide vaccines. *Rev Infect Dis* **1989**, *11* Suppl 3, S598-602.
- Gold, R.; Artenstein, M. S., Meningococcal infections. 2. Field trial of group C meningococcal polysaccharide vaccine in 1969-70. *Bull World Health Organ* **1971**, *45* (3), 279-82.
- Artenstein, M. S., Control of meningococcal meningitis with meningococcal vaccines. *Yale J Biol Med* **1975**, *48* (3), 197-200.
- Anderson, P.; Peter, G.; Johnston, R. B., Jr.; Wetterlow, L. H.; Smith, D. H., Immunization of humans with polyribophosphate, the capsular antigen of Hemophilus influenzae, type b. *J Clin Invest* **1972**, *51* (1), 39-44.
- Schneerson, R.; Barrera, O.; Sutton, A.; Robbins, J. B., Preparation, characterization, and immunogenicity of Haemophilus influenzae type b polysaccharide-protein conjugates. *J Exp Med* **1980**, *152* (2), 361-76.
- Sato, Y.; Sato, H., Development of acellular pertussis vaccines. *Biologicals* **1999**, *27* (2), 61-9.
- Maassab, H. F.; DeBorde, D. C., Development and characterization of cold-adapted viruses for use as live virus vaccines. *Vaccine* **1985**, *3* (5), 355-69.
- Clark, H. F.; Offit, P. A.; Plotkin, S. A.; Heaton, P. M., The new pentavalent rotavirus vaccine composed of bovine (strain WC3)-human rotavirus reassortants. *The Pediatric infectious disease journal* **2006**, *25* (7), 577-583.
- Plotkin, S., History of vaccination. *Proc Natl Acad Sci USA* **2014**, *111* (34), 12283-7.
- Kirnbaier, R.; Booy, F.; Cheng, N.; Lowy, D. R.; Schiller, J. T., Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. *Proc Natl Acad Sci USA* **1992**, *89* (24), 12180-4.
- Serruto, D.; Bottomley, M. J.; Ram, S.; Giuliani, M. M.; Rappuoli, R., The new multicomponent vaccine against meningococcal serogroup B, 4CMenB: immunological, functional and structural characterization of the antigens. *Vaccine* **2012**, *30* Suppl 2 (0 2), B87-97.
- Organization, W. H. WHO Coronavirus (COVID-19) Dashboard. <https://COVID19.who.int/> (accessed 5-25-23).
- Hinman, A. R.; Orenstein, W. A.; Mortimer, E. A., When, where, and how do immunizations fail? *Annals of Epidemiology* **1992**, *2* (6), 805-812.
- Fine, P. E., Variation in protection by BCG: implications of and for heterologous immunity. *Lancet* **1995**, *346* (8986), 1339-45.
- Sabin, A. B.; Boulger, L. R., History of Sabin attenuated polio virus oral live vaccine strains. *Journal of Biological Standardization* **1973**, *1* (2), 115-118.
- Moro, P.; Baumblatt, J.; Lewis, P.; Cragan, J.; Tepper, N.; Cano, M., Surveillance of Adverse Events After Seasonal Influenza Vaccination in Pregnant Women and Their Infants in the Vaccine Adverse Event Reporting System, July 2010-May 2016. *Drug Saf* **2017**, *40* (2), 145-152.
- James D. Nordin, E. O. K., Gabriela Vazquez Benitez, Heather L

- ipkind, Claudia Vellozzi, Frank DeStefano, Maternal Influenza Vaccine and Risks for Preterm or Small for Gestational Age Birth Vaccine Safety Data. *The Journal of Pediatrics* **2014**, *164* (5), P1051-1057.E2.
31. Fauci, A. S., The story behind COVID-19 vaccines. *Science* **2021**, *372* (6538), 109.
32. Gouglas, D.; Thanh Le, T.; Henderson, K.; Kaloudis, A.; Daniels, T.; Hammersland, N. C.; Robinson, J. M.; Heaton, P. M.; Røttingen, J. A., Estimating the cost of vaccine development against epidemic infectious diseases: a cost minimisation study. *Lancet Glob Health* **2018**, *6* (12), e1386-e1396.
33. Slaoui, M.; Hepburn, M., Developing Safe and Effective COVID Vaccines - Operation Warp Speed's Strategy and Approach. *N Engl J Med* **2020**, *383* (18), 1701-1703.
34. Sadoff, J.; Gray, G.; Vandebosch, A.; Cárdenas, V.; Shukarev, G.; Grinsztejn, B.; Goepfert, P. A.; Truyers, C.; Fennema, H.; Spiessens, B.; Offergeld, K.; Scheper, G.; Taylor, K. L.; Robb, M. L.; Treanor, J.; Barouch, D. H.; Stoddard, J.; Ryser, M. F.; Marovich, M. A.; Neuzil, K. M.; Corey, L.; Cauwenberghs, N.; Tanner, T.; Hardt, K.; Ruiz-Guiñazú, J.; Le Gars, M.; Schuitemaker, H.; Van Hoof, J.; Struyf, F.; Douoguih, M., Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against COVID-19. *N Engl J Med* **2021**, *384* (23), 2187-2201.
35. Meo, S. A.; Bukhari, I. A.; Akram, J.; Meo, A. S.; Klonoff, D. C., COVID-19 vaccines: comparison of biological, pharmacological characteristics and adverse effects of Pfizer/BioNTech and Moderna Vaccines. *Eur Rev Med Pharmacol Sci* **2021**, *25* (3), 1663-1669.
36. Vogel, G.; Kupferschmidt, K., Side effect worry grows for AstraZeneca vaccine. *Science* **2021**, *372* (6537), 14-15.
37. Wise, J., COVID-19: Rare immune response may cause clots after AstraZeneca vaccine, say researchers. *BMJ* **2021**, *373*:n954.
38. Mahase, E., AstraZeneca vaccine: Blood clots are "extremely rare" and benefits outweigh risks, regulators conclude. *Bmj* **2021**, *373*, n931.
39. Greinacher, A.; Thiele, T.; Warkentin, T. E.; Weisser, K.; Kyrle, P. A.; Eichinger, S., Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination. *N Engl J Med* **2021**, *384* (22), 2092-2101.
40. Gargano JW, W. M., Hadler SC, et al., Use of mRNA COVID-19 Vaccine After Reports of Myocarditis Among Vaccine Recipients: Update from the Advisory Committee on Immunization Practices — United States. *MMWR Morb Mortal Wkly Rep* **2021**, *70*, 977-982.
41. Sadoff, J.; Struyf, F.; Douoguih, M., A plain language summary of how well the single-dose Janssen vaccine works and how safe it is. *Future Virol* **2021**, *16* (11), 725-739.
42. Sadoff, J.; Gray, G.; Vandebosch, A.; Cárdenas, V.; Shukarev, G.; Grinsztejn, B.; Goepfert, P. A.; Truyers, C.; Van Dromme, I.; Spiessens, B.; Vingerhoets, J.; Custers, J.; Scheper, G.; Robb, M. L.; Treanor, J.; Ryser, M. F.; Barouch, D. H.; Swann, E.; Marovich, M. A.; Neuzil, K. M.; Corey, L.; Stoddard, J.; Hardt, K.; Ruiz-Guiñazú, J.; Le Gars, M.; Schuitemaker, H.; Van Hoof, J.; Struyf, F.; Douoguih, M., Final Analysis of Efficacy and Safety of Single-Dose Ad26.COV2.S. *N Engl J Med* **2022**, *386* (9), 847-860.
43. Chandra, S. Why India's Second COVID Surge Is So Much Worse Than the First. June 9th, 2021. 2021. (accessed 5-25-23).
44. Purohit, N.; Chugh, Y.; Bahuguna, P.; Prinja, S., COVID-19 management: The vaccination drive in India. *Health Policy Technol* **2022**, *11* (2), 100636.
45. Klobucista, C., A Guide to Global COVID-19 Vaccine Efforts. <https://www.cfr.org/background/guide-global-COVID-19-vaccine-efforts>, 2022.
46. News., B., "Coronavirus vaccine: UK signs deal with GSK and Sanofi" BBC News. 29 July 2020. 29 July 2020.
47. Sanofi, Immunization Action Coalition 2022.
48. Waltz, E., China, and India approve nasal COVID vaccines — are they a game changer? *Nature* **2023**, 609.

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Investigating Herding Behavior in The Cryptocurrency Market Post Covid-19

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ABSTRACT: This study investigates whether the cryptocurrency market exhibits herding behavior during events following COVID-19. These include unusual volatility, volume, and noteworthy events in the cryptocurrency space between 2021 and 2022. The notable events include the collapse of FTX, the ban on cryptocurrency in China, and El Salvador's legalization of Bitcoin. Herding occurs when cryptocurrency users collaborate and imitate one another without using rational thought. This study analyzes data from the top 30 cryptocurrencies by market capitalization based on the cci30 index. This study used the cross-sectional standard deviation (CSSD) and cross-sectional absolute deviation (CSAD) regression models. The results indicate that investors in the cryptocurrency market might be more likely to act irrationally and exhibit herding behavior during periods of low volatility, high volatility, and high volume. This can be explained since cryptocurrencies tend to move uniformly during these periods and do not necessarily reflect the fundamentals of investing in the cryptocurrency market. However, due to the amount of noise in the results, there are uncertainties with this conclusion.

KEYWORDS: Behavioral and Social Sciences; Sociology and Social Psychology; Herd Behavior; Cryptocurrencies.

■ Introduction

Cryptocurrency is often defined as a digital currency where records are maintained by a decentralized system based on cryptography instead of a centralized authority. Since the Bitcoin cryptocurrency paper was published, over 22,000 new cryptocurrencies have been released and are currently available for trade.¹ The decentralized nature of cryptocurrencies produced a change in the way in which ownership, transactions, and the process of creating money are monitored.

The act of herding is often defined as when an investor follows. It imitates the investment decisions of others without reference to fundamentals and without using their own rationale when making investment decisions.² Exploration into herding behavior empirically in classic financial markets has been thoroughly researched;^{3,4} However, much about herding behavior in the cryptocurrency market needs to be explored. Due to high volatility, cryptocurrency as an asset class is usually considered relatively risky and speculative.⁵ Studying the behavior of investors to look for herding is important because the state of the cryptocurrency market relies heavily on the investors' actions. For example, herding behavior in financial markets has often been associated with high volatility, explosivity, and even crises.⁶ By examining herding behavior, investors can be better guided during investment.

Herding behavior is assumed to occur when all cryptocurrencies move in lockstep on any given day. Thus, examining the cross-dispersion of returns can be used as a proxy for analyzing the amount of herding behavior that exists during that period.

■ Literature Review

There are two leading methodologies applied to evaluate herding behavior. They both consist of modifications to clas-

sical herding behavior determination approaches. The first method is the cross-sectional standard deviation (CSSD) of returns developed by Christie and Huang.⁷ The second is a cross-sectional absolute deviation (CSAD), which was the approach of Chang *et al.* (2000) from the stock returns distribution.⁸

More recently, CSAD has been more prominently applied in the presence of outliers in the dataset; these extreme values may influence CSSD and not accurately reflect the true spread of the data. Amirat and Alfawi asked: "Does herding behavior exist in the cryptocurrency market?"⁹ Their data consisted of daily closing prices of the 20 largest cryptocurrencies by market cap from January 1, 2015, to January 31, 2019. They applied CSAD using the MVIS 100 index (a market-cap-weighted index that tracks the performance of the 100 largest digital assets) and returns from the daily closing prices of the 20 largest cryptocurrency coins. Using CSAD, Amirat, and Alfawi found no evidence of herding behavior. Silva *et al.* explored herding behavior and contagion in the cryptocurrency market.¹⁰ They used both CSSD and CSAD to investigate herding behavior, and the daily closing prices of cryptocurrencies included in their dataset were chosen based on liquidity, market cap, and data availability from March 2015 to November 2018. Their results revealed herding behavior, which demonstrated extreme periods of adverse herding behavior. Susana *et al.* observed whether or not herding behavior among traders increased during the COVID-19 pandemic.¹¹ Their dataset included the daily closing prices from the time period July 29, 2019, to July 28, 2020. Their results reported evidence of herding in normal market conditions but not during a market upswing or downswing. Bouri *et al.* also investigated the existence of herding

behavior for two years using the 14 leading cryptocurrencies at the time.⁶ Their results did not reveal any significant herding.

Both methods have also been used to test herding behavior in traditional asset classes. For example, Jiang *et al.* investigated herding behavior triggered by the COVID-19 outbreak in 2020 by analyzing six typical Asian stock markets.¹² They detected herding in both upward and downward markets in Singapore and Japan and downward markets in Taiwan and Hong Kong. Ampofo *et al.* also investigated COVID-19 effects on herding behavior in USA and UK stock markets.¹³ They employed CSAD to detect herding behavior but found no evidence of herding during the COVID-19 period.

The uniqueness of almost all research papers on herding behavior is based mainly on their recent data. Therefore, this paper adds to the literature in this field by analyzing specific abnormal time periods post-COVID-19, such as low volatility and noteworthy events, and providing the most recent data available—data from the 2022 calendar year.

■ Methods

This study focuses on the daily closing prices of the 30 leading cryptocurrencies, excluding stablecoins, by market capitalization according to the cci30 index (Bitcoin, Ethereum, Binance, Ripple, Cardano, Dogecoin, Solana, Polygon, Litecoin, TRON, Polkadot, Bitcoin Cash, Toncoin, Shiba Inu, Avalanche, UNUS SED LEO, Chainlink, Cosmos, Uniswap, Monero, Ethereum Classic, Stellar, OKB, Filecoin, Internet Computer, Lido Dao, Aptos, Hedera, Cronos, and Near Protocol) at the time in which this research was performed. All historical data for the daily closing prices of each cryptocurrency was obtained through coinmarketcap.com. All sample time periods were chosen to represent a wide range of scenarios in the cryptocurrency market. These include high and low volume periods, high and low volatility, and noteworthy events (Table 1). These events include the ban on cryptocurrencies in China, the collapse of FTX, and the legalization of Bitcoin in El Salvador. Another unique feature of this paper is that it contains the sample period for the entirety of the year 2022, representing the most recent data.

Table 1: Summary of the sample periods analyzed in this study.

Sample Period	Start Date	End Date
High Volume	January 1st 2021	May 31st, 2021
Low Volume	August 12th, 2022	November 8th, 2022
Low Volatility in 2021	March 21st, 2021	May 21st, 2021
Low Volatility in 2022	March 25th, 2022	May 7th, 2022
High Volatility in 2021	January 17th, 2021	March 17th, 2021
High Volatility in 2022	June 12th, 2022	September 14th, 2022
China Crypto Ban	October 1st, 2021	December 31, 2022
FTX Collapse	November 1, 2022	December 31, 2022
El Salvador Bitcoin Legalization	June 6th 2021	December 31, 2021
Entire Year 2022	January 1, 2022	December 31, 2022

Statista.com's web page, "Overall cryptocurrency 24-hour trade volume from July 1, 2020, to November 10, 2022," determined the high and low volume time periods. The periods of high and low volatility were determined by cvi.finance's Crypto Volatility index. The cci30 cryptocurrency index, based on the 30 largest cryptocurrencies by market capitalization, was used to represent the market behavior over all the above-listed time periods.

Firstly, returns were calculated as follows:

$$R_{c,t} = \frac{P_{c,t}}{P_{c,t-1}}$$

(1)

Where c signifies the cryptocurrency, t represents the day of the closing price, and P denotes the closing price for that cryptocurrency.

The CSSD herding detection method is a way to quantify the dispersion of beliefs across different assets, cryptocurrencies, in this case, designed by Christie and Huang:

$$CSSD_t = \sqrt{\frac{\sum_{i=1}^N (R_{i,t} - \bar{R})^2}{N-1}}$$

(2)

Where $R_{i,t}$ is the observed return on asset i , and \bar{R} is the average market return of the asset n .

To model the behavior of CSSD using the Up and Down status, we can estimate the relationship between CSSD and the market volatility.

$$CSSD = b_0 + b_1 \text{Down} + b_2 \text{UP} + \varepsilon \quad (3)$$

where:

Down = 1 if the market return on day t lies in the extreme lower tail of the return distribution

Down = 0 if the market return on day t lies in the extreme upper tail or middle of the return distribution, and

UP = 1 if the market return on day t lies in the extreme upper tail of the return distribution

UP = 0 if the market return on day t lies in the extreme lower tail or middle of the return distribution.

The coefficient b_0 represents the sample average dispersion between the upper and lower extreme tails.

In summary,

- If herding exists $b_1 < 0$ and $b_2 < 0$

- If no herding exists, $b_1 > 0$ and $b_2 > 0$

Since outliers can have a large and direct impact and increase the spread and variability of CSSD, it is hard to regard the detected herding behavior in this method as conclusive. Thus, Chang *et al.* suggest a better and modified model for detecting herding behavior. It is defined as:

$$CSAD_t = \frac{1}{N} \sum_{i=1}^N |R_{i,t} - R_{m,t}|$$

(4)

Where $R_{i,t}$ represents the return on asset i , and $R_{m,t}$ represents the weighted average of all 30 cryptocurrency returns for period t weighted on its percentage of total market capitalization. N represents the number of cryptocurrencies in the portfolio at time t .

We can estimate the following polynomial model for return dispersion.

$$CSAD_t = a_0 + a_1 |R_{m,t}| + a_2 R_{m,t}^2 + \epsilon_t$$

As indicated by Christie and Huang and common belief, the likelihood of herd behavior increases during time periods of considerable price fluctuations and substantial market stress.

In summary, when testing for herding using the model above:

- If herding exists, $a_2 < 0$
- If no herding behavior exists, $a_2 = 0$, and $a_1 > 0$
- If the opposite of herding exists, then $a_2 > 0$.

The results are deemed statistically significant if the p-value is less than the conventional 5%. As multiple statistical tests are performed simultaneously, it is worth noting that the overall false positive risk, in other words, Type 1 Error, could have been inflated. Because of the exploratory nature of this work, p-values are reported without multiple testing adjustments. Caution must be considered while interpreting the significance of these p-values.

■ Results and Discussion

Equation (3) was estimated using 5% criteria to define extreme up and down market movements. Table 2 provides the CSSD regression estimates across the different time periods associated with the recent significant events.

Table 2: Regression Results of CSSD.

Significant Event	Time Period	Intercept	b1	p-value for b1	b2	p-value for b2
High Volume	January 1, 2021 - May 31, 2021	0.193	-0.12	75%	-0.069	86%
Low Volume	August 12, 2022 - November 8, 2022	0.027	0.008	20%	0.016	1%
Low Volatility 2021	March 21, 2021 - May 21, 2021	0.112	-0.043	58%	-0.013	87%
Low Volatility 2022	March 25, 2022 - May 7, 2022	0.028	0.001	89%	0.014	3%
High Volatility 2021	January 17, 2021 - March 17, 2021	0.339	-0.258	78%	-0.233	80%
High Volatility 2022	June 12, 2022 - September 14, 2022	0.036	0.011	18%	0.021	1%
China Crypto Ban	October 1, 2021 - December 31, 2022	0.033	0.001	1%	0.018	0%
FTX Collapse	November 1, 2022 - December 31, 2022	0.023	0.034	0%	0.025	0%
El Salvador Bitcoin Legalization	June 5, 2021 - December 31, 2021	0.044	0.019	0%	0.006	35%
Entire 2022 Year	January 1, 2022 - December 31, 2022	0.03	0.01	0%	0.02	0%

Table 2 shows the results for Equation (3). Christie and Huang state that the probability of herding behavior increases during times when there are large price movements and market stress. The result is deemed statistically significant if the p-value is less than 5%

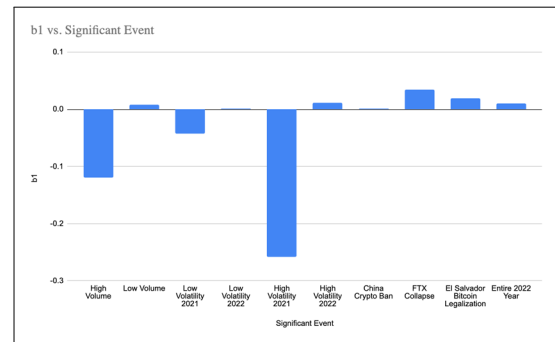


Figure 1: Dispersion of b_1 results for CSSD.

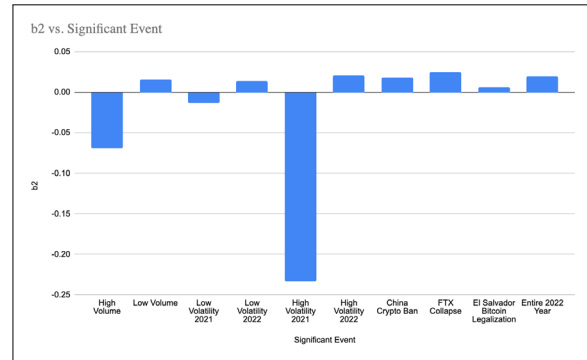


Figure 2: Dispersion of b_2 results for CSSD.

As explained in the previous section, significantly positive coefficients of the Left and Right extreme values (b_1 and b_2) suggest the absence of herding behavior. In contrast, significantly negative coefficients of b_1 and b_2 are consistent with the presence of herding behavior. b_1 and b_2 are negative for the following time periods in Table 2, which indicates the existence of herding:

- Low Volatility in both 2021 and 2022
- High Volatility period in 2021 and 2022
- High Volume period

However, their p-values are all above the usual rule of thumb of 5%; as such, we can't rule out that these values are negative due to random chance.

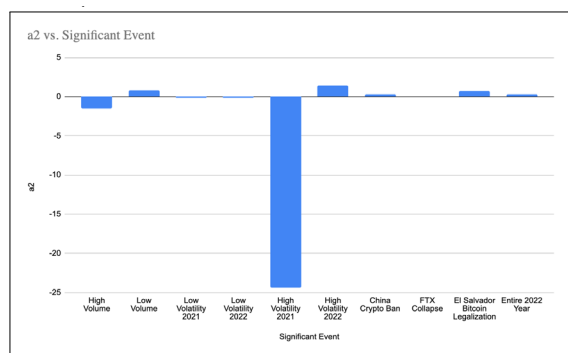
All other time periods indicate the presence of rational decision-making with positive coefficients of the Left and Right extreme values. For example, following the China Crypto ban and FTX collapse, both b_1 and b_2 are positive and significant, indicating rational behavior even with large market fluctuation. Looking at the entire year of 2021, both b_1 and b_2 are positive and significant, suggesting that investors in the cryptocurrency market are generally relatively rational.

In addition to CSSD, a modified CSAD model was also applied to the same data. Table 3 provides the CSAD regression estimates using Equation (5) across the different time periods associated with the recent significant events.

Table 3: Regression Results of CSSD.

Significant Event	Time Period	a_2	p-value of a_2	a_1	a_0	Result
High Volume	January 1, 2021-May 31, 2021	-1.475	71%	0.299	6%	Herding Behavior Demonstrated, Inconclusive
Low Volume	August 12, 2022-November 8, 2022	0.788	32%	0.058	2%	No Herding Behavior Demonstrated
Low Volatility 2021	March 21, 2021 - May 21, 2021	-0.161	85%	0.056	5%	Herding Behavior Demonstrated, Inconclusive
Low Volatility 2022	March 25, 2022 - May 7, 2022	-0.155	90%	0.138	1%	Herding Behavior Demonstrated, Inconclusive
High Volatility 2021	January 17, 2021 - March 17, 2021	-24.354	43%	3.08	4%	Herding Behavior Demonstrated, Inconclusive
High Volatility 2022	June 12, 2022 - September 14, 2022	1.407	7%	0.013	2%	No Herding Behavior Demonstrated
China Crypto Ban	October 1, 2021 - December 31, 2022	0.255	37%	0.098	2%	No Herding Behavior Demonstrated
FTX Collapse	November 1, 2022 - December 31, 2022	-0.005	99%	0.2	1%	Herding Behavior Demonstrated, Inconclusive
El Salvador Bitcoin Legalization	June 5, 2021 - December 31, 2021	0.681	10%	-0.007	3%	No Herding Behavior Demonstrated
Entire 2022 Year	January 1, 2022 - December 31, 2022	0.252	27%	0.131	2%	No Herding Behavior Demonstrated

Table 3 shows the results for Equation (5). The result is deemed significant if the p-value is less than 5%. The coefficient a_2 relates CSAD to squared market return and checks for nonlinearities of herding behavior.

**Figure 3:** Dispersion of a_2 results from CSAD.

As explained above, a negative a_2 suggests the presence of herding, whereas a positive a_2 indicates the opposite of herding. Table 3 shows that in the high-volume period and the period following the collapse of FTX, the coefficient a_2 is negative in the following sample periods:

- High Volume
- Low Volatility in both 2021 and 2022
- High Volatility in 2021
- Following the FTX Collapse

However, their p-values also all exceed the usual rule of thumb of 5%; as such, we can't rule out that these values are negative due to random chance.

Discussion

Upon analyzing the result for both the CSSD and CSAD regression models, the high-volume period, low-volatility period in 2021, and high-volatility period in 2021 all contained evidence of herding behavior in both regression models. However, the lack of statistical significance means the evidence could be stronger. This suggests that while herding is possible, the data does not reflect any statistically significant evidence of herding behavior. Herding is also to be expected, especially considering the number of sample periods analyzed in this study. Furthermore, the characteristics of the cryptocurrency market itself are expected to exhibit herding behavior. Amateur traders often expect quick and positive outcomes despite having access to little concrete quality information on the performances of specific coins, and the market is hardly ever regulated based on its decentralized nature. Based on this hypothesis, of the analyzed sample periods, herding is most expected during periods of high volatility and high volume and least expected during periods of low volume and low volatility, as well as following the collapse of FTX. While this study includes sample periods based on daily closing prices, it could be helpful to investigate herding behavior based on different time intervals, such as hourly or even monthly closing prices.

Conclusion

This paper investigated whether herding behavior exists in the cryptocurrency market post-Covid-19. This paper contributes to the literature in the field by exploring specified time periods in the cryptocurrency market instead of a single sample period spanning a few years. Furthermore, the 2022 calendar year data was used as the most recent data.

The results report that while there appear to be trends indicative of herding behavior during time periods of high, low volatility, and high volume, the lack of statistical significance suggests that these observations could be attributed to random chance instead of definitive patterns. Through using the cross-sectional standard deviation (CSSD) and cross-sectional absolute deviation (CSAD) regression models, the results of all the above-listed sample periods revealed negative b_1 and b_2 coefficients in the CSSD model, as well as negative a_2 coefficients in the CSAD model. Both of these results indicate the possibility of herding behavior in the cryptocurrency market.

This paper used data from the closing prices for all 30 cryptocurrency coins. The next step is to investigate herding behavior at different intervals, such as hourly or monthly closing prices.

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References

1. Nakamoto, Satoshi. Bitcoin: A Peer-to-Peer Electronic Cash System. **2008**, Available at <https://Bitcoin.org/Bitcoin.pdf>
2. Hwang, Soosung.; Salmon, Mark. Market stress and herding. *Journal of Empirical Finance*. **2004**, *11*(4), 585-616. DOI: <https://doi.org/10.1016/j.jempfin.2004.04.003>
3. Economou, Fotini.; Katsikas, Epameinondas.; Vickers, Gregory. Testing for herding in the Athens Stock Exchange during the crisis

- is period. *Finance Research Letters*. **2016**, *18*, 334-341. DOI: <http://dx.doi.org/10.1016/j.frl.2016.05.011>
4. Yao, Juan.; Ma, Chuanchan.; He, William P. Investor herding behavior of Chinese stock market. *International Review of Economics & Finance*, **2014**, *29*, 12-29. DOI: <http://dx.doi.org/10.1016/j.iref.2013.03.002>
 5. Makarov, Igor.; Schoar, Antoinette. Trading and arbitrage in cryptocurrency markets. *Journal of Financial Economics*. **2020**, *135*(2), 293-319. DOI: <https://doi.org/10.1016/j.jfineco.2019.07.001>
 6. Bouri, Elie.; Gupta, Rangan.; Roubad, David. Herding behavior in cryptocurrencies. *Finance Research Letters*. **2019**, *29*, 216-221. DOI: <https://doi.org/10.1016/j.frl.2018.07.008>
 7. Christie, William G.; Huang, Roger D. Following the Pied Piper: Do Individual Returns Herd around the Market? *Financial Analysts Journal*. **1995**, *51*(4), 31-37. DOI: 10.2469/faj.v51.n4.1918
 8. Chang, Eric C.; Cheng, Joseph W.; Khorana, Ajay. An examination of herd behavior in equity markets: An international perspective. *Journal of Banking & Finance*. **2000**, *24*(10), 1651-1679. DOI: [https://doi.org/10.1016/S0378-4266\(99\)00096-5](https://doi.org/10.1016/S0378-4266(99)00096-5)
 9. Amirat, Amina.; Alwafi, Wafa. Does Herding Behavior Exist in the Cryptocurrency Market? *Cogent Economics & Finance*. **2020**, *8*(1). DOI: <https://doi.org/10.1080/23322039.2020.1735680>
 10. Da Gama Silva, Paulo V. J.; Klotzle, Marcelo C.; Pinto, Antonio C. F.; Gomes, Leonardo L. Herding behavior and contagion in the cryptocurrency market. *Journal of Behavioral and Experimental Finance*. **2019**, *22*, 41-50. DOI: <https://doi.org/10.1016/j.jbef.2019.01.006>
 11. Susana, D.; Kavisamathi, J. K.; Sreejith, S. Does Herding Behavior Among Traders Increase During Covid 19 Pandemic? Evidence from the Cryptocurrency market. *Re-imagining Diffusion and Adoption of Information Technology and Systems: A Continuing Conversation*. 2020, 617, 178-189. DOI: https://doi.org/10.1007/978-3-030-64849-7_17
 12. Jiang, R.; Wen, C.; Zhang, R.; Cui, Y. Investor's herding behavior in Asian equity markets during COVID-19 period. *Pacific-Basin Finance Journal*. **2022**, *73*. DOI: 10.1016/j.pacfin.2022.101771
 13. Ampofo, Richard T.; Aidoo, Eric N.; Ntiamoah, Bernard O.; Frimpong, O.; Sasu, D. An empirical investigation of COVID-19 effects on herding behavior in USA and UK stock markets using a quantile regression approach. *Journal of Economic Finance*. **2023**, *47*(2), 517-540. DOI: 10.1007/s12197-022-09613-8

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Computational Analysis of Autism-Associated Genetic Mutation on the Interaction Between Neurexin and Neuroligin

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ABSTRACT: The two cell-adhesion molecules, *Neurexin-1* and *Neuroligin-1*, are essential for synapse function in neural networks. Interruptions to the function of these two proteins are associated with predestination for cognitive disorders, such as Autism spectrum disorder (ASD). In this paper, we study the effects of genetic missense mutations located on the cell interface between *NRXN-1* and *NLGN-1* on the development of neurodevelopmental disorders by identifying and determining the effects of mutations on bond strengths between *NRXN-1* and *NLGN-1* on the synaptic interface by building a computational platform. This was done by mapping all ASD-associated point mutations found in a web-based database on the computationally modeled structure of *NRXN-1*, then determining which mutations interact with *NLGN-1*. Then, the binding strength of the two proteins without the influence of the mutations was compared to the binding strength with the mutations so that the effects of the mutation were established. Based on our structural models and calculations of binding free energy, we found that these mutations can change the binding affinity of Neurexin-neuroligin interaction. Thus, we suggest that the changes in bond strength between Neurexin and Neuroligin at neural synapses can affect neural circuits, leading to autism-associated symptoms.

KEYWORDS: Biological Sciences; Neural Synapses; Genetic Mutation; Neurexin-1.

■ Introduction

A developmental disability, autism spectrum disorder (ASD), is common and caused by brain-related differences. ASD affects 1 in 100 children worldwide,¹ and its main signs include problems with social interaction, communication, interests, or behavior.

Although the etiology of autism is likely multifactorial, it is primarily considered a complex genetic disorder with high heritability, which involves synaptic dysfunction. Synapses connect neurons to one another and are central to neural networks, as well as crucial to process sensory input and generate a motor output that increases the chance of organism survival.² Well-recognized high-risk protein candidates for ASD susceptibility include the cell adhesion family of Neurexins (*NRXN-1*), Neuroligins, and members of the SHANK family of postsynaptic scaffolding proteins.^{3,4}

The Neurexin protein family is a class of cell-adhesion molecules located mainly on the presynaptic membrane. Alternative splicing in Neurexin results in over two thousand possibilities in splice variants, indicating Neurexin's important role in the function of synaptic specificity and synaptogenesis. Additional research shows Neurexin's crucial role in Ca²⁺-triggered neurotransmitter release.⁵ Genetic mutations within the *NRXN-1* isoform have historically been associated with neurodevelopmental disorders, including autism spectrum disorder.⁶

Furthermore, Neurexin acts as a presynaptic receptor for many related extracellular ligand binding partners, including Neuroligin (*NLGN*), highlighting its role in presynaptic organization.⁷ With its binding partner *NLGN-1*, *NRXN-1* works to specify synaptic roles and functions in neural networks

and intercede signaling between synapses by binding to each other and interacting with PDZ-domain receptors and other intracellular proteins.² The two cell-adhesion molecules are essential for synapse function. Interruptions to the function of these two proteins are associated with a predestination for cognitive disorders, as the binding between the proteins is important to process information in neural networks.

This study will mainly focus on one of Neurexin's alpha-type isoforms, Neurexin-1a, and analyze its cell-surface binding with one of its partners, transmembrane protein Neuroligin-1. Prior literature emphasizes a general overview of *NRXN-1*'s mutations as a possible genetic marker of autism-associated symptoms but does not study mutation-specific effects on protein interaction. A platform is needed to be built to investigate the role of genetic mutations in neuronal protein binding affinity. Thus, the goal of this research is to build a platform to study the effects of genetic point missense mutations located on the cell interface between *NRXN-1* and *NLGN-1* on the development of neurodevelopmental disorders, specifically autism spectrum disorder, by identifying and determining the effects of mutations on bond strengths between *NRXN-1* and *NLGN-1* on the synaptic interface. This was done by mapping all point missense mutations found on *NRXN-1*, then determining which mutations interact with *NLGN-1* to initiate neurotransmission. Then, the bond strength of the two proteins was determined with or without the influence of mutations, and the effects of the mutation were established. The creation of a platform that can systematically yet efficiently determine the effects of mutations on neuronal binding affinities is a step in streamlining the often time-consuming and

complex experimental approaches to genetic analysis of protein binding affinity.

■ Methods

In our research, we first established which genetic mutations associated with autism existed on the *NRXN-1* protein. Then, we cross-referenced these mutations against nucleotides on the binding interface between *NRXN-1* and *NLGN-1*. Then, three genetic mutations on *NRXN-1* were determined to be located on two interfaces of the *NRXN-1* and *NLGN-1* binding affinity. The binding strength with and without mutation was determined for each interface, and we were able to analyze subsequent changes in binding free energy.

The database VariCarta was consulted to determine the mutations historically correlated with the presence of autism spectrum disorder.⁸ VariCarta is a web-based database containing a comprehensive archive of scholarly literature-based genomic variants associated with ASD. Two hundred forty-three variant events were reported in *NRXN-1*, with 32 identified as missense point mutations (Table 1). All forms of inheritance and contexts were considered.

Uniprot, a web-based database containing protein sequence information, was consulted to find the structure of *Neurexin-1* with Uniprot ID Q9ULB1. The predicted alpha-fold structure was then downloaded as a PDB file. The 32 missense point mutations identified were then graphed using Visual Molecular Dynamics (VMD), a program used to model, display, and analyze biological systems. In this particular study, VMD was used to visualize the structure of *NRXN-1* using the downloaded alpha-fold model from Uniprot, as well as demonstrate the physical location of each missense mutation (Figure 1).⁹

After the missense mutations were graphed on VMD (Figures 2, 3), the HDOCK server created a bonding model between *NRXN-1* and *NLGN-1*. HDOCK is a web-based server used to create protein-protein and protein-nucleic acid docking.¹⁰

The new model's PDB file was then loaded into Atom, a text and source code editor. This allowed for the visualization of specific nucleotide locations. When cross-referenced with the 32-point mutations identified by VariCarta, three specific mutations were determined to be located on residues of the interface between *NRXN-1* and *NLGN-1*. Models of the interface with each missense mutation were generated using the open-source tool FASPR.¹¹

Models of the interface with and without the mutations were then uploaded to Prodigy,¹² a web-based server allowing for prediction of binding affinity in biological compounds. This server was used to determine the differences in binding strength between *NRXN-1* and *NLGN-1*'s first two interfaces with and without the mutation.

Table 1: VariCarta ID and amino acid mutation of all 32 missense mutations found on *Neurexin-1*.

VariCarta ID	Nucleotide Mutation
10-05671	p.A660T
AU1165302	p.R816W
M27849	p.G87C
GD0072.p1	p.A375T
SX0080.p1	p.G947R
SD0017.p1	p.Y964N

HEN0045.p1	p.G833D
LI2017:23778	p.M1111I
IHART1890	p.E474X
12501.p1	p.Y915X
211-5211-3	p.R116I
AU2054301	p.Y398X
61188249	p.A716E
60372238	p.A757V
M23715	p.D889N
AU0614312	p.R132Q
M26820	p.G818E
BK742-01	p.G1074R
138	p.T289S
386431	p.M725I
84840610	p.R585Q
2-1116-003	p.H845Y
08C73161	p.E222X
DEASD_0107_001	p.V180I
NDAR_INVCG000TG1_wes1	p.N359H
M02013	p.R813H
LI2017:18488	p.G704R
M27891	p.P1063L
JASD_Fam0198	p.G22R
SF0140821.p1	p.H577Q
SF0069869.p1	p.D480H
SX0014.p1	p.R745Q

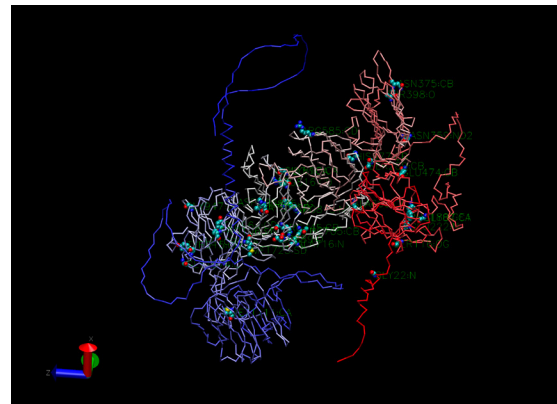


Figure 1: VMD model of the *NRXN-1* protein isoform with all mutations identified mapped.

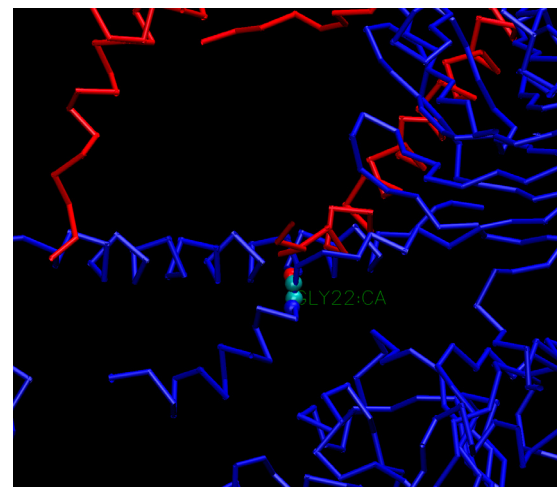


Figure 2: VMD model of Interface 1 binding between *NRXN-1* (blue) and *NLGN-1* (red) with location of mutation *G22R* graphed.

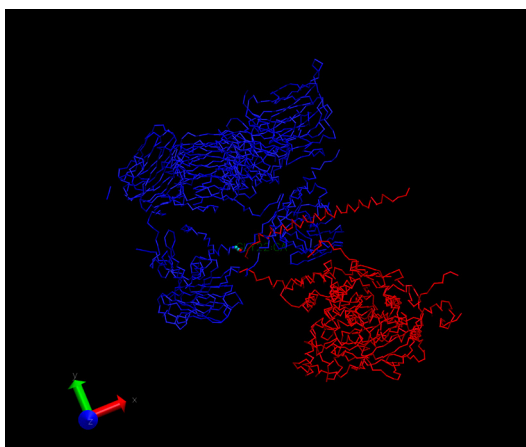


Figure 3: VMD model of Interface 1 binding between *NRXN-1* (blue) and *NLGN-1* (red) with location of mutation G22R graphed.

■ Results and Discussion

Results:

Out of the thirty-two mutations identified on *NRXN-1*, only three mutations were determined to be located on the interface between *NRXN-1* and *NLGN-1*. Hence, they might affect the neuronal protein binding affinity between *NRXN-1* and *NLGN-1*. For Interface 1, one mutation on the binding between *NRXN-1* and *NLGN-1* was identified, *G22R*. For Interface 2, two mutations were identified: *H577Q* and *N359H*. In Interface 1, residue 22 glycine mutated into arginine. In Interface 2, residue 577 histidine mutated into glutamine. Residue 359 asparagine was also found to have been mutated into histidine.

After running both the mutated forms and natural forms of both interface files into Prodigy, the binding free energy was found.

Table 2 shows that the binding free energy from the original protein to the *G22R* mutation on Interface 1 was decreased from -10.8 kcal/mol⁻¹ to -11.0 kcal/mol⁻¹. Table 3 shows that the binding free energy of Interface 2 was increased with both of its mutations, *H577Q* and *N359H*, from -15.2 kcal/mol⁻¹ to -14.2 kcal/mol⁻¹ and -14.0 kcal/mol⁻¹ respectively. A decreased binding free energy means the bond strength between *NRXN-1* and *NLGN-1* increases. Alternatively, an increased binding free energy means that the bond strength between *NRXN-1* and *NLGN-1* is decreased. Using the platform we built, we demonstrated that genetic mutations located on the interface between *NRXN-1* and *NLGN-1* are associated with an altered binding affinity between *NRXN-1* and *NLGN-1*.

Discussion:

Genetic-based testing and modeling are essential to define the basis of neurodevelopmental disorders like autism; their importance should not be understated. This study, which focused on the ligand-receptor binding of a pair of proteins essential to the formation and functionality of synaptic connections, will help further the genetic research front to neurodevelopmental disorders by providing a platform to investigate ASD-related mutations on protein interactions. This research will also add a focus on bond-strength analysis to already existing research of *NRXN-1* and *NLGN-1*, as well as provide a framework for

future understanding of genetic foundations of neurodevelopmental disorders, including autism.

Various databases and programs allowed for identifying specific mutations existing on the interface between *NRXN-1* and *NLGN-1*. After graphing and visualizing these mutations, they were run through a web-based server to determine their effect on the bond strength between *NRXN-1* and *NLGN-1*.

Table 2: Effect of *G22R*, *H577Q*, and *N359H* mutation on binding free value predicted by PRODIGY web server for Interface 1 and 2.

	Original ΔG	<i>G22R</i> Mutated ΔG	<i>H577Q</i> Mutated ΔG	<i>N359H</i> Mutated ΔG
Interface 1	-10.8	-11.0	X	X
Interface 2	-15.2	X	-14.2	-14.0

Since *G22R* does not appear on Interface 2, and *H577Q* and *N359H* do not appear on Interface 1, we respectively marked them off in Table 2.

Our results showed that some mutations of residues in Neurexin are on the binding interface with Neuroigin. Based on our structural models and calculations of binding free energy, we found that these mutations can change the binding affinity of Neurexin-neuroigin interaction. Thus, we suggest that the changes in bond strength between Neurexin and Neuroigin at neural synapses can affect the formation, maturation, or plasticity of neural circuits, leading to autism-associated symptoms. The above results demonstrated that our computational platform is an effective and convenient tool to investigate the effect of genetic mutation on protein binding affinity and will likely lead to a better understanding of neurodevelopmental diseases in the future.

One limitation of this study is that our conclusions are based on computational analysis. The insights provided by our results need to be experimentally validated by future studies. For instance, the structure of the complex formed between *NRXN-1* and *NLGN-1* is currently unavailable and thus was generated by a docking algorithm. Only the best five complex models were selected to search for the mutations located at the interface between *NRXN-1* and *NLGN-1*. These two proteins likely bind to each other in a different mode. Therefore, other mutations involved at their binding interface might exist. Similarly, the binding free energy was also predicted computationally. Whether specific mutations can strengthen or weaken the interaction between *NRXN-1* and *NLGN-1* should be validated in the future.

■ Conclusion

In summary, the computational framework applied in this study serves as a proof-of-concept platform to test the impacts of ASD-related mutations on protein-protein interactions in the neural system. Compared to the time-consuming and labor-intensive experimental approaches, our platform is more convenient to be used as a tool to test specific hypotheses that cause ASD. The platform can be potentially extended to study the interactions between *NRXN-1* and its other binding partners, as well as many other proteins containing mutations associated with ASD.

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■ References

1. Zeidan, J.; Fombonne, E.; Scora, J.; Ibrahim, A.; Durkin, M. S.; Saxena, S.; Yusuf, A.; Shih, A.; Elsabbagh, M. Global Prevalence of Autism: A Systematic Review Update. *Autism Research* **2022**, *15* (5), 778–790. DOI:10.1002/aur.2696.
2. Südhof, T. C. Neuroligins and Neurexins Link Synaptic Function to Cognitive Disease. *Nature* **2008**, *455* (7215), 903–911. DOI:10.1038/nature07456.
3. Sauer, A. K.; Stanton, J. E.; Hans, S.; Grabrucker, A. M. Autism Spectrum Disorders: ETIOLOGY AND PATHOLOGY. *Autism Spectrum Disorders* **2021**, 1–16. DOI:10.36255/exonpublications.autismspectrumdisorders.2021.etiology.
4. Dexter Hadley, M. Genetics of Autism Spectrum Disorders. <https://emedicine.medscape.com/article/2024885-overview> (accessed 2023-07-07).
5. Missler, M.; Zhang, W.; Rohlmann, A.; Kattenstroth, G.; Hammer, R. E.; Gottmann, K.; Südhof, T. C. A-Neurexins Couple Ca²⁺ Channels to Synaptic Vesicle Exocytosis. *Nature* **2003**, *423* (6943), 939–948. DOI:10.1038/nature01755.
6. Vaags, A. K.; Lionel, A. C.; Sato, D.; Goodenberger, M.; Stein, Q. P.; Curran, S.; Ogilvie, C.; Ahn, J. W.; Drmic, I.; Senman, L.; Chrysler, C.; Thompson, A.; Russell, C.; Prasad, A.; Walker, S.; Pinto, D.; Marshall, C. R.; Stavropoulos, D. J.; Zwaigenbaum, L.; Fernandez, B. A.; Fombonne, E.; Bolton, P. F.; Collier, D. A.; Hodge, J. C.; Roberts, W.; Szatmari, P.; Scherer, S. W. Rare Deletions at the Neurexin 3 Locus in Autism Spectrum Disorder. *The American Journal of Human Genetics* **2012**, *90* (1), 133–141. DOI:10.1016/j.ajhg.2011.11.025.
7. Gomez, A. M.; Traunmüller, L.; Scheiffele, P. Neurexins: Molecular Codes for Shaping Neuronal Synapses. *Nature Reviews Neuroscience* **2021**, *22* (3), 137–151. DOI:10.1038/s41583-020-00415-7.
8. Belmadani, M.; Jacobson, M.; Holmes, N.; Phan, M.; Nguyen, T.; Pavlidis, P.; Rogic, S. Varicarta: A Comprehensive Database of Harmonized Genomic Variants Found in Autism Spectrum Disorder Sequencing Studies. *Autism Research* **2019**, *12* (12), 1728–1736. DOI:10.1002/aur.2236.
9. Humphrey, W.; Dalke, A.; Schulten, K. VMD: Visual Molecular Dynamics. *Journal of Molecular Graphics* **1996**, *14* (1), 33–38. DOI:10.1016/0263-7855(96)00018-5.
10. Yan, Y.; Zhang, D.; Zhou, P.; Li, B.; Huang, S.-Y. HDock: A Web Server for Protein–Protein and Protein–DNA/RNA Docking Based on a Hybrid Strategy. *Nucleic Acids Research* **2017**, *45* (W1). DOI:10.1093/nar/gkx407.
11. Huang, X.; Pearce, R.; Zhang, Y. FASPR: An Open-Source Tool for Fast and Accurate Protein Side-Chain Packing. *Bioinformatics* **2020**, *36* (12), 3758–3765. DOI:10.1093/bioinformatics/btaa234.
12. Xue, L. C.; Rodrigues, J. P.; Kastriitis, P. L.; Bonvin, A. M.; Vangone, A. Prodigy: A Web Server for Predicting the Binding Affinity of Protein–Protein Complexes. *Bioinformatics* **2016**, *32* (23), 3676–3678. DOI:10.1093/bioinformatics/btw514.

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Biomimicry as an Approach to Sustainable Architecture

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ABSTRACT: Biomimicry is defined as a solution to design problems inspired by natural elements and processes. It offers ideas to enhance sustainability and potentially create a regenerative structure. Moving towards sustainable design is becoming increasingly crucial for the building sector, as most current architecture contributes negatively to climate change. As a result, interest in biomimicry as an approach in architecture has increased, though terminology remains fragmented, creating challenges for efficient research. In both conceptual and built architecture, biomimicry has been used in numerous contexts and for various purposes. This study aims to provide a thorough review by clarifying terminology, explaining the different approaches and classifications of biomimicry, and examining built case studies of successful biomimicry in architecture implementation. Furthermore, the discussion section will note future recommendations to avoid unnecessary challenges, identify differences between biomimicry approaches, and describe steps to encourage future studies.

KEYWORDS: Environmental engineering; sustainable design; sustainable architecture; biomimicry in architecture; biomimicry design approaches.

■ Introduction

In a 2022 press release by the UN environmental programme, the buildings and construction sector is said to account for over 34 percent of energy demand and around 37 percent of CO₂ emissions in 2021.¹ The looming threats of environmental disasters urges action, and architecture can play a massive part in the solution. Biomimicry is viable for solving many issues, including energy efficiency, emissions, and materials.

Biomimicry is a promising, emerging field of research defined to be the solution to design challenges inspired by natural elements and processes.² Janine M. Benyus popularized the term in her seminal book *Biomimicry: Innovation Inspired by Nature* (1997), and it has been increasingly used in research. As an interdisciplinary approach, biomimicry holds the potential to create innovative and sustainable solutions through collaboration between architects, biologists, engineers, and experts across scientific and design-related fields. The final goal of today's sustainability approaches is to move from a depleting linear economy, where raw materials are used to create products and later discarded as waste, to a circular economy, where all forms of waste are returned to the economy or used more efficiently. Thus, using nature as a model, biomimicry can create solutions that enhance sustainability or generate a restorative approach. As a result, biomimicry has become increasingly relevant in architecture, used in numerous implementations for various purposes, from providing aesthetic inspiration to providing thermal comfort, energy efficiency, passive or natural ventilation, and more. However, the increasing fragmentation in the field accompanies the increase in relevance, owing much to the constantly changing and increasing terminologies.

This paper will first provide an overview of the history and evolution of biomimicry, including explanations of contemporary terminologies to resolve confusion and misinterpretation.

This section will clarify the differences between biomimicry-related keywords and identify how keywords will be used throughout this paper. Then, the report will describe the two general approaches to biomimicry and provide a framework for understanding further classifications of methodologies. This section will differentiate between levels of biomimicry and provide examples to help visualize biomimicry in architecture. Last, before the discussion and conclusion section, the paper will analyze established case studies of successful implementations of biomimicry in architecture. This section looks at existing design methods and highlights the benefits and limits of current implementations. Finally, the discussion section will identify issues that arose during the research process, suggest steps to avoid unnecessary challenges in the future, compare the analyzed case studies to show current biomimicry's contribution to sustainability, and describe how biomimicry as an approach to sustainability in architecture should be promoted and encouraged.

Definition and History:

Biomimicry, bionics, biomimetics, and similar terms all refer to mimicking nature in one way or another.³ For approximately 70 years, biomimicry, and its aliases, have been used by academics with a general definition of taking lessons from nature and incorporating them into practical solutions to human problems. However, numerous related terminologies were created due to the diverse backgrounds of researchers (biologists, architects, engineers, etc.) and the development of various design methods.⁴ The lack of clarity and consistency in definitions makes it challenging to find and understand research reviews and can hinder further advances in this promising field of research. Thus, this section seeks to clarify some important keywords by identifying common interpretations among researchers.

Every biomimicry-related keyword has a different focus. To start, the prefixes “bio” and “eco” have distinct differences: “bio” refers to the description and science of life while “eco” concerns the relationship between organisms and their environment.^{5,6} When combining “bio” and “eco” with “morphism” or “mimetic,” the focus shifts to the imitation of living things.³ Architects are frequently inspired by nature through symbolic associations without considering innovation or sustainability.⁷ “Mimicry” and “mimetics” then go beyond the simple form to focus on representing models on more profound levels for various purposes.³ For instance, biomimetics solve practical problems through interdisciplinary approaches that transform natural processes into solutions for human systems.⁸ It is also often used as an adjective form of biomimicry.³ Then, bionics intend to enhance mechanical systems through nature-inspired technologies and is found most often in products but also structural or MEP (mechanical, electrical, and plumbing) systems of buildings.⁹ Lastly, biomorphism and organic design relate more to shape and form; the latter bridges over to bionics as well.³ To avoid unnecessary complications, this paper will use biomimetic in a largely synonymous context to biomimicry.

The idea of biomimicry has a long history. Critics and philosophers have been looking to natural organisms as models for a harmonious balance between parts of a design since ancient Greece. More than 1,700 years ago, the first Chinese umbrellas were invented by Lu Ban, a revered inventor in Chinese history whose idea formed when he saw children using lotus leaves to shield themselves from rain.¹⁰ By mimicking the flexibility and repellency of the leaf, Lu Ban created a successful, innovative product of his own. The well-known Leonardo da Vinci was also, in many ways, a pioneer of biomimicry: he studied the forms of birds’ wings in hopes of finding solutions to enable human flight. In addition, many architects and designers looked to biology for inspiration in the early nineteenth century: Le Corbusier and Frank Lloyd Wright are among the most famous examples.

The history of the terminologies can be traced to 1957, when Otto Schmitt, a bioengineer and physicist, first proposed the word “biomimetics” to define his device that imitated the electrical action of a nerve.⁸ Following closely after, in 1958, Jack Steele introduced and defined bionics as the science of natural systems or their analogs.³ This was reinforced in the 1960s under the heavy impact of cybernetics. At this time, the word “bionik” was introduced; it merged the German words “biologie” and “technik” and referred to the transfer of ideas from biology to technology.¹¹ The term was decreasingly used for the following 30 years. However, the word was brought back and popularized in 1997 through Janine Benyus’s book *Biomimicry: Innovation Inspired by Nature*. Benyus offers the most widely known definition of biomimicry in her book: a “new science that studies nature’s models and then imitates or takes inspiration from these designs and processes to solve human problems”.¹²

Approaches to Biomimicry:

Designers have adopted two general approaches to biomimicry: solution-based (or “bottom-up approach” or “biology to

design”) and problem-based (or “top-down approach” or “design to biology”).¹³ The two approaches are distinguished by their different starting points of development. In a solution-based approach, biological knowledge influences human design; the research starts with a biological discovery that presents possible technical applications.^{3,12} In a problem-based approach, a designer recognizes a technical problem and looks at laws in nature for solutions; the research starts with a specific technical question.^{3,12} In addition, a third approach can be identified as an extended solution-based approach. This approach utilizes an iterative process driven by a returning question: “What if nature has an even better solution?”. Though this approach often requires more time and research, it has the potential to generate innovative and out-of-the-box outcomes.¹⁴

Either way, biomimicry requires designers to approach their usual processes differently. Designers traditionally look for solutions within their expertise, but a biomimicry-based approach is interdisciplinary. Most designers need to be educated in biology and would benefit from collaborating with biologists for creative and sustainable findings. Depending on the level of biomimicry, collaboration may be the key to literally or metaphorically translating biological strategies. Moreover, collaboration could help non-biology experts through the most challenging abstraction phase.^{14,15}

Levels of Biomimicry:

Under the two approaches, biomimicry can be divided into three levels: organism, behavior, and ecosystem.⁵ These levels are applied to all fields and determine which aspect of biology is being mimicked. A design can mimic some parts of an organism, an organism’s behaviors or responses in its context, or a function of an ecosystem.⁵ Under these three levels, five additional dimensions directed to architectural applications can be identified: form, material, construction, process, and function.¹⁶ To describe these classifications, Pedersen Zari developed a comprehensible chart in which she uses a building mimicking termites as an example, providing imagery that helps with understanding the differences between levels of biomimicry (Table 1).¹⁶

Table 1: A framework adopted from Zari for understanding biomimicry uses a building that mimics termites as an example.

Level of Biomimicry	Example: A building that mimics termites	
Organism Level (Mimicry of a specific organism)	Form	The building looks like a termite
	Material	The building is made from the same material as a termite; a material that mimics termite exoskeleton / skin for example.
	Construction	The building is made in the same way as a termite; it goes through various growth cycles for example.
	Process	The building works in the same way as an individual termite; it produces hydrogen efficiently through meta-genomics for example.
	Function	The building functions like a termite in a larger context; it recycles cellulose waste and creates soil for example.
Ecosystem Level (Mimicry of an ecosystem)	Form	The building looks like it was made by a termite; a replica of a termite mound for example.
	Material	The building is made from the same materials that a termite builds with; using digested fine soil as the primary material for example.

Behaviour Level (Mimicry of how an organism behaves or relates to its larger context)	Construction	The building is made in the same way that a termite would build in; piling earth in certain places at certain times for example.
	Process	The building works in the same way as a termite mound would; by careful orientation, shape, materials selection and natural ventilation for example, or it mimics how termites work together
	Function	The building functions in the same way that it would if made by termites; internal conditions are regulated to be optimal and thermally stable. It may also function in the same way that a termite mound does in a larger context.
	Form	The building looks like an ecosystem (a termite would live in).
Level of Biomimicry		Example: A building that mimics termites
Ecosystem Level (Mimicry of an ecosystem)	Material	The building is made from the same kind of materials that (a termite) ecosystem is made of; it uses naturally occurring common compounds, and water as the primary chemical medium for example.
	Construction	The building is assembled in the same way as a (termite) ecosystem; principles of succession and increasing complexity over time are used for example.
	Process	The building works in the same way as a (termite) ecosystem; it captures and converts energy from the sun, and stores water for example.
	Function	The building is able to function in the same way that a (termite) ecosystem would and forms part of a complex system by utilizing the relationships between processes; it is able to participate in the hydrological, carbon, nitrogen cycles etc. in a similar way to an ecosystem for example.

Buildings are inherently complex, and some can have biomimicry strategies on different levels.³ For instance, the Cairo Gate Residence project functions on both the ecosystem level (stores water and uses solar power) and the behavior level (via its careful orientation, form, and use of natural ventilation).¹⁷ In general, mimicry at the organism level primarily inspires a building's form, shape, or structure, whereas mimicking behaviors focus on the interactions between the building and its surroundings. Although biomimicry can be separated into levels and specific dimensions, it is expected that overlap exists between different kinds of biomimicry and that each type is not mutually exclusive.¹⁶

Biomimicry in Architecture:

Modern-day requirements for architecture are often complex and necessitate a comprehensive approach to inventive, functional, and sustainable building systems. These growing demands for the built environment have made architects increasingly interested in the development of biomimicry in architecture for ameliorating the technological, aesthetic, and environmental impacts of a building.³ High-strength polymers, super-efficient structures, zero-waste systems, and materials made from atmospheric carbon are all naturally existing structures that architects can learn from to create architecture better suited for the needs of this age.² The biosystem adapts naturally to environmental changes, and, in mimicking this adaptability of nature, designers find answers to creating highly resilient and environmentally sustainable built environments. Unlike many current sustainable designs, biomimicry points to a new model based on optimizing positives rather than mitigating negatives.² Thus, biomimicry-based designs emerge as a revolutionary trend in architecture.¹⁸ Fortunately, the great possibilities of biomimicry are becoming increasingly tangible with the development of new building materials and

construction techniques.¹⁹ Additionally, the advancements in design software with digital fabrication and manufacturing of composite materials and 3D printing technology have further realized biomimetic designs.^{2,15}

On the other hand, there is still a gap between biomimicry's focus on the biosystem, a natural structure, and architecture's human-made nature. While humans create a built environment, biomimicry is a natural evolution process; this limits the cross-domain information for biologists and architects alike and has been identified as one of the most significant constraints to applying biomimicry to architecture. In addition, it should be noted that built case studies of biomimicry in architecture are still scarce even if built and conceptual examples can be found.³ Despite limitations, scholars widely agreed that biomimicry is a practical methodology for answering sustainability issues in architectural designs. The potential uses of biomimicry in architecture include but are not limited to integrating building elements with a life cycle in nature, supporting lightweight structures, and optimizing the building envelope. Furthermore, biomimicry provides sustainable heating, cooling, lighting, and insulation ideas.¹⁹ In the following subsections, this paper will review built case studies on current architectural implementations by discussing three levels of biomimicry: organism, behavior, and ecosystem.

Organism Level:

For billions of years, organisms have adapted and evolved to changes around them. Other organisms have already addressed many problems humans struggle with today. Thus, architects have numerous examples to draw solutions from, especially for optimizing energy and materials. Organism-level mimicry can further the understanding of human activities' environmental impact.¹²

The Lotus Temple in New Delhi, India, is a religious building that utilizes organism-level biomimicry to inspire form. The temple is known for its distinctive, flowerlike shape with twenty-seven petals evenly divided into the entrance, outer, and inner leaves (Figure 1). This deliberate design reflects the beauty and symmetry of a lotus flower, which makes important connections to many Eastern and Indian faiths.²⁰ The building considers its environmental impact by installing solar panels, which generate 20 percent of the needed electricity, and by implementing structures that encourage natural ventilation and lighting.²¹ Although the building makes an effort to reflect sustainability, it should be noted that the flower petals were made with concrete and marble and, to maintain a pristine appearance, were reinforced with 300 tons of galvanized bar to avoid rusting and cracking.²¹ Overall, though, the temple is incredibly successful for its excellence in religious art and architecture and for attracting visitors. It is the most visited building in India, with over 10,000 visitors per day.²⁰

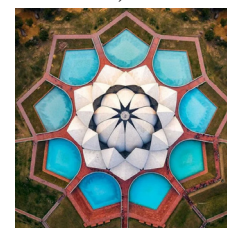


Figure 1: The lotus-inspired form of the temple adds aesthetic value and reflects the wonderful symmetry of a lotus flower.

The Gherkin Tower, also known as 30 St Mary Axe, is another example of mimicry on the organism level. Situated in London, UK, it represents a successful implementation beyond imitating form for aesthetics. This remarkable skyscraper takes inspiration from the Venus Flower Basket Sponge: an aquatic organism with a lattice-like exoskeleton and a round form (Figure 2). The structure of the sponge allows it to withstand the high-water currents of its habitat by dispersing stresses in various directions.³ The tower replicates these properties through an aluminum-coated steel diagrid structure and a similarly curved shape.



Figure 2: The Gherkin Tower draws inspiration from the Venus Flower Basket's form.

The curved, cylindrical form of the tower allows wind to pass smoothly around it (Figure 3). This presents multiple advantages. For one, more air flows around the side of a cylinder than around the corners of a rectangle, thus creating a higher negative air potential at the back of the building, which architect Norman Foster used to drive a natural ventilation system.¹² Less wind circulation exists on the ground, reducing wind turbulence at plaza level and allowing for a social living space around the ring structure.^{12,22} The efficiency of the diagrid construction, which mimics the sponge's lattice structure, has also allowed the Gherkin tower to use 20% less structural steel than a conventional moment-framed building.²² The tower's 5-degree rotation between each floor plate incorporates wedge-shaped lightwells, allowing light and air to reach each floor.¹² To add, the building adopted a double skin facade to maximize sun exposure; horizontal shading devices are placed between the walls and venting flaps direct hot air up and out of the building.¹² In this study, biomimicry-based implementations contributed to passive cooling, heating, natural ventilation, and lighting techniques. With its innovative design, the Gherkin Tower is considered the first ecological skyscraper in London and has been deemed worthy of many architectural awards, such as the Stirling Award.²²

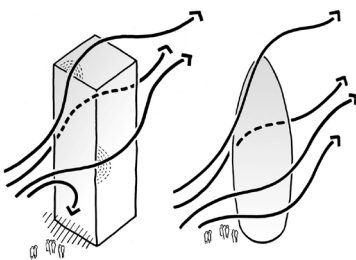


Figure 3: The drawing by Norman Foster shows the differences between wind interacting with a standard rectangular skyscraper versus the Gherkin Tower, demonstrating the advantages of the cylindrical form.

Behavior Level:

Behavior level bases mimicry on the understanding that because organisms live in the same environment as humans, they experience similar issues; thus, humans can learn from how these organisms operate within their limited conditions and available materials.¹²

Rolling a planar surface is a simple way to turn it into a protective structure. Research has found that many organisms use the behavior of wrapping leaves into tubes to support their metamorphosis process (Figure 4).² This process is then mimicked in architecture projects such as the Shadow Pavilion by PLY Architecture, which utilizes sheets of laser-cut aluminum rolled into cones and assembled in phyllotactic geometry (Figure 5).²

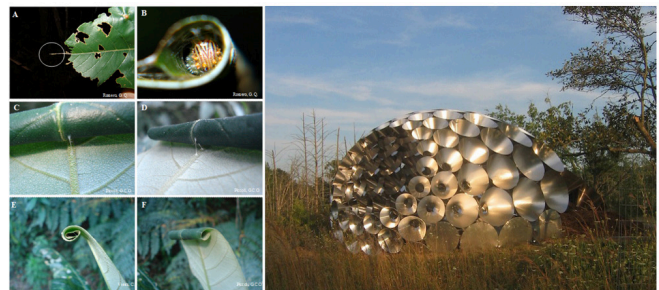


Figure 4: A leaf roller caterpillar (genus *Aenea*) shapes flat leaves into tubes and secures them with silk, creating leaf shelters.

Figure 5: The Shadow Pavilion is built with cone-shaped elements to generate a distinctive form.

A deeper level of mimicry on the behavior level can be represented by the Eastgate Centre in central Harare, designed by Mick Pearce. The building opened in 1996 and stands as an iconic, pioneering example of biomimicry in architecture. Eastgate is a commercial and office building in Harare, containing two buildings linked with a glass roof.²³ Lead architect Mick Pearce wanted the building to be inspired by local nature, which was filled with organisms living comfortably in Zimbabwe's tropical climates.²⁴ Pearce was inspired by African termites and their remarkable mounds, specifically its flow principle and natural temperature-controlling abilities (Figure 6).²⁴ Rather than using conventional air-conditioning or heating, the building is ventilated and thermoregulated passively (Figure 7).²⁵ Fresh air is captured in the lower levels, injected into collective spaces, and led out through the chimneys.^{23,25} With forty-eight brick funnels lining the roof and thirty-two banks of low and high-volume fans, the building successfully mimics the natural systems of the mounds.²³ At night, the heavier hot air stored during the day is attracted upwards and led out through the 48 large chimneys, allowing restoration of freshness.²⁵ The building stores heat during the day, uses high thermal inertia materials which termites use (clay bricks), and diffuses heat at night.²⁵

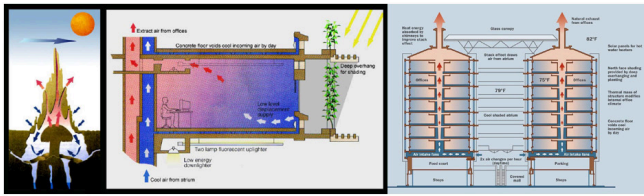


Figure 6: Comparison of termite mounds' flow principles and that of Eastgate.

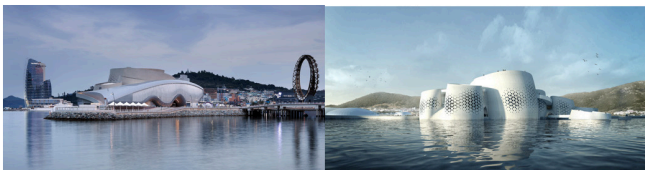
Figure 7: Passive thermoregulation of Eastgate.

With its effective implementations of biomimicry, the building reduces energy consumption by 35% and saves 10% on the capital cost in comparison to the average of six other conventional buildings with full HVAC (heating, ventilation, and air conditioning) in Harare.^{23,25}

Ecosystem Level:

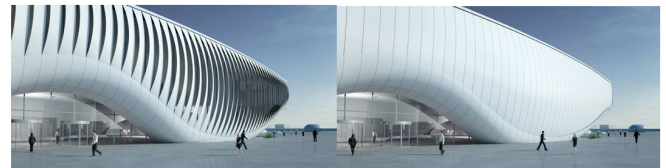
The mimicking of ecosystems is described as an integral part of biomimicry.⁵ An advantage provided by this level is its ability to be used in conjunction with the other two levels. Mimicry at this level branches into a "circular economy" where there should essentially be no by-product.²⁶ The ecosystem level is primarily used to ameliorate the urban metabolism, mimicking an entire ecosystem and its functions helps to positively shift the ecological impact of buildings in the urban setting.²⁷

The One Ocean building in Yeosu, South Korea, has a 140 meters long facade, ranging from three to thirteen meters high. The building is integrated into its urban context and surrounding nature without sacrificing its aesthetics as an iconic landmark (Figures 8-9).²⁸



Figures 8-9: View of the One Ocean building in its urban and natural context.

Its spatial and organizational concept takes inspiration from the ocean's duality of its endless surface and its immersive depth.²⁸ The buildings' continuous and twisting surface defines the interior space, and its kinetic adaptive facade system, structured with moveable lamellas (Figures 10-11), allows light control to specific areas of the building.^{25,28} These lamellas are made with glass fiber-reinforced polymer, which, by making use of its material properties, allows for movement.²⁵ The shutter, which controls light input, moves individually through actuators at the ends by mimicking the opening and closing system of a Bird-Of-Paradise flower's stamen, demonstrating mimicry at the organism level on top of its ecosystem level mimicry.²⁵ In addition to light, the adaptable facade, with its strategic orientation, enhances natural ventilation by capturing and guiding winds through the building.²⁹ Other than its functional purposes, the movement builds on the sensuous experiences of One Ocean and is a major attraction to visitors.



Figures 10-11: Opened and closed lamellas of the One Ocean building.

Regarding energy use, photovoltaic panels in the roof landscape generate solar electricity responsible for two-thirds of the total consumption.²⁹ In all, the building successfully and seamlessly relates form, material, light, and movement with its innovative approach to biomimicry.

Although innovative, the One Ocean building primarily falls under the biomimicry level of form and does little in demonstrating ecosystem-level biomimicry's potential to facilitate regenerative design at the urban scale. An example that better illustrates this ability would be the Lloyd Crossing Project in Oregon, USA, which was a visionary plan designed for an existing, mixed-use 35 block area in Northeast Portland (Figure 12).



Figure 12: The Lloyd Crossing urban design plan.

The plan was inspired by local ecosystem patterns and its abilities to catalyze regeneration at the district level.³⁰ Mithūn and Greenworks designers based their plan on a functional concept of "Pre-development Metrics," which embodied a theoretical baseline illustrating the ecological profile of the site before human presence.³¹ The plan establishes specific performance goals in three areas: wildlife habitat, water and usage quality, and energy consumption.³¹ The designers assessed both the pre-development conifer forest ecosystem and the existing urban ecosystem to identify gaps, which were then used to define ecological performance targets and long-term urban development strategies (Figure 13).³⁰ Four principles were derived from these pre-development metrics: restore habitat and vegetation canopy, rely on on-site rainwater, fully utilize locally available solar energy, and create overall carbon neutrality.

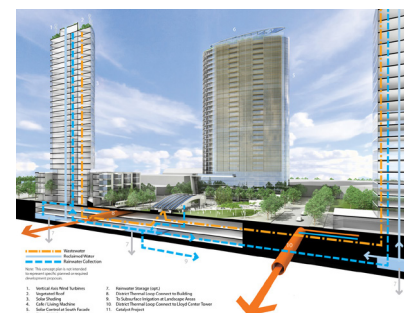


Figure 13: Examples of strategies planned for high-rise buildings.

Starting with habitat restoration, the plan compares the predevelopment metric of 90 percent tree cover to 14.5 percent at the time of design and attempts to reestablish 25 to 30 percent tree cover.^{30,31} An abstracted mixed-conifer forest would be achieved, involving native “forest patches,” rooftop gardens, green streets, and habitat corridors.³¹ Another important aspect was water optimization. The plan looks at the 64 million gallons of rainwater received per year and proposed water consumption reduction, rainwater treatment and harvesting, and wastewater reuse.³¹ In terms of energy, the plan focuses on utilizing the 161 million kilowatt hours per year of solar energy available to the neighborhood.³¹ To reach its goal of creating an overall carbon-neutral strategy, the design recognizes the construction industry’s high consumption of raw materials and gives preference to materials that employ renewable resources, are available within 300–500 miles of the site, and have low embodied energy and a positive Live Cycle Assessment.³¹ Lastly, the plan builds on successful qualities of the older core neighborhoods and argues that its new features will lead to a unique district identity with new open spaces, mixed use residential units, set-back towers to allow sun light at street level and more.³¹

■ Discussion

During the research process, terminologies become an apparent problem. It takes substantial effort to identify which definition of biomimicry-related terminology an article uses, and when referencing multiple papers, understanding the material becomes challenging as one would need to switch between different authors’ use of terminology. This issue is especially evident in the research of biomimicry, where numerous related keywords and variations of definitions are generated constantly as the field expands. Confusion surrounding consistent terminology can discourage and delay further studies on the topic; thus, a solution should be proposed. Furthermore, it is unlikely and unnecessary that all researchers agree on a single definition, especially in this context where biomimicry is still an emerging concept that researchers should continue to debate, explore, and expand upon. Thus, another proposal should be made; it is easy and effective if the paper notes how it will use specific terms at the beginning of the article. For instance, in the section immediately following the introduction, this paper stated that biomimetic would be used as an adjective for biomimicry: “in a largely synonymous context.” Following this example may help to make future research more efficient.

In terms of biomimicry, and related approaches, in architecture, it is evident that the discipline is implemented with various focuses, including symbolic associations, aesthetic concerns, and sustainability. In studying established case studies, this paper has shown that biomimicry only sometimes addresses sustainability concerns. For instance, the Lotus Temple used biomimicry mainly to produce its visually striking aesthetics. Although it does show consideration for sustainability, its implementations of openings for ventilation and solar panels for electricity stem from ideas unrelated to biomimicry and are separate from its different biomimetic approaches. The design used nature primarily to inspire formal and symbolic associations, which do not automatically constitute sustainable forms

of architecture, abandoning further investigation to develop resource-efficient structures or materials that may have contributed to better addressing the sustainability aspect.

In contrast, the Gherkin Tower, which also adopted organism-level biomimicry, investigated the Venus Basket Flower Sponge’s form more in-depth; the sponge’s ability to disperse water currents was put into consideration and replicated in the building through a cylindrical shape and a diagrid structure. By extending the analysis beyond aesthetics and symbolic associations, the Gherkin Tower reduced structural steel and utilized natural ventilation along with other passive control systems. Although biomimicry is not always used as a solution to sustainability, it is clear that it has enormous potential and evident successes in doing so. Thus, this paper encourages future biomimetic implementations and further research regarding this discipline.

Looking at the Lloyd Crossing project, we see the potential biomimicry has in optimizing resources and creating regenerative strategies. Unlike many other examples, this project was able to devise a holistic approach addressing multiple factors such as biodiversity, material flow, and carbon neutrality. Their method of using the complex ecosystem as the base of its performance goals proved successful. Understanding and integrating more in-depth ecological knowledge into the design was the key to developing this regenerative plan, and this raises the importance of such knowledge in advancing biomimicry. To move forward with ecosystem-level biomimicry as an approach to regenerative urban design, it is crucial to integrate local ecological factors into design phases. More can be achieved with more extensive comprehension on these ecosystems. This further encourages interdisciplinary collaborations to utilize the advantages of having thorough understanding of both design methods and biological details.

A few questions should be asked in promoting biomimicry as an approach in architecture. What drives sustainable practices and pushes architects and designers to utilize biomimicry-based implementations? Research has found that the primary motivation behind sustainable design was autonomous motivations, which emerged from individuals.³² This refers to a person’s moral imperatives, personal commitments, and similar ideas. Of course, extrinsic motivators play a role as well; for instance, client demand seems to be the initial motivator in many cases, though many elaborated that the perceived demands for sustainability were surprisingly little, and it was often the architects themselves that became the primary driver to instigate inclusion of sustainable elements.³² Interestingly, the self-determination theory also suggests that extrinsic factors may be internalized over time; in other words, the perceived autonomous motivations may be developed through external factors.³³

Since this internalization may drive architects and designers, external motivators such as legislation should promote designs that address sustainability issues, especially innovations such as biomimetic approaches. Some also argue that the solution to fostering sustainable designs lies in governmental relief directed to low and zero-carbon building investment activities through financial and non-financial incentives.³³

Another way to encourage greater adoption of biomimicry is to increase awareness of the field. An unexpected discovery was made during the research process: few people, including architects, knew what biomimicry was. Even if one was familiar with famous examples of biomimicry in architecture, such as the Bird's Nest of China, the building was broadly not identified as an example of biomimicry. This is mainly for two reasons: for one, biomimicry is still a relatively new concept in architecture; thus a limited number of people have come in contact with the term at all, let alone made an effort to learn more about it; for another, older architectures did not promote or note themselves as examples of biomimetic implementations, mostly because biomimicry was not a popular term in its age. To help biomimicry reach a wider audience, researchers should try to bring up biomimicry in related conversations and participate in interdisciplinary collaborations, buildings that use a biomimetic approach should actively include the concept in their descriptions and advertisements, and training programs or school curriculums should consider including biomimicry in its education. To start, researchers must help "spread the word." Otherwise, only those actively researching the field will come across the term.

Additionally, collaborations between different fields will help generate novel solutions; since biomimicry is an interdisciplinary study, biological knowledge combined with design methods is the key to creating innovative results. Of course, the idea of biomimicry, if continuously proven successful, will popularize itself with time. But why should we wait instead of aiding this process? In the same context, new and older architecture that now understand its approaches as biomimetic should make the biomimicry aspect of their building known. Not only does this help encourage future implementation, but it also sets helpful models for architects and designers and becomes easily identifiable as potential case studies for researchers. For the building itself, biomimicry can be a selling point. Lastly, education is the most direct way architects can come into contact with biomimicry. If schools start to include biomimicry as a part of the course curriculum, studying and understanding it becomes natural. The first step would be developing biomimicry-focused courses for architecture students. Then, it can be required that students take at least one credit of said course to complete an architecture degree. These three proposed methods can help stimulate biomimicry as an approach to sustainable architecture.

■ Conclusion

The growing interest in biomimicry in architecture has led to increasing fragmentation due to scholars' different interpretations of terminologies. To clarify, the paper reviewed the history of terminologies, telling how terms developed into what we know today, and summarized common variations of keywords among researchers today. To a certain level, there is a consensus on what biomimicry is, and the similar associations to nature-drawn inspirations of many related terms help to organize general definitions. The paper recommends that authors state the definition they will utilize at the beginning of an article to avoid confusion for researchers.

With Benyus' classification system of ecosystem, behavior, and organism, and Zari's further division of form, material, construction, process, and function, the complexity of biomimicry can be reduced by a certain degree, and understanding the concept becomes more straightforward. Across all frameworks, it is implied that sufficient knowledge of biology increases the potential and impact of biomimicry in architecture; in some cases, depending on the design complexity, biomimicry may not be realized without a certain level of biological understanding. In agreement with this trend, this paper encourages collaboration between disciplines to maximize biomimicry's potential in producing successful, creative, and innovative solutions. Developing a joined and unified database of classification systems, design methods, and biological information may help make biomimicry more accessible to researchers and designers.

While sustainability is stately included in some biomimicry-related terminologies and definitions, the paper has revealed that not all cases address this. In some instances, biomimicry-related keywords are directed at symbolic associations; in others, terms are explicitly used to describe technological aspects. The general agreement amongst scholars is that biomimicry is built on two key components: drawing lessons from nature and using them to develop practical solutions to human design problems. The well-rounded nature of biomimicry approaches pushes it in numerous directions, creating distinctive architectural implementations. In the current built environment, biomimicry has been used to drive a natural ventilation system, provide passive control systems, and ameliorate aesthetic components. However, the field is still relatively new and abstract. Thus, further research is needed to reveal biomimicry's specifics and hidden possibilities.

In conclusion, the building sector must become environmentally conscious and actively reduce its contribution to global climate change. By taking nature as the source of inspiration, biomimicry is suitable for addressing these urgent demands. However, biomimicry still needs unification in methodology and research, underscoring the need for further investigation. Consistent terminology will help increase accessibility and efficiency in research, thus prompting development in the field, generating more design solutions that may contribute to the reduction of climate change. Having a shared understanding of terms will ease the process of spreading information; this will then increase awareness. Finally, though biomimicry does not guarantee a high level of sustainability, it can do so and go beyond the reduction of negatives by optimizing positives with regenerative solutions.

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■ References

1. El Sheikh, S. *CO2 emissions from buildings and construction hit new high, leaving sector off track to decarbonize by 2050*. UN. UN Environment. <https://www.unep.org/news-and-stories/press-release/co2-emissions-buildings-and-construction-hit-leaving-sector>.

2. Pawlyn, M. *Biomimicry in Architecture*; Routledge, 2019.
3. Verbrugghe, N.; Rubinacci, E.; Khan, A. Z. Biomimicry in Architecture: A Review of Definitions, Case Studies, and Design Methods. *Biomimetics* **2023**, *8* (1), 107. <https://doi.org/10.3390/biomimetics8010107>.
4. Verma, S. K.; Punekar, R. M. Gaining Insights into the Creative Process of Designing Nature Inspired Product Forms. *International Journal of Technology and Design Education* **2022**. <https://doi.org/10.1007/s10798-022-09749-y>.
5. Benyus, J. M. *Biomimicry: Innovation Inspired by Nature*; Perennial: New York, NY, 2002.
6. eco- | *Meaning of prefix eco- by etymonline*. www.etymonline.com. <https://www.etymonline.com/word/eco->.
7. Chayaamor-Heil, N.; Hannachi-Belkadi, N. Towards a Platform of Investigative Tools for Biomimicry as a New Approach for Energy-Efficient Building Design. *Buildings* **2017**, *7* (4), 19. <https://doi.org/10.3390/buildings7010019>.
8. Vincent, J. F. V.; Bogatyreva, O. A.; Bogatyrev, N. R.; Bowyer, A.; Pahl, A.-K. Biomimetics: Its Practice and Theory. *Journal of the Royal Society, Interface* **2006**, *3* (9), 471–482. <https://doi.org/10.1098/rsif.2006.0127>.
9. Yuan, Y.; Yu, X.; Yang, X.; Xiao, Y.; Xiang, B.; Wang, Y. Bionic Building Energy Efficiency and Bionic Green Architecture: A Review. *Renewable and Sustainable Energy Reviews* **2017**, *74*, 771–787. <https://doi.org/10.1016/j.rser.2017.03.004>.
10. Schreiner, W. Biomimicry: *A History* | eHISTORY. osu.edu. <http://ehistory.osu.edu/exhibitions/biomimicry-a-history>.
11. *Architectural Design Theme: BIOINSPIRATION*. issuu.com/banush.shyqeriu/docs/design_studio_3b_bioinspiration_-_portfolio_2021/s/12623421 (accessed 2023-04-18).
12. Nkandu, M.; Alibaba, H. *Biomimicry as an Alternative Approach to Sustainability*. Research Gate. https://www.researchgate.net/profile/Halil-Alibaba/publication/323573055_Biomimicry_as_an_Alternative_Approach_to_Sustainability/links/5a9e65e70f7e9bc35fd01e94/Biomimicry-as-an-Alternative-Approach-to-Sustainability.pdf (accessed 2023-04-17).
13. Hehenkamp, M. BIOMIMICRY as a TOOL for SUSTAINABLE ARCHITECTURAL DESIGN towards MORPHOGENETIC ARCHITECTURE. www.academia.edu.
14. Brebbia, C. A. *Design & Nature IV: Comparing Design in Nature with Science and Engineering*; WIT Press, 2008.
15. Faragalla, A. M. A.; Asadi, S. Biomimetic Design for Adaptive Building Façades: A Paradigm Shift towards Environmentally Conscious Architecture. *Energies* **2022**, *15* (15), 5390. <https://doi.org/10.3390/en15155390>.
16. Maibritt Pedersen Zari. *Biomimetic approaches to architectural design for increased sustainability*. Sustainable Building Conference (SB07), Auckland, New Zealand. https://www.academia.edu/9509268/Biomimetic_approaches_to_architectural_design_for_increased_sustainability.
17. Othmani, N. I.; Mohamed, S. A.; Abdul Hamid, N. H.; Ramlie, N.; Yeo, L. B.; Mohd Yunos, M. Y. Reviewing Biomimicry Design Case Studies as a Solution to Sustainable Design. *Environmental Science and Pollution Research* **2022**, *29* (46), 69327–69340. <https://doi.org/10.1007/s11356-022-22342-z>.
18. Kuru, A.; Oldfield, P.; Bonser, S.; Fiorito, F. Biomimetic Adaptive Building Skins: Energy and Environmental Regulation in Buildings. *Energy and Buildings* **2019**, *205*, 109544. <https://doi.org/10.1016/j.enbuild.2019.109544>.
19. Jamei, E.; Vrcelj, Z. Biomimicry and the Built Environment, Learning from Nature's Solutions. *Applied Sciences* **2021**, *11* (16), 7514. <https://doi.org/10.3390/app11167514>.
20. *Explore the Lotus Temple's Architecture Style and History*. MasterClass. <https://www.masterclass.com/articles/lotus-temple-design-a-nd-history-explained> (accessed 2023-05-06).
21. Mohammed Sahil. Case Study on Architecture of Lotus Temple. *International Journal of Engineering Research and* **2020**, *V9* (05). <https://doi.org/10.17577/ijertv9is050907>.
22. Küçük, M.; Ibrahim Arslan, H. Investigation of Diagrid Structures over Gherkin Tower. *Proceedings Article* **2020**. <https://doi.org/10.38027/n12020iccaa316291>.
23. Pearce, M. *Eastgate Building Harare*. mickpearce.com. <https://www.mickpearce.com/Eastgate.html>.
24. Zillante, G.; Pullen, S.; Wilson, L.; Davidson, K.; Chileshe, N.; Zuo, J.; Arman, M. <https://core.ac.uk/download/pdf/79077468.pdf#page=247>.
25. Cruz, E.; Hubert, T.; Chancoco, G.; Naim, O.; Chayaamor-Heil, N.; Cornette, R.; Menezo, C.; Badarnah, L.; Raskin, K.; Aujard, F. Design Processes and Multi-Regulation of Biomimetic Building Skins: A Comparative Analysis. *Energy and Buildings* **2021**, *246*, 111034. <https://doi.org/10.1016/j.enbuild.2021.111034>.
26. Saluja, T. *Understanding Biomimicry The three levels of Mimicry*. R TF | Rethinking The Future. <https://www.re-thinkingthefuture.com/2020/07/15/a1285-understanding-biomimicry-the-three-levels-of-mimicry/#:~:text=THE%20THIRD%20LEVEL%3A%20ECOSYSTEM>. (accessed 2023-05-01).
27. Pedersen Zari, M.; Hecht, K. Biomimicry for Regenerative Built Environments: Mapping Design Strategies for Producing Ecosystem Services. *Biomimetics* **2020**, *5* (2), 18. <https://doi.org/10.3390/biomimetics5020018>.
28. *soma architecture - theme pavilion*. www.soma-architecture.com. https://www.soma-architecture.com/index.php?page=theme_pavilion&parent=2# (accessed 2023-05-01).
29. *One Ocean - Pavilion EXPO 2012 | Transsolar | KlimaEngineering*. transsolar.com. <https://transsolar.com/projects/one-ocean-pavillon-expo-2012>.
30. Blanco, E., Pedersen Zari, M., Raskin, K., & Clergeau, P. Urban Ecosystem-Level Biomimicry and Regenerative Design: Linking Ecosystem Functioning and Urban Built Environments. *Sustainability*, *13*(1), 404. <https://doi.org/10.3390/su13010404>.
31. Hayter, J. A. (2005). Lloyd Crossing Sustainable Urban Design Plan and Catalyst Project - Portland, Oregon [2005 EDRA/Places Award -- Planning]. Retrieved from <https://escholarship.org/content/qt53g4r22c/qt53g4r22c.pdf>.
32. Murtagh, N.; Roberts, A.; Hind, R. The Relationship between Motivations of Architectural Designers and Environmentally Sustainable Construction Design. *Construction Management and Economics* **2016**, *34* (1), 61–75. <https://doi.org/10.1080/01446193.2016.1178392>.
33. Ryan, R. M.; Deci, E. L. Self-Determination Theory and the Facilitation of Intrinsic Motivation, Social Development, and Well-Being. *American Psychologist* **2000**, *55* (1), 68–78. <https://doi.org/10.1037/0003-066x.55.1.68>.

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Screening Of Naturopathic Hits in The Treatment of Receptor Tyrosine Kinase Mediated Cancers

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ABSTRACT: Cancer is one of the most prevalent diseases and encompasses three main points of treatment: Cure, Control, and Palliation. The control stage of various cancer treatments often leads to severe side effects and significantly increases costs due to the extensive resources needed. This study used initial region-based dietary factors and current naturopathic data to develop an effective, safe, and accessible pharmaceutical for cancer therapy. Six compounds were found through region-based statistics and molecular pathways, with Receptor Tyrosine Kinase (RTK) being identified as the primary pathway. Currently, many RTK Inhibitors (RTKIs) are expensive and contribute to various side effects, limiting their use in different regions worldwide. Therefore, two modeling software programs, AutoDock-Vina and Desmond, were employed to compare the probability of natural metabolites inhibiting RTKs. The metabolite EGCG demonstrated convincing binding possibilities to the active sites, offering a potential route to inhibit their cancerous functions. Comparing natural metabolites with RTKIs revealed strong correlations between binding affinity and active site placements. The novel method, along with its various results, demonstrates the potential for fostering further pharmaceutical development. This study aims to translate the primary findings into an effective treatment that can be accessible and widely used around the globe.

KEYWORDS: Computational Biology and Bioinformatics; Computational Pharmacology; Cancer Treatment; Natural Metabolites; Receptor Tyrosine Kinases; High Throughput Screening.

■ Introduction

Background:

Cancer is the leading cause of death worldwide, accounting for one-sixth of all global mortalities.¹ It is characterized by the accumulation of mutations within DNA, resulting in abnormal cell growth and tumor formation in most patients. These mutations can take various paths to become oncogenic, with a significant portion identified in cancer patients arising from misfolded proteins. Specifically, mutations occurring during the transcription or translation steps produce dysfunctional or nonfunctional proteins.² Proteins are present throughout the human body, playing crucial roles in various cell signaling pathways. Cell signaling pathways encompass a multitude of pathways found in the human body, facilitating the reception and response to signals. The first stage of cell signaling pathways, the reception stage, is mediated by a class of proteins called cell receptors. These receptors are located within or on the surface of cells and serve as the regulatory component of signaling pathways. They relay signals to activate specific transduction stages. Mutations in these receptor proteins can disrupt proper regulation and contribute to the development of various forms of cancer.³ Cell surface receptors are divided into three categories, each with its own reception stage and functional properties. The first category is G Protein-Coupled Receptors, cell-surface receptors with diverse human peripheral nervous system functions. The second category is Ion Channel Receptors, which primarily regulate the flow of ions to maintain physiological equilibrium in systems such as digestion, cognition, and the heart. Lastly, Receptor Tyrosine Kinases

(RTKs) are transmembrane proteins that function as protein kinases. They work in pairs and dimerize upon activation, allowing them to regulate highly specific cellular functions.⁴

Common Knowledge:

Unhealthy dietary factors play a significant role in cancer development worldwide. The consumption of substances such as glucose and refined carbohydrates, which promote rapid cell growth, is strongly associated with an increased risk of cancer.⁵ Therefore, one identified approach to significantly reduce the risk of cancer is to adopt diets that are low in substances promoting cell growth while emphasizing the consumption of foods that are low in calories and fat and rich in phytochemicals and antioxidants.⁶ By avoiding unhealthy foods, individuals can lower their chances of developing cancer. Furthermore, certain foods containing specific chemicals and metabolites have demonstrated significant roles in cancer regulation and prevention. These foods are highly recommended as part of daily diets due to their potential benefits in combating cancer.⁷

Project Objective:

This project aimed to identify naturally occurring metabolites that exhibit strong capabilities in inhibiting the functions of misfolded cell receptors during cancer's prevention and therapy stages. Established data on foods and chemical types with potential cancer-preventive properties were cross-referenced to achieve this. Each subcategory was thoroughly examined to understand their involvement in cell signaling pathways and chemical interactions within the human body. Pathways associated with Receptor Tyrosine Kinases (RTK) were specifically chosen due to their frequent malfunction in cancer cases.⁸ The

identified chemical types were then cross-tested against RTKs and existing FDA-approved chemotherapy options. The collected data were analyzed to identify potential candidates for new chemotherapy drugs. The goal was to discover alternatives that could provide therapeutic benefits similar to existing drugs but with reduced costs and minimize side effects. The findings aimed to contribute to developing improved chemotherapy options for cancer treatment.

Project Pathogenesis:

Cancer can manifest in various ways within the human body, affecting multiple pathways and organ systems. This project focuses explicitly on targeting malfunctioning and carcinogenic cells during the secondary stage of cancer treatments. To identify potential targets, empirical studies on cancer pathways were analyzed. Cancer pathways involving Receptor Tyrosine Kinases (RTKs) were chosen among the three common types of receptors due to their widespread association with cancers. RTKs are necessary for diverse processes such as proliferation, differentiation, gene transcription, and metabolic regulation.⁸ RTKs are activated by ligands, signaling molecules that bind to the protein's active site, initiating transduction pathways. They are the most complex receptor type in the body, with various forms and intricate negative feedback loops that regulate their activity. This complexity in structure and function contributes to a wide range of complications, including altered gene transcription and feedback regulation. RTKs are a class of transmembrane proteins with enzymatic activity, catalyzing the phosphate group transfer from Adenosine Triphosphate (ATP) to tyrosine residues. The extracellular region of RTKs is known as the ligand-binding domain, responsible for binding and signaling ligands, while the cytoplasmic regions contain the tyrosine kinase region.⁹ This study focused on vascular endothelial growth factor receptor (VEGFR) for testing purposes due to its significant role in cancer development, promising preliminary data, and specific functional factors. VEGFR is typically present in endothelial, glial, and smooth muscle cells. Mutations in the P53 gene have been observed to cause misfolding and amplification of VEGFR, commonly associated with glioma, lung, and breast cancers. A positive feedback loop exists within VEGFR, where P53 relies on VEGF expression for activation. Mutations in both VEGFR and P53 lead to rapid progression and malfunction. It has been shown that VEGF expression and overexpression can increase the expression of STAT3 and MYC, resulting in unique cell growth patterns.¹⁰ Finally, due to VEGFRs previously discussed prevalence in cancer models, proper documentation and files are present in *In-Silico* databases.

Materials and Methods

Screening Tools:

In-silico docking techniques have received significant attention for their advanced approaches, simulations, and software programs. These molecular dynamic software programs compare and analyze interactions between ligands and proteins. Each program utilizes different datasets, including binding efficiencies, distances, conformability, stability, and other factors relevant to ligand-protein interactions. In this study, two software programs, Autodock Vina and Desmond, were uti-

lized with various visual programs to finalize the data analysis process. These software programs were crucial in examining and understanding the research context's interactions between ligands and proteins.

Autodock Vina:

Autodock Vina, separate from Autodock 4, is widely recognized as one of the field's most commonly used modeling software programs. Its popularity stems from its user-friendly interface and accessibility. The program employs a scoring function that enables the prediction of noncovalent binding between ligands and proteins. By predicting and analyzing these interactions, users can screen many drugs and ligands without a physical laboratory setup by predicting and analyzing these interactions. Gibbs free energy is involved during the binding process. Autodock utilizes the intermolecular part of the lowest-scoring conformation of the protein-ligand interaction to provide the Gibbs free energy estimate. Various optimization algorithms are employed to ensure accurate data generation. The final results from Autodock Vina include the Gibbs free energy values, which indicate the likelihood of interaction and the distances from specific bond locations. The software is typically configured to provide the top eight binding probabilities based on free energy calculations. It requires a minimum of thirty minutes for preparation and running, offering relatively quick results and generating a larger dataset than a traditional laboratory experiment. It's important to note that Autodock Vina is a computational dynamics software; its results are only considered partially accurate due to potential inconsistencies between the software and the natural environment. Therefore, caution should be exercised in the interpretation of the results obtained.

Desmond:

Schrödinger's molecular dynamics software, Desmond 2022-3, is renowned for its capability to simulate and analyze a wide range of data points. It stands out as a powerful tool due to its ability to configure a cellular environment that closely resembles real cells. Desmond can replicate real-world pathways by considering structural changes influenced by environmental factors, such as the cell's cytoplasm. It considers solvent concentrations, the thermodynamic environment, and pressure gradients for a comprehensive analysis. The program utilizes real-world variables and compares the positions and velocities of the initial ligand atoms over a time frame measured in nanoseconds. It also considers the molecular mechanic's force field for accurate modeling. Desmond encompasses six key areas of investigation: Forces, Particles, Force fields, Space, Time, and Dynamics. This comprehensive approach enables Schrödinger's software to produce highly accurate results, making it a valuable tool in drug discovery processes. Due to the computational complexity, each simulation in Desmond requires a considerable amount of time to complete and run, typically around 7 hours. The need for meticulous analysis of each nanosecond of a simulation further limits the number of Desmond trials that can be performed. Once a simulation is finished, Desmond provides a detailed analysis spanning 12 pages, covering various aspects such as amino acid interactions, stability, time progression, ligand and protein movement, and

binding confirmation. This extensive analysis aids in drawing in-depth conclusions from the simulation results.

Initial Literature Compounds Identification:

The initial chemical compounds used in this study were identified based on information from the American Institute for Cancer Research (AICR) and empirical studies highlighting their relevance to similar processes within the human body. These compounds belonged to prominent chemical families and served as the foundation for conducting in-depth investigations in the later stages of the study. Furthermore, these chemical compounds were selected based on their relevance to cancer prevention and their presence in natural sources. The study aimed to investigate the potential inhibitory effects of malfunctioning cell receptors and their interactions within cellular pathways. By selecting these compounds, the researchers sought to focus on substances with potential cancer-preventive properties and explore their effects and interactions in greater detail.

1. Flavan-3-ols: Flavan-3-ols are a family of compounds found in many fruits and teas. They are single-molecule monomers with a molecular formula of $C_{15}H_{14}O_5$.¹¹

2. Tetrahydrocurcumin: Tetrahydrocurcumin belongs to the curcuminoid family of compounds and is a natural curcumin derivative. It has a structure derived from a beta-diketone and is classified as a polyphenol and diarylheptanoid. Its chemical formula is $C_{21}H_{24}O_6$.¹²

3. Curcumin: Curcumin is a compound found in the Zingiberaceae family, most commonly extracted from turmeric. It is a single-molecule monomer classified as a polyphenol and exhibits antioxidant properties. Its chemical formula is $C_{21}H_{20}O_6$.¹³

4. Chlorogenic Acid: Chlorogenic acid is a family of compounds that refers to different esters. It is found in various fruits and herbs and is a small organic oxygen compound. Its structure is representative of aromatic homocyclic compounds. Chlorogenic acid has a chemical formula of $C_{16}H_{18}O_9$.¹⁴

5. Coumarin: Coumarin belongs to a class of compounds with a benzene structure. It is an aromatic organic chemical compound found in various plants and herbs. Due to its small molecular weight and size, it can act as a target for inhibition within cell signaling pathways. Coumarin has a molecular formula of $C_9H_6O_2$.¹⁵

6. L-theanine: L-theanine is an amino acid derivative commonly found in teas and mushrooms. It is closely related to the amino acid glutamate. L-theanine is primarily associated with the regulation of brain functions. It has a chemical formula of $C_9H_{11}NO_2$.¹⁶

In-Silico Compound Identification:

After screening the chemical groups using Autodock with the protein VEGFR, the data ranked the family classes in the following order of effectiveness: 1. Flavan-3-ols, 2. Tetrahydrocurcumin, 3. Curcumin, 4. Chlorogenic Acid, 5. Coumarin, 6. L-theanine. Although initial empirical studies suggest promising results for all six chemical groups, the analysis disproved this hypothesis as none showed a high affinity towards the active site of the RTK VEGFR. As a result, the focus of the study shifted to the chemical families with the highest affinity

towards the active site and the strongest binding affinities. The chemical compounds curcumin and flavan-3-ols were proposed for further investigation. They exhibited the greatest affinity towards the active site with three poses within 7 Å and the highest binding affinity resulting in increased entropy during simulation. Within the category of teas, the study identified a subclass known as catechins, which are natural phenols and antioxidants belonging to the flavan-3-ols family. One prominent catechin is Epigallocatechin gallate (EGCG), commonly found in green tea. EGCG is a single-molecule compound with various expressions and regulations in cell signaling pathways. The study then focused on studying the most prominent cell signaling pathways associated with EGCG, utilizing empirical studies and Kegg pathways for analysis.

Autodock Compound Comparison against RTKI Lenvatinib:

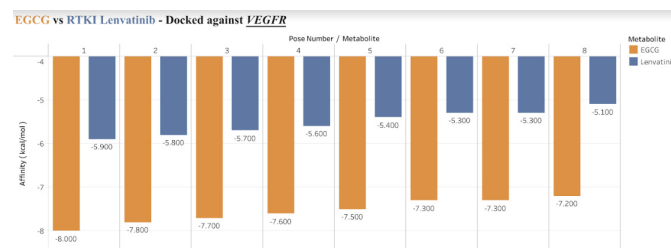


Figure 1: Gibbs Free Energy for EGCG vs. Lenvatinib; EGCG outperforms RTKI Lenvatinib in all eight binding poses provided by Autodock.

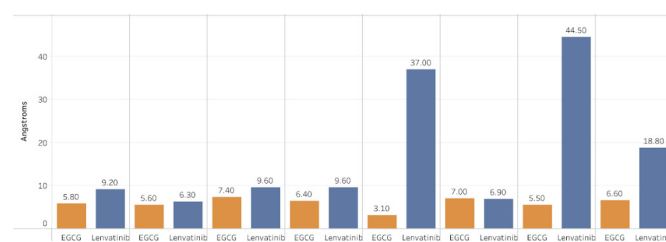


Figure 2: Bond Distance from Active Sight for EGCG vs. Lenvatinib; EGCG exhibits binding positions closer and more consistent towards the active sight over the eight binding poses compared to RTKI Lenvatinib.

The analysis of VEGFR revealed that it functions as a heterodimer requiring dimer formation for activation. The active site, located towards the north of the protein, serves as the binding site for both tested molecules. Lenvatinib exhibited most of its poses anchored towards the labeled active sites, although a few poses were found to bind through alternative means. On the other hand, EGCG demonstrated a 100% placement rate within the active site amino acids, indicating a strong affinity for the binding site. Furthermore, the relative position of the molecules within the active site was examined, with EGCG showing closer proximity to the identified active site amino acids compared to Lenvatinib, as shown in Figure 2. The binding affinity analysis supported this position within the active site, which revealed that EGCG exhibited a considerably stronger attraction than Lenvatinib, as indicated by the Gibbs free energy, as shown in Figure 1. When comparing the visual binding conformability, it was seen that both metabolites bind in the

same region, supporting proper activation placement. However, the results overwhelmingly favored EGCG in terms of its binding characteristics and affinity towards the active site. These findings provide compelling evidence for the need to conduct further molecular dynamics testing with EGCG as a potential therapeutic candidate.

Demond Molecular Dynamics Compound Comparison against RTKI Lenvatinib:

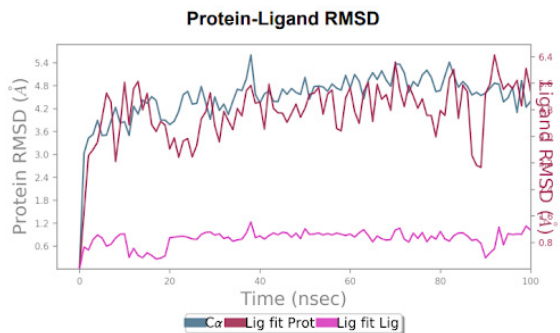


Figure 3: Root Mean Square Deviation for EGCG; EGCG showcases stable binding positions over 100 nanoseconds.

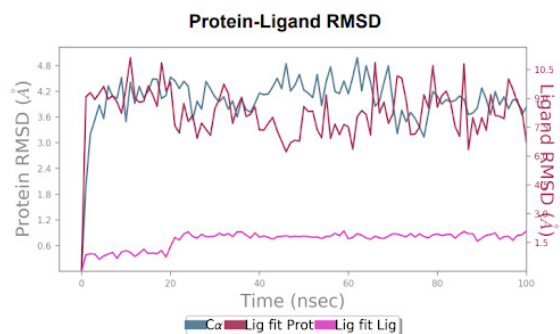


Figure 4: Root Mean Square Deviation for Lenvatinib; RTKI Lenvatinib highlights stable binding positions similar to EGCG, further proving EGCG's effectiveness.

Desmond, a powerful ligand-protein simulation tool, was used in this study to obtain in-depth and correct results. The docking in Desmond was performed using reference poses identified within Autodock Vina, with each simulation being approximately 12 times longer than the average Autodock trial. This precision simulation in Autodock was necessary before transferring the results to Desmond. Desmond provided root mean square deviation (RMSD) results for each simulation, although only four were analyzed and discussed in detail. These data sets comprehensively analyzed various key points related to ligand-protein interactions.

The analysis of VEGFR ligand-protein contacts showed a substantial overlap between EGCG and Lenvatinib, highlighting their efficacy in inactivating the active site through direct blocking. Within EGCG, amino acids Valine 21, Glutamic Acid 95, Asparagine 92, Leucine 99, and Serine 97 were identified within the active site region and exhibited interactions with EGCG and VEGFR. Glutamic Acid formed a direct hydrogen bond, while other amino acids participated in water-mediated bridges induced by the solvent-filled environment. Similar results were observed for Lenvatinib,

with amino acids Serine 97 and Asparagine 92 forming direct active site hydrogen bonds and Leucine 11 and Arginine 13 forming water-mediated bridges. The root mean square fluctuation (RMSF) analysis of the protein and ligand demonstrated effective stability by the end of the 100 nanoseconds of testing. A comparison between EGCG and Lenvatinib revealed that EGCG exhibited greater stability with minimal ligand or protein movement, while Lenvatinib showed more significant movement. This difference could be attributed to the time constraints of the simulations, with more extended tests potentially revealing greater stability. Nonetheless, it was clear that EGCG reached a stable reference pose faster than Lenvatinib. Protein-ligand contacts further supported the notion of proper inhibition, as bonds were formed with active site amino acids throughout the simulation. Overall, the simulation results obtained through Desmond provided valuable insights into the interactions between the ligands (EGCG and Lenvatinib) and the VEGF protein. These findings further support current *in-vitro* studies for the potential of EGCG as a promising candidate for further investigation as an inhibitor of VEGFR.¹⁷

Conclusion

This study aimed to identify a natural metabolite that shares analogous features with FDA-approved receptor tyrosine kinase inhibitors (RTKIs) by employing two different ligand interaction software tools to analyze various aspects of ligand-protein interactions. The focus was on a subclass of cell receptor proteins called RTKs, which play a role in cancer regulation. The study utilized chemical compounds from subclasses identified by AICR and subjected them to investigations using Autodock Vina, Desmond, and drug efficacy measurements. EGCG emerged as the most promising metabolite for further investigation among the compounds tested. Contrary to initial expectations based on the effectiveness of its family class, EGCG exhibited results supporting its efficiency, comparable to the tested RTKIs. The study observed highly effective inhibition rates of EGCG, making it a compelling compound for drug investigation and potential modification for use. The identification of EGCG as a potential candidate opens avenues for further research and potential optimization to enhance its drug-like properties that must be further tested in clinical studies. *In-silico* software cannot fully replicate *in-vitro* and *in-vivo* environments and must be validated. Overall, this study highlights EGCG as a metabolite with promising inhibitory properties similar to FDA-approved RTKIs.

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References

1. "Cancer." World Health Organization, www.who.int/health-topic/cancer#tab=tab_1.
2. "The Genetics of Cancer." National Cancer Institute, www.cancer.gov/about-cancer/causes-prevention/genetics.
3. Panel, Author links open overlay, and AbstractSignal transduction describes how a cell receives a signal. "Cell Signaling Events." Goodman's Medical Cell Biology (Fourth Edition), 10 July 2020, ww

- www.sciencedirect.com/science/article/abs/pii/B9780128179277000090?via%3Dihub.
4. Chen Yunfeng. "Receptor-Mediated Cell Mechanosensing." *Molecular Biology of the Cell*, 7 Nov. 2017, www.ncbi.nlm.nih.gov/pmc/articles/PMC5687017/.
 5. Donaldson, Michael S. "Nutrition and Cancer: A Review of the Evidence for an Anti-Cancer Diet." *Nutrition Journal*, 20 Oct. 2004, www.ncbi.nlm.nih.gov/pmc/articles/PMC526387/.
 6. Tsai, Hsueh-Han "The Risk of Breast Cancer between Western and Mediterranean dietary patterns." *MDPI*, 25 Apr. 2023, www.mdpi.com/2072-6643/15/9/2057.
 7. Anand, Preetha "Cancer Is a Preventable Disease That Requires Major Lifestyle Changes." *Pharmaceutical Research*, Sept. 2008, www.ncbi.nlm.nih.gov/pmc/articles/PMC2515569/.
 8. Du, Zhenfang, and Christine M Lovly. "Mechanisms of Receptor Tyrosine Kinase Activation in Cancer." *Molecular Cancer*, 19 Feb. 2018, www.ncbi.nlm.nih.gov/pmc/articles/PMC5817791/.
 9. JH; Hubbard SR; Till. "Protein Tyrosine Kinase Structure and Function." *Annual Review of Biochemistry*, pubmed.ncbi.nlm.nih.gov/10966463/. Accessed 11 June 2023.
 10. Shibuya, Masabumi. "Vascular Endothelial Growth Factor (VEGF) and Its Receptor (VEGFR) Signaling in Angiogenesis: A Crucial Target for Anti- and pro-Angiogenic Therapies." *Genes & Cancer*, Dec. 2011, www.ncbi.nlm.nih.gov/pmc/articles/PMC3411125/.
 11. JA; Aron PM; Kennedy. "Flavan-3-Ols: Nature, Occurrence and Biological Activity." *Molecular Nutrition & Food Research*, pubmed.ncbi.nlm.nih.gov/18081206/. Accessed 11 June 2023.
 12. Lee, Wing-Hin, "Curcumin and Its Derivatives: Their Application in Neuropharmacology and Neuroscience in the 21st Century." *Current Neuropharmacology*, July 2013, www.ncbi.nlm.nih.gov/pmc/articles/PMC3744901/.
 13. "Curcumin." National Center for Biotechnology Information. PubChem Compound Database, pubchem.ncbi.nlm.nih.gov/compound/Curcumin. Accessed 11 June 2023.
 14. Smeriglio, Antonella "Proanthocyanidins and Hydrolysable Tannins: Occurrence, Dietary Intake and Pharmacological Effects." *British Journal of Pharmacology*, June 2017, www.ncbi.nlm.nih.gov/pmc/articles/PMC5429339/.
 15. Napolitano HB; Silva M; Ellena J; Rodrigues BD; Almeida AL; Vieira PC; Oliva G; Thiemann OH; "Aurapten, a Coumarin with Growth Inhibition against Leishmania Major Promastigotes." *Brazilian Journal of Medical and Biological Research = Revista Brasileira de Pesquisas Medicas e Biologicas*, pubmed.ncbi.nlm.nih.gov/15558191/. Accessed 11 June 2023.
 16. Saeed, Muhammad "L-Theanine: An Astounding Sui Generis Amino Acid in Poultry Nutrition." *Poultry Science*, Nov. 2020, www.ncbi.nlm.nih.gov/pmc/articles/PMC7647716/.
 17. Basini, Giuseppina, et al. "EGCG, a Major Component of Green Tea, Inhibits VEGF Production by Swine Granulosa Cells." *BioFactors*, vol. 23, no. 1, 2005, pp. 25–33, https://doi.org/10.1002/biof.5520230104.

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Remediation of Soils Contaminated with Per- and Polyfluoroalkyl Substances (PFAS): A Review

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ABSTRACT: Per- and polyfluoroalkyl substances (PFAS) are man-made chemicals extensively used worldwide in numerous applications and, therefore, are continuously being introduced to the environment through various anthropogenic activities. This is problematic since PFAS are characterized by their long-term persistence in the environment, and concerns have been growing regarding their toxicity to human and ecological health. Here, we present the different pathways whereby PFAS contaminates soils and the various applicable remediation methods. These can be grouped into three categories: Immobilization, mobilization, and degradation. Immobilization approaches trap PFAS within the soils, reducing their mobility and bioavailability. Mobilization techniques enhance the mobility of PFAS from the soil into another medium (e.g., water, sorbents, plants, etc.). Degradation methods involve the partial or complete destruction of PFAS in place. Future studies should consider investigating hybrid methods (i.e., coupling two or more approaches), such as the *in-situ* mobilization of PFAS coupled with either immobilization, thermal destruction or chemical oxidation. Such combined remediation approaches might result in increasing treatment efficiencies and decreasing costs.

KEYWORDS: Environmental Engineering; Soil Remediation; Immobilization; Degradation; Mobilization.

■ Introduction

Per- and polyfluoroalkyl substances (PFAS) are classified as “Fluorochemicals,” which is a general term that describes a universe of organic and inorganic substances that contain at least one fluorine atom (F).^{1, 2} A subgroup of fluorochemicals contains the perfluoroalkyl fraction C_nF_{2n+1} (n is a positive integer), whereby all of the hydrogen atoms (H) on the carbon atoms (C) have been replaced by F atoms. Consequently, these compounds are referred to as “perfluoroalkyl and polyfluoroalkyl substances”, abbreviated as PFAS.³ For perfluoroalkyl substances, all carbon atoms in the chain are fully saturated with fluorine atoms. Hence, only C–F bonds exist, while in the case of polyfluoroalkyl substances, the carbon chain is saturated mostly with fluorine since it contains some C–H bonds, as shown in Figure 1. The partial or fully fluorinated alkyl chain is usually connected to a functional group, such as carboxylates, sulfonates, sulphonamides, phosphonates, and alcohols.^{2, 3}

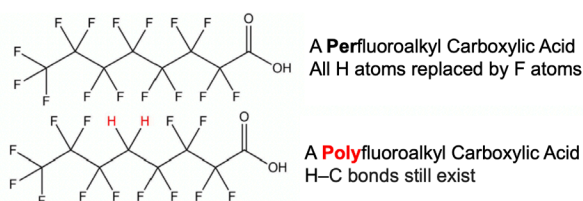


Figure 1: Difference between perfluoroalkyl and polyfluoroalkyl substances for the case of fluoroalkyl carboxylic acid.

Since their invention in the 1930s, PFAS have been widely used in numerous industrial and commercial applications and were the main ingredients in nonstick and waterproof coatings.⁴

Currently, PFAS are extensively used for the production of various commercial and industrial products,⁴ including, but not limited to, textile stain and soil repellents, food-contact paper, coatings, paints, personal care products, surfactant agents, and aqueous film-forming foams (AFFFs). Buck, *et al.*³ thoroughly explained the terminology, history, and structure of PFAS. The most detected compounds of PFAS in environmental systems are PerFluoroOctane Sulfonic acid (PFOS, $C_8HF_{17}O_3S$) and PerFluoroOctanoic Acid (PFOA, $C_8HF_{15}O_2$).⁵ This was expected since, among all PFAS compounds, PFOA and PFOS (Figure 2) have been manufactured the longest.⁴

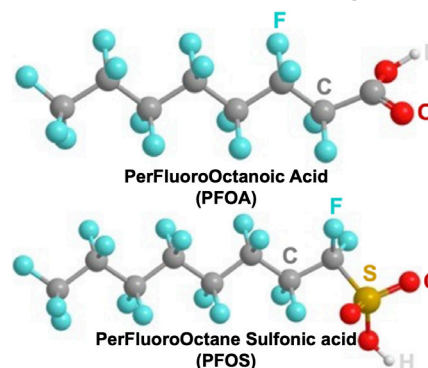


Figure 2: Chemical structure of PFOA and PFOS.

Environmental PFAS contamination is problematic since these chemicals are extremely hard to decompose under normal environmental conditions due to their strong C–F bonds. Hence, they are characterized by their long-term persistence in various ecosystems – sometimes, they are referred to as the

“forever chemicals.” Because PFAS can bioaccumulate in organisms, concerns have been raised regarding their toxicity to human and ecological health.⁶ For instance, the half-life of PFOA and PFOS in human blood ranges from two to five years,⁷ and several illnesses and dangerous side effects have been linked to the presence of PFAS in the human body, including, but not limited to, high cholesterol, lower birth weight, ulcerative colitis, pregnancy-induced hypertension, thyroid disease, testicular cancer, kidney cancer, and decreased response to vaccines, among others.⁶ Such concerns have led the United States Environmental Protection Agency to set a limit on PFAS concentration in water not exceeding 70 parts per trillion (ppt).⁸

Numerous studies and investigations have led to the development of technologies that can remediate PFAS-contaminated ecosystems.⁹⁻¹¹ Most of the current PFAS remediation methods are predominantly used for the treatment of contaminated water (e.g., drinking water, wastewater, groundwater, etc.) and are not readily available for the efficient remediation of PFAS-contaminated soils.^{9, 10, 12} Soil contamination by these fluorinated substances is very problematic, primarily because of the continuous leaching (i.e., release) of PFAS from the polluted soil to the groundwater flow.¹³ Here, we review the occurrence of PFAS in soils, the different treatment techniques that can be adopted for remediating PFAS-contaminated soils, and the possible coupling of different remediation methods for treating PFAS contamination in such sedimentary ecosystems more efficiently and at lower costs.

Occurrence of PFAS in Soils:

Soils can be contaminated with PFAS from various applications or sources, such as the use of aqueous film-forming foams (AFFFs) for fighting fires, effluents from wastewater treatment plants, leachate discharge from landfills, and seepage from contaminated wastes such as biosolids.¹⁴ Depending on the pollution source, PFAS concentrations in soils vary from a few ppt to thousands of parts per billion (ppb).¹⁵

Firefighting Foams:

PFAS from AFFFs are usually introduced to the environment during the storage, handling, use, and post-use cleaning stages.¹⁶ The common release of AFFF occurs during firefighting operations, resulting in a large volume of PFAS entering the environment. Soil contamination from firefighting foams at training sites (Figure 3) has also been reported.¹⁷ In addition, leakage and lateral overflow from provisional ponds, which store AFFF-contaminated water following fire training operations, was found to be an important contamination source.¹⁷ About 26,000 PFAS-contaminated firefighting training sites exist across the United States, impacting millions of people through drinking water contamination.¹⁸



Figure 3: Using AFFFs in firefighting training areas. Source: https://www.flickr.com/photos/marine_corps/8413440131/

Wastewater Effluents and Sludge:

Municipal wastewater usually contains a low concentration of PFAS, which ends up accumulating in sludge after going through the different stages of wastewater treatment plants.^{19,20} Venkatesan and Halden²¹ found a mean concentration in biosolids of 403 ppb and 34 ppb of PFOS and PFOA, respectively, after analyzing samples collected from 94 wastewater treatment plants in the United States. Other studies reported similar findings in sewage sludge from wastewater treatment plants in different countries, such as Switzerland,²² Sweden,²³ Australia,^{19,20} and Nordic countries.²⁴ It is estimated that around 2,000 kg of PFAS ends up every year in agricultural lands in the United States through the use of biosolids from wastewater treatment plants for soil enhancement.²¹

Landfills:

Landfills usually contain various wastes rich in PFAS, such as municipal wastes containing hydrophobic and stain-resistant coatings,²⁰ and industrial wastes composed of fabrics, building, and coating materials.²⁵ Consequently, PFAS can contaminate soils through the release of leachates from landfills, which could also contaminate the surrounding lands if no appropriate lining is in place. Previous studies have found high concentrations of PFAS (> 1,000 ppb) in leachate from landfill sites.²⁶

Remediation Approaches

Immobilization:

Immobilization approaches are by far the most studied and applied for remediating PFAS-contaminated soils.¹⁴ The technique involves the redistribution of PFAS from the porewater solution and sediments to a solid phase, thereby restricting their mobility and bioavailability by preventing the continuous leaching of PFAS from the soil to the groundwater. However, a significant issue with applying such “trapping” methods is that the concentration of PFAS in the soil remains practically unaffected. Therefore, the long-term stability of immobilized PFAS still requires further investigation.¹⁰ The main techniques for immobilizing PFAS in soil are sorption and stabilization/solidification.

Sorption. This involves using a sorbent whereby PFAS adsorbs onto it, thereby restricting their mobility. Materials used for the sorption of PFAS in contaminated soils mainly include carbon-based and clay-based materials, ionic surfactants, and anion exchange resins.²⁷ Various amendments have been tested for immobilizing PFAS in soils by sorption, such as activated carbon,²⁸ organic matter,²⁹ biochar,³⁰ modified clay,³¹ and titanium dioxide,³² among others. Applying a combination of these amendments was also investigated and was found in many cases to enhance PFAS immobilization further.³³

Stabilization/solidification. This is achieved by applying cementitious binders and additives to the contaminated soils.³⁴ The added agents would immobilize contaminants via (1) physical protection, where contaminant leaching is prevented by reducing the hydraulic conductivity of the system, or (2) chemical protection in which contaminants are stabilized by reducing their aqueous solubility through precipitation, redox alteration, and sorption reactions. The stabilization/solidification techniques undertaken *in situ* or *ex situ* are usually

efficient in terms of performance and costs when treating a relatively large area.³⁴

Mobilization:

This technique involves moving the PFAS from the bulky soil into a medium (e.g., water, sorbents, plants, etc.) that can be sequentially treated or properly disposed of. PFAS mobilization from soils is relatively the least studied,¹⁴ and a critical review discussing the mobilization of various hazardous materials (including PFAS) from contaminated soils has been recently published.³⁵ Several approaches exist for mobilizing PFAS from soils.³⁶

Soil flushing. This is conducted *in situ* by pumping water or other suitable aqueous solutions into polluted soils to solubilize contaminants and impurities,³⁷ such as acids,³⁸ heavy metals,³⁹ inorganic ions,⁴⁰ herbicides,⁴¹ polychlorinated biphenyls,⁴² and hydrocarbons.⁴³ Then, the collected water solution is appropriately recycled or disposed of.⁴⁴ Soil flushing is most effective in permeable soils such as sandy soils with low clay contents.^{37, 45} The main advantage of soil flushing is that large quantities of soil can be treated without the need for excavation and transport.^{37, 46}

Soil washing. This method is similar to soil flushing in the sense that the contaminants are mobilized by using water with the possible addition of amendments. Unlike soil flushing, however, soil washing is usually conducted *ex situ*, by excavating the bulk soil from the contaminated setting and transporting it onto a specific site where it first goes through screening (i.e., to remove soils with large particles) and then scrubbing using a water-based process.⁴⁷ *In situ* soil washing can be carried out, however, by using mobile washing plants (Figure 4). Regardless of which water-based application is selected, soil flushing and washing produce a significant amount of contaminated water that must be appropriately treated for reuse or safe disposal. Several water treatment methods can be adopted for decontaminating aqueous solutions after flushing/washing PFAS-contaminated soils, such as sorption, ion exchange, and filtration, among others.⁹ Note that many case studies indicated that soil washing and flushing fails in adequately removing PFAS from the clay fraction, thus creating a secondary waste stream that requires further handling.⁴⁵



Figure 4: Mobile washing plant for treating contaminated soils and sediments. Source: https://www.flickr.com/photos/marine_corps/8413440131/

Phytoremediation. This is a plant-based approach whereby plants are used to remove pollutants or lower their bioavailability in contaminated soils. Phytoremediation involves several mechanisms (Figure 5), which are rhizofiltration (i.e., absorption into the roots), phytostabilization (i.e., adsorption onto the roots), phytoextraction (i.e., removal by plant uptake), phytovolatilization (i.e., conversion of contaminants to volatile form), and phytodegradation (i.e., participation of root exudates and microbial populations in degrading the contaminants). A wide range of organic pollutants can be removed from soils by phytoremediation.⁴⁸ Given that common PFAS are relatively water-soluble,⁴⁹ they can remain dissolved in the soil's pore water and, therefore, can be removed via plant uptake (i.e., phytoextraction and rhizofiltration), whereby PFAS bioaccumulate in the plants without undergoing notable phytodegradation due to the strength and resilience of the C-F bonds.⁵⁰

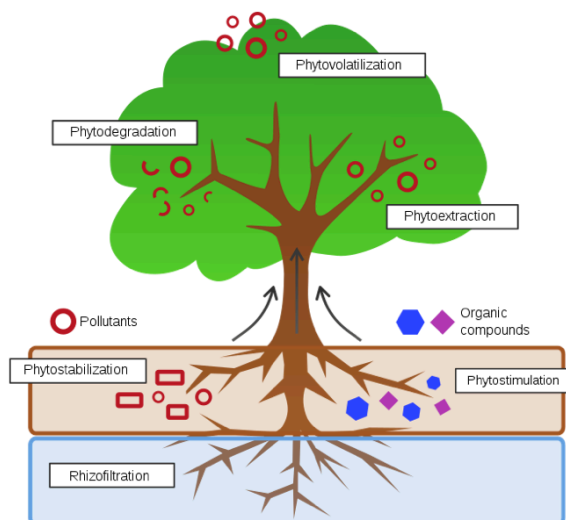


Figure 5: Phytoremediation of contaminated soils.

Source: https://commons.wikimedia.org/wiki/File:Phytoremediation_Process.svg

Electrokinetic remediation. This is a relatively inexpensive mobilization technique whereby charged contaminants migrate towards the electrode of opposite charge under the effect of a low-intensity electric field (i.e., anions towards the anode and cations towards the cathode).⁵¹ This method involves the insertion of electrodes into the contaminated soil and applying an electric current (i.e., 100 to 1,500 mA) and a low voltage (i.e., 20 to 60 V). Electrokinetic remediation can be conducted *in-situ*⁵² and has several reported advantages over other treatment methods, such as low cost and a good ability to treat cohesive soils with a clay content of up to 80%.^{46, 53, 54, 49} Electrokinetics has mainly been used for removing heavy metals (e.g., As, Cu, Pb, and Cr) from soils.⁵⁰ Additional applications include the removal of non-aqueous phase liquids by delivering surfactants and cosolvents.⁵³⁻⁵⁵ Since common PFAS compounds (e.g., PFOA and PFOS) are anionic under normal environmental conditions,⁵⁶ they are expected to migrate toward the positively charged electrode (i.e., the anode).⁵⁴

Soil liquefaction. This method comprises the *in-situ* or *ex-situ* extraction of PFAS from impacted soils by combining foam fractionation and soil liquefaction. Foam fractionation is a well-developed water treatment technology for separating surface-active compounds from a solution by exploiting their affinity to the air-water interface. The process is carried out by injecting air microbubbles into the water and removing the contaminants from the risen bubbles by suction (e.g., OPEC Systems⁵⁷), after which the collected aqueous concentrate is treated. Recently, Buckley, *et al.*⁵⁸ thoroughly reviewed the effectiveness of foam fractionation for treating various pollutants in different types of industries, such as textiles, dyes, heavy metals, proteins in food processing waste, and PFAS. In the case of PFAS-contaminated soils, however, applying foam fractionation requires fluidizing or liquifying the soil to create a slurry, hence the term liquefaction.

Degradation:

This remediation approach involves the removal of PFAS compounds from soils through total or partial destruction, which can be accomplished through bioremediation,⁵⁹ chemical oxidation,⁶⁰ or thermal treatment.⁶¹ Although degradation is particularly attractive since PFAS is totally removed from the environment, it could involve very high costs (e.g., high usage of chemicals for oxidation) and substantial energy usage requirements (e.g., requiring elevated temperatures for complete mineralization of PFAS).

Biodegradation. Microbial degradation can be an effective natural attenuation method to remediate soils and ground-water contaminated with various organic pollutants.⁶² Nevertheless, common PFAS, such as PFOS and PFOA, are strongly resistant to microbial degradation. Some studies, however, identified a few microbial species that can degrade PFAS.^{63, 59} Nonetheless, it is most likely that soils might not contain the appropriate microbial populations that can degrade PFAS, and hence relying on natural attenuation does not appear to be a suitable option for treating PFAS-contaminated soils.¹⁴ Nonetheless, many reports documented the transformation of some fluorinated species into PFAS by microorganisms. These fluorinated compounds are usually reported as precursor PFAS, which includes partially fluorinated compounds, fluorotelomer alcohols, and PFAS with amine head groups, among others. Precursor PFAS can transform into terminal PFAS compounds (e.g., PFOA and PFOS) by microbial degradation.^{63, 59} In such cases, the concentration of terminal PFAS increases due to microbial interactions, but the amount of total PFAS (i.e., precursors and terminal) usually remains the same.^{63, 59}

Chemical treatment. The chemical degradation of PFAS using common water oxidative/disinfection methods, such as chlorination, ozonation, and ultraviolet treatment, is usually ineffective.¹⁴ The removal of PFAS compounds from soil, however, can be achieved through chemical redox reactions.⁶⁰ Nonetheless, such oxidative methods may be practically applied to large-scale remediation of PFAS-contaminated soils under field conditions since they are expensive and result in residual chemicals which may cause environmental degradation and issues with safe disposal.^{14, 64} In addition, the degree of

PFAS defluorination by various advanced oxidation processes remains questionable.¹⁴

Thermal treatment. It involves the partial or total degradation of PFAS while subject to high temperatures, up to 1,000°C, and even higher in some cases.⁶⁵ Unfortunately, this results in substantial operational costs, in addition to the generation of environmentally harmful products of incomplete destruction, such as volatile organofluorine compounds.⁶¹ Therefore, some thermal methods rely on a two-step process whereby PFAS are first separated from the soils at relatively low temperatures (i.e., to volatilize the PFAS at the respective boiling points), after which the resulting flue gas is introduced into an afterburner set to high temperatures (>1,000°C) to destroy the vaporized fluorinated compounds. Generally, there are several processes available to achieve such high temperatures: (1) electrical, conducted *in-situ* and involves constructing a zone encircled by graphite electrodes inserted in the ground which pass energy through the soil, and (2) thermal, conducted *ex-situ* and is generally carried out in a rotary kiln.⁶⁶

Combined Treatment:

Although some of the existing remediation processes are considered effective in treating PFAS-contaminated soils (e.g., immobilization, chemical oxidation, thermal methods, etc.), applying them can be associated with numerous drawbacks (e.g., elevated energy consumption, high costs, extreme operating conditions, etc.). This considerably suppresses their commercialization for *in-situ* applications. Combining treatment processes, however, could alleviate the treatment costs while optimizing the removal efficiency of PFAS from polluted soils by using multiple synergetic technologies simultaneously. Lu, *et al.*⁶⁷ conducted an extensive review of the recently reported water treatment train (i.e., combined processes) studies while discussing their innovative designs, remediation performances, present limits, and possible improvements, in addition to proposing a novel remediation method consisting of three individual technologies, namely, nanofiltration, electrochemical anodic oxidation, and electro-Fenton degradation, to optimize the economic and environmental benefits of treating PFAS-contaminated waters. Unfortunately, such remediation processes are not readily available for treating PFAS-contaminated soils, mainly because the physicochemical interactions of PFAS in sedimentary systems differ from those in aquatic matrices. Consequently, future research should address the applicability of combined treatment methods for efficiently remediating PFAS-contaminated soils with appropriate field applications and cost-to-benefit analyses.

Combining mobilization techniques with immobilization or degradation methods should be particularly studied since such synergy would allow the allocation of PFAS from the contaminated soil into a water solution that can be sequentially treated more efficiently and possibly at a lower cost. For instance, Niarchos, *et al.*⁶⁸ investigated the treatment efficiency of electrokinetic coupled with immobilization by activated carbon when treating an AFFF-impacted soil, which resulted in removing up to 75% of PFAS from the soil. Another combination could be treating PFAS-impacted soils by phytoremediation, then applying thermal conversion of

the PFAS-containing plant part into biochar, such as using pyrolysis,⁶⁹ thus resulting in either the destruction of PFAS compounds or their stabilization onto the resulting carbonaceous matter.

■ Conclusion

The article addressed the common occurrence of PFAS in soils and the various remediation technologies for treating them, which include immobilization, mobilization, and degradation. It was found that conventional techniques for remediating PFAS-contaminated soils, such as soil washing, electrokinetics, sorption, and thermal treatment, can be effective in many scenarios. Still, there are situations where hybrid technologies should be considered. Therefore, investigating the possible combination of *in-situ* mobilization techniques with other remediation methods (i.e., immobilization, thermal destruction, chemical oxidation, etc.) is particularly interesting since it could result in increasing treatment efficiencies while possibly decreasing the costs, thereby enhancing their regulatory acceptance through sound scientific advances and demonstrations while providing more options to decision-makers for treating PFAS-contaminated soils.

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■ References

1. Banks, R. E.; Smart, B. E.; Tatlow, J. *Organofluorine chemistry: principles and commercial applications*; Springer Science & Business Media, 2013.
2. Meegoda, J. N.; Kewalramani, J. A.; Li, B.; Marsh, R. W. A review of the applications, environmental release, and remediation technologies of per- and polyfluoroalkyl substances. *International journal of environmental research and public health* **2020**, *17* (21), 8117. DOI: <https://doi.org/10.3390/ijerph17218117>.
3. Buck, R. C.; Franklin, J.; Berger, U.; Conder, J. M.; Cousins, I. T.; De Voogt, P.; Jensen, A. A.; Kannan, K.; Mabury, S. A.; van Leeuwen, S. P. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. *Integrated environmental assessment and management* **2011**, *7* (4), 513-541. DOI: <https://doi.org/10.1002/ieam.258>.
4. Kissa, E. Fluorinated surfactants and repellents (revised and expanded) (Surfactant science series 97). *New York, NY* **2001**. DOI: <https://doi.org/10.1021/ja015260a>.
5. Abunada, Z.; Alazaiza, M. Y.; Bashir, M. J. An Overview of Per- and Polyfluoroalkyl Substances (PFAS) in the Environment: Source, Fate, Risk and Regulations. *Water* **2020**, *12* (12), 3590. DOI: <https://doi.org/10.3390/w12123590>.
6. Betts, K. PFOS and PFOA in humans: new study links prenatal exposure to lower birth weight. National Institute of Environmental Health Sciences: 2007. DOI: <https://doi.org/10.1289/ehp.115-a550a>.
7. Li, Y.; Fletcher, T.; Mucs, D.; Scott, K.; Lindh, C. H.; Tallving, P.; Jakobsson, K. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. *Occupational and environmental medicine* **2018**, *75* (1), 46-51. DOI: <http://dx.doi.org/10.1136/oemed-2017-104651>.
8. EPA. 2022 *Interim Updated PFOA and PFOS Health Advisories*. 2022. <https://www.epa.gov/sdwa/drinking-water-health-advisories-pfoa-and-pfos> (accessed 2022 13 September).
9. Verma, S.; Varma, R. S.; Nadagouda, M. N. Remediation and mine realization processes for per- and polyfluoroalkyl substances (PFAS) in water: A review. *Science of The Total Environment* **2021**, *794*, 148987. DOI: <https://doi.org/10.1016/j.scitotenv.2021.148987>.
10. Mahinroosta, R.; Senevirathna, L. A review of the emerging treatment technologies for PFAS contaminated soils. *Journal of environmental management* **2020**, *255*, 109896. DOI: <https://doi.org/10.1016/j.jenvman.2019.109896>.
11. Kewalramani, J. A.; de Souza, B. B.; Marsh, R. W.; Meegoda, J. N. Contributions of reactor geometry and ultrasound frequency on the efficiency of sonochemical reactor. *Ultrasonics Sonochemistry* **2023**, *106529*. DOI: <https://doi.org/10.1016/j.ultsonch.2023.106529>.
12. Kewalramani, J. A.; Wang, B.; Marsh, R. W.; Meegoda, J. N.; Freire, L. R. Coupled high and low-frequency ultrasound remediation of PFAS-contaminated soils. *Ultrasonics Sonochemistry* **2022**, *88*, 106063. DOI: <https://doi.org/10.1016/j.ultsonch.2022.106063>.
13. Hale, S. E.; Arp, H. P. H.; Slinde, G. A.; Wade, E. J.; Bjørseth, K.; Breedveld, G. D.; Straith, B. F.; Moe, K. G.; Jartun, M.; Høisæter, Å. Sorbent amendment as a remediation strategy to reduce PFAS mobility and leaching in a contaminated sandy soil from a Norwegian firefighting training facility. *Chemosphere* **2017**, *171*, 9-18. DOI: <https://doi.org/10.1016/j.chemosphere.2016.12.057>.
14. Bolan, N.; Sarkar, B.; Yan, Y.; Li, Q.; Wijesekara, H.; Kannan, K.; Tsang, D. C.; Schauer, M.; Bosch, J.; Noll, H. Remediation of poly- and perfluoroalkyl substances (PFAS) contaminated soils—To mobilize or to immobilize or to degrade? *Journal of hazardous materials* **2021**, *401*, 123892. DOI: <https://doi.org/10.1016/j.jhazmat.2020.123892>.
15. Brusseau, M. L.; Anderson, R. H.; Guo, B. PFAS concentrations in soils: Background levels versus contaminated sites. *Science of the Total Environment* **2020**, *740*, 140017. DOI: <https://doi.org/10.1016/j.scitotenv.2020.140017>.
16. Cousins, I. T.; Goldenman, G.; Herzke, D.; Lohmann, R.; Miller, M.; Ng, C. A.; Patton, S.; Scheringer, M.; Trier, X.; Vierke, L. The concept of essential use for determining when uses of PFASs can be phased out. *Environmental Science: Processes & Impacts* **2019**, *21* (11), 1803-1815. DOI: <https://doi.org/10.1039/C9EM00163H>.
17. Houtz, E. F.; Higgins, C. P.; Field, J. A.; Sedlak, D. L. Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil. *Environmental science & technology* **2013**, *47* (15), 8187-8195. DOI: <https://doi.org/10.1021/es4018877>.
18. Darlington, R.; Barth, E.; McKernan, J. The challenges of PFAS remediation. *The Military Engineer* **2018**, *110* (712), 58. DOI: <https://doi.org/10.1021/es4018877>.
19. Gallen, C.; Eaglesham, G.; Drage, D.; Nguyen, T. H.; Mueller, J. A mass estimate of perfluoroalkyl substance (PFAS) release from Australian wastewater treatment plants. *Chemosphere* **2018**, *208*, 975-983. DOI: <https://doi.org/10.1016/j.chemosphere.2018.06.024>.
20. Gallen, C.; Drage, D.; Kaserzon, S.; Baduel, C.; Gallen, M.; Banks, A.; Broomhall, S.; Mueller, J. Occurrence and distribution of brominated flame retardants and perfluoroalkyl substances in Australian landfill leachate and biosolids. *Journal of hazardous materials* **2016**, *312*, 55-64. DOI: <https://doi.org/10.1016/j.jhazmat.2016.03.031>.
21. Venkatesan, A. K.; Halden, R. U. National inventory of perfluoroalkyl substances in archived US biosolids from the 2001 EPA National Sewage Sludge Survey. *Journal of hazardous materials* **2013**, *252*, 413-418. DOI: <https://doi.org/10.1016/j.jhazmat.2013.03.016>.
22. Sun, H.; Gerecke, A. C.; Giger, W.; Alder, A. C. Long-chain perfluorinated chemicals in digested sewage sludges in Switzerland. *Environmental Pollution* **2011**, *159* (2), 654-662. DOI: <https://doi.org/10.1016/j.envpol.2010.09.020>.
23. Haglund, P.; Olofsson, U. Miljöövervakning av slam: Redovisning av resultat från 2004 och 2005 års provtagningar. Umeå universitet

- itet, kemiska institutionen; Naturvårdsverket: 2009.
24. Kallenborn, R. *Perfluorinated alkylated substances (PFAS) in the Nordic environment*; Nordic Council of Ministers, 2004.
 25. Janousek, R. M.; Lebertz, S.; Knepper, T. P. Previously unidentified sources of perfluoroalkyl and polyfluoroalkyl substances from building materials and industrial fabrics. *Environmental Science: Processes & Impacts* **2019**, *21* (11), 1936-1945. DOI: <https://doi.org/10.1039/C9EM00091G>.
 26. Knutsen, H.; Mæhlum, T.; Haarstad, K.; Slinde, G. A.; Arp, H. P. H. Leachate emissions of short- and long-chain per- and polyfluoroalkyl substances (PFASs) from various Norwegian landfills. *Environmental Science: Processes & Impacts* **2019**, *21* (11), 1970-1979. DOI: <https://doi.org/10.1039/C9EM00170K>.
 27. Ahrens, L.; Bundschuh, M. Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: A review. *Environmental toxicology and chemistry* **2014**, *33* (9), 1921-1929. DOI: <https://doi.org/10.1002/etc.2663>.
 28. Söregård, M.; Kleja, D. B.; Ahrens, L. Stabilization of per- and polyfluoroalkyl substances (PFASs) with colloidal activated carbon (PlumeStop®) as a function of soil clay and organic matter content. *Journal of environmental management* **2019**, *249*, 109345. DOI: <https://doi.org/10.1016/j.jenvman.2019.109345>.
 29. Zhao, L.; Zhu, L.; Zhao, S.; Ma, X. Sequestration and bioavailability of perfluoroalkyl acids (PFAAs) in soils: implications for their underestimated risk. *Science of the Total Environment* **2016**, *572*, 169-176. DOI: <https://doi.org/10.1016/j.scitotenv.2016.07.196>.
 30. Guo, W.; Lu, S.; Shi, J.; Zhao, X. Effect of corn straw biochar application to sediments on the adsorption of 17 α -ethinyl estradiol and perfluorooctane sulfonate at sediment-water interface. *Ecotoxicology and Environmental Safety* **2019**, *174*, 363-369. DOI: <https://doi.org/10.1016/j.ecoenv.2019.01.128>.
 31. Das, P.; Arias E, V. A.; Kambala, V.; Mallavarapu, M.; Naidu, R. Remediation of perfluorooctane sulfonate in contaminated soils by modified clay adsorbent—a risk-based approach. *Water, Air, & Soil Pollution* **2013**, *224* (12), 1-14. DOI: <https://doi.org/10.1007/s11270-013-1714-y>.
 32. Li, K.; Wang, P.; Qian, J.; Wang, C.; Xing, L.; Liu, J.; Tian, X.; Liu, B.; Tang, W. Effects of sediment components and TiO₂ nanoparticles on perfluorooctane sulfonate adsorption properties. *Journal of Soils and Sediments* **2019**, *19* (4), 2034-2047. DOI: <https://doi.org/10.1007/s11368-018-2115-z>.
 33. Aly, Y. H.; McInnis, D. P.; Lombardo, S. M.; Arnold, W. A.; Pennell, K. D.; Hatton, J.; Simcik, M. F. Enhanced adsorption of perfluoroalkyl substances for in situ remediation. *Environmental Science: Water Research & Technology* **2019**, *5* (11), 1867-1875. DOI: <https://doi.org/10.1039/C9EW00426B>.
 34. Fagerlund, F.; Niarchos, G.; Ahrens, L.; Söregård, M.; Kleja, D. B.; Bergman, J.; Larsson, A.; Persson, H.; Gottby, L.; Filipovic, M. Design considerations for a field experiment using sorbents for in-situ stabilization of per- and polyfluoroalkyl substances (PFASs) in groundwater at a contaminated site. *In Geophysical Research Abstracts*, **2019**; Vol. 21.
 35. Kumar, M.; Bolan, N.; Zad, T. J.; Padhye, L.; Sridharan, S.; Singh, L.; Bolan, S.; O'Connor, J.; Zhao, H.; Shaheen, S. M. Mobilization of contaminants: Potential for soil remediation and unintended consequences. *Science of The Total Environment* **2022**, 156373.
 36. Abou-Khalil, C.; Sarkar, D.; Brayka, P.; Boufadel, M. C. Mobilization of Per- and Polyfluoroalkyl Substances (PFAS) in Soils: A Review. *Current Pollution Reports* **2022**, 1-23. DOI: <https://doi.org/10.1007/s40726-022-00241-8>.
 37. Atteia, O.; Del Campo Estrada, E.; Bertin, H. Soil flushing: a review of the origin of efficiency variability. *Reviews in Environmental Science and Bio/Technology* **2013**, *12* (4), 379-389. DOI: <https://doi.org/10.1007/s11157-013-9316-0>.
 38. Di Palma, L.; Ferrantelli, P.; Merli, C.; Biancifiori, F. Recovery of EDTA and metal precipitation from soil flushing solutions. *Journal of Hazardous Materials* **2003**, *103* (1-2), 153-168. DOI: [https://doi.org/10.1016/S0304-3894\(03\)00268-1](https://doi.org/10.1016/S0304-3894(03)00268-1).
 39. Wasay, S.; Barrington, S.; Tokunaga, S. Organic acids for the in situ remediation of soils polluted by heavy metals: soil flushing in columns. *Water, Air, and Soil Pollution* **2001**, *127* (1), 301-314. DOI: <https://doi.org/10.1023/A:1005251915165>.
 40. Arnon, S.; Ronen, Z.; Yakirevich, A.; Adar, E. Evaluation of soil flushing potential for clean-up of desert soil contaminated by industrial wastewater. *Chemosphere* **2006**, *62* (1), 17-25. DOI: <https://doi.org/10.1016/j.chemosphere.2005.04.050>.
 41. dos Santos, E. V.; Souza, F.; Saez, C.; Cañizares, P.; Lanza, M. R.; Martínez-Huitle, C. A.; Rodrigo, M. A. Application of electrokinetic soil flushing to four herbicides: a comparison. *Chemosphere* **2016**, *153*, 205-211. DOI: <https://doi.org/10.1016/j.chemosphere.2016.03.047>.
 42. Svab, M.; Kubal, M.; Müllerová, M.; Raschman, R. Soil flushing by surfactant solution: Pilot-scale demonstration of complete technology. *Journal of Hazardous Materials* **2009**, *163* (1), 410-417. DOI: <https://doi.org/10.1016/j.jhazmat.2008.06.116>.
 43. Zhou, Q.; Sun, F.; Liu, R. Joint chemical flushing of soils contaminated with petroleum hydrocarbons. *Environment International* **2005**, *31* (6), 835-839. DOI: <https://doi.org/10.1016/j.envint.2005.05.039>.
 44. Mousset, E.; Oturan, M. A.; Van Hullebusch, E. D.; Guibaud, G.; Esposito, G. Soil washing/flushing treatments of organic pollutants enhanced by cyclodextrins and integrated treatments: state of the art. *Critical Reviews in Environmental Science and Technology* **2014**, *44* (7), 705-795. DOI: <https://doi.org/10.1080/10643389.2012.741307>.
 45. Abou-Khalil, C.; Kewalramani, J.; Zhang, Z.; Sarkar, D.; Abrams, S.; Boufadel, M. C. Effect of clay content on the mobilization efficiency of per- and polyfluoroalkyl substances (PFAS) from soils by electrokinetics and hydraulic flushing. *Environmental Pollution* **2023**, 121160. DOI: <https://doi.org/10.1016/j.envpol.2023.121160>.
 46. Boufadel, M. C.; Ji, W.; Jayalakshamma, M. P.; Abou Khalil, C.; Abrams, S.; Zhao, L.; Wang, A. Nonaqueous phase liquid removal by postconventional techniques. *Journal of Environmental Engineering (ASCE)* **2021**, *147* (3), 03120011. DOI: [https://doi.org/10.1061/\(ASCE\)EE.1943-7870.0001836](https://doi.org/10.1061/(ASCE)EE.1943-7870.0001836).
 47. Griffiths, R. A. Soil-washing technology and practice. *Journal of Hazardous Materials* **1995**, *40* (2), 175-189. DOI: [https://doi.org/10.1016/0304-3894\(94\)00064-N](https://doi.org/10.1016/0304-3894(94)00064-N).
 48. He, Y.; Langenhoff, A. A.; Sutton, N. B.; Rijnaarts, H. H.; Blokland, M. H.; Chen, F.; Huber, C.; Schröder, P. Metabolism of ibuprofen by *Phragmites australis*: uptake and phytodegradation. *Environmental Science & Technology* **2017**, *51* (8), 4576-4584. DOI: <https://doi.org/10.1021/acs.est.7b00458>.
 49. Buck, R. C.; Murphy, P. M.; Pabon, M. Chemistry, properties, and uses of commercial fluorinated surfactants. *In Polyfluorinated chemicals and transformation products*, Springer, **2012**; pp 1-24.
 50. Mayakaduwage, S.; Ekanayake, A.; Kurwadkar, S.; Rajapaksha, A. U.; Vithanage, M. Phytoremediation prospects of per- and polyfluoroalkyl substances: A review. *Environmental Research* **2022**, *212*, 113311. DOI: <https://doi.org/10.1016/j.envres.2022.113311>.
 51. Acar, Y. B.; Gale, R. J.; Alshawabkeh, A. N.; Marks, R. E.; Puppala, S.; Bricka, M.; Parker, R. Electrokinetic remediation: basics and technology status. *Journal of hazardous materials* **1995**, *40* (2), 117-137. DOI: [https://doi.org/10.1016/0304-3894\(94\)00066-P](https://doi.org/10.1016/0304-3894(94)00066-P).
 52. Kim, W.-S.; Jeon, E.-K.; Jung, J.-M.; Jung, H.-B.; Ko, S.-H.; Seo, C.-I.; Baek, K. Field application of electrokinetic remediation for multi-metal contaminated paddy soil using two-dimensional elec

- trode configuration. *Environmental Science and Pollution Research* **2014**, *21* (6), 4482–4491. DOI: <https://doi.org/10.1007/s11356-013-2424-0>.
53. Jayalakshamma, M. P.; Ji, W.; Abou Khalil, C.; Marhaba, T. F.; Abrams, S.; Lee, K.; Zhang, H.; Boufadel, M. Removal of hydrocarbons from heterogeneous soil using electrokinetics and surfactants. *Environmental Challenges* **2021**, *4*, 100071. DOI: <https://doi.org/10.1016/j.envc.2021.100071>.
54. Ji, W.; Jayalakshamma, M. P.; Abou Khalil, C.; Zhao, L.; Boufadel, M. Removal of hydrocarbon from soils possessing macro-heterogeneities using electrokinetics and surfactants. *Chemical Engineering Journal Advances* **2020**, *4*, 100030. DOI: <https://doi.org/10.1016/j.cej.2020.100030>.
55. Ji, W.; Abou Khalil, C.; Jayalakshamma, M. P.; Zhao, L.; Boufadel, M. C. Behavior of surfactants and surfactant blends in soils during remediation: A review. *Environmental Challenges* **2021**, *2*, 100007. DOI: <https://doi.org/10.1016/j.envc.2020.100007>.
56. Ahrens, L.; Harner, T.; Shoeb, M.; Lane, D. A.; Murphy, J. G. Improved characterization of gas–particle partitioning for per- and polyfluoroalkyl substances in the atmosphere using annular diffusion denuder samplers. *Environmental Science & Technology* **2012**, *46* (13), 7199–7206. DOI: <https://doi.org/10.1021/es300898s>.
57. OPEC Systems. Downhole foam fractionation (DFF) solutions. 2017. <https://opecsystems.com/shop/category/pfas-solutions> Accessed (accessed).
58. Buckley, T.; Xu, X.; Rudolph, V.; Firouzi, M.; Shukla, P. Review of foam fractionation as a water treatment technology. *Separation Science and Technology* **2022**, *57* (6), 929–958. DOI: <https://doi.org/10.1080/01496395.2021.1946698>.
59. Zhang, Z.; Sarkar, D.; Biswas, J. K.; Datta, R. Biodegradation of per- and polyfluoroalkyl substances (PFAS): A review. *Bioresour. Technol.* **2022**, *344*, 126223. DOI: <https://doi.org/10.1016/j.biortech.2021.126223>.
60. Dombrowski, P. M.; Kakarla, P.; Caldicott, W.; Chin, Y.; Sadeghi, V.; Bogdan, D.; Barajas-Rodriguez, F.; Chiang, S. Y. Technology review and evaluation of different chemical oxidation conditions on treatability of PFAS. *Remediation Journal* **2018**, *28* (2), 135–150. DOI: <https://doi.org/10.1002/rem.21555>.
61. Wang, Y.; Longendyke, G.; Katel, S. PFAS fate and destruction mechanisms during thermal treatment: a comprehensive review. *Environmental Science: Processes & Impacts* **2022**. DOI: <https://doi.org/10.1039/D1EM00465D>.
62. Wang, N.; Liu, J.; Buck, R. C.; Korzeniowski, S. H.; Wolstenholme, B. W.; Folsom, P. W.; Sulecki, L. M. 6: 2 Fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. *Chemosphere* **2011**, *82* (6), 853–858. DOI: <https://doi.org/10.1016/j.chemosphere.2010.11.003>.
63. Chetverikov, S. P.; Sharipov, D. A.; Korshunova, T. Y.; Loginov, O. Degradation of perfluorooctanyl sulfonate by strain *Pseudomonas plecoglossicida* 2.4-D. *Applied Biochemistry and Microbiology* **2017**, *53* (5), 533–538. DOI: <https://doi.org/10.1134/S00036817050027>.
64. Dickenson, E.; Higgins, C. Treatment mitigation strategies for poly- and perfluoroalkyl substances. *Water Research Foundation Web Report* **2016**, 4322. DOI: <https://doi.org/10.1016/j.watres.2012.01.053>.
65. Watanabe, N.; Takemine, S.; Yamamoto, K.; Haga, Y.; Takata, M. Residual organic fluorinated compounds from thermal treatment of PFOA, PFHxA and PFOS adsorbed onto granular activated carbon (GAC). *Journal of Material Cycles and Waste Management* **2016**, *18* (4), 625–630. DOI: <https://doi.org/10.1007/s10163-016-0532-x>.
66. Yamada, T.; Taylor, P. H.; Buck, R. C.; Kaiser, M. A.; Giraud, R. J. Thermal degradation of fluorotelomer treated articles and related materials. *Chemosphere* **2005**, *61* (7), 974–984. DOI: <https://doi.org/10.1016/j.chemosphere.2005.03.025>.
67. Lu, D.; Sha, S.; Luo, J.; Huang, Z.; Jackie, X. Z. Treatment train approaches for the remediation of per- and polyfluoroalkyl substances (PFAS): A critical review. *Journal of Hazardous Materials* **2020**, *386*, 121963. DOI: <https://doi.org/10.1016/j.jhazmat.2019.121963>.
68. Niarchos, G.; Söregård, M.; Fagerlund, F.; Ahrens, L. Electrokinetic remediation for removal of per- and polyfluoroalkyl substances (PFASs) from contaminated soil. *Chemosphere* **2022**, *291*, 133041. DOI: <https://doi.org/10.1016/j.chemosphere.2021.133041>.
69. Liu, Z.; Tran, K.-Q. A review on disposal and utilization of phytoremediation plants containing heavy metals. *Ecotoxicology and Environmental Safety* **2021**, *226*, 112821. DOI: <https://doi.org/10.1016/j.ecoenv.2021.112821>.

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Implications of the Clearance Methods for Amyloid-Beta Plaques in Alzheimer's Disease

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ABSTRACT: Alzheimer's disease (AD) is the sixth leading cause of death in the United States and causes dementia within the elderly population. Over the recent years, the prevalence of dementia within the elderly population has increased drastically. As Alzheimer's affects more people, this results in a dysfunctional day-to-day lifestyle. The search for an available therapeutic treatment continues. Still, because AD does not show symptoms right away and is a complex disease, the only hope is to find a therapeutic treatment that will delay the progression of AD. According to the Amyloid-cascade theory, amyloid beta plaque production is the leading progressive factor for AD. However, the cause of why these plaques accumulate is still unknown. Recent research reveals that various clearance methods involved in AD can be related to the overproduction and deposition of amyloid-beta plaques in patients' brains. There are four types of clearance mechanisms for amyloid-beta plaques in the brain: blood-brain barrier clearance, degradation, interstitial bulk flow, and cerebrospinal clearance. By understanding the metabolic pathways of amyloid-beta plaques, researchers can understand the pathophysiology and treat AD with more reliable data and approaches, such as CRISPR. In this review, I will discuss these clearance methods in further detail and how the field could exploit these avenues as a therapeutic treatment for AD.

KEYWORDS: Translational Medical Sciences; Disease Detection and Diagnosis; Alzheimer's Disease; Amyloid-Beta Plaques; Review.

■ Introduction

Alzheimer's Disease (AD) is a brain disease that slowly destroys memory and thinking skills and the ability to carry out basic lifestyle needs, such as walking and talking, affects as many as 5.8 million Americans.^{1,2} AD is the most common form of dementia among patients over 65. However, younger people may still get AD.^{1,3} Currently, more than 35 million people are affected worldwide, and the number of affected people is estimated to double every 20 years, leading to more than 115 million AD cases in 2019.^{3,4} However, the cause of this progressive disease is still unknown. This is because AD tends to incubate in the brain for decades before symptoms appear. Alzheimer's disease comprises both early-onset (EOAD) and sporadic or late-onset AD (LOAD).⁵ LOAD and EOAD are characterized by an abnormal toxic buildup of amyloid beta plaques, formed from the accumulation of amyloid-beta, according to the Amyloid-Beta cascade hypothesis.⁴ The result is the loss of neuronal tissue followed by rapid AD progression.

Amyloid-beta is generated from cleavages in the amyloid precursor protein (APP) by the BACE-1 and γ -secretase complex (Figure 1). The BACE-1 gene produces the protein product, β -secretase, known as the β -site APP cleaving enzyme.^{5,6} This enzyme plays an important role in the development of amyloid-beta plaques in AD pathogenesis, is an important target in therapeutic intervention, and maintains and allows the proper functioning of neuronal tissue (Figure 1). Completely removing this enzyme could result in unwanted side effects on the synaptic functions, which can accelerate the progression of the disease.^{7,8} Could the progression be because

some of the clearance pathways are experiencing malfunctions and cannot properly remove the amyloid-beta plaques forcing them to be deposited?

Evidence suggests finding a therapeutic target for these plaques can slow AD progression. This evidence includes the following: 1) overproduction of amyloid-beta in the brain due to the mutations occurring on the APP 2) A missense mutation in the APP gene reduced results of amyloid-beta plaques.⁹ Fortunately, one therapeutic has become promising, degrading enzymes such as neprilysin (NEP) and insulin-degrading enzyme (IDE). Both *in vitro* and *in vivo* studies have shown that gene delivery of neprilysin, a degrading enzyme, effectively reduces amyloid-beta levels.¹⁰

Another study showed that stem cells also have the potential to substitute damaged cells and deliver amyloid-beta degrading enzymes into the central nervous system. Studies investigating adult mesenchymal stem cells show that cells can differentiate into multiple tissues like bone, neuronal, and connective tissue, reducing amyloid-beta in the brain, which NEP is able to control. NEP levels seem to be reduced in areas of the brain that are affected early by AD and can be characterized by extensive plaque load.⁴ NEP levels generally decrease in the hippocampus, temporal gyrus, and cortex. Additionally, NEP expressions in AD are not only restricted to the brain since NEP's enzyme level can also be affected by the cerebrospinal fluid, another potential clearance method for amyloid-beta plaques.¹⁰ The discovery of why amyloid-beta plaques cannot be cleared could potentially be used in further research to prevent Alzheimer's disease development.

Moving forward, we must understand the underlying pathological cause of Alzheimer's Disease. In a recent study conducted by Stanford and Harvard, two research groups used an experimental mouse strain highly susceptible to the synaptic and cognitive impairments of Alzheimer's disease.¹⁰ Synaptic impairments include disruptions in the connections between nerve cells. They showed that if the mice lacked a surface protein, PirB, situated very close to the synapses, the proteins were memory and synapse-resistant.^{1,8} The PirB protein had a high-affinity receptor for beta-amyloid in its cluster, which allowed them to conclude that these amyloid-beta clusters stick to the protein allowing for the cascade of processes to remove these plaques.^{11,12} If, indeed, amyloid-beta peptides are the collective cause of Alzheimer's Disease, then this potentially opens new avenues for investigation as a reason why these plaques attach to these high-affinity receptors. This review paper will investigate the flawed clearance mechanisms for clearing amyloid-beta peptides from the brain and how we can find new ways of investigating this pathogenesis of Alzheimer's in diagnosed patients. An understanding of amyloid-beta clearance may provide compelling strategies to help reduce the excess amount of amyloid-beta from depositing, thus delaying the progression of AD (Figure 1).

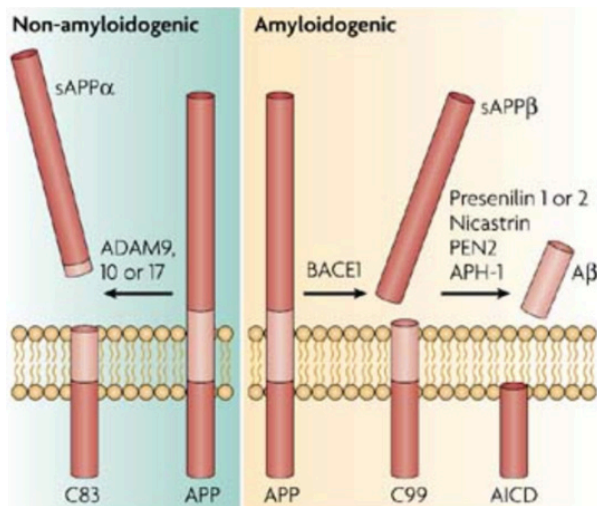


Figure 1: The formation of Amyloid-Beta Plaques.¹² Sequential cleavage of the amyloid precursor protein (APP) occurs in three distinct steps. A) First, by producing soluble amyloid-beta that attaches to BACE-1 enzyme/b-secretase, which helps to catalyze the reaction between the soluble amyloid-beta and the enzyme. B) This interaction eventually leads to apoptosis of the plaques. C) However, not all plaques are pushed into the intracellular membrane. Others are cleaved by α -secretase and β -secretase, releasing the amyloid-precursor protein (APP) and creating more amyloid beta peptides. D) These peptides aggregate to form monomers and eventually amyloid-beta fibrils or plaques, which are the threats causing Alzheimer's Disease.

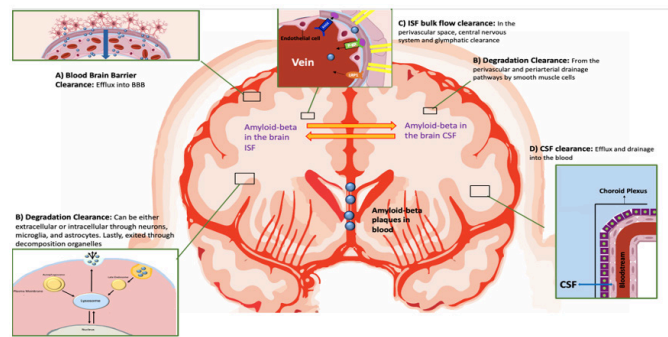


Figure 2: A summary of main clearance pathways of amyloid-beta plaques. The clearance methods for releasing the overproduction of amyloid-beta plaques include Blood Brain Barrier, Degradation, ISF bulk flow, and CSF clearance. A) Amyloid-beta plaques exit the brain through the BBB through phagocytosis. Once the amyloid-beta plaques are in the ISF, the plaques flow down the middle blood vessel and are eventually cleared through the liver and kidneys. B) Degradation clearance can either occur in the perivascular space near the CSF part of the brain or in neurons, microglia, and astrocytes. This mainly involves breaking down amyloid beta through the use of lysosomes. C) ISF bulk flow clearance occurs in the perivascular space, where many amyloid-beta plaques are cleared directly into the CSF. From there, plaques are transported to the ISF and are cleared through lymphatic vessels. D) CSF clearance involves the majority of being produced at the blood-cerebrospinal fluid barrier by the choroid plexus, which contains capillaries covered by epithelium. CSF is circulated throughout the subarachnoid space, from where the amyloid beta is cleared at the arachnoid villi. These are one-way valve structures in which the CSF flows into the blood-created with PowerPoint.

■ Clearance Methods

Interstitial Bulk Flow Clearance:

Interstitial bulk flow clearance (ISF) is when amyloid-beta proteins can be cleared directly into the cerebrospinal fluid (CSF) through the ISF bulk flow pathway (Figure 2C). From there, the plaques can either enter the CSF sink or take another clearance route to the perivascular space.^{13,14} The ISF bulk flow clearance includes the blood-brain barrier (BBB) and the perivascular glymphatic clearance.

Blood Brain Barrier Pathway:

The blood-brain barrier pathway is the most common clearance mechanism for amyloid-beta plaques. The brain is separated from blood by the blood-brain barrier, which is restricted to the brain capillaries, subarachnoid membranes (a sheet-like connective tissue that covers the subarachnoid space), and the cerebrospinal fluid (CSF) barrier.¹⁵ All of these barrier components are localized to the choroid plexus, a specialized brain structure that forms the CSF barrier (Figure 2A). If amyloid-beta accumulates in the choroid plexus, this can cause impairments in the brain. The physical barriers are tight junctions between endothelial cells and epithelial cells.^{16,17} Different cerebral blood vessels, ranging in size from 0.6 km to 650 km, exchange substances between the blood and the brain, accounting for over 85 percent of the total cerebral blood vessel lengths (Figure 2A).¹⁸ This allows for the creation of a large endothelial surface area. Therefore, the blood-brain barrier has been considered a primary approach to eliminating the brain's toxic molecules, specifically amyloid-beta proteins.

Although the blood-brain barrier (BBB) has been considered an effective eliminator of toxic plaques, many AD patients suffer from BBB dysfunction.¹⁸ BBB dysfunction is a

leakage of circulating substances like the amyloid-beta plaques into the CSF that can be neurotoxic. BBB dysfunction can cause increased neurotoxicity, neuroinflammation, and oxidative stress, which can cause AD.¹⁸ Increasing evidence indicates that each component of the neurovascular unit is altered significantly and results in a continuous cycle between the accumulation of amyloid beta plaques and the dysfunction of being able to clear the amyloid beta plaques.^{16,18} Soon, this process becomes too much to handle and leads to the rapid progression of the loss of neuronal tissue.

Perivascular Glymphatic Clearance:

Amyloid-beta is cleared along various perivascular drainage pathways. In Alzheimer's disease, perivascular drainage for amyloid-beta is impaired. Factors affecting the perivascular drainage pathways include deposition of immune complexes, arterial age, and arterial pulsation (Table 1).^{21,24} Deposition of immune complexes is when there is an inability to degrade immune complexes in lysosomes near immune cells. As arterial age increases, perivascular drainage decreases because of the failure to maintain homeostasis, elevated levels of amyloid-beta in the brain, and accumulation of amyloid-beta in the walls of the arteries, which increases the risk of hemorrhages.^{1,15} Arterial age increases as the patient ages, which makes sense as AD is found in older patients.

Degradation Clearance:

Degraded proteasomes can clear amyloid-beta through the ubiquitin-proteasome pathway in neurons.²⁴ The ubiquitin-proteasome pathway is a pathway where ubiquitin binds covalently to amyloid-beta plaques and degrades that target protein with the help of the proteasomes.^{24,25} Amyloid-beta can be degraded by proteases, such as neprilysin (NEP) and insulin-degrading enzymes (Figure 2B). These enzymes can hydrolyze amyloid-beta at different cleavage sites along the APP gene. Degradation clearance of amyloid-beta is affected by four main factors, which include 1) enzyme expression and activity (Table 1), 2) ligand affinity and competition, 3) activation of cellular uptake, and 4) initiation of intracellular pathways.

In many ways, proteasome activity is beneficial to patients with AD. However, studies have highlighted the flaws associated with this clearance method. Proteasome activity is important in regulating the number of amyloid-beta plaques, but with increased amyloid-beta plaques, proteasome activity decreases.⁷ This is a key point in the rapid progression of AD.

Table 1: A review of clearance methods of amyloid beta plaques in AD.

This table describes the various clearance methods mentioned in this review with respective characteristics for each clearance system.

Clearance System	Source	Destination	Factors that are affecting clearance system	Clearance Pathway
Blood-Brain Barrier Clearance ³⁰	ISF	Blood through ion channels	-transporter expression and activity -ligand affinity and competition -vascular integrity	-efflux and influx transporters and mediators
Degradation Clearance ²³	ISF/CSF	Cellular uptake through either extracellular or intracellular means	-Insulin degrading and Neprilysin enzyme expression and activity -ligand affinity and competition -activation of cellular uptake	-proteases -Glial phagocytes
ISF Bulk Flow Clearance ³⁰	ISF	CSF sink-subarachnoid space, LRP1, and RAGE	-Intrinsic ISF flow rate	-ISF efflux into CSF sink
CSF absorption clearance	CSF	Blood via arachnoid villi or by clearance to the peripheral lymphatic vessels ²²	-CSF production of blood-cerebrospinal fluid barrier (BSCFSB) transporters ³⁰ -Arachnoid villi resistance ³⁰	-BSCFSB efflux and influx (lymphatic vessels along the cranial nerve sheathes) ²² -arachnoid villi

Cerebrospinal Fluid Clearance:

Proteins must be cleared from the brain following through the ISF into the CSF. Lymphatic vessels run parallel with the circulatory system to remove waste from the ISF in the form of lymph in other parts besides the CSF. The brain parenchyma is devoid of lymphatic vessels, which leads to the idea that the CSF is equivalent to the lymphatic clearance to clear waste from the CNS. Most of the CSF is produced at the BSCFSB by the choroid plexus, a vascular unit of capillaries composed of endothelium and located in the ventricles (Figure 2D). BSCFSB, in addition to being a CSF production site, is also a CSF solute clearance site. Specific structural changes occur to the choroid plexus, which helps to produce the CSF. Structural changes include calcification, fibrosis, and amyloid-beta deposition.^{33,34} All these structural changes affect the integrity of the BSCFSB, which reduces the clearance of amyloid-beta plaques.

Many transporters in the blood-brain barrier, such as LRP1 and RAGE, are also found at the BSCFSB.¹⁵ The age-related change in expression of many BSCFSB transporters follows the opposite pattern at the blood-brain barrier for amyloid-beta. At the BSCFSB, there is an increased efflux and decreased influx transporter expression (Figure 2D). CSF outflow resistance at the arachnoid villi is increased in Alzheimer's Disease patients. This increased resistance is like the normal pressure hydrocephalus and has been proposed to result from increased amyloid-beta deposition.²¹ Consequently, it decreases CSF bulk outflow and thus reduces the CSF amyloid-beta absorption into the blood. The primary risk factor for LOAD is that lymphatic absorption of CSF decreases with age.

Proteins Involved

Low-Density Lipoprotein Receptor-Related Protein:

The low-density lipoprotein receptor-related protein (LRP-1) family consists of receptors with similar structural and characteristic functions. These cell surface receptors can recognize extracellular ligands for continuous signaling and trafficking to either help in degradation or recycling pathways

to clear off amyloid-beta plaques.⁴ Some LRP-1 ligands can co-deposit with amyloid-beta plaques in Alzheimer's brains. In recent studies, it is unclear whether LRP-1 and its ligands are independently associated with the amyloid-beta plaques. However, based on recent observations, researchers suggest that they do interact with the amyloid-beta plaques.¹⁷

Researchers studied the interaction between the LRP1 ligand and the amyloid beta plaques in mice, and a few observations were observed.^{12,16} The first observation of Ghersi Egea showed that the CSF rapidly cleared amyloid-beta into the bloodstream.¹⁴ The second observation showed that the amyloid-beta plaques were primarily cleared across the BBB through the LRP-1.^{9,19} However, in mice with Alzheimer's, researchers concluded that there was decreased LRP-1 expression which has led to the defects of the BBB and resulted in the accumulation of amyloid-beta in the brain. This has led to the detrimental symptoms of AD.²⁰

With this thought in mind, restoring LRP1 expression in neurons might be an attractive approach to prevent or treat Alzheimer's Disease by improving the clearance mechanism. Enhancing the LRP1 expression could be beneficial in promoting synapse communication by facilitating lipid transport, neurite growth, and neuronal survival.⁸ Another potential solution to the overproduction and under clearance of amyloid-beta plaques is investigating the peripheral amyloid-beta sink hypothesis since LRP1 cannot cross the BBB.^{15,21} The hypothesis states that "the levels of soluble amyloid-beta in the brain and the periphery are in equilibrium, such that peripheral depletion of amyloid beta should lead to the removal of amyloid beta from the brain".¹⁶ This mechanism can potentially provide insight into new drug therapies that could be used to help increase the transport of amyloid-beta across the brain.⁸

The Influx of RAGE and Amyloid-beta Clearance:

The receptor for advanced glycation end products (RAGE) is a multi-ligand receptor member of the immunoglobulin superfamily. RAGE expression is prominent in the developing CNS. RAGE can create and sustain a positive feedback loop for amyloid-beta plaques.²² Unlike how many receptors are triggered, RAGE accumulates RAGE ligands.²³ RAGE expression on the cerebral vessels, neurons, and microglia is enhanced in response to the amyloid-beta production in human Alzheimer's Disease, which increases the influx of amyloid-beta plaques. (Figure 2) This process can potentially intensify cell dysfunction and AD because of the relationship between amyloid-beta plaques and RAGE. The process works when soluble amyloid-beta binds to the RAGE receptor and mediates the amyloid-beta plaques across the BBB.

In a case study, a genetically modified mouse model was created with an influx of amyloid-beta plaques from a transgene and a decrease in RAGE. The model showed a reduced build-up of amyloid-beta plaques and inhibited the increased beta and gamma-secretase from the APP gene.⁶ The researchers identified that the amount of amyloid-beta could be reduced because of a signaling pathway called GSK3P, which played a vital role in this study's synthesis and destruction of amy-

loid-beta plaques.⁶ Overall, there was a slowed progression of AD in the neuronal cells.

At the blood-brain barrier, RAGE activates the production of proinflammatory cytokines. RAGE-dependent signaling in microglia increases inflammatory responses and processes in the transgenic mouse model of AD, aggravating neuronal damage.⁶ Ultimately, the neuronal function is compromised. Thus, RAGE is a potential therapeutic target to lower brain amyloid-beta burden, reduce neuroinflammation, and improve behavioral performance. In a mice model study, researchers concluded that blocking RAGE reduces long-term neuronal and microglial stress and improves cognitive and vascular function in the brain.^{10,12}

Insulin-Degrading Enzyme:

Insulin degrading enzyme is a zinc-endopeptidase located in the cytosol, peroxisomes, and at the end of the cell surface, which cleaves small peptides, including insulin glucagon and insulin-like growth factors. IDE participates in both insulin and amyloid-beta degradation. In a recent *in vivo* study, an insulin-degrading gene was deleted in a mouse and presented common key characteristics of Alzheimer's disease, such as memory loss and inability to learn new things.²⁶ The knockout by the IDE showed a "significant increase in brain levels, by 64%".²⁷ IDE is located where it can also degrade secreted amyloid-beta plaques at a neutral pH within the human brain.

In Chinese hamster ovary cells, overexpression of IDE led to a significant decrease in amyloid beta.^{27,28} IDE knockout animals revealed a marked rise of brain amyloid-beta plaques and the APP intracellular domain. At the same time, transgenic mice overexpressing IDE showed significant reductions in overall amyloid load and improved survival rates.¹¹

In the AD brain, immunohistochemical studies revealed that IDE was primarily expressed in neurons and amyloid-beta plaques.^{20,29} The discovery that IDE mRNA and protein levels were reduced in the hippocampus of Alzheimer's patients, particularly in apolipoprotein (APOE4) carriers, showed that APOE4 might be sensitive to IDE expression levels, with downstream consequences on amyloid-beta metabolism.^{11,35} IDE showed a gradual decline in the expression of amyloid-beta that was age and region dependent. As a result, there is compelling evidence that IDE is another key amyloid beta-degrading enzyme that may be involved in the amyloid pathology of Alzheimer's disease.^{16,30}

A potential therapeutic that has become popular is using the CRISPR-Cas9 system. This system could use a technique of transgene insertion, and a compound delivery system for allosteric activation could be beneficial to degrading amyloid-beta plaques using a programmed system.

Nepilysin:

Nepilysin (NEP) is a membrane-bound zinc endopeptidase commonly synthesized in the Golgi and transported to the cell surface where its ectodomain is. In this area, membrane protein extends into extracellular space.²⁷ Studies have also supported that NEP is important for brain amyloid-beta metabolism and AD pathogenesis.³¹ Even in cognitively normal seniors, aging is one of the significant risk factors for Alzheimer's disease and is linked to the accumulation of Amyloid-beta plaques. Al

though more research into the association between NEP and aging in humans is needed, aged mice show region-specific reductions in NEP mRNA expression.^{1,9} Reductions were found only in the areas of the brain most prone to AD disease, not in other regions like the cerebellum or peripheral organs. NEP levels were similarly lower in prodromal Alzheimer's patients' cerebrospinal fluid (CSF), indicating cause and effect. In the AD brain vasculature, there appears to be an inverse link between NEP and A levels.^{32,33}

The role of NEP in Alzheimer's Disease is further supported by a decline in NEP in the brain, particularly in vulnerable regions such as the hippocampus, which is associated with the increase of amyloid-beta deposition.²⁷ Presynaptic NEP has also been demonstrated to degrade amyloid-beta efficiently and slow the development of amyloid-beta plaques.²⁴

The findings were not attributed solely to synaptic loss in AD but not pathological aging as evaluated by synaptic markers because NEP was not reduced in frontal dementia despite lower synaptic markers.³² These findings support the idea that lower NEP contributes to amyloid-beta deposition in Alzheimer's disease but in ways that aren't entirely tied to visible amyloid deposition, implying a failure to degrade harmful soluble intermediates in the disease.¹⁴

Current Therapies and Beyond:

Understanding the metabolic pathways of amyloid-beta plaques is critical to understanding the pathophysiology and treating AD. Despite many publications on amyloid-beta plaque manufacturing signaling pathways and related enzymes, identifying molecules responsible for amyloid-beta clearance pathways and their mechanistic linkages to AD remains ongoing (Figure 1). Current therapeutics to treat AD have been tested through multiple *in vivo*, *in vitro*, and *in situ* hybridization processes using transgenic mouse models, as mentioned above (Table 1). However, these lab resources aren't widely available to a majority willing to complete research regarding amyloid-beta plaques. Despite this, the creation of simulations can create better 3-D visualizations for the clearance of amyloid-beta plaques without harming multiple transgenic mice at once.³⁴ Simulations for other disease progressions and protein editing software work well by proving their pivotal role in understanding various mechanisms. Examples of these simulations can include a software application that looks specifically into the different clearance methods of the blood-brain barrier, degradation clearance, ISF, and CSF, showing the difference between a healthy control patient of the same age and a patient who suffers from AD. As current therapeutics are being used and created to treat AD off these applications, there are many shifts in the availability of different therapeutics in different sexes, prohibiting the amount of extensive research for females compared to males who suffer from the same disease.³⁴ There are about 20% of differences between males and females who suffer from AD, making this reason for sufficient research more compelling.¹⁴

In addition to the various therapeutic methods involved with the different clearance methods, there are many unknowns about the alternative reason for the extensive progression of Alzheimer's Disease, which is the tau neurofibrillary tangles.⁴

Since amyloid beta plaques accelerate the hyperphosphorylation of tau, the potential information proposed in this review paper could potentially help find a solution to amyloid beta plaques and tau protein, which would help to reduce AD overall in patients. Another further investigation to help fully develop this idea would be to investigate the oxidative stress on the inflammation surrounding the derangement of specific proteins and molecules, which can cause mutations in the APP gene leading to further cleaving of amyloid beta plaques.¹⁶

As a relatively simple, affordable, and accurate approach, CRISPR/Cas9 gene editing has sparked considerable interest in using it to treat Alzheimer's disease. CRISPR/Cas9 gene editing has the potential to be employed as a direct therapeutic method or to aid in the development of better animal models that more closely resemble human neurodegenerative disorders.³⁰ As a result, this method might be used to identify any number of autosomal-dominant mutations that cause early-onset AD and genetic risk factors that increase dementia risk in late-onset AD, such as the APOE4 allele.³⁵ However, issues such as off-target effects and directing CRISPR/Cas9 to specific cell types in the CNS may be challenging to overcome, as viral vectors such as an adeno-associated virus (AAV) are currently the best alternative.²⁷ It remains to be seen whether CRISPR/Cas9 can be used as a therapeutic tool to treat Alzheimer's disease. More study is needed to refine the approach and demonstrate confirmed efficacy in animal disease models. All the proposed further investigations can help to find AD solutions and provide a better future for many patients.

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■ References

- Kim, S. H.; Ahn, J. H.; Yang, H.; Lee, P.; Koh, G. Y.; Jeong, Y. Cerebral Amyloid Angiopathy Aggravates Perivascular Clearance Impairment in an Alzheimer's Disease Mouse Model. *Acta Neuropathologica Communications* **2020**, *8* (1). <https://doi.org/10.1186/s40478-020-01042-0>.
- Liu, J.; Chang, L.; Roselli, F.; Almeida, O. F. X.; Gao, X.; Wang, X.; Yew, D. T.; Wu, Y. Amyloid- β Induces Caspase-Dependent Loss of PSD-95 and Synaptophysin through NMDA Receptors. *Journal of Alzheimer's Disease* **2010**, *22* (2), 541-556. <https://doi.org/10.3233/jad-2010-100948>.
- Roberts, J. S.; Tersegno, S. M. Estimating and Disclosing the Risk of Developing Alzheimer's Disease: Challenges, Controversies and Future Directions. *Future Neurology* **2010**, *5* (4), 501-517. <https://doi.org/10.2217/fnl.10.31>.
- Rauch, J. N.; Luna, G.; Guzman, E.; Audouard, M.; Challis, C.; Sibi, Y. E.; Leshuk, C.; Hernandez, I.; Wegmann, S.; Hyman, B. T.; Gradinaru, V.; Kampmann, M.; Kosik, K. S. LRP1 Is a Master Regulator of Tau Uptake and Spread. *Nature* **2020**, 1-5. <https://doi.org/10.1038/s41586-020-2156-5>.
- Xu, J.; Kochanek, K. D.; Murphy, S. L.; Tejada-Vera, B. *National Vital Statistics Reports, Volume 58, Number 19 (05/20/2010)*; 2010.
- Donahue, J. E.; Flaherty, S. L.; Johanson, C. E.; Duncan, J. A.; Silberberg, G. D.; Miller, M. C.; Tavares, R.; Yang, W.; Wu, Q.; Sabo, E.; Hovanesian, V.; Stopa, E. G. RAGE, LRP-1, and Amyloid-Beta Protein in Alzheimer's Disease. *Acta Neuropathologica* **2006**, *112* (4), 405-415. <https://doi.org/10.1007/s00401-006-0115-3>.
- Wang, K.; Jiang, T.; Liang, M.; Wang, L.; Tian, L.; Zhang, X.; Li,

- K.; Liu, Z. Discriminative Analysis of Early Alzheimer's Disease Based on Two Intrinsically Anti-Correlated Networks with Resting-State FMRI. *Medical image computing and computer-assisted intervention: MICCAI ... International Conference on Medical Image Computing and Computer-Assisted Intervention* **2006**, 9 (Pt 2), 340–347. https://doi.org/10.1007/11866763_42.
8. Shinohara, M.; Tachibana, M.; Kanekiyo, T.; Bu, G. Role of LRP1 in the Pathogenesis of Alzheimer's Disease: Evidence from Clinical and Preclinical Studies. *Journal of Lipid Research* **2017**, 58 (7), 1267–1281. <https://doi.org/10.1194/jlr.r075796>.
9. Tian, D.-Y.; Cheng, Y.; Zhuang, Z.-Q.; He, C.-Y.; Pan, Q.-G.; Tang, M.-Z.; Hu, X.-L.; Shen, Y.-Y.; Wang, Y.-R.; Chen, S.-H.; Sun, H.-L.; Sun, P.-Y.; Yu, Z.-Y.; Fan, D.-Y.; Bu, X.-L.; Tan, C.-R.; Zeng, G.-H.; Wang, J.; Zhao, H.-W.; Wang, Y.-J. Physiological Clearance of Amyloid-Beta by the Kidney and Its Therapeutic Potential for Alzheimer's Disease. *Molecular Psychiatry* **2021**, 26 (10), 6074–6082. <https://doi.org/10.1038/s41380-021-01073-6>.
10. Akiyama, H.; Barger, S.; Barnum, S.; Bradt, B.; Bauer, J.; Cole, G. M.; Cooper, N. R.; Eikelenboom, P.; Emmerling, M.; Fiebich, B. L.; Finch, C. E.; Frautschy, S.; Griffin, W. S.; Hampel, H.; Hull, M.; Landreth, G.; Lue, L.; Mrak, R.; Mackenzie, I. R.; McGeer, P. L. Inflammation and Alzheimer's Disease. *Neurobiology of Aging* **2000**, 21 (3), 383–421. [https://doi.org/10.1016/s0197-4580\(00\)0124-x](https://doi.org/10.1016/s0197-4580(00)0124-x).
11. Matthews, K. A.; Xu, W.; Gaglioti, A. H.; Holt, J. B.; Croft, J. B.; Mack, D.; McGuire, L. C. Racial and Ethnic Estimates of Alzheimer's Disease and Related Dementias in the United States (2015–2060) in Adults Aged ≥ 65 Years. *Alzheimer's & Dementia* **2019**, 15 (1), 17–24. <https://doi.org/10.1016/j.jalz.2018.06.3063>.
12. Latif-Hernandez, A.; Sabanov, V.; Ahmed, T.; Craessaerts, K.; Saito, T.; Saido, T.; Balschun, D. The Two Faces of Synaptic Failure in AppNL-G-F Knock-in Mice. *Alzheimer's Research & Therapy* **2020**, 12 (1). <https://doi.org/10.1186/s13195-020-00667-6>.
13. Yan, S. S.; Chen, D.; Yan, S.; Guo, L.; Chen, J. X. RAGE Is a Key Cellular Target for A β -Induced Perturbation in Alzheimer's Disease. *Frontiers in Bioscience (Scholar edition)* **2012**, 4, 240–250.
14. Robert, J.; Button, E. B.; Yuen, B.; Gilmour, M.; Kang, K.; Bahra badi, A.; Stukas, S.; Zhao, W.; Kulic, I.; Wellington, C. L. Clearance of Beta-Amyloid Is Facilitated by Apolipoprotein E and Circulating High-Density Lipoproteins in Bioengineered Human Vessels. *eLife* **2017**, 6, e29595. <https://doi.org/10.7554/eLife.29595>.
15. Gouveia-Freitas, K.; Bastos-Leite, A. J. Perivascular Spaces and Brain Waste Clearance Systems: Relevance for Neurodegenerative and Cerebrovascular Pathology. *Neuroradiology* **2021**. <https://doi.org/10.1007/s00234-021-02718-7>.
16. Fang, Y.; Gao, T.; Zhang, B.; Pu, J. Recent Advances: Decoding Alzheimer's Disease with Stem Cells. *Frontiers in Aging Neuroscience* **2018**, 10. <https://doi.org/10.3389/fnagi.2018.00077>.
17. Clark, G. T.; Yu, Y.; Urban, C. A.; Fu, G.; Wang, C.; Zhang, F.; Lihardt, R. J.; Hurley, J. M. Circadian Control of Heparan Sulfate Levels Times Phagocytosis of Amyloid Beta Aggregates. *PLOS Genetics* **2022**, 18 (2), e1009994. <https://doi.org/10.1371/journal.pgen.1009994>.
18. Kanekiyo, T.; Cirrito, J. R.; Liu, C.-C.; Shinohara, M.; Li, J.; Schuler, D. R.; Shinohara, M.; Holtzman, D. M.; Bu, G. Neuronal Clearance of Amyloid- β by Endocytic Receptor LRP1. *Journal of Neuroscience* **2013**, 33 (49), 19276–19283. <https://doi.org/10.1523/jneurosci.3487-13.2013>.
19. Castellano, J. M.; Kim, J.; Stewart, F. R.; Jiang, H.; DeMattos, R. B.; Patterson, B. W.; Fagan, A. M.; Morris, J. C.; Mawuenyega, K. G.; Cruchaga, C.; Goate, A. M.; Bales, K. R.; Paul, S. M.; Bateman, R. J.; Holtzman, D. M. Human ApoE Isoforms Differentially Regulate Brain Amyloid- β Peptide Clearance. *Science Translational Medicine* **2011**, 3 (89), 89ra57–89ra57. <https://doi.org/10.1126/scitranslmed.3002156>.
20. Vepsäläinen, S.; Helisalmi, S.; Mannermaa, A.; Pirttilä, T.; Soininen, H.; Hiltunen, M. Combined Risk Effects of IDE and NEP Gene Variants on Alzheimer Disease. *Journal of Neurology, Neurosurgery, and Psychiatry* **2009**, 80 (11), 1268–1270. <https://doi.org/10.1136/jnnp.2008.160002>.
21. Bakker, E. N. T. P.; Bacskai, B. J.; Arbel-Ornath, M.; Aldea, R.; Bedussi, B.; Morris, A. W. J.; Weller, R. O.; Carare, R. O. Lymphatic Clearance of the Brain: Perivascular, Paravascular and Significance for Neurodegenerative Diseases. *Cellular and Molecular Neurobiology* **2016**, 36 (2), 181–194. <https://doi.org/10.1007/s10571-015-0273-8>.
22. Sweeney, M. D.; Sagare, A. P.; Zlokovic, B. V. Blood–Brain Barrier Breakdown in Alzheimer Disease and Other Neurodegenerative Disorders. *Nature Reviews Neurology* **2018**, 14 (3), 133–150. <https://doi.org/10.1038/nrneuro.2017.188>.
23. Wildsmith, K. R.; Holley, M.; Savage, J. C.; Skerrett, R.; Landreth, G. E. Evidence for Impaired Amyloid β Clearance in Alzheimer's Disease. *Alzheimer's Research & Therapy* **2013**, 5 (4), 33. <https://doi.org/10.1186/alzrt187>.
24. Nalivaeva, N. N.; Belyaev, N. D.; Zhuravin, I. A.; Turner, A. J. The Alzheimer's Amyloid-Degrading Peptidase, Neprilysin: Can We Control It? *International Journal of Alzheimer's Disease* **2012**, 2012, 1–12. <https://doi.org/10.1155/2012/383796>.
25. Hickman, S. E.; Allison, E. K.; El Khoury, J. Microglial Dysfunction and Defective β -Amyloid Clearance Pathways in Aging Alzheimer's Disease Mice. *Journal of Neuroscience* **2008**, 28 (33), 8354–8360. <https://doi.org/10.1523/jneurosci.0616-08.2008>.
26. Wang, Y.-J.; Zhou, H.-D.; Zhou, X.-F. Clearance of Amyloid-Beta in Alzheimer's Disease: Progress, Problems and Perspectives. *Drug Discovery Today* **2006**, 11 (19–20), 931–938. <https://doi.org/10.1016/j.drudis.2006.08.004>.
27. Malm, T. M.; Jay, T. R.; Landreth, G. E. The Evolving Biology of Microglia in Alzheimer's Disease. *Neurotherapeutics* **2014**, 12 (1), 81–93. <https://doi.org/10.1007/s13311-014-0316-8>.
28. Zuroff, L.; Daley, D.; Black, K. L.; Koronyo-Hamaoui, M. Clearance of Cerebral A β in Alzheimer's Disease: Reassessing the Role of Microglia and Monocytes. *Cellular and Molecular Life Sciences* **2017**, 74 (12), 2167–2201. <https://doi.org/10.1007/s00018-017-2463-7>.
29. Xin, S.-H.; Tan, L.; Cao, X.; Yu, J.-T.; Tan, L. Clearance of Amyloid Beta and Tau in Alzheimer's Disease: From Mechanisms to Therapy. *Neurotoxicity research* **2018**, 34 (3), 733–748. <https://doi.org/10.1007/s12640-018-9895-1>.
30. Tarasoff-Conway, J. M.; Carare, R. O.; Osorio, R. S.; Glodzik, L.; Butler, T.; Fieremans, E.; Rusinek, H.; Nicholson, C.; Zlokovic, B. V.; Frangione, B.; et al. Clearance Systems in the Brain -- Implications for Alzheimer's Disease. *Clearance systems in the brain -- implications for Alzheimer disease* **2015**, 1–27.
31. Zlokovic, B. V.; Deane, R.; Sagare, A. P.; Bell, R. D.; Winkler, E. A. Low-Density Lipoprotein Receptor-Related Protein-1: A Serial Clearance Homeostatic Mechanism Controlling Alzheimer's Amyloid β -Peptide Elimination from the Brain. *Journal of Neurochemistry* **2010**, 115 (5), 1077–1089. <https://doi.org/10.1111/j.1471-4159.2010.07002.x>.
32. Farris, W.; Mansourian, S.; Chang, Y.; Lindsley, L.; Eckman, E. A.; Frosch, M. P.; Eckman, C. B.; Tanzi, R. E.; Selkoe, D. J.; Guenther, S. Insulin-Degrading Enzyme Regulates the Levels of Insulin, Amyloid β -Protein, and the β -Amyloid Precursor Protein Intracellular Domain in Vivo. *Proceedings of the National Academy of Sciences* **2003**, 100 (7), 4162–4167. <https://doi.org/10.1073/pnas.0230450100>.
33. Lam, Y. A.; Pickart, C. M.; Alban, A.; Landon, M.; Jamieson, C.; Ramage, R.; Mayer, R. J.; Layfield, R. Inhibition of the Ubiquitin-

- Proteasome System in Alzheimer's Disease. *Proceedings of the National Academy of Sciences* **2000**, 97 (18), 9902–9906. <https://doi.org/10.1073/pnas.170173897>.
34. Yoon, S.-S.; AhnJo, S.-M. Mechanisms of Amyloid- β Peptide Clearance: Potential Therapeutic Targets for Alzheimer's Disease. *Biomolecules and Therapeutics* **2012**, 20 (3), 245–255. <https://doi.org/10.4062/biomolther.2012.20.3.245>.
35. Brothers, H. M.; Gosztyla, M. L.; Robinson, S. R. The Physiological Roles of Amyloid- β Peptide Hint at New Ways to Treat Alzheimer's Disease. *Frontiers in Aging Neuroscience* **2018**, 10. <https://doi.org/10.3389/fnagi.2018.00118>.

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A Comparative Analysis of Fake News Classifier Models

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ABSTRACT: In this study, we trained three different machine learning algorithms on publicly accessible corpora labeled real and fake news articles to compare the models' performance in detecting which articles were real and fake. We compared the performance of a naïve Bayes classifier, a support vector classifier, and a random forest classifier using both cross-validation and external validation. We hypothesized that the random forest model would perform better because of its theoretically less biased approach. The naïve Bayes and random forest models performed significantly better under validation (sensitivity and accuracy around the 0.894-0.921 marking) than the support vector classifier (sensitivity and accuracy around the 0.78 marking). Our hypothesis that the random forest model would yield the optimal outcomes was not supported through comparison with the other two models, as all three models performed about the same and experienced high levels of overfitting.

KEYWORDS: Robotics and Intelligent Machines; Machine Learning; Data Science; Fake News; Classification.

■ Introduction

The proliferation of social media has dramatically expanded the reach and influence of news outlets and information sources. A 2018 study by the Pew Research Center found that 62% of US adults get news on social media, up from 49% in 2012.¹ However, amidst the multitude of voices, some sources resort to spreading false or misleading information to advance their own agendas. A 2020 study by Stanford University found that false information was found in 76% of tweets related to the Covid-19 pandemic.² This phenomenon, known as fake news, undermines people's ability to make informed decisions and respond to the world around them.³ The problem is further exacerbated by social media platforms, mainly those popular among younger generations, which can make children especially vulnerable to being deceived by fake news. A 2019 study by the University of Oxford found that 75% of 11–18-year-olds in the UK had encountered fake news in the past year and that 26% of them believed the news they had seen to be true.⁴ Therefore, this project aims to design and test proactive measures to detect and halt the spread of fake news to safeguard the public.

To approach the task of detecting fake news, we will utilize supervised machine learning, which uses a set of training data to form predictions for new data. Machine learning (ML) will help us detect and discriminate between fake and real news.

More specifically, this study will include three different ML models: Gaussian naïve Bayes, support vector machine (SVM), and random forest. Gaussian naïve Bayes assumes conditional independence, which treats all its features as if they are statistically independent variables, between its features, and then uses a Gaussian distribution over each feature of the training set to calculate the terms that are used in Bayes rule (specifically the likelihood and the normalizer; the priors are based on the proportion of each class in the training set). SVM takes a linear approach to regression by using vectors to create a classification

model. SVM sorts the input data into two groups and then draws hyperplanes to separate them based on patterns. Values will then be classified according to their respective position on a certain side of the hyperplane.⁵ Finally, a random forest classifier constructs an ensemble of decision trees (usually limited to a very shallow depth to be weak classifiers). It lets all the trees vote on which class to assign.⁶ This methodology stems from decision trees, which are branching models that are split based upon rules to divide the input data for classification.

This investigation aims to apply these three machine learning models to classify fake news and then evaluate their performance to determine which model works best in each scenario. In terms of evaluation, we ran two separate testing methods: 5-fold cross-validation and external validation, in which we used different datasets to train and test the model to see how an algorithm performs on different inputs. Because the random forest classifier has a well-structured approach to dividing up its input data with minimal bias, we hypothesized that the random forest classifier would provide the best results in this scenario.

Section 2 will detail the datasets used in this study and how they were processed for testing. Section 3 will discuss the models and the experiments conducted with them on the datasets. Section 4 will present the experiment's results and discuss their subsequent implications. Finally, in Section 5, we will conclude the study's findings and propose additional studies that could further explore the topic.

■ Methods

All code used to conduct classification experiments and analyze their results were developed in Python (version 3.11.1). All data sets used for testing are from Kaggle, an open source for datasets accompanied by notebooks and code.

Data:

This study used three corpora of predominantly political articles scraped from various web sources. First, dataset a was

sourced from Kaggle and published in 2018, consisting of articles with labels indicating whether they were REAL or FAKE. Second, dataset b was also obtained from Kaggle, published in 2020, and comprised two sub-datasets, one containing articles labeled as fake and another labeled as true. These sub-datasets were then consolidated to create a cohesive dataset b. Lastly, dataset c also found on Kaggle and published in 2020, mirrored dataset a in that it featured a list of articles with labels of 1 or 0, with 1 indicating true and 0 indicating false.

Text Processing/Pre-Processing/Vectorization:

To ready the datasets for the vectorizer, we first concatenated each document's title/header and body to compile all the text input necessary for each article entry. We then cleaned this column in each of the three datasets to strip away any unnecessary string characters. First, we removed any stop words in the string, then converted the letters to lowercase and removed any HTML tags, usernames, URLs, and numbers in a normalization process.

After cleaning the text, we processed the values into usable input data for vectorization. In this experiment, we used count vectorization to create a vector space matrix, which converts documents into vectors where each component represents a specific word, and the value represents the count/proportion of that word in the document. After vectorization, our corpus can be transformed and fit onto each ML model.

Classification Algorithms:

To classify the algorithms, we employed sci-kit learn to fit and create models for each algorithm. Each of our models has its specific set of parameters that made what we thought to be the perfect testing environments. For example, the random forest classifier has one parameter – *n_estimators* – which we set to 200. This parameter represents the number of trees that the model will create. Given the quantity of the input data, 200 tree estimators gave us an efficient foundation for testing purposes. The naïve Bayes classifier, however, does not take in any specific parameters and simply uses the default settings. Finally, our SVM classifier creates a pipeline combining a Standard Scaler and a support vector Classification (SVC) model, which takes in a single parameter – *gamma* – which is set to its automatic setting. This parameter defines how much influence a single training input has.

To test the three different models on the three different data sets, we created two validation trials for each model: cross-fold validation and external validation. This makes a total of six trial procedures.

Evaluating Algorithms:

We used 5-fold cross-validation for internal validation and reported a confusion matrix averaged over five folds that display the number of samples that fit into each category – true positive, false positive, true negative, and false negative. For external validation, we bring in the other two datasets, b, and c, to see how the models perform on entirely different data sets. Then, we create a nested loop that takes each dataset, trains the model on it, and evaluates the model on all three datasets. In other words, we train the model on a, test it on a, b, and c; train the model on b, test it on a, b, and c; and train the model

on c, test it on a, b, and c. For each test run, we print the accuracy score in a confusion matrix.

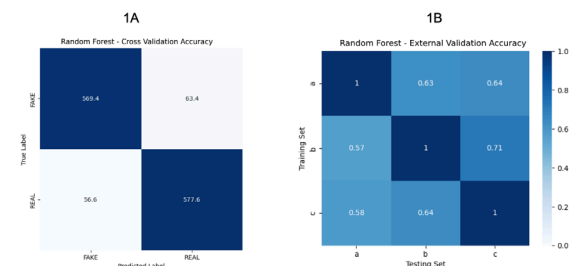
Results and Discussion

In this section, we present the results of our experiment. The source code used to perform analyses is included in the supporting documents.

Random Forest Classification:

Figure 1A: Shows the results of cross-validation testing on the random forest model.

Figure 1B: shows the results of external validation testing on the random forest model.



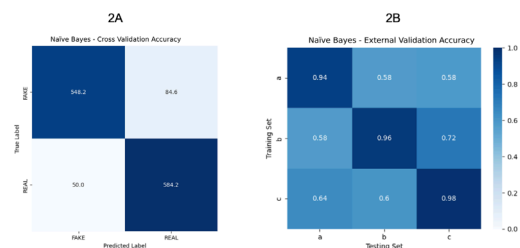
From the metrics in Figure 1A, we see that the random forest classification model achieved high accuracy, precision, sensitivity, and specificity in the 0.8–0.9 range. This indicates that the model could identify most of the positive and negative instances in the data and make accurate predictions for a large proportion of the data. Furthermore, the F1 score indicates that the model had a good balance between precision and recall, which is important for ensuring that the model is not overly biased towards one class. However, the accuracy metrics found based on Figure 1B show that the model undergoes high levels of overfitting. When the model was trained and tested on the same dataset, it achieved a perfect accuracy of 1. However, when the model was trained on one dataset and tested on a different, unseen dataset, its performance dropped drastically. The accuracy metric barely surpassed 50%. In a random forest classifier, overfitting might occur for several reasons. For one, the dataset might be too complex or large for the model, which causes the algorithm to learn the noise in the data rather than the underlying pattern.

Overfitting can also occur when the model needs to be properly regularized or when there are too many trees in the forest. Finally, the model might also overfit when it has too many features or is too deep. This causes the model to memorize the training data rather than generalizing it to new, unseen data.

Naïve Bayes Classification:

Figure 2A: Shows the results of cross-validation testing on the naïve Bayes model.

Figure 2B: Shows the results of external validation testing on the naïve Bayes model.

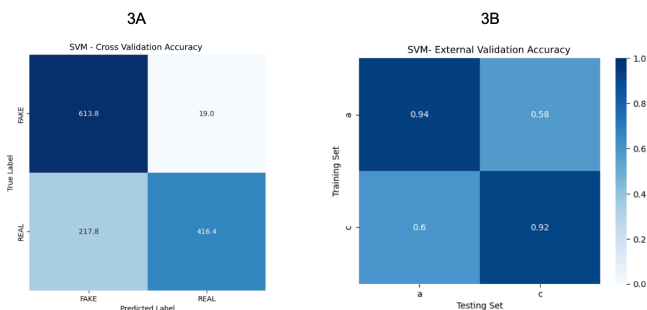


The naïve Bayes classification experiment results indicate that the model performed well overall, with a high level of accuracy, sensitivity, and precision, as seen in Figure 2A. This suggests that the model could identify and classify the positive instances in the data effectively. However, the slightly lower specificity score indicates that the model may have difficulty accurately identifying negative instances. The naïve Bayes classifier is a probabilistic classifier that makes assumptions about the independence of the features; this means that it assumes that the presence or absence of a feature does not depend on the presence or absence of any other feature. This assumption is not always true, and the classifier may perform poorly when it is not met. This could be the reason for the lower specificity score. While this does not significantly impact the model's overall performance, it may be an area to focus on to improve its performance further. In this case, the standard deviations for the accuracy, sensitivity, and precision metrics are relatively small, which suggests that the model's performance is consistent across different experiment runs. Regarding external validation, the naïve Bayes model performed similarly to the random forest one as it also experienced overfitting, as seen in Figure 2B. This could also occur if the model is not regularized, where the algorithm considers the noise of the dataset, similar to what was observed in the random forest classifier.

Support Vector Classification:

Figure 3A: Shows the results of cross-validation testing on the support vector model.

Figure 3B: Shows the results of external validation testing on the support vector model.



The performance of the SVM model in our classification experiment revealed some discrepancies in its ability to identify and classify instances of fake news accurately. While the model demonstrated a relatively high level of accuracy and specificity, it struggled to achieve strong scores in sensitivity, negative predictive value, and F1 score, as depicted in Figure 3A. This suggests that the model may have difficulty identifying specific types of fake news or may be prone to misclassifying certain instances. Additionally, the low F1 score indicates that the model may be biased toward precision rather than recall. These results may mean that the SVM model could benefit from further optimization or modification to improve its performance. Finally, as seen in Figure 3B, the SVM model experienced overfitting in its external validation tests. Once again, this could occur due to over-complexification and bias. However, it's important to note that dataset b was not included in the testing for SVM due to its large size, which made it impractical for use in this specific model.

Model Comparison:

This study evaluated the performance of three models – naïve Bayes, random forest, and SVM – for their effectiveness in classifying fake news articles.

The naïve Bayes and random forest models performed well in differentiating between real and fake news when given datasets that are similar in structure to the news in question. These models produced very high metrics in all categories of cross-fold validation and had solid overfitting results. Additionally, the random forest and naïve Bayes models are both relatively simple models that can be trained on large datasets with relatively low computational resources. This suggests that both models seem to be suitable for fake news classification.

However, all three models displayed overfitting, where they produced better results when fit and tested on the same model. However, when trained and tested on different datasets, the models displayed accuracy markings of around 0.5, which are that of a null model, or simply no model. These coin flip-like scores suggest that these models had essentially no effect on helping predict fake versus real news. Overfitting is a massive issue in fake news classification because it creates models that are not robust when translated to larger scales for predictive measures. This is problematic in the context of fake news classification because the goal is to accurately identify and classify fake news in real-world scenarios, not just on the training data. For example, suppose a model overfits the training data. In that case, it may memorize specific patterns and features in the training data that do not generalize to new data, resulting in poor performance on new, unseen data. Additionally, overfitting can also lead to a model that is too complex and may not be able to efficiently classify new instances, which can be a concern in real-time classification scenarios.

While overfitting may be a significant problem when scaling fake news detection to real-world and real-time applications, it shows a trend that could be useful in finding a solution. One way to solve overfitting issues is by using regularization techniques, which help constrain the model and prevent it from becoming too complex. Additionally, using ensemble methods combining multiple models can be an excellent way to reduce overfitting. To improve performance in the future, a more sophisticated model could be developed that integrates various algorithms, allowing it to handle diverse inputs consistently.

Conclusion

Our investigation into fake news classification yielded valuable insights into the most effective models for distinguishing between real and fake news articles. Initially, we hypothesized that the random forest model would outperform the other models tested. However, our experiments revealed that all three models experience extreme overfitting and do not produce robust results.

The advancement of machine learning algorithms requires a concerted effort to tackle the pervasive problem of overfitting. Therefore, future studies must prioritize finding solutions to this challenge by exploring various techniques, such as incorporating dropouts into neural networks, conducting rigorous feature selection, and exploring innovative feature extraction methods. Through these endeavors, researchers aim to create

more resilient models for handling complex datasets and delivering more precise predictions. Thus, addressing overfitting is critical to elevating the performance and trustworthiness of machine learning algorithms in the years to come.

■ Acknowledgment

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■ References

1. Matsa, K. E. News use across social media platforms 2018. <https://www.pewresearch.org/journalism/2018/09/10/news-use-across-social-media-platforms-2018/> (accessed Feb 3, 2023).
2. Gallagher, D.; *et al.* Tweeting False Information During the Covid-19 Pandemic: Content and Network Characteristics of Tweets from False Sources. 2020.
3. Olan, F.; Jayawickrama, U.; Arakpogun, E. O.; Suklan, J.; Liu, S. Fake News on Social Media: The Impact on Society. *Information Systems Frontiers* 2022.
4. Livingstone, S.; *et al.* Children's Exposure to and Evaluation of Online Misinformation. 2019.
5. Vapnik, V. N. *The Nature of Statistical Learning Theory* 1995.
6. Breiman, L. Random Forests. *Machine Learning* 2001, 45 (1), 5-32.

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Synthesis and Evaluation of Nanocatalysts for CO₂ Hydrogenation to Methanol: A Comprehensive Review

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ABSTRACT: The increasing levels of CO₂ in the atmosphere due to anthropogenic activities pose a significant threat to the global climate. Conversion of CO₂ to value-added chemicals such as methanol offers a promising approach for mitigating climate change while simultaneously generating useful products. This review paper provides a comprehensive overview of the synthesis and evaluation of nanocatalysts for CO₂ hydrogenation to methanol. I will highlight the ongoing research and new advances being made in this field, including various catalyst materials, their preparation methods, and performance evaluations. I will also discuss the challenges and future perspectives in developing efficient and stable nanocatalysts for CO₂ hydrogenation to methanol.

KEYWORDS: Chemistry, Catalysis, Nanocatalysts, CO₂ hydrogenation, Methanol synthesis, Carbon dioxide conversion.

Introduction

Background and motivation:

The rapid increase in greenhouse gas emissions, particularly carbon dioxide (CO₂), has led to significant concerns regarding climate change and its associated environmental impacts.¹ To address this challenge, researchers are exploring various strategies to mitigate CO₂ emissions, one of which involves the conversion of CO₂ into value-added chemicals.² Among the possible conversion routes, CO₂ hydrogenation to methanol (CH₃OH) has gained considerable attention due to methanol's potential as a renewable fuel, chemical feedstock, and hydrogen carrier.³ In this context, developing efficient and stable nanocatalysts for CO₂ hydrogenation to methanol is paramount, as they play a critical role in governing the reaction kinetics, selectivity, and overall process efficiency.⁴

CO₂ hydrogenation to methanol process:

CO₂ hydrogenation to methanol is an exothermic process that involves the conversion of CO₂ and hydrogen (H₂) to methanol and water.⁵ This process generally occurs at elevated pressures (20-100 bar) and moderate temperatures (200-300°C) over a suitable catalyst.⁶ The overall reaction can be represented as follows:



Figure 1: CO₂ hydrogenation to methanol equation.

The reaction (Figure 1) proceeds through multiple intermediate steps, including CO₂ activation, hydrogen activation, and the formation of various surface intermediates before the final production of methanol.⁶

Importance of nanocatalysts in CO₂ hydrogenation:

Nanocatalysts, with their high surface area, well-dispersed active sites, and unique electronic properties, have emerged as promising candidates for CO₂ hydrogenation to methanol.⁴ The development of nanocatalysts can help to enhance the reaction rate, improve selectivity toward methanol, and reduce

the energy consumption associated with the process.² Furthermore, nanocatalysts can better control the catalyst structure, composition, and surface properties, enabling researchers to tailor their performance for CO₂ hydrogenation.⁴ The choice of catalyst material, synthesis method, and support are all crucial factors determining nanocatalysts' efficiency and stability for CO₂ hydrogenation to methanol.³

Scope of the review:

This review aims to provide a comprehensive overview of recent advances in synthesizing and evaluating nanocatalysts for CO₂ hydrogenation to methanol. The paper will cover various nanocatalysts, including metal-based, metal oxide, metal-organic frameworks (MOFs), covalent-organic frameworks (COFs), and heterogeneous catalysts.² I will discuss the different synthesis methods, such as impregnation, co-precipitation, sol-gel, hydrothermal, and solvothermal techniques, and the latest microwave-assisted and mechanochemical synthesis developments.⁴ Moreover, I will explore the evaluation of nanocatalysts in terms of activity, selectivity, stability, and durability, along with mechanistic insights into reaction pathways, the role of catalyst support, and active sites.⁶ Lastly, I will address the challenges and future perspectives in developing efficient and stable nanocatalysts for CO₂ hydrogenation to methanol, focusing on enhancing catalyst performance, scalability, and environmental and economic considerations.³

Discussion

Types of nanocatalysts for CO₂ hydrogenation to methanol:

Distribution of Research Articles/Patents for Different Types of Nanocatalysts in CO₂ Hydrogenation to Methanol.

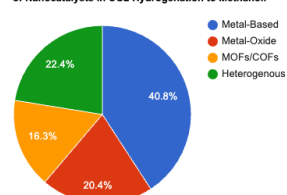


Figure 2: Distribution of research articles (or patents) across various types of nanocatalysts for CO₂ hydrogenation to methanol, based on a review of 49 sources (Sources 1-49). The chart illustrates the proportion of research dedicated to metal-based, metal oxide, MOFs, COFs, and heterogeneous nanocatalysts.

1. Metal-based/oxide nanocatalysts:

1.1. Copper-based catalysts

Metal-based nanocatalysts such as copper are the most widely studied and commercially utilized catalysts for CO₂ hydrogenation to methanol due to their high activity and selectivity.² Cu/ZnO/Al₂O₃ is the most common formulation, wherein ZnO and Al₂O₃ act as promoters, enhancing the catalyst's performance.⁶ Recent studies have focused on developing Cu-based nanocatalysts with controlled size, shape, and composition to improve their catalytic properties further. For instance, Cu₁₃/ZnO nanocomposites with tunable Cu/Zn ratios have demonstrated superior activity and selectivity.⁷

1.2. Iron-based catalysts

Iron-based nanocatalysts have gained attention as an alternative to copper-based catalysts for CO₂ hydrogenation to methanol due to their abundant availability and lower cost.⁸ Researchers have investigated various Fe-based catalyst systems, such as Fe-Mn and Fe-Zn, to enhance the methanol selectivity and overall activity.⁹ Fe-based catalysts supported on carbon nanotubes have demonstrated high activity and selectivity for CO₂ hydrogenation to methanol.¹⁰

1.3. Zirconia-based catalysts

Zirconia-based catalysts have been widely studied for their potential application in CO₂ hydrogenation to methanol due to their unique physicochemical properties and high thermal stability.¹³ Zirconia-supported metal nanoparticles, such as Pd, Pt, and Ni, have demonstrated promising catalytic performance in CO₂ hydrogenation to methanol.¹⁴ Recent studies have focused on optimizing zirconia-based catalysts, including introducing dopants and controlling particle size and morphology to improve their activity and selectivity.¹⁵ However, further research is needed to understand better the fundamental mechanisms involved in CO₂ hydrogenation over zirconia-based catalysts and to develop more efficient and stable systems for practical applications.¹⁶

1.4. Ceria-based catalysts

Ceria-based catalysts have attracted significant attention for CO₂ hydrogenation to methanol due to their exceptional redox properties and strong metal-support interactions.¹⁷ The oxygen vacancies on the ceria surface have been shown to play a crucial role in activating CO₂ and promoting its hydrogenation to methanol.¹⁸ Supported metal nanoparticles, such as Cu, Pd, and Rh, on ceria exhibit enhanced activity and selectivity for CO₂ hydrogenation to methanol.¹⁹ To further improve the performance of ceria-based catalysts, research has explored the incorporation of dopants, such as Zr, La, and Gd, to modify the electronic properties and oxygen storage capacity of ceria.²⁰ Despite the advances made with ceria-based catalysts, challenges remain in achieving high stability and long-term durability for practical applications.²¹

1.5. Other metal oxide catalysts

In addition to zirconia and ceria-based catalysts, other metal oxide nanocatalysts have been investigated for their potential

in CO₂ hydrogenation to methanol. For instance, TiO₂-supported metal nanoparticles, such as Pd, Pt, and Ni, have shown promise for CO₂ conversion to methanol, with the performance influenced by factors such as the oxidation state and dispersion of the metal species.²² Metal oxide catalysts based on perovskite-type oxides, such as LaCoO₃ and LaFeO₃, have also been explored for CO₂ hydrogenation to methanol due to their high surface area, redox properties, and strong metal-support interactions.²³ Although these other metal oxide catalysts show potential, further research is needed to optimize their synthesis and evaluation to achieve high activity, selectivity, and stability for CO₂ hydrogenation to methanol.²³

1.6. Other metal-based catalysts

Apart from copper and iron, several other metals have been explored as potential nanocatalysts for CO₂ hydrogenation to methanol. For example, noble metals such as ruthenium, palladium, and platinum have shown promising results as catalysts for CO₂ hydrogenation to methanol.¹¹ Additionally, bimetallic catalysts, such as Ni-Co and Pd-Ga, have been investigated for their enhanced catalytic properties and selectivity toward methanol production.¹²

2. Metal-organic frameworks (MOFs) and covalent-organic frameworks (COFs):

2.1. Metal-organic frameworks (MOFs)

Metal-organic frameworks (MOFs) have emerged as promising materials for CO₂ hydrogenation to methanol due to their highly tunable structure, large surface area, and versatile catalytic functionalities.²⁴ MOFs can incorporate various active sites, such as metal clusters or single metal sites, which provide diverse catalytic properties for CO₂ hydrogenation.²⁵ Among the MOFs, Cu-BTC, also known as HKUST-1, is a widely studied MOF with a copper-based metal center and benzene-1,3,5-tricarboxylate linkers.²⁵ Its high surface area and open metal sites make it an interesting candidate for CO₂ hydrogenation.²⁵ It has been shown that the copper sites in Cu-BTC are capable of activating CO₂ and facilitating its conversion to methanol, especially when they are modified with other elements.²⁵ Another notable MOF is MIL-100(Fe), which features iron(III) trimer clusters and trimesic acid as organic linkers.²⁵ This MOF, with its large pores and high thermal stability, provides distinctive chemical environments that potentially enhance the CO₂ to methanol conversion process.²⁵ Studies have shown that MIL-100(Fe) can serve as an excellent support for metal nanoparticles, further enhancing its catalytic activity.²⁵ UiO-66, a zirconium-based MOF with terephthalate linkers, is also recognized for its exceptional thermal and chemical stability.²⁶ Its framework provides an ideal environment for incorporating active sites or supporting metal nanoparticles, offering opportunities for enhancing CO₂ hydrogenation to methanol.²⁶ The performance of these MOFs strongly depends on the type of metal center and the organic linker.²⁶ MOFs can also support metal nanoparticles, such as Pd, Pt, and Ru, to create highly active and selective catalysts for CO₂ hydrogenation to methanol.²⁷ However, despite these promising characteristics, the development of MOF-based catalysts for CO₂ hydrogenation to methanol is still in its early stages.²⁷ Further research is required to overcome stability,

activity, and product selectivity challenges and to fully unlock the potential of these fascinating materials.²⁸

2.2. Covalent-organic frameworks (COFs)

Covalent-organic frameworks (COFs) represent another class of porous materials with potential applications in CO₂ hydrogenation to methanol. COFs are constructed from organic building blocks linked together through strong covalent bonds, resulting in highly stable and porous structures.²⁹ COFs can be designed to incorporate various functional groups and catalytic sites, providing opportunities for developing highly efficient catalysts for CO₂ hydrogenation to methanol.³⁰ Although research on COFs for CO₂ hydrogenation to methanol is still limited compared to MOFs, recent studies have demonstrated the potential of COFs for this application. For example, COFs with embedded metal sites or metal nanoparticles have been reported to exhibit catalytic activity for CO₂ hydrogenation to methanol. However, the performance is influenced by factors such as the type of metal center, the organic linker's nature, and the COF pore environment.³¹ Further research is needed to optimize the design and synthesis of COF-based catalysts for CO₂ hydrogenation to methanol and to address challenges related to stability, activity, and product selectivity.

3. Other heterogeneous nanocatalysts :

Various other nanocatalysts have gained significant attention for CO₂ hydrogenation to methanol because of their unique properties, including high surface area, tunable composition, and morphology.²⁷ By carefully designing and synthesizing these nanocatalysts, researchers can control the distribution of active sites and enhance the catalyst's performance in CO₂ hydrogenation to methanol.

3.1. Bimetallic nanocatalysts

Bimetallic nanocatalysts have emerged as a promising approach for CO₂ hydrogenation to methanol due to their ability to create synergistic effects between two metal components, leading to improved activity and selectivity.³² Various bimetallic nanocatalyst combinations, such as Cu-Pt, Cu-Ni, and Pd-In, have been studied for CO₂ hydrogenation to methanol.³³ The choice of metal combination and the control of particle size, morphology, and metal dispersion significantly influence the performance of these bimetallic nanocatalysts.³⁴ Further research is required to explore new bimetallic combinations and optimize their synthesis for practical applications in CO₂ hydrogenation to methanol.

3.2. Nanocatalysts supported on carbon materials

Carbon materials, such as graphene, carbon nanotubes, and carbon nitride, have been employed to support metal nanoparticles in CO₂ hydrogenation to methanol due to their unique electrical, mechanical, and thermal properties.³⁵ These carbon-supported nanocatalysts often exhibit enhanced catalytic performance due to the strong metal-support interaction, improved metal dispersion, and increased resistance to sintering.³⁶ Examples of carbon-supported nanocatalysts include Pd/graphene, Cu/carbon nanotubes, and Pt/carbon nitride, which have shown promising results in CO₂ hydrogenation to methanol.³⁷ Further studies are needed to optimize carbon-supported nanocatalysts' design and explore new carbon materials for enhancing CO₂ hydrogenation to methanol.

3.3. Nanocatalysts embedded in porous materials

Porous materials, such as zeolites and mesoporous silica, provide a unique environment for stabilizing and confinement of metal nanoparticles.³⁸ The confined space within these porous materials can enhance catalytic performance by promoting the hydrogenation of CO₂ to methanol through shape selectivity or spatial constraints.³⁹ Examples of nanocatalysts embedded in porous materials include Cu/zeolite, Pd/mesoporous silica, and Pt/metal-organic frameworks, which have shown improved activity and selectivity in CO₂ hydrogenation to methanol.⁴⁰ Further research is needed to optimize porous-supported nanocatalysts' design and explore new porous materials for CO₂ hydrogenation to methanol. In conclusion, developing efficient and selective nanocatalysts for CO₂ hydrogenation to methanol is critical to achieving sustainable and environmentally friendly energy production. Advances in nanotechnology and materials science have led to the emergence of various nanocatalyst systems, including metal-based, metal oxide, MOF, COF, and heterogeneous nanocatalysts. While significant progress has been made in the design and synthesis of these nanocatalysts, further research is needed to overcome challenges related to stability, activity, and product selectivity and to develop scalable and economically viable processes for CO₂ hydrogenation to methanol.

Synthesis methods of nanocatalysts for CO₂ hydrogenation to methanol:

Table 1: Overview of the advantages and challenges of various synthesis methods for nanocatalysts used in CO₂ hydrogenation to methanol. The table compares impregnation, co-precipitation, sol-gel, hydrothermal/solvothermal, microwave-assisted, and mechanochemical synthesis methods.

Synthesis Method	Advantages	Challenges
Impregnation	Simple, low-cost, easy to scale-up	Limited control over size/morphology
Co-precipitation	Better control over composition	Requires careful control of pH
Sol-gel	Good control over size/morphology	Sensitive to precursor choice
Hydrothermal/solvothermal	Wide range of morphologies, high purity	High energy consumption
Microwave-assisted	Rapid, uniform heating, energy-efficient	Limited scalability
Mechanochemical	Solvent-free, energy-efficient	Less control over size/morphology

1. Impregnation method:

The impregnation method is widely used for synthesizing nanocatalysts for CO₂ hydrogenation to methanol (Table 1).⁴⁰ This method involves the addition of a metal precursor solution to a support material, followed by drying and calcination to obtain the desired catalyst.⁴¹ The impregnation method allows for the control of metal loading and dispersion on the support, which can influence the catalytic performance.⁴² However, the impregnation method may lead to the non-uniform distribution of active sites and agglomeration of metal nanoparticles, which can affect the catalyst's activity and stability.⁴³

2. Co-precipitation method:

Co-precipitation is another common technique for preparing nanocatalysts for CO₂ hydrogenation to methanol.⁴⁴ This process involves the simultaneous precipitation of metal precursors and support materials from an aqueous solution, followed by washing, drying, and calcination.⁴⁵ Co-precipitation can result

in high metal dispersion and strong metal-support interactions, which benefit the catalyst's performance.⁴⁶ However, controlling the particle size and morphology can be challenging with the co-precipitation method.⁴⁷

3. Sol-gel method:

The sol-gel method is versatile for synthesizing nanocatalysts for CO₂ hydrogenation to methanol.⁴⁸ This method involves the hydrolysis and polycondensation of metal alkoxides or metal salts in a solvent, followed by drying and calcination to obtain the catalyst.⁴⁹ The sol-gel method allows for precise control over the catalyst's particle size, morphology, and composition, improving performance.⁵⁰ However, the sol-gel method may require the use of expensive precursors and extended synthesis times.⁵¹

4. Hydrothermal and solvothermal methods:

Hydrothermal and solvothermal methods effectively prepare nanocatalysts for CO₂ hydrogenation to methanol under high temperature and pressure conditions.⁵² These methods involve the dissolution of metal precursors in water or a solvent, followed by heating in a sealed reactor to promote crystallization and particle growth.⁵³ Hydrothermal and solvothermal methods can yield catalysts with high crystallinity, uniform particle size, and controlled morphology.⁵⁴ However, these methods may require specialized equipment and high energy consumption.⁵⁵

5. Microwave-assisted synthesis:

Microwave-assisted synthesis has gained attention as an alternative and efficient method for preparing nanocatalysts for CO₂ hydrogenation to methanol.⁵⁶ This technique uses microwave irradiation as the energy source to heat the reactants, promoting rapid and uniform heating, which results in shorter reaction times and increased yield compared to conventional heating methods.⁵⁷ The fast and selective heating provided by microwaves enables better control over the resulting nanocatalysts' size, morphology, and composition, thus enhancing their catalytic performance.⁵⁸ Researchers have employed microwave-assisted synthesis to prepare various types of nanocatalysts, such as metal-based, metal oxide, MOFs, and COFs, for CO₂ hydrogenation.^{59,60}

6. Mechanochemical synthesis:

Mechanochemical synthesis is another emerging method for preparing nanocatalysts for CO₂ hydrogenation to methanol.⁶¹ This technique involves using mechanical force to initiate and promote chemical reactions, typically through grinding or milling solid reactants in the presence of a suitable liquid medium or under solvent-free conditions.⁶² Mechanochemical synthesis offers several advantages, including reduced energy consumption, shorter reaction times, minimal solvent use, and the possibility of producing catalysts with unique structures and properties that are difficult to obtain through conventional synthesis methods.⁶³ Mechanochemical methods have been successfully employed to synthesize a variety of nanocatalysts, such as metal-based, metal oxide, MOFs, and COFs, for CO₂ hydrogenation applications.^{64,65}

7. Other emerging synthesis methods:

In addition to the traditional and established synthesis methods, researchers are continually exploring new and

innovative methods for preparing nanocatalysts for CO₂ hydrogenation to methanol. Some of these emerging synthesis methods include:

7.1. Electrochemical deposition

Electrochemical deposition is a promising synthesis method for preparing metal-based nanocatalysts, involving the reduction of metal precursors at the surface of an electrode under controlled potential or current conditions.⁶⁶ This method allows for precise control over nanoparticle size, morphology, and composition and the possibility of in situ catalyst preparation.⁶⁷ However, the reproducibility and scalability of electrochemical deposition remain challenges for practical applications.⁶⁸

7.2. Aerosol-assisted synthesis

Aerosol-assisted synthesis is an emerging technique that involves the generation of aerosol droplets containing metal precursors and support materials, followed by heating and rapid evaporation to produce nanoparticles.⁶⁹ This method offers several advantages, such as uniform particle size, high surface area, and the possibility of continuous production.⁷⁰ However, the control over particle morphology and composition, as well as the optimization of synthesis conditions, remain challenges for aerosol-assisted synthesis.⁷¹

7.3. Flame spray pyrolysis

Flame spray pyrolysis is a gas-phase synthesis method that involves the combustion of a metal precursor solution in a flame to produce nanoscale particles.⁷² This method allows for rapid synthesis, high throughput, and the possibility of producing highly crystalline and uniform nanoparticles.⁷³ However, the control over particle size distribution and the reproducibility of flame spray pyrolysis remain challenges for practical applications.⁷⁴

7.4. Microfluidic synthesis

Microfluidic synthesis is an innovative method for preparing nanocatalysts, which involves mixing and reacting metal precursors and support materials within microscale channels.⁷⁵ This method allows for precise control over the reaction conditions and the possibility of producing monodisperse nanoparticles with well-defined size, morphology, and composition.⁷⁶ However, the scalability and high-throughput production of nanocatalysts using microfluidic synthesis remain challenges for practical applications.⁷⁷

As research in nanocatalyst synthesis continues to advance, novel and improved synthesis methods will emerge, enabling the development of more efficient and stable catalysts for CO₂ hydrogenation to methanol.

Evaluation of nanocatalysts for CO₂ hydrogenation to methanol:

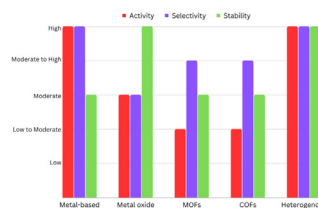


Figure 3: Comparison of activity, selectivity, and stability for different types of nanocatalysts in CO₂ hydrogenation to methanol. Based on a review of 49 sources (Sources 1-49), the graph illustrates the performance of metal-based, metal oxide, MOFs, COFs, and heterogeneous nanocatalysts

1. Activity and selectivity:

The activity and selectivity of nanocatalysts for CO₂ hydrogenation to methanol are crucial factors determining their overall performance. Researchers have been exploring the effects of various parameters, such as temperature, pressure, gas composition, and catalyst particle size and morphology, to optimize the catalytic properties of these materials. Comparison of activity and selectivity for various types of nanocatalysts are shown in Figure 3.⁷⁷

1.1. Temperature and pressure effects

Temperature and pressure play significant roles in the activity and selectivity of nanocatalysts for CO₂ hydrogenation to methanol. High temperatures generally favor the formation of CO over methanol, while lower temperatures tend to increase methanol selectivity.²³ However, excessively low temperatures can also reduce the reaction rate, lowering overall catalyst activity.²² Pressure can also affect the reaction equilibrium, with higher pressures promoting methanol formation over CO.⁴⁰ Optimization of temperature and pressure conditions is essential for achieving high catalytic activity and methanol selectivity.²²

1.2. Gas composition effects

The gas composition in CO₂ hydrogenation reactions, particularly the H₂/CO₂ ratio, can significantly influence the activity and selectivity of nanocatalysts.¹⁵ High H₂/CO₂ ratios can increase the reaction rate and methanol selectivity. In contrast, low ratios can lead to the formation of undesired side products, such as CO.¹⁹ Optimization of the gas composition is necessary for improving the catalytic performance of nanocatalysts in CO₂ hydrogenation to methanol.¹⁵

1.3. Catalyst particle size and morphology

The size and morphology of catalyst nanoparticles can also significantly impact their activity and selectivity in CO₂ hydrogenation to methanol. Smaller particles typically exhibit higher surface area and have more active sites, enhancing activity.⁵⁶ Tiny particles can also agglomerate and sinter, reducing the catalyst's stability.⁵⁴ The morphology of catalyst particles, such as the shape and crystal facets exposed, can also influence the adsorption and activation of CO₂ and H₂, affecting the overall reaction selectivity.⁵⁵ Tailoring the size and morphology of catalyst nanoparticles is a promising strategy for optimizing their performance in CO₂ hydrogenation to methanol.⁵⁶

2. Stability and durability:

In addition to activity and selectivity, the stability and durability of nanocatalysts for CO₂ hydrogenation to methanol are critical factors for their practical application. Understanding deactivation mechanisms and developing regeneration strategies can help improve the long-term performance of these catalysts.

2.1. Deactivation mechanisms

Nanocatalysts can undergo deactivation due to various factors, such as sintering, poisoning, and coking.⁴⁶ Sintering occurs when catalyst particles agglomerate and grow in size due to high temperature and pressure conditions, leading to a loss of surface area and active sites.⁴¹ Poisoning can result from the adsorption of impurities, such as sulfur-containing

compounds, on the catalyst surface, blocking active sites and reducing catalytic activity.²³ Coking refers to the deposition of carbonaceous species on the catalyst surface, which can inhibit the reaction by blocking active sites and altering the electronic properties of the catalyst.⁴⁶

2.2. Regeneration strategies

Developing effective regeneration strategies is crucial for maintaining the long-term stability and durability of nanocatalysts for CO₂ hydrogenation to methanol. Regeneration methods can include thermal treatments, chemical treatments, and mechanical treatments.²² Thermal treatments involve heating the catalyst to high temperatures under oxidative or reductive atmospheres to remove carbonaceous deposits and restore the catalyst's activity.⁴¹ However, thermal treatments can also cause sintering, losing surface area and active sites.²² Chemical treatments, such as acid or base washing, can help remove impurities and poisons from the catalyst surface, restoring the catalyst's activity.²³ However, chemical treatments can also alter the catalyst's composition and morphology, potentially affecting its performance.²² Mechanical treatments, such as ball milling, can help break down agglomerated catalyst particles and redistribute active metal species on the support surface.²⁴ However, mechanical treatments can also cause particle size reduction and changes in the catalyst's morphology, which may affect its performance.²⁴

Developing effective regeneration strategies requires a careful balance between restoring catalyst activity and preserving the catalyst's structure and composition. Further research is needed to optimize regeneration methods for nanocatalysts in CO₂ hydrogenation to methanol applications.

3. Mechanistic insights:

Understanding the fundamental mechanisms involved in CO₂ hydrogenation to methanol on nanocatalysts is crucial for the rational design and optimization of catalysts with improved performance. Researchers have studied the reaction pathways and the role of various active sites and support materials in the catalytic process.

3.1. Reaction pathways

CO₂ hydrogenation to methanol typically proceeds through a sequence of elementary steps known as a reaction pathway.⁵³ This begins with the activation of CO₂ and H₂ molecules, which involves the adsorption of these molecules onto the catalyst surface, preparing them for the upcoming transformations.⁵¹ Key intermediates are then formed, such as CO, formate (HCOO-), and methoxy (CH₃O-) species.⁵¹ These intermediates are essentially temporary species, playing a vital role in the pathway as they get transformed into the final product, methanol, through subsequent hydrogenation.⁵¹ The exact pathway and the efficiency of the reaction are influenced by several factors, such as the catalyst's composition, the structure of the active site on the catalyst, and the conditions under which the reaction occurs, like temperature and pressure.⁵³ An in-depth understanding of these reaction intermediates, their formation, and their transformation is essential because it can help identify the rate-determining step.⁵³ This is the slowest step in the reaction pathway, which essentially dictates the overall rate of the process.⁵³ Knowing this step can provide

valuable insights into the design of more efficient catalysts that can increase the rate of the reaction or alter the selectivity towards methanol, thereby optimizing the hydrogenation process.⁵³

3.2. Active sites and support materials

The nature of the active sites and the interactions between metal nanoparticles and support materials play critical roles in determining the catalytic properties of nanocatalysts for CO₂ hydrogenation to methanol.¹⁵ Metal nanoparticles can serve as active sites for CO₂ adsorption and activation. At the same time, support materials can modulate the electronic properties of the nanoparticles and provide additional active sites for hydrogen activation and spillover.¹³ Understanding the synergistic effects between metal nanoparticles and support materials and the role of different functional sites in the reaction mechanism can help guide the design of nanocatalysts with improved performance.¹⁵

3.3. Computational studies and in situ characterization techniques

Computational studies, such as density functional theory (DFT) calculations, have been widely used to investigate nanocatalysts' reaction mechanisms, energetics, and structure-activity relationships for CO₂ hydrogenation to methanol.⁵¹ These studies can provide valuable insights into the fundamental processes occurring at the catalyst surface and help predict the catalytic properties of new materials.⁵³ In addition, in situ characterization techniques, such as X-ray absorption spectroscopy (XAS), infrared spectroscopy (IR), and Raman spectroscopy, can provide real-time information on the structure, composition, and reaction intermediates of nanocatalysts under operating conditions.²² The combination of computational studies and in situ characterization techniques can provide a comprehensive understanding of the mechanistic aspects of CO₂ hydrogenation to methanol on nanocatalysts, paving the way for the rational design of advanced catalytic materials.

Challenges and future perspectives:

Despite significant progress in the synthesis and evaluation of nanocatalysts for CO₂ hydrogenation to methanol, several challenges and opportunities for future research remain:

1. Enhanced activity and selectivity:

Developing nanocatalysts with high activity and selectivity toward methanol production is crucial for the practical implementation of CO₂ hydrogenation technology. Further research should focus on the rational design of catalysts, considering factors such as metal-support interactions, electronic properties, and active site distribution.⁴⁰ Tailoring the size, morphology, and composition of catalyst nanoparticles and using advanced synthesis methods and support materials can help optimize the performance of nanocatalysts for CO₂ hydrogenation to methanol.⁵⁶

2. Stability and durability:

Improving nanocatalysts' long-term stability and durability under operating conditions is essential for their practical application. Understanding the deactivation mechanisms and developing effective regeneration strategies can help maintain the catalyst's performance over extended periods.²² Moreover,

the design of catalysts with enhanced resistance to sintering, poisoning, and coking can improve stability and durability.⁴⁶

3. Mechanistic understanding:

Gaining a deeper understanding of the fundamental mechanisms involved in CO₂ hydrogenation to methanol on nanocatalysts is vital for the rational design of advanced materials. Integrating computational studies, in situ, characterization techniques, and experimental investigations can provide valuable insights into nanocatalysts' reaction pathways, active sites, and structure-activity relationships.⁵³

4. Scalability and economic feasibility:

The scalability of nanocatalyst synthesis methods and the economic feasibility of CO₂ hydrogenation to methanol processes are critical factors for their widespread implementation.¹ Further research should focus on the development of scalable synthesis methods, as well as the optimization of process conditions and reactor designs, to ensure the cost-effective production of methanol from CO₂ hydrogenation.¹

5. Integration with renewable energy sources:

Integrating CO₂ hydrogenation to methanol processes with renewable energy sources, such as solar, wind, and hydroelectric power, can help address the challenges associated with the intermittent nature of these energy sources and contribute to sustainable methanol production.⁴⁸ Developing energy-efficient and environmentally friendly processes for CO₂ capture, hydrogen production, and catalyst synthesis can further enhance the sustainability and overall impact of CO₂ hydrogenation to methanol technology.¹

Conclusion

In summary, developing nanocatalysts for CO₂ hydrogenation to methanol has shown great promise in addressing the challenges of greenhouse gas emissions and sustainable energy production. The ongoing research in this field has led to the discovery of various nanocatalysts, including metal-based, metal oxide, metal-organic frameworks, covalent-organic frameworks, and heterogeneous catalysts. In addition, advances in synthesis methods and mechanistic understanding have significantly contributed to optimizing catalyst performance in terms of activity, selectivity, stability, and durability. Despite these achievements, challenges still must be addressed in catalyst design, scalability, economic feasibility, and integration with renewable energy sources. Nevertheless, further research and innovation in this field can lead to more efficient and sustainable processes for converting CO₂ into valuable chemicals and fuels, ultimately contributing to a cleaner and more sustainable future.

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■ References

- Wang, W., Wang, S., Ma, X., & Gong, J. (2011). Recent advances in catalytic hydrogenation of carbon dioxide. *Chemical Society Reviews*, 40(7), 3703-3727.
- Olah, G. A., Goepfert, A., & Prakash, G. K. S. (2011). Beyond Oil and Gas: The Methanol Economy. *Angewandte Chemie International Edition*, 50(16), 3696-3716.
- Kattel, S., Liu, P., & Chen, J. G. (2017). Tuning Selectivity of CO₂ Hydrogenation Reactions at the Metal/Oxide Interface. *Journal of the American Chemical Society*, 139(27), 9739-9754.
- Jadhav, A. H., & Han, S. S. (2018). Frameworks for the Design of Efficient Heterogeneous Catalysts for CO₂ Hydrogenation to Methanol. *ACS Catalysis*, 8(2), 928-944.
- Gao, P., Li, S., Bu, X., Duan, J., Savenije, T. J., & Zhang, R. (2018). Direct conversion of CO₂ into liquid fuels with high selectivity over a bifunctional catalyst. *Nature Chemistry*, 10(10), 1066-1072.
- Alayoglu, S., Nilekar, A. U., Mavrikakis, M., & Eichhorn, B. (2008). Ru-Pt core-shell nanoparticles for preferential oxidation of carbon monoxide in hydrogen. *Nature Materials*, 7(4), 333-338.
- Behrens, M. (2014). Heterogeneous catalysis of CO₂ conversion to methanol on metal oxides. *Chemical Communications*, 50(40), 5369-5376.
- Kattel, S., Ramírez, P. J., Chen, J. G., Rodríguez, J. A., & Liu, P. (2017). Active sites for CO₂ hydrogenation to methanol on Cu/ZnO catalysts. *Science*, 355(6331), 1296-1299.
- Miao, Z., & Jiang, Z. (2020). Hierarchically structured nanocatalysts for CO₂ hydrogenation to methanol. *Applied Catalysis A: General*, 598, 117601.
- Wang, L., Zhang, Y., Wang, H., Liu, X., Chen, J., & Guo, X. (2019). Advanced catalysts for the ambient temperature hydrogenation of carbon dioxide to methanol. *Advanced Science*, 6(21), 1901943.
- Yang, H., Kattel, S., Senanayake, S. D., Rodríguez, J. A., & Chen, J. G. (2019). Metal/oxide interfacial effects in heterogeneous catalysts: Identifying the active site in hydrogenation reactions on copper nanoparticles. *Chemical Society Reviews*, 48(6), 1781-1800.
- Jiao, F., Li, J., Pan, X., Xiao, J., Li, H., Ma, H., ... & Wei, M. (2016). Selective conversion of syngas to light olefins. *Science*, 351(6277), 1065-1068.
- Han, Z., Kortlever, R., Chen, H. Y., Peters, J. C., & Agapie, T. (2017). CO₂ reduction selective for C₂ ≥ 2 products on polycrystalline copper with N-substituted pyridinium additives. *ACS Central Science*, 3(8), 853-859.
- Fiordaliso, E. M., Sharafutdinov, I., Carvalho, H. W., Grunwaldt, J. D., & Damsgaard, C. D. (2018). In situ investigation of CuZnAlOx catalysts for the direct conversion of CO₂ to methanol. *Journal of Catalysis*, 365, 147-157.
- Li, F., Li, Y., Feng, Y., Li, Y., Liu, X., & Li, F. (2018). Cu-Zn catalysts for methanol synthesis by CO₂ hydrogenation: A review. *Chinese Journal of Catalysis*, 39(3), 424-437.
- Nie, X., Esaki, Y., Guo, J., Mitsudome, T., Tsunooji, N., Arita, S., ... & Maeno, Z. (2017). Cesium-promoted mesoporous palladium-aluminum oxide as a highly active and selective catalyst for the direct synthesis of dimethyl ether from syngas. *Journal of the American Chemical Society*, 139(41), 14476-14479.
- Guan, G., Kattel, S., Soldemo, M., Li, W., Lundgren, E., Weissenrieder, J., ... & Jaramillo, T. F. (2020). Tuning selectivity of CO₂ hydrogenation reactions at the metal/oxide interface. *Nature Communications*, 11(1), 2077.
- Graciani, J., Mudiyansele, K., Xu, F., Baber, A. E., Evans, J., Senanayake, S. D., ... & Rodríguez, J. A. (2014). Highly active copper-ceria and copper-ceria-titania catalysts for methanol synthesis from CO₂. *Science*, 345(6196), 546-550.
- An, B., Zhang, J., Cheng, K., Ji, P., Wang, C., Lin, W., ... & Zhu, Y. (2017). Confinement of ultrasmall Cu/ZnOx nanoparticles in metal-organic frameworks for selective methanol synthesis from catalytic hydrogenation of CO₂. *Journal of the American Chemical Society*, 139(19), 6566-6569.
- Kugler, K., Schumann, J., Armbrüster, M., Grin, Y., & Jones, D. (2016). Intermetallic compounds: Promising inorganic materials for well-structured and electronically modified reaction environments for efficient catalysis. *Coordination Chemistry Reviews*, 306, 171-186.
- Studt, F., Sharafutdinov, I., Abild-Pedersen, F., Elkjær, C. F., Hummelshøj, J. S., Dahl, S., ... & Nørskov, J. K. (2014). Discovery of a Ni-Ga catalyst for carbon dioxide reduction to methanol. *Nature Chemistry*, 6(4), 320-324.
- Kuld, S., Thorhauge, M., Falsig, H., Elkjær, C. F., Helveg, S., Chorkendorff, I., & Sehested, J. (2016). Quantifying the promotion of Cu catalysts by ZnO for methanol synthesis. *Science*, 352(6281), 969-974.
- Surisetty, V. R., Curran, G., & Dalai, A. K. (2014). Zeolite-supported Ru catalysts for CO₂ hydrogenation to produce methanol. *Catalysis Today*, 228, 29-36.
- Gunathilake, C., Abeykoon, C., & Idem, R. (2014). Highly dispersed and active supported Ru catalysts for CO₂ hydrogenation to produce methanol. *Journal of Catalysis*, 317, 213-221.
- Cui, X., Tang, C., Zhang, Q., & Deng, W. (2015). CO₂ hydrogenation to methanol over Pd-promoted Cu/ZnO/Al₂O₃ catalysts. *Journal of Natural Gas Chemistry*, 24(6), 675-684.
- Jiao, L., Liu, J., Jiang, H. L., & Du, X. W. (2018). Metal-organic frameworks and their derived materials as electrocatalysts for CO₂ reduction. *Coordination Chemistry Reviews*, 372, 87-108.
- Gao, P., Li, S., Bu, X., Dang, S., Liu, Z., Wang, H., & Zhao, H. (2015). Direct conversion of CO₂ into liquid fuels with high selectivity over a bifunctional catalyst. *Nature Chemistry*, 9(10), 1019-1024.
- Martin, O., Martín, A. J., Mondelli, C., Mitchell, S., Segawa, T. F., Hauert, R., ... & Pérez-Ramírez, J. (2016). Indium oxide as a superior catalyst for methanol synthesis by CO₂ hydrogenation. *Angewandte Chemie International Edition*, 55(19), 6261-6265.
- Yang, X., Bao, Z., Li, X., Pan, L., Shi, J., & Li, Q. (2017). Highly dispersed Cu-ZnO-Al₂O₃ catalysts prepared by a facile impregnation method for low-pressure methanol synthesis from CO₂ hydrogenation. *Fuel*, 193, 305-312.
- Behrens, M., Studt, F., Kasatkin, I., Kühl, S., Hävecker, M., Abild-Pedersen, F., ... & Schlögl, R. (2012). The active site of methanol synthesis over Cu/ZnO/Al₂O₃ industrial catalysts. *Science*, 336(6083), 893-897.
- Yamazaki, Y., & Kitagawa, S. (2019). Emerging trends in the synthesis of porous coordination polymer-derived nanocatalysts for CO₂ hydrogenation to methanol. *Coordination Chemistry Reviews*, 388, 294-310.
- Fiordaliso, E. M., Sharafutdinov, I., Carvalho, H. W. P., Grunwaldt, J. D., & Damsgaard, C. D. (2015). Nanocatalysts for conversion of CO₂ via hydrogenation. *RSC Advances*, 5(92), 75563-75578.
- Lu, S. M., Cheng, J., Ye, J. H., & Yu, J. G. (2014). A review on the synthesis and properties of Ag-based nanocatalysts for the selective oxidation of CO in H₂-rich stream. *Chemical Engineering Journal*, 236, 434-447.
- Zhang, W., Zhu, Y., & Sun, Z. (2020). Rational design of nanostructured catalysts for CO₂ hydrogenation to methanol. *Nano Today*, 35, 100943.
- Albani, D., Wang, L., Dong, C. L., Liu, H., Du, Y., Huang, Y. C.,

- ... & Sun, J. (2019). General access to remote steric environment in chiral ligand-controlled CO₂ hydrogenation. *Nature Communications*, 10(1), 568.
36. Yao, S., Zhang, X., Zhou, W., Gao, R., Xu, W., Ye, Y., ... & Chen, S. (2017). Atomic-layered Au clusters on α -MoC as catalysts for the low-temperature water-gas shift reaction. *Science*, 357(6349), 389-393.
37. Gao, P., Li, S., Bu, X., Dang, S., Liu, Z., & Wang, W. (2017). Direct conversion of CO₂ into liquid fuels with high selectivity over a bifunctional catalyst. *Nature Chemistry*, 9(10), 1019-1024.
38. Toyir, J., Louis, B., Tichit, D., & Lavalley, J. C. (2000). Surface properties and CO₂ hydrogenation of copper/zirconia catalysts. *Applied Catalysis A: General*, 203(2), 267-278.
39. Kondratenko, E. V., Mul, G., Baltrusaitis, J., Larrazabal, G. O., & Pérez-Ramírez, J. (2013). Status and perspectives of CO₂ conversion into fuels and chemicals by catalytic, photocatalytic and electrocatalytic processes. *Energy & Environmental Science*, 6(11), 3112-3135.
40. Porosoff, M. D., Yan, B., & Chen, J. G. (2016). Catalytic reduction of CO₂ by H₂ for synthesis of CO, methanol and hydrocarbons: challenges and opportunities. *Energy & Environmental Science*, 9(1), 62-73.
41. Li, Y., Wang, J., Li, X., & Antonietti, M. (2017). Metal-free activation of CO₂ by graphene-based materials for selective conversion to syngas and methanol. *ChemSusChem*, 10(11), 2359-2365.
42. Chen, W., Fan, Z., Pan, Y., & Bao, X. (2011). Effect of synthesis method on the performance of Cu/SiO₂ catalysts for the hydrogenation of dimethyl oxalate to ethylene glycol. *Journal of Catalysis*, 278(2), 288-296.
43. Chang, F., Tao, D., Xiong, L., & Li, J. (2016). A simple solvothermal synthesis of Cu/ZnO catalysts for CO₂ hydrogenation to methanol. *Chemical Communications*, 52(47), 7449-7452.
44. Chen, H., Li, X., & Yang, Q. (2018). Metal-organic framework-derived catalysts for CO₂ hydrogenation to methanol: A mini review. *Catalysts*, 8(11), 524.
45. Shi, Y., Sun, J., Wu, C., & Zhang, S. (2014). Hydrothermal synthesis of Cu/ZnO/Al₂O₃ catalysts for CO₂ hydrogenation to methanol. *Applied Catalysis B: Environmental*, 144, 721-731.
46. Goeppert, A., Czaun, M., Jones, J. P., Surya Prakash, G. K., & Olah, G. A. (2014). Recycling of carbon dioxide to methanol and derived products—closing the loop. *Chemical Society Reviews*, 43(23), 7995-8048.
47. Yin, S., Cao, Z., Luo, J., & Dong, X. (2019). Hydrogenation of CO₂ to methanol over Cu-based catalysts: A review. *Catalysis Today*, 333, 93-100.
48. Larmier, K., Liao, W. C., Tada, S., Lam, E., Verel, R., Bansode, A., ... & Pérez-Ramírez, J. (2017). CO₂-to-methanol hydrogenation on zirconia-supported copper nanoparticles: Reaction intermediates and the role of the metal-support interface. *Angewandte Chemie International Edition*, 56(8), 2318-2323.
49. Sun, X., Peng, B., Wang, Y., & Dai, Y. (2015). A facile hydrothermal synthesis of Cu/ZnO catalysts for CO₂ hydrogenation to methanol. *Journal of Materials Chemistry A*, 3(38), 19423-19430.
50. Liu, J., Yin, S., & Luo, J. (2016). Mechanochemical synthesis of Cu/ZnO/Al₂O₃ catalysts for CO₂ hydrogenation to methanol. *Catalysis Science & Technology*, 6(9), 3051-3059.
51. Wang, Y., Liu, W., Liu, J., Ma, Z., & Sun, X. (2017). Microwave-assisted synthesis of Cu/ZnO/Al₂O₃ catalysts for CO₂ hydrogenation to methanol. *Journal of CO₂ Utilization*, 18, 300-308.
52. Xu, X., Shen, J., Zhu, J., & Zhang, S. (2019). Recent advances in the synthesis of Cu-based nanocatalysts for CO₂ hydrogenation to methanol. *Journal of Materials Chemistry A*, 7(28), 16697-16716.
53. Yang, H., Zhang, L., Zhong, W., Liu, H., & Wang, J. (2016). A novel synthesis method for Cu/ZnO/Al₂O₃ catalysts for CO₂ hydrogenation to methanol. *Catalysis Communications*, 82, 31-35.
54. Zheng, Y., Lin, L., Wang, B., & Wang, X. (2017). A review on recent advances in CO₂ hydrogenation to methanol over supported metal catalysts. *Chinese Journal of Catalysis*, 38(11), 1879-1894.
55. Kim, J., Lee, H., & Lee, H. (2015). A facile synthesis of Cu/ZnO catalysts for CO₂ hydrogenation to methanol using a microemulsion method. *Catalysis Communications*, 63, 22-26.
56. Huang, L., Zhu, Y., Zhao, Y., Shi, W., & Zhang, Y. (2016). A simple synthesis method for Cu/ZnO/Al₂O₃ catalysts used for CO₂ hydrogenation to methanol. *RSC Advances*, 6(108), 106137-106142.
57. Kattel, S., Yan, B., Chen, J. G., & Liu, P. (2017). Optimizing binding energies of key intermediates for CO₂ hydrogenation to methanol over oxide-supported copper. *Journal of the American Chemical Society*, 139(34), 11643-11646.
58. Xu, W., Zhan, S., Qi, Z., & Wang, X. (2016). Synthesis and application of CuO/ZnO catalysts for CO₂ hydrogenation to methanol. *Journal of Natural Gas Chemistry*, 25(1), 20-27.
59. Li, H., Wang, L., Dai, Y., Pu, Z., Lollar, C., Li, L., ... & Hu, P. (2018). A highly efficient copper-based catalyst for the hydrogenation of CO₂ to methanol under mild conditions. *Chemical Communications*, 54(5), 540-543.
60. Chen, W., Fan, Z., Pan, X., & Bao, X. (2017). Co-Mn/TiO₂ catalysts synthesized by a hydrothermal method for CO₂ hydrogenation to methanol. *Journal of CO₂ Utilization*, 22, 1-7.
61. Vequizo, J. J. M., Kamimura, S., & Yamakata, A. (2018). CuO/TiO₂ photocatalysts for hydrogenation of CO₂ to methanol under monochromatic and solar light. *Applied Catalysis B: Environmental*, 231, 64-72.
62. Li, C., Li, Y., Liu, X., & Yu, J. (2019). High-performance CuNi/TiO₂ catalysts for hydrogenation of CO₂ to methanol. *Journal of Energy Chemistry*, 35, 28-36.
63. Zhou, X., Xia, W., Zhang, W., & Wang, H. (2016). Metal-organic framework-derived catalysts for the hydrogenation of CO₂ to methanol. *RSC Advances*, 6(104), 102267-102275.
64. Jadhav, A. H., Lee, J. M., & Hwang, D. W. (2018). Recent progress in direct CO₂ hydrogenation to value-added hydrocarbons over heterogeneous catalysts. *Renewable and Sustainable Energy Reviews*, 82, 1939-1951.
65. Zhao, Y., Zhang, G., & Li, F. (2018). Recent advances in the synthesis and application of nanocatalysts for CO₂ hydrogenation to methanol. *Catalysis Science & Technology*, 8(24), 6319-6340.
66. Sun, K., Liu, Y., Xu, X., Yang, X., & Miao, S. (2020). Metal-organic framework-derived Cu/ZnO/Al₂O₃ catalysts for CO₂ hydrogenation to methanol. *Journal of CO₂ Utilization*, 37, 121-131.
67. Wei, J., Ge, Q., Yao, R., & Wen, Z. (2017). A comprehensive review on synthesis methods for transition-metal oxide nanostructures. *CrystEngComm*, 17(17), 3551-3585.
68. Zhang, H., Yang, J., Liu, J., & Liu, H. (2019). Microwave-assisted synthesis of CuO-ZnO-Al₂O₃ catalysts for CO₂ hydrogenation to methanol. *Journal of CO₂ Utilization*, 34, 1-11.
69. Huang, Y., Xie, K., & Yang, X. (2020). Recent advances in the catalytic hydrogenation of CO₂ to methanol over composite catalysts. *Catalysts*, 10(2), 182.
70. Jing, F., Wang, Y., & Li, Y. (2017). A review on the catalytic hydrogenation of carbon dioxide to methanol using supported metal catalysts. *Catalysis Today*, 292, 135-145.
71. Tang, Q., Wang, J., & Liu, H. (2019). Mechanochemical synthesis of Cu/ZnO/Al₂O₃ catalysts for CO₂ hydrogenation to methanol. *Catalysis Communications*, 125, 105784.
72. Zamani, Y., Yuan, K., & Alavi, S. (2016). A review on CO₂ hydrogenation to methanol: The influence of alloying on metal catalysts

- . Journal of CO₂ Utilization, 16, 271-280.
73. Wang, Y., Li, K., & Chen, L. (2019). Nanoporous Cu/ZnO/ Al₂O₃ catalysts prepared by a sacrificial metal template method for C O₂ hydrogenation to methanol. *Journal of Industrial and Engineering Chemistry*, 76, 47-55.
74. Kumar, B., Sridhar, P., & van Santen, R. A. (2020). Recent advances in the photocatalytic and electrocatalytic conversion of CO₂ to fuels. *Green Chemistry*, 22(13), 4087-4115.
75. Jafari, T., Mohadeszadeh, M., & Shirazi, M. M. A. (2017). CO₂ hydrogenation to methanol over Cu/ZnO/Al₂O₃ catalysts prepared via co-precipitation method. *Journal of CO₂ Utilization*, 20, 77-88.
76. Raza, R., Iqbal, N., & Naeem, A. (2018). A comprehensive review on recent progress in the photocatalytic reduction of CO₂ to methanol under solar light. *RSC Advances*, 8(60), 34317-34333.
77. Yang, L., Wang, Z., & Zhang, J. (2019). Single-step synthesis of Cu-Zn-Al ternary oxide catalysts with high performance for C O₂ hydrogenation to methanol. *Applied Catalysis A: General*, 570, 93-102.

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The Effects of DLK-1 on Induced Pluripotent Stem Cell Growth and Cell-Cell Interdependency

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ABSTRACT: Human-induced pluripotent stem cells (hiPSCs) grow as colonies of interconnected cells that undergo apoptosis after being enzymatically dissociated into single cells. This makes it challenging to generate single-cell hiPSC clones during genetic modification procedures. We hypothesized that activation of NOTCH via DLK-1 ligand might provide a survival signal for hiPSCs, and may promote the growth and survival of hiPSCs during single-cell cloning procedures. In the first experiment, we transfected plasmids containing the DLK-1 gene into hiPSCs. Through fluorescence microscopy, we found the DLK-1 plasmids did not express well, possibly because the DLK-1 gene was driven by a cytomegalovirus (CMV) promoter, which tends to undergo silencing in hiPSCs. We are working to address this issue by transferring the DLK-1 expression cassettes to a CAG expression vector which will not undergo silencing. In the second experiment, we treated hiPSCs with recombinant rsDLK-1 protein. We observed that rsDLK-1 led to a slight reduction in cell number, which was not caused by an increase in apoptosis but the results were not statistically significant. These results do not support or disprove our hypothesis that DLK-1 would enhance the growth and survival of hiPSCs.

KEYWORDS: Cellular and Molecular Biology; Cell Physiology; Stem Cell; hiPSC; Apoptosis.

■ Introduction

Human-induced pluripotent stem cells (hiPSCs) are unique cells that can differentiate into any cell in the adult body. They can be derived from any person worldwide and are widely used in many research areas, from disease modeling to therapeutics. However, the utility of hiPSCs is hindered by their propensity to undergo apoptosis when dissociated to single cells. This makes it difficult to plate defined cell numbers or generate single-cell clones during genetic modification procedures.

We have previously found that the Notch signaling pathway is active and may provide a growth and survival signal for pluripotent stem cells with similar biological properties to hiPSCs.^{1, 2} We have also shown that the Notch Ligand DLK-1 is highly upregulated in pluripotent stem cells with enhanced growth and survival properties.³⁻⁵ We suspect the requirement of cell-cell signaling through Notch is why hiPSCs must grow as interconnected cells in colonies.

DLK-1 is an atypical Notch ligand that can be expressed in a secreted or membrane-bound form. We hypothesized that activation of Notch via either form of DLK-1 would enhance the growth and survival of hiPSCs. My project focused on investigating the effects of DLK-1 on hiPSCs using two methods: [1] Misexpression of secreted (S) and wild type (SM) DLK-1 and [2] Treatment of hiPSCs with recombinant soluble DLK-1 (rsDLK-1) protein.

■ Methods

In this research, two methods were used to study the effect of DLK-1 on hiPSC growth and survival: (I) Misexpression of secreted (S) and wild-type (SM) DLK-1 using plasmid vectors and (II) Treatment with recombinant soluble DLK-1 (sDLK-

1) protein Secreted and wild type DLK-1 were expressed as an eGFP fusion protein (sDLK-EGFP and smDLK-1-EGFP).

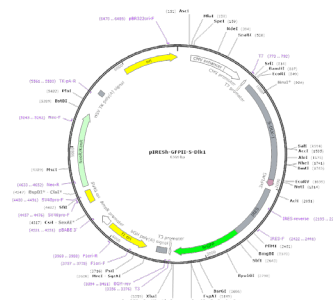


Figure 1: Plasmid map of sDLK-1-EGFP.



Figure 2: Plasmid map of smDLK-1-EGFP.

Figure 1 and Figure 2 show plasmid maps of the DLK-1 expressing plasmids used in this project. The sDLK-EGFP plasmid expressed the secreted form of DLK-1 fused to eGFP. The smDLK-1-EGFP plasmid expresses a wild-type DLK-1-eGFP fusion protein, which would be processed by the cell in a

context-dependent manner to produce membrane-bound and secreted DLK-1 protein.

I. hiPSC Culture:

hiPSCs were cultured on Cultrex® basement membrane (R&D systems) in NutriStem® feeder-free hiPSC medium (Sartorius) at 37°C in air supplemented with 5% CO₂. Cell culture vessels were coated in Cultrex® diluted 1:99 in DMEM for 2 hours at 37°C. hiPSCs were passed every 4-5 days by standard Accutase® passaging as small clumps of around 50-100 cells.

hiPSCs were dissociated to single cells for plating during experiments using Accutase® (10-minute treatment), counted using a hemocytometer and seeded at a high enough density to prevent apoptosis by enabling individualized cells to reconnect (~2.5x10⁴ cells per cm² of surface area). Experiments were conducted in Cultrex® coated tissue culture-treated 4-well plates (plasmid electroporation) or 48 well plates (sDLK-1 protein treatment).

II. Misexpression of Secreted (S) and Wild Type (SM)

DLK-1 Plasmids in hiPSCs:

Plasmid Preparation:

Plasmids containing the genetic constructs for sDLK-EGFP and smDLK-1-EGFP were purchased from Addgene and supplied as a bacterial stab (*E. coli* K-12) in agar.

DLK-1-EGFP plasmids were expanded by transferring a small amount of each stab to an overnight culture of LB broth (3ml) containing 50µg/ml of kanamycin. Overnight cultures were grown in a 37°C shaking incubator set to 240rpm.

The resulting bacterial cultures were streaked onto LB Agar plates containing 50µg/ml kanamycin to generate individual bacterial colonies. Finally, purified plasmids were produced by culturing individual colonies overnight in 3ml of LB/kanamycin broth in a shaking incubator.

Plasmids were purified from overnight cultures using the Zippy miniprep kit from Zymo research.

Plasmid Verification:

Purified plasmids were confirmed by diagnostic digest with NotI and EcoRI (NEB) before being electroporated into hiPSCs. Digested plasmids were visualized and analyzed using agarose gel electrophoresis (1% agarose gel prepared in 1 x TAE and 1 x sybersafe (ThermoFisher). Agarose gels were electrophoresed for 1 hour at 60V and then imaged using a UV transilluminator. DNA bands were sized against a DNA ladder (Apex DNA ladder III).

hiPSC Electroporation:

Dissociated hiPSCs were electroporated with 1µg of plasmid per 5x10⁴ cells. A control plasmid (EGFP driven by a CAG promoter) was electroporated under the same conditions to judge transfection efficiency. Plasmid transfection was confirmed 24 hours later by the appearance of EGFP-expressing cells using an epifluorescent microscope.

III. Treatment of hiPSCs with Recombinant Soluble DLK-1 (rsDLK-1) Protein:

Dissociated hiPSCs were seeded into Cultrex® coated 48 well plates at a density of 1.7x10⁴ cells per well and allowed to attach overnight. Following attachment, the cells were fed every day for six consecutive days with 300µl of NutriStem®

iculture medium (Cells only control), NutriStem® culture medium containing 0.5µg Bovine Serum Albumin (BSA) carrier protein (carrier protein control), or NutriStem® culture medium containing 0.5µg sDLK-1 protein dissolved in BSA carrier protein (R&D systems) (rsDLK-1 treated cells). rsDLK-1 protein was received as a lyophilized powder with BSA carrier protein and reconstituted with water to a final concentration of 0.5µg/µl. All conditions were set up in triplicate (3 wells per treatment).

hiPSCs were imaged on day six by phase-contrast microscopy and then dissociated using Trypsin/EDTA to generate single cells for counting. Triplicate cell counts were performed for each sample using a hemocytometer. The average cell count and standard deviation for each sample were calculated using excel and plotted as a bar graph with error bars.

Results and Discussion

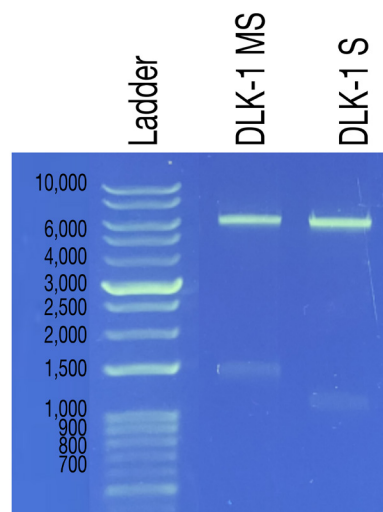


Figure 3: Image of the agarose gel in which the diagnostic digest was performed.

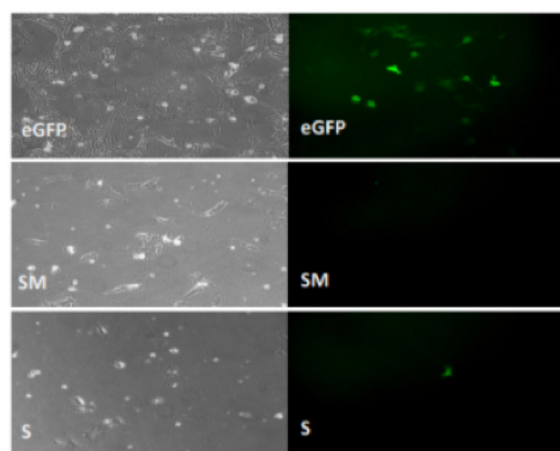


Figure 4: Image grid comparing cell numbers with transfected cell numbers. First row is of the eGFP transfected cells. Second row is of the SM (Wild Type) transfected cells. Third row is of the S (Secreted Type) transfected cells.

Figure 4. shows that the DLK-1 – eGFP fusion constructs did not express well when electroporated into hiPSCs compared to a control plasmid where eGFP is driven by a stronger

promoter (CAG promoter).

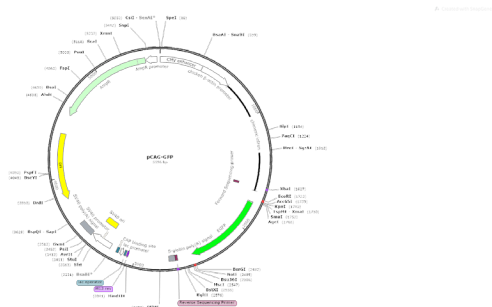


Figure 5: Plasmid map of CAG-eGFP.

The plasmid map in Figure 5 represents the control eGFP plasmid used, in this study and successfully transfected into the cells, as seen by the green cells in the first row (eGFP) of Figure 4. Therefore, we suspect the CMV promoter driving the DLK-1-GFP fusion constructs underwent silencing, preventing the DLK-1 eGFP constructs from being expressed.

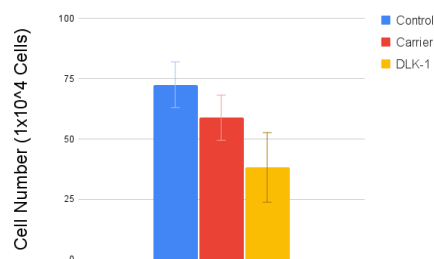


Figure 6: Cell Numbers After Dlk 1 Protein Experiment.

We found that treatment with rsDLK-1 resulted in a slight reduction in cell number (Figure 6); however, the reduction in growth is only statistically significant when compared to the cells-only control. Figure 6 was graphed using the mean cell counts of 72.5, 58.83, 38.25 cells respectively. Error bars for the respective bars were based on the standard deviations derived; 9.47, 9.39, and 14.42 respectively. The clear overlap of the error bars exhibited by the histogram above signified the non-significance of data.

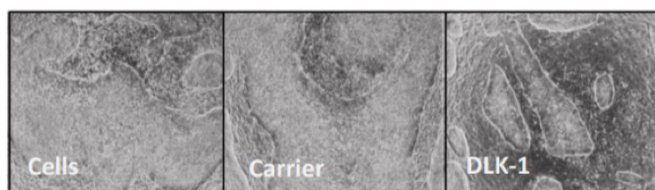


Figure 7: Cells viewed using a phase-contrast microscope.

This reduction in growth could also be seen visually when the cells were viewed using a phase-contrast microscope (Figure 7).

Conclusion

hiPSCs have great potential to revolutionize medicine and research science. This potential cannot be fully achieved until methods for creating genetically modified cells are optimized. It is currently difficult to modify hiPSCs because they apoptose when dissociated into single cells for cloning. The experiments

in this research aimed to overcome this issue by identifying signaling molecules that could improve hiPSC survival. Our study investigated the effects of DLK-1 on hiPSCs using two methods: [1] Misexpression of the secreted (S) and wild type (SM) type forms of DLK-1 and [2] Treatment of hiPSCs with recombinant soluble DLK-1 (rsDLK-1) protein.

The study results show that treatment of hiPSCs with rsDLK-1 protein led to a slight reduction in cell number compared to untreated control cells but not to the control cells treated with BSA carrier protein. (An increase in cell death did not accompany this reduction in cell number.) A slight decrease in cell growth was also observed in the BSA-treated control cells compared to the untreated cells for reasons we do not understand. We are currently under the suspicion that the BSA was not of great quality and may have adversely affected the growth of the cells on its own. BSA is known to be lot-variable. Low-grade BSA can have negative effects on the growth of hiPSC.

We could not assess the effect of misexpression of DLK-1 as a wild type or secreted protein due to silencing of the plasmid construct they were expressed from. We are currently working to overcome this issue by moving the DLK-1 constructs to a CAG expression plasmid.

Our current data do not support or disprove the hypothesis that stimulation of Notch signaling by DLK-1 protein improves the growth or survival of hiPSCs. However, we are still working on repeating these experiments and confirming our results.

There are other Notch ligands and pathways that can play a role in isolated hiPSC survival. It has been shown previously that Delta-like-3 (DLL3) seems incapable of activating Notch signaling⁶; however, ligands Delta-like-1 (DLL1), Delta-like-4 (DLL4), Jagged-1 (JAG1), and Jagged-2 (JAG2) have been implicated in stem cell survival and merit further study.^{7,8} Apart from the Notch signaling pathway, additional studies are needed to elucidate the roles of PI3K-AKT signaling pathway and WNT/ β -catenin signaling pathway in the survival of isolated hiPSCs.^{9,10}

Acknowledgment

I want to thank my PI and the director of Pathways to Stem Cell Science, Dr. Fox for making this experience possible. I would also like to thank my mentor Kenta Shimizu for guiding me through this internship and research.

References

1. Fox, V.; Gokhale, P. J.; Walsh, J. R.; Matin, M.; Jones, M.; Andrews, P. W. Cell-cell signaling through notch regulates human embryonic stem cell proliferation. *Stem Cells* **2007**, *26*(3), 715–723. DOI: 10.1634/stemcells.2007-0368
2. Ferrón, S. R.; Charalambous, M.; Radford, E.; McEwen, K.; Wildner, H.; Hind, E.; Morante-Redolat, J. M.; Laborda, J.; Guillemot, F.; Bauer, S. R.; Fariñas, I.; Ferguson-Smith, A. C. Postnatal loss of DLK1 imprinting in stem cells and niche astrocytes regulates neurogenesis. *Nature* **2011**, *475*, 381–385. DOI: 10.1038/nature10229
3. Grassi, E. S.; Pietras, A. Emerging Roles of DLK1 in the Stem Cell Niche and Cancer Stemness. *Journal of Histochemistry & Cytochemistry* **2021**, *70* (1), 17–28. DOI:10.1369/00221554211048951.

4. Traustadóttir, G. Á.; Lagoni, L. V.; Ankerstjerne, L. B.; Bisgaard, H. C.; Jensen, C. H.; Andersen, D. C. The Imprinted Gene Delta like Non-Canonical Notch Ligand 1 (DLK1) Is Conserved in Mammals, and Serves a Growth Modulatory Role during Tissue Development and Regeneration through Notch Dependent and Independent Mechanisms. *Cytokine & Growth Factor Reviews* **2019**, *46*, 17–27. DOI:10.1016/j.cytogfr.2019.03.006.
5. Falix, F. A.; Aronson, D. C.; Lamers, W. H.; Gaemers, I. C. Possible Roles of DLK1 in the Notch Pathway during Development and Disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **2012**, *1822* (6), 988–995. DOI:10.1016/j.bbadis.2012.02.003.
6. Van de Walle, I.; De Smet, G.; Gärtner, M.; De Smedt, M.; Waegemans, E.; Vandekerckhove, B.; Leclercq, G.; Plum, J.; Aster, J. C.; Bernstein, I. D.; Guidos, C. J.; Kyewski, B.; Taghon, T. Jagged2 Acts as a Delta-like Notch Ligand during Early Hematopoietic Cell Fate Decisions. *Blood* **2011**, *117* (17), 4449–4459. DOI:10.1182/blood-2010-06-290049.
7. Dontu, G.; Jackson, K. W.; McNicholas, E.; Kawamura, M. J.; Abdallah, W. M.; Wicha, M. S. Role of Notch Signaling in Cell-Fate Determination of Human Mammary Stem/Progenitor Cells. *Breast Cancer Research* **2004**, *6* (6). DOI:10.1186/bcr920.
8. Karanu, F. N.; Murdoch, B.; Gallacher, L.; Wu, D. M.; Koremoto, M.; Sakano, S.; Bhatia, M. The Notch Ligand Jagged-1 Represents a Novel Growth Factor of Human Hematopoietic Stem Cells. *Journal of Experimental Medicine* **2000**, *192* (9), 1365–1372. DOI:10.1084/jem.192.9.1365.
9. Hossini, A. M.; Quast, A. S.; Plötz, M.; Grauel, K.; Exner, T.; Küchler, J.; Stachelscheid, H.; Eberle, J.; Rabien, A.; Makrantonaki, E.; Zouboulis, C. C. PI3K/Akt Signaling Pathway Is Essential for Survival of Induced Pluripotent Stem Cells. *PLOS ONE* **2016**, *11* (5). DOI:10.1371/journal.pone.0154770.
10. Yang, K.; Wang, X.; Zhang, H.; Wang, Z.; Nan, G.; Li, Y.; Zhang, F.; Mohammed, M. K.; Haydon, R. C.; Luu, H. H.; Bi, Y.; He, T.-C. The Evolving Roles of Canonical Wnt Signaling in Stem Cells and Tumorigenesis: Implications in Targeted Cancer Therapies. *Laboratory Investigation* **2016**, *96* (2), 116–136. DOI:10.1038/labinvest.2015.144.

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Agricultural Waste Bio-Absorbent for Oil-Polluted Water

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Mentor: David Newell

ABSTRACT: Oil pollution has become a serious environmental concern. Many methods have emerged to counter this pollution, and agricultural wastes have recently been developed as absorbents for oil. To improve the oleophilic/hydrophobic properties, the sorbents are usually modified through physical or chemical treatment. However, the advantages of agricultural wastes are lost once the cost of treatment outweighs the benefits due to energy-intensive and environmentally unfriendly processes. This project aims to determine whether the pomelo peel (PP) in its raw form can be an effective oil absorbent and a filter media to separate oil from water. This project tested the oil absorption capacity of PP at different temperatures, dosages, and sample types and investigated the relationship between these factors and oil absorption capacity. The results of this work show that the gradient porous structure and the hydrophobic property of PP allow it to absorb oil effectively; oil absorbed varied with the sample type. PPs can soak up oil spills like a sponge or separate oil from water as a filter media while also reducing agricultural waste. Therefore, the pomelo peel is a non-toxic, energy-saving, eco-friendly and effective bio-sorbent for oil-polluted water.

KEYWORDS: Materials science; Biomaterials; Agriculture waste; Bio-absorbent; Oil pollution; Pomelo peel.

■ Introduction

Oil pollution continues to threaten the environment and public health. Water sources can be contaminated by oil resulting from marine spills, illegal discharges, and various industries such as food, petrochemical, and metal processing. In addition, an oil spill would probably lead to the destruction of nearly everything in a given biological community, with recovery times that could be decades long.¹

Various techniques based on physical, chemical, and biological properties have been developed to address the issues arising from oil pollution, such as *in situ* burning, biodegradation, flotation, physical absorption etc.² A variety of materials have been used for this purpose including inorganic mineral sorbents, synthetic organic sorbents, and natural organic sorbents.³ Due to low environmental impact, biodegradability, and low cost, porous materials derived from natural resources, especially agricultural wastes, have recently emerged as absorbents for oil. These are things like rice husks, corn cobs, banana peels, orange peels, and polypore Mushrooms.⁴⁻⁸ To improve the oleophilic/hydrophobic properties, the sorbents are usually modified using some physical and chemical treatment.³ The problem is that most modification processes are energy-intensive and environmentally unfriendly due to additional chemical reagents and process.⁹ Cheap and straightforward solutions become saturated with chemicals and difficult to manufacture field.¹⁰ Moreover, some chemical modifiers may introduce potentially toxic substances to water, which is not worth the risk.

The pomelo is the largest citrus fruit. As one can imagine, this fruit has a large pomelo peel (PP). Pomelo peels can be used for insect pest management because their essential oils are toxic to various insect species. Thanks to its excellent absorption capacity, it can also be used as an absorbent for many pollutants such as dyeing sewage. However, the largest amounts

of pomelo peels are thrown away after consumption. PPs are light, full of tiny pores like sponges and very thick. This project aims to determine whether the PP with minimal treatment can effectively absorb and filter out oil from water. In addition, the effects of time, temperature, dosage, and sample type of PP on oil absorption capacity will also be considered. The initial hypotheses of this project are as follows:

(1) PP has oleophilic/ hydrophobic properties and will demonstrate a high absorption capacity for oil;

(2) The sample affects the absorption capacity. Specifically, the oil absorption capacity of dried PP is higher than that of granulated PP.

■ Methods

Materials:

Pomelo, beetroot, canola oil, and distilled water were purchased at markets in Victoria, BC. PH test paper, 1M NaOH, 1M HCl, and Ethanol were provided by the science department at Mount Douglas Secondary. According to the literature, the most encountered oil types in spills are crude oil (35%), diesel oil (20%), marine oil (10%), and gasoline (8%).³ Therefore, diesel and gasoline were bought due to their availability. The morphology of the samples was observed with a MAX 20 watts, type T microscope and a wireless digital microscope. The weights of materials were measured with an Ohaus Cent-O-Gram model 311 mechanical balance. Pictures were taken with a cellphone.

Pomelo peel pre-treatment:

The pre-treatment procedure of PP is illustrated in Figure 1. First the external yellow skin was peeled off; then the remaining pith was cut into samples of around 4x4x2 cm. The samples were thoroughly washed several times and immersed in distilled water for 24 hours to eliminate interference from other particles. Then, the cleaned pomelo peels were placed in

After drying, samples of absorbents were divided into three groups: 1) Sample A: dried PP; 2) Sample B: Granular PPs were made using a blender and passed through a 1mm sieve to remove particles too fine to use; 3) Sample C: Sample A was immersed in 0.1M NaOH for 12 hours to theoretically remove impurities. After that, the product was thoroughly washed in distilled water until the runoff was colorless and transparent (pH =7). The obtained material was dried. NaOH was neutralized with HCl and disposed of properly. All samples were stored in airtight containers with desiccants to avoid atmospheric moisture absorption.

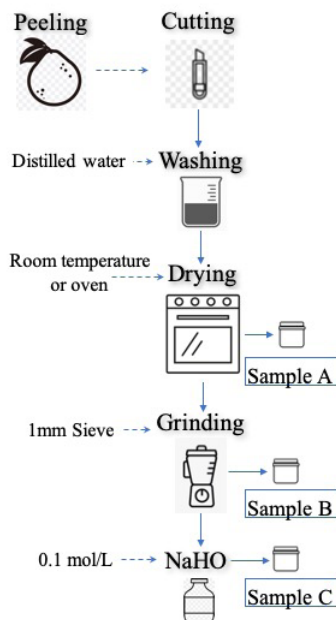


Figure 1: Schematic diagram of sample processing.

Oil absorption capacity:

PPs used in this study were cut to be 0.2 g or 0.4g while dry (initial weight m_0). An appropriate amount of oil was poured into a beaker; then a sample was placed. The sample was taken out at intervals of 5 seconds, and oil adsorbed on the surface was carefully scraped with a plastic card because only the weight of oil adsorbed was wanted. The sample weight (m_1) was recorded after each test. This process was repeated several times until the sample reached saturation. Each test was performed twice, and the average value was used as the final record. The oil absorption capacity Q could be calculated using the formula (1).

$$Q = \frac{(m_1 - m_0)}{m_0} \quad (1)$$

where Q is the oil absorption capacity of the sample (g/g), m_0 represents the weight of samples before absorption, and m_1 represents the weight of samples after absorption.

Oil-water separation apparatus:

An apparatus was constructed to separate oil from water (Figure 2). The system comprised storage tank A, funnel B, a filtration column, and an effluent collection tank C. The volumes of A and B were 800ml and 200ml, respectively. The filtration column had a height of 6.00 cm, and an inner diameter of 2.50 cm. Collection tank C had a volume of 500ml.

If the amount of liquid needed to be separated is large, oil and water can first be separated by density in storage tank A. Oil and water outs were controlled by valves at the lower end. Then, the residual oil-water mixture that the first step cannot treat can be introduced into funnel B.

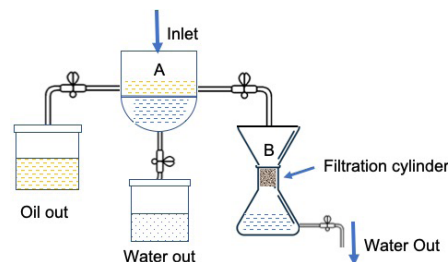


Figure 2: Oil-water separation apparatus.

Results

Structure and morphology of a typical pomelo fruit:

Figure 3 shows the anatomy of the two pomelos used in this project. The peel of pomelo fruit is about 2 cm thick and accounts for approximately 30%-50% of the total fruit weight. Figures 4 and 5 show the fresh PP and the three types of samples. PP has a natural gradient porous structure made up of wrinkled pores. The pore sizes of PPs vary from 10–1000 μm and increase closer to the skin, with the average pore size being $151 \pm 31 \mu\text{m}$.^{2,11}

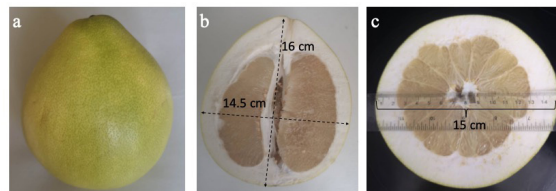


Figure 3: Anatomy of a typical pomelo (a) Pomelo fruit; (b) Longitudinal section; (c) Cross section.

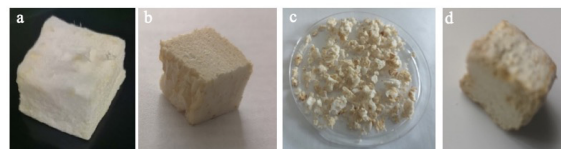


Figure 4: Fresh PP and the sample types (a) Fresh PP; (b) Sample A; (c) Sample B; (d) Sample C.



Figure 5: Microscope images of the PP (a) Fresh PP; (b) Sample A and B; (c) Sample C.

Wettability of the PP:

Wettability is the ability of water to spread over a surface. Water contact angles (WCA) between liquids and surfaces are used to evaluate wettability. The material is classified as wettable if the WCA is less than 90° . If greater than 90° , it is non-wettable.³ Figure 6 (a) shows that the PP floated above the water indicating its low density. In Figure 6 (b), water

(colored red with beetroot) formed a drop on the surface of the dried PP (sample A), while oil was rapidly and completely absorbed. For example, at a temperature of 10 °C, a drop of canola oil was absorbed in about 2.3 seconds, and diesel and gasoline in less than 1 seconds. A drop of water, in comparison, will stay on the surface of the PP for up to 9 hours.

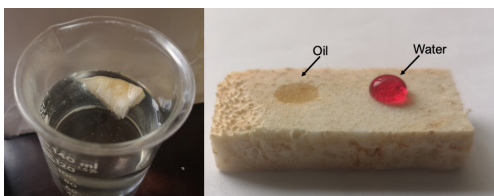


Figure 6: Wettability of PP.

(a) Behavior of PP in water; (b) Wettability of oil and water on the surface of a sorbent

This project estimated the WCA using a protractor on a picture (Figure 7). Since the surfaces were not perfectly flat, the angles on each side varied. The two WCAs were 105° and 115°. Therefore, dried PP could be classified as non-wettable.

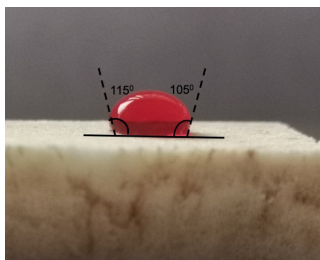


Figure 7: The contact angle of water on PP.

Oil water separation:

1 gram of canola oil was put into a beaker containing water (colored with beetroot), and then a PP sample of 0.2 grams was added to the beaker. The PP completely absorbed the oil within 5 seconds. Then, 50 ml of water was mixed with 2 grams of canola/diesel oil and the mixture was directly dumped into funnel B and passed through the cylinder containing 0.4 grams of sample B. Only water flowed into the effluent tank below. The oil and water were completely separated.

The absorption capacity of oil:

Many factors, including time, temperature, dosage, and sample type, could influence how much oil is absorbed. First, taking sample A as an example, the effects of time, temperature, and dosage were analyzed. Then, the effect of sample types was analyzed by comparing the absorption capacity of samples A, B, and C at 10 °C. First, the weights of samples were recorded, and then the absorption capacities at different time intervals were calculated using the formula (1).

The kinetic curves of all tests were drawn using Excel (Figure 8). The overall trend was that oil would be rapidly absorbed in the first five seconds, with the amount then gradually increasing over time until it reached saturation. The absorption capacities (AC) when diesel and gasoline at 40°C were higher than when at 10°C, which shows AC increases with temperature. However, higher temperatures were not conducive to the AC for canola oil.

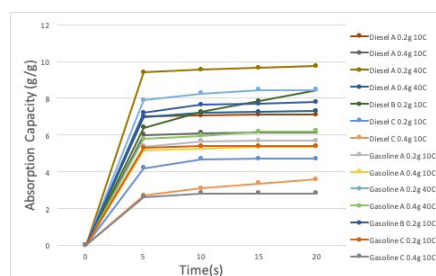


Figure 8: Temperature effect (canola oil). Little change occurred after 20 seconds.

Taking diesel and gasoline as examples, Figure 9 illustrates the absorption capacity of different doses of each sample at different temperatures. For example, the absorption capacity of a 20g sample is higher than that of a 40g one at both 10 °C and 40 °C.

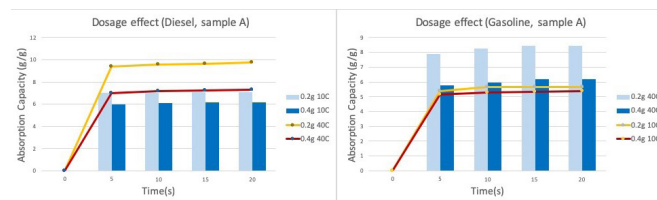


Figure 9: The effect of sample amount on the oil absorption capacity of PP.

Figure 10 shows the absorption capacity of different samples for canola oil, diesel and gasoline at 10°C. For all types of oils, sample B had the highest absorption capacity. At 10°C, 0.2g samples of B could absorb diesel, gasoline and canola oil with an AC of 8.45 g/g, 7.8 g/g and 8.3 g/g, respectively. Sample A had capacities of 7.1g/g, 5.68g/g and 6.6g/g. The absorption capacity of NaOH-treated sample C is less than that of other samples.

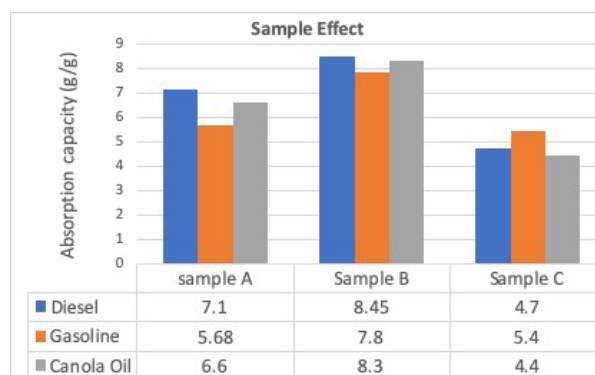


Figure 10: The absorption capacity of all samples is at 10 °C.

Absorption capacity for water:

While dried PPs are non-wettable, their pores could still collect water in the process of absorbing oil. Therefore, the AC of PP was actually lower than the values in the above tests. To see the effect that water had on the oil AC, the AC of water was tested. The results showed that within the first 60s, the absorption capacity for water was about 0.6g/g and increased over time (Figure 11).

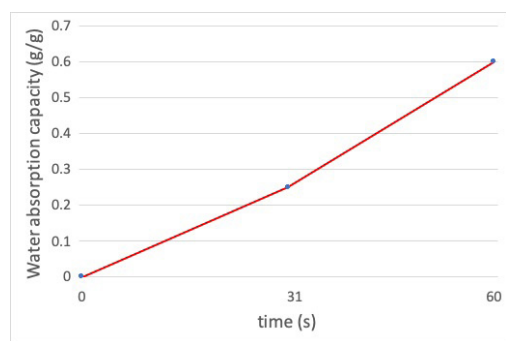


Figure 11: Absorption kinetic curves for water.

Discussion

The properties of pp and their contributions:

The porous structure and the non-wettable property of PPs significantly contribute to their high ACs for oil, which is consistent with our hypothesis. In addition, the various pore sizes of PP, especially macro-pores, may significantly enhance that capacity.² Also, the main components of PP are pectin, cellulose, hemicellulose, and lignin. Lignin is relatively hydrophobic in nature and plays a more important role in oil adsorption than cellulose and hemicellulose components.¹² The presence of functional groups on the surface of PP was the main adsorption mechanism.⁷ The hydrophobic property of PP prevents most water absorption and improves the efficiency of oil adsorption because there is no competition between water and oil molecules.³

The effects of temperature, dosage, and sample type on the absorption capacity:

Many factors, including time, dosage, and sample size, influenced the absorption capacity of PP. First, unlike diesel and gasoline, higher temperatures did not aid in the absorption of canola oil. The reason might be that high fluidity reduces absorption. Since viscosity varies with temperature, the viscosity of canola oil decreased dramatically with the increase in temperature. In addition, the absorption capacity of other samples could have been better than that of NaOH-modified sample C. Finally, in trying to remove impurities from the pores, NaOH might have ruined the fragile structure.

Moreover, the phenomenon that sample B had high absorption capacities did not support the hypothesis. That may be attributed to the granular PP's higher surface area to volume ratio. This can also explain why sample B's absorption capacity is higher than sample A's. Thus, it is better to use sample B as the adsorbent to soak up oil.

Compared to other types of peel:

In this work, at 10°C, 0.2g of sample A could absorb canola oil, diesel and gasoline with a sorption capacity of 6.6g/g, 7.1g/g and 5.68g/g, respectively. Comparable to other natural materials, the AC of PP is similar to or even higher. For example, gasoline's AC of carbonized rice husks is about 3.7g/g at 20°C.² The AC of corn cobs for crude oil is 2.5 g/g; raw orange peel for crude, diesel, and used engine oil is 3-5g/g;⁵ and raw banana peels had capacities of 5.31, 6.35, and 6.63 g/g for gas oil, 1day, and 7-day weathered crude oil, respectively.⁴ It is

inferior to some modified materials, such as acetylated banana fibers (18.12g/g).¹²

Limitations and further research:

This project had some limitations that may have affected the final results. First, hydrophobic does not mean completely waterproof. The absorption capacity of PP for oil is likely less than the results suggest because PP may absorb a specific (even though minimal) amount of water simultaneously. Moreover, the high volatility of gasoline will affect the accuracy of the measurement. Finally, this project analyzed the oil adsorption of PP for three oil types and future research could analyze the absorption capacity for other types of oil.

Conclusion

Oil pollution is a serious issue. As a part of the pomelo that usually goes to waste, peels are cheap, abundant, and biodegradable. In this work, the morphology, wettability, and oil adsorption capacity of PP were investigated. The results indicated that PP has a low-density, gradient porous structure. Without modification, the contact angle at the water-dried PP boundary remained large, indicating a strong hydrophobic character. The PP treatment process without chemical modification or carbonization reduces unnecessary treatment processes, saves energy and does not introduce harmful substances to water. Due to its pores structure and surface hydrophobicity, PP demonstrated high oil adsorption capacity. It could clean up oil spills as an ultra-light sponge or separate oil from water as a filter media while also reducing agricultural waste. Therefore, PP can be an attractive candidate for nontoxic, energy-saving, eco-friendly, easy-to-operate and effective bio-sorbent for oil-polluted water. The countries with the highest pomelos production volumes are China, Vietnam, United States, Mexico, South Africa, and Thailand. This method of using pomelo peels to absorb oil pollution can be used in many countries.

Acknowledgment

I want to thank my teacher (David Newell) for his invaluable advice and help in doing my project by supplying vital equipment. I would also like to thank my mom for her support and encouragement. Finally, thanks to the Editors and the reviewers who have made this article the best it can be.

References

- Baker, J. M., Oil Pollution. Encyclopedia of Ocean Sciences (Third Edition) 2001, 6, 350-358.
- Shi, G.; Qian, Y.; Tan, F.; Cai, W.; Li, Y.; Cao, Y., Controllable synthesis of pomelo peel-based aerogel and its application in adsorption of oil/organic pollutants. *Royal Society Open Science* **2019**, 6 (181823), 1-13.
- Zamparas, M.; Tzivras, D.; Dracopoulos, V.; Ioannides, T., Application of Sorbents for Oil Spill Cleanup Focusing on Natural-Based Modified Materials: A Review. *Molecules* **2020**, 25 (4522), 1-22.
- Angelova, D.; Uzunov, I.; Uzunova, S.; A.Gigova; Minchev, L., Kinetics of oil and oil products adsorption by carbonized rice husks. *Chemical Engineering Journal* **2011**, 172, 306-311.
- Nwadiogbu, J.; Okoye, P.; Ajiwe, V.; Nnaji, N., Hydrophobic treatment of corn cob by acetylation: Kinetics and thermodynamics studies. *Journal of Environmental Chemical Engineering* **2014**, 2, 169-1704.

6. El-Din, A.; Amer, A.; Malsh, G.; Hussein, M., Study on the use of banana peels for oil spill removal. *Alexandria Engineering Journal* **2018**, *57*, 2061–2068.
7. Gheriany, I.; Saqa, F. A.; Amer, A. A.; Hussein, M., Oil spill sorption capacity of raw and thermally modified orange peel waste. *Alexandria Engineering Journal* **2020**, *59* (2), 925–932.
8. Balzamo, G.; Singh, N.; Wang, N., Vladislavljević GT, Bolognesi G, Mele E. 3D Arrays of Super-Hydrophobic Microtubes from Polypore Mushrooms as Naturally-Derived Systems for Oil Absorption. *Materials (Basel)*. **2019**, *12*(132),1-11.
9. Tocmo, R.; Pena-Fronteras, J.; Calumba, K. F.; Mendoza, M.; Johnson, J. J., Valorization of pomelo (*Citrus grandis* Osbeck) peel: A review of current utilization, phytochemistry, bioactivities, and mechanisms of action. *Comprehensive Review in Food Science and Food Safety* **2020**, *19*, 1969-2012.
10. Yu, X.; He, Y., Optimal ranges of variables for an effective adsorption of lead(II) by the agricultural waste pomelo (*Citrus grandis*) peels using Doehlert designs. *Scientific Reports* **2018**, *8* (729), 1- 9.
11. Looyrach, J.; Methacanon, P.; Gamonpilas, C., Pomelo (*Citrus maxima*) Peel-Inspired Property for Development of Eco-Friendly Loose-Fill Foam. *Key Engineering Materials* **2015**, *659*, 279-283.
12. Sadeek, S.; Negm, N.; Hefni, H.; Wahab, M., Metal adsorption by agricultural biosorbents: Adsorption isotherm, kinetic and biosorbents chemical structures. *International Journal of Biological Macromolecules* **2015**, *81*, 400–409.
13. Teli, M. D.; Valia, S., Acetylation of banana fibre to improve oil adsorbency. *Carbohydrate Polymers* **2013**, *92*, 328– 333.

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An Analysis of AP Classes Growth and the Effects on Student Stress

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ABSTRACT: Many high school students take accelerated courses, and past research has shown that this can lead to higher stress. The Advanced Placement program (AP) is currently one of the most ubiquitous accelerated course programs across the United States. To understand how AP enrollment changes over time, this study analyzed AP data reports from 2002-2021 provided by the College Board. To further understand how AP classes affect students' academic stress and well-being factors, this study surveyed 176 students at a North Central Florida high school. The results show that the AP exams per student exhibited a significant increase for high school students in the past two decades, with faster increases in the lower grades (9th and 10th graders) and higher expectation students. In addition, the survey indicates that when the average student takes more APs, they tend to spend more time on homework, get less sleep, and have higher self-perceived stress. However, students who took one to three AP classes generally had the best median mental well-being score. Though the sample distribution may have certain biases, these results provide useful information for students and families to develop optimal academic strategies based on students' study habits, stress-coping ability, and expectations.

KEYWORDS: Behavioral and Social Sciences; Sociology and Social Psychology; Advanced Placement class; mental stress; high school student, eustress.

■ Introduction

High school plays a significant role in the development of adolescents, a period of important changes in responsibilities, physique, and mental functions.¹ However, high-stress levels have long been an issue for high school students. Many factors, including social, academic, and family reasons, usually contribute to this stress. Among these, academic factors are among the top reasons for student stress.² As past studies have suggested, the impacts of normative stressors, or those that are frequent and widespread, like academic's stress, which is experienced by many students frequently, profoundly affect mental health.³ Consequently, high stress could often lead to harmful coping behaviors, a key reason for student stress to be studied. Furthermore, student stress is increasing, and the changing academic world is a major reason for its rise.⁴

Currently, accelerated curricular courses play an important role in high school education. Higher enrollments in accelerated curricula like International Baccalaureate (IB) and Advanced Placement (AP) programs exist for high school students than in past decades. The AP program, offered in about 70% of public high schools across the U.S.⁵ with over 4.5 million exams registered in 2021,⁶ is one of the most ubiquitous accelerated course programs in the United States. The increased popularity of taking AP courses in high school was partly triggered by government support,^{7, 8} such as financial support, easier accessibility, and higher Grade Point Average (GPA) weight. Another critical factor contributing to its popularity is AP's potential advantages, such as earning college credits, enriched learning opportunities, and increased chances to win scholarships.⁹

Indeed, AP science course participants performed better than regular course group participants regarding their end-of-year assessment of scientific abilities,⁹ post-secondary education grades, graduation rates,¹⁰ and the chance to get accepted to universities.¹¹ These advantages have become important reasons for the rapid increase in AP enrollment. However, the research found that students in AP classes often experienced higher stress levels than students in general classes.^{12,13} Since AP courses are more challenging than regular courses because of their difficulty and workload, students must devote more time and effort to them. Students may need help to accomplish the demanding academic requirements of AP courses.¹⁴ The pressure to perform well in AP exams, or the end-of-course exam for each AP course, is another source of stress. Students who are high achievers or with higher expectations are especially prone to such academic stress.¹⁵ Furthermore, students are often sleep-deprived due to busy and demanding schedules.^{16,17}

While most of the previous studies do focus on AP courses' effects on adolescents' mental health, stress, and ways to cope with this stress, they are mainly qualitative, using methods such as interviewing students and inquiring if students are taking AP rather than how many APs students are taking. As such, the current body of knowledge does not provide a quantitative measure of how many AP classes benefit students, cause the most stress, or lead to the best mental health. Furthermore, there is minimal research on how student stress is changing in relation to the changes in AP enrollment. These are important gaps to cover and are especially valuable information to students, teachers, and guardians. With the rapid increase

AP courses, there is a need to understand how AP enrollment will affect teens' stress and the tradeoff between academic expectations and stress levels. Through the 20 years of data provided by the College Board, this study hopes to document any changes or trends in AP enrollment and correspond them with the changes in the stress of students. With a perspective from students on how the continued development in AP classes has affected students' stress, mental health, and sleep schedule, this study aims to cover the gap of how student stress in relation to AP classes is changing and how an equilibrium of stress and AP enrollment can be reached.

■ Methods

This study uses quantitative approaches: a trend analysis of the College board's datasets and an analysis of a survey conducted at F.W. Buchholz High School, a North Central Florida public high school. Besides the histogram of SWEMWBS scores in Figure 8, created with Python 3.9, all graphs and data analysis were completed with Excel 2016. The first section of the method, using statistical analysis, is the analysis of datasets composed of yearly reports of AP classes published by the College Board.¹⁸ The second part consists of surveying 176 public high school students and analyzing those responses (Questionnaire, See Appendix A).

College Board Datasets:

The College Board provides annually reported data on the AP program from 2002 to 2021.¹⁸ The datasets include four categories: annual AP summary reports, annual AP score distributions, annual AP exam volume, and yearly AP other information and data. This study utilized five statistical information from 2002 to 2021, including the yearly total number of exams taken, the total number of students, the number of AP students in each grade, the number of exams for each grade, and the frequency of students taking each number of AP exams for each four-year administrative period.

To identify how the AP volume is changing per student, the study calculated the average number of AP exams per student, the proportion of AP students from each grade, and the 50th, 75th, 95th, and 99th percentile of students based on number of exams they are taking for each 4-year administration period.

However, the analysis of AP enrollment only gives an idea of how students' academic workloads may be changing. There is no direct connection to students' stress levels, sleep hours, and mental well-being. Therefore, this study conducted a survey at the school inquiring about students' AP schedules and various stress indicators to better understand the relationship between the two variables.

Survey:

Questionnaire Design

The study used a cross-sectional survey administered online at one point in time. The questionnaire focused on AP course numbers, students' physical responses to academic stress (e.g., self-perceived stress, homework hour, sleep time), and mental health. The survey questions were divided into two sections: one inquiring about students' number of APs taken this year and their AP enrollment history; and another section on students' self-perceived stress, sleep habits, and homework hours. For example, to self-reported general stress levels, students

answered the prompt "I would consider myself to usually be" with one of five categories: very relaxed, somewhat relaxed, equal parts relaxed and stressed, somewhat stressed, and very stressed. To measure students' mental well-being, the study used the Short Warwick-Edinburgh Mental Wellbeing Scale (SWEMWBS), a mental well-being scale created by the Warwick Medical School. Since longer surveys may discourage students from responding to the survey, the 7-question scale (SWEMWBS) instead of the 14-question version – WEMWBS – was used.¹⁹ Past research shows that the SWEMWBS is a valuable scale for educational, developmental, and physiological uses on adolescents' mental well-being.^{20, 21} The scores for all of the seven questions on the SWEMWBS, which are graded from 1 to 5, are added to attain the final score, which ranges from 7 to 35, with higher scores indicating higher mental wellness.

Survey collection

In January 2023, a school-wide email was sent with an attached Google Forms survey. Participation was sought from all students in a public high school in Florida - F.W. Buchholz High School. The email included a short explanation of the study and a link to participate in the online survey. The survey was sent to the email addresses provided by the school, ensuring all students had access to this email. Students could access the link from their email at any time, and all students had access to a computer to fill out the survey, either through personal computers or at the school library. The author's email was provided for students in case of any questions about the survey. Names were never collected to ensure confidentiality, but limiting one response per email was activated. This avoided duplicate responses from the same student. The surveys could only be submitted when all the questions were answered. No incentives or inducements for participation were offered to students beyond the opportunity to contribute to the current body of knowledge.

Survey analysis

To understand the general background of the survey population, univariate analysis was done on students' AP background, SWEMWBS scores, and sleep hours, and corresponding pie charts and histograms were made. These graphs helped provide background analysis of the student survey population, allowing for an easier understanding of the correlational analysis between the number of AP classes and stress variables. The stress variables used for analysis were homework hours, self-perceived stress, and SWEMWBS score. These variables were correlated with the number of AP classes students took for this 2022-2023 school year. The significance of the relationship was based on the value of Pearson's correlation coefficient, calculated by Google Sheets and Excel. Most of the scatterplots had very low correlations. They did not lead to a significant relationship because of the high variability of student scores for all stress variables regardless of AP classes. Thus, the stress variables were calculated into medians. The median was chosen over the mean because the median is more resistant to outliers in the data. Thus, the median homework hours, self-perceived stress, and SWEMWBS score were correlated with the number of AP classes students took, and the

relationship was documented. A further correlation between self-perceived stress was also conducted to determine if stress may benefit mental well-being.

Results

Changes in AP exam number:

The changing trend of the average AP examination volume

A significant, strong, and positive linear relationship exists between the number of AP exams per student and the year from 2002 to 2021 ($r = 0.96, f = 2.79 \times 10^{-10}$) (Figure 1). Average AP exams per student increased from 1.70 in 2002 to 1.80 in 2021, corresponding to an increase of 2.9% per 10 years. The fastest growth occurred in 2010–2015, with a peak in 2018 and then a decrease afterward, possibly due to the Covid-19 pandemic. Though this result indicates that the potential academic pressure on AP students across the United States may be continuously increasing, as the number of AP classes per student increases, the increase is only 0.0065 AP exams per student each year.

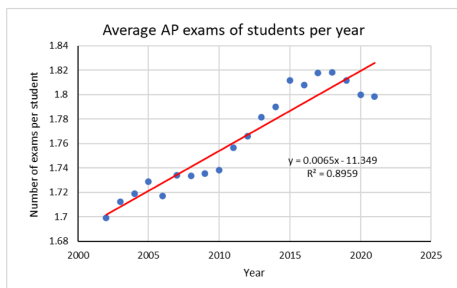


Figure 1: A scatterplot of the average number of APs per student from 2002 to 2021.

AP course number changes among grades

Figure 2 demonstrates a pronounced shift of AP exam takers from higher- to lower-grade students. In 2002, 49.8% of the AP exams were taken by the 12th graders, representing the largest grade groups and followed by 11th graders. However, this number gradually decreased to 31.5% in 2021, giving the largest grade groups to juniors since 2016. Furthermore, AP exams taken by 9th and 10th exhibited a rapid increase, with 0.6% (2002) to 9.1% (2021) for the 9th graders and 10.1% (2002) to 22.4% (2021) for the 10th graders, respectively. This change indicates increasing academic pressure for the younger students; this elevated stress level due to accelerated courses, according to Suldo *et al.*,²² is present for students as early as 9th grade.

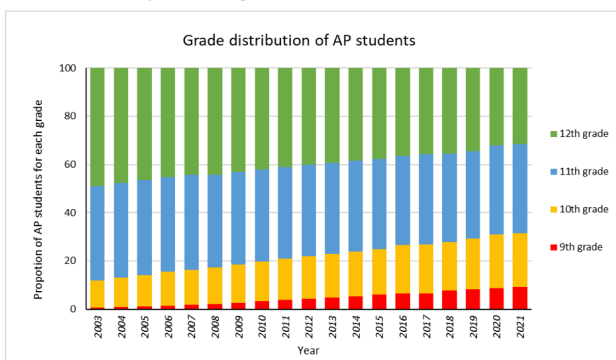


Figure 2: The proportion of students from each grade taking AP classes for each prospective year from 2002 to 2021.

Change of students' AP exam numbers for four administrative periods

To investigate the overall stress level for students during high school, this study looked at the AP exam changes for different student groups by dividing them into four groups based on the number of AP exams they take in four-year administered periods. This division clusters the students into four groups, representing the different levels of academic pressure, defined as pressure experienced by students due to the work and expectations of their academics, from high to low (high: ~95th percentile, middle high: 75–95th percentile, middle low: 50–75th percentile, and low: ~50th percentile). For instance, students in the 75th percentile have taken more AP classes in their four-year period than 75% of AP students who are in the same four-year period have. All four student groups exhibited a linear increase in AP exam numbers for the past two decades. However, students in groups with higher academic pressures experienced a faster growth rate than those with lower pressures. For example, the AP exams taken by the 95th percentile students increased roughly six times faster than the 50th percentile students. This implies that while most students experienced a gradual increase in their AP classes, the students with the highest academic expectation experienced the fastest increases in AP enrollment, from 8 to 11 and a change of 38%. This suggests that students with high ambitions could have more stress, consistent with Blazer's claims¹⁵ that high achievers are more susceptible to academic stress.

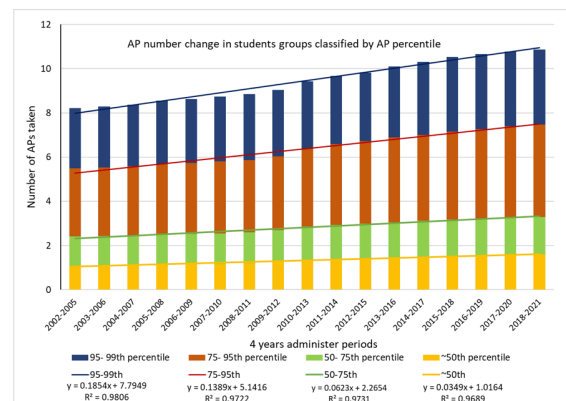


Figure 3: The number of students' AP exams increased for each 4-year administrative period from 2002 - 2005 to 2018 - 2021.

AP vs. Stress and mental health based on the survey:

School background and survey result

Buchholz high school is a school located in North Central Florida. With a student population of 2,472 for the school year 2022–2023, it is the most populated school in the Alachua school district. This school offers 29 AP classes for the 2022–2023 school year.²³ Buchholz has a 40% AP enrollment rate and a 76% AP exam pass rate for the 2022–2023 school year.²⁴ Though most classes are available to all grade levels, some classes are only available once pre-requisite classes are completed, meaning that some of the courses are reserved for juniors and seniors. Students can enroll in an AP class by signing up with a school counselor, who will work with the student

to confirm the AP class and determine the schedule (such as checking if prerequisites are completed).

This survey collected responses until early February 2023, receiving 180 responses, around 7.4% of the school population. Four responses were removed due to contradicting answers or nonsense responses. The distribution of valid responses by grade and the number that are taking at least 1 AP class for this year is shown in Table 1.

Table 1: Participant sample and academic background of AP characteristics.

Grade	Responses distribution by grade		The number of students taking AP classes	
	Num.	% in sample	Number of students taking AP classes in that grade	% taking AP Classes in that grade in the sample
9th	27	15.34	18	66.67
10th	46	26.14	40	86.96
11th	52	29.54	48	92.31
12th	51	28.98	50	98.04
Total	176	100%	156	88.64

APs background of respondents

Figure 4a shows the number of AP courses survey participants took for the 2022-2023 academic year. Among them, 21.6% of students participated in two AP courses, accounting for the largest proportion, while 28.4% chose to take four or more AP courses. Figure 4b shows the grades of the surveyed students who participated in the first AP course. More than half (59.7%) of the students took their first AP class in 9th grade, followed by about 22.7% in 10th grade, indicating once again that high school students have been under academic pressure since the beginning of their freshman year. This shows some consistency with the College Board's data as a significant number of students take their first AP in 9th or 10th grade.

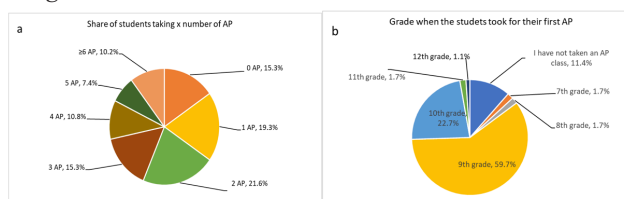


Figure 4: Pie charts of surveyed student background in AP.

Sleep habits and homework hours

Sleep habits:

Students reported their average sleeping hours per night during school days. Overall, most of the students (35.6%) reported a sleeping hour between 7-8 hours, followed by 6-7 hours (31.1%), greater than 8 hours (19.8%), and less than 6 hours (13.6%). The results showed that most students' sleep time was lower than the health standard recommended by the Centers for Disease Control and Prevention (CDC; Teens 13-18 years old, 8-10 hours of sleep per hour). In addition, according to students' reports on bedtime, 35.0% of the students said they usually sleep between 11 pm and 12 am, and 29.4% said they typically sleep between 12 am and 2 am. Previous studies showed that late and short sleep increased the risks of diseases.^{25, 26} The key reasons for going to bed late are homework (107 students, 60.5%), entertainment (e.g., games) (73 students, 41.2%), and social media (60 students, 33.8%), suggesting academic pressures are one of the key drivers affecting students' sleep habits.

A one-tailed t-test was conducted at the alpha level of 0.05 to determine if there was a significant difference in sleep hours between students with varying AP classes. This study found a significant difference between students who planned to take (or have taken if they are seniors) 0 - 12 AP classes and students who planned to take 12+ AP classes in their four-year high school. The students who planned/took 0-12 AP classes had an average of 6.87 hours of sleep per night ($n=136$, $sd=1.131$), while this number was 6.55 hours for the students who planned to take 12+ AP ($n=40$, $sd=1.019$). The hours between the two groups are significantly different, with a p-value of 0.048, indicating that significant evidence suggests that students who took or planned to take more AP tended to sleep less than those who took or planned to take less.

An individual's stress experience also depends on their appraisal of their ability to cope with the stressor.³¹ Because students who have higher expectations tend to take more AP courses, this study classified the students into three groups based on the total number of AP during the four administrative years and examined their mean stress characteristics (Table 2). Groups with an AP number between 4-14 exhibited the highest stress level, indicated by sleep time, stress level, and SWEMWBS scores. Although the difference in values between groups was insignificant ($P>0.05$) for most indicators, students with AP courses greater than 14 tended to have lower stress levels than those with 4-14 APs, as indicated by the SWEMWBS score.

Table 2: Mean survey values in sleep time, stress level, SWEMWBS, and homework for different student groups divided by the expected total number of AP throughout high school.

AP Group	Sleep time	Stress level	SWEMWBS score	Homework hour
14+	6.47a	3.58a	22.88ab	19.37a
4-14	6.89a	3.68a	21.93a	14.71ab
0-3	6.65a	3.26b	23.79b	13.49b

Note: Means in each column that were significantly different ($P<0.05$) based on the Least Significant Difference (LSD) analysis are labeled with different letters (a, b, ab).

Homework hours:

The time spent on daily homework reported by students is distributed from less than 1 hour to more than 5 hours after school each school day. The largest proportion of students (36.7%) reported 1-2 homework hours per day, followed by 0-1 hours (24.9%) and 2-3 hours (19.8%). Many factors contributed to the differences in homework hours, including students' learning habits, homework efficiency, homework difficulty, homework pressure, and after-school activities. Reported homework time on weekends is more scattered than the time on the school day, ranging from less than 1 hour to more than 10 hours. Most students (24.3%) work 1-2 hours on weekends, followed by less than 1 hour by 20.9%.

The study calculated the total hours per week that students spent on academics for the whole week (school day + weekends) outside of school. This was done by summing up five times the hours spent on a weekday plus the total number of hours spent on the weekends. Then, extreme outliers or unexpected data points (i.e., >20 hours per day) were removed, and the median number of hours spent correlated to the number of APs students took for that year.

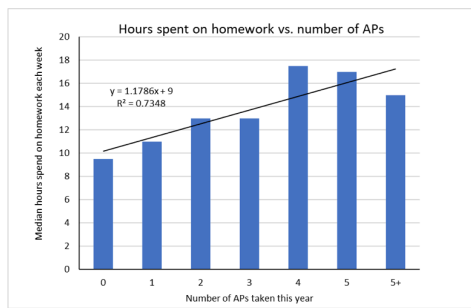


Figure 5: Relationship between median hours spent on homework and number of AP classes taken.

Figure 5 shows that the median number of hours spent on homework per week has a strong significant linear positive relationship ($r = 0.857$, F-value for the F-test is 0.0137 when the confidence level is 95%, which is significant as $F < 0.05$) with the number of AP classes taken that year. Students taking more AP classes tend to have a higher amount of homework to complete, supporting previous findings that a high AP course load increases students' workload and learning pressure.

AP vs. self-perceived stress and SWEMWBS

The study classified the evaluation results of students' self-reported stress levels into five groups: never, rarely, sometimes, often, and always. In the self-stress level reported by students, the group of "often stressed" took the largest proportion (44.6%), followed by the group of "sometimes stressed" (28.8%) and "always stressed" (13.6%) (Figure 6).

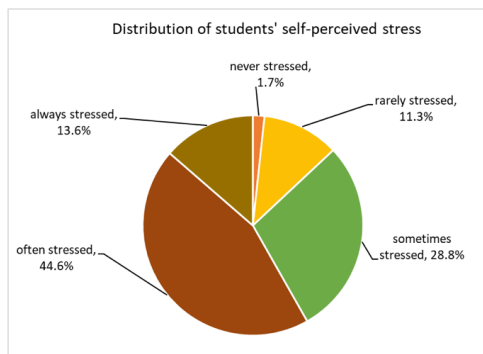


Figure 6: The proportion of students' self-perceived stress level.

Figure 7 shows the relationship between students' median perceived stress and the number of AP taken per year. This relationship is significant under the F-test (significance $F = 0.00411 < 0.05$ at the confidence level of 95%). The perceived stress was classified into five categories, with 1 to 5, respectively, representing 'never stress' to 'always stress.' Perceived stress increases as the number of APs increase but levels off after about 2 to 3 APs. This indicates that students who take more AP classes turn out to have higher stress levels, but only sometimes, as the AP number exceeds a certain threshold, in line with the findings by Conger *et al.*⁹

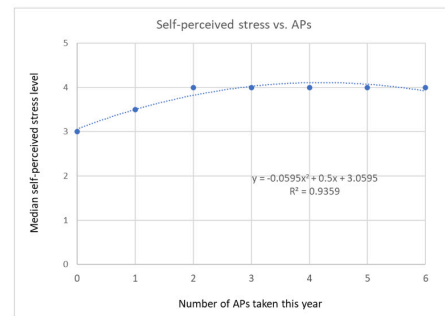


Figure 7: The relationship between the student's median perceived stress and the number of AP classes taken yearly.

It can be seen from Figure 8 that the SWEMWBS scores of students conform to the normal distribution, although with a slight and insignificant right skewness. The mean score is 22.5, and then a score of 22 is the score with the highest frequency. Regarding the score for every seven questions in SWEMWBS, the question "I've been feeling relaxed" received the lowest score of 2.77 on the 0-5 scale. This low score may imply the widespread stress status among students, which is also reflected in students' self-reported stress status. The second lowest score is for the question "I've been feeling useful" with an average of 3.028, followed by the score for "I've been dealing with problems well" with an average of 3.14. These two questions, to some extent, may embody students' status of self-confidence, in which an excess number of AP exams and associated pressure can likely increase the chance of students losing their self-confidence.²⁸

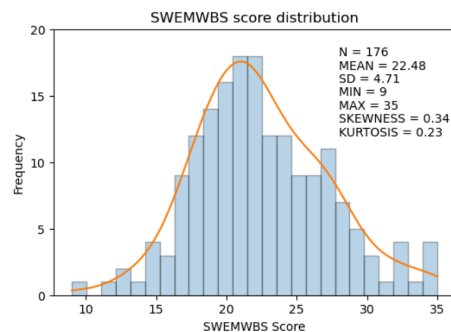


Figure 8: The distribution of the SWEMWBS scores received in the survey.

A correlation analysis was conducted between the number of AP courses per year and the SWEMWBS score (the median for all the AP groups) (Figure 9). There is a non-linear relationship between the AP course number and the SWEMWBS score, with an optimum AP number of around 2 for the highest score. The result of the F-test with a confidence level of 95% leads to a significance F of 0.0242, which is significant as it is less than the alpha level of 0.05. This result may indicate that taking one to three AP courses may benefit students' mental health the most. The median SWEMWBS score goes down at both ends when students take fewer or more AP courses, suggesting that fewer than one or more than three APs could increase student stress. This result may alternatively indicate that students with higher median mental health tend to choose one to three AP classes per year.

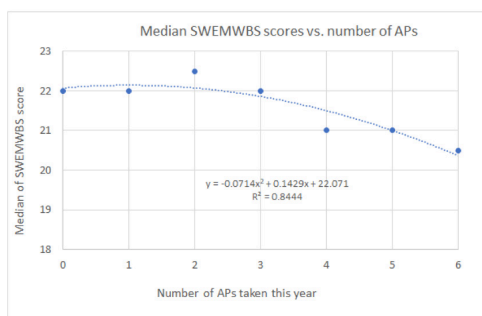


Figure 9: Relationship between the number of AP courses taken by students in the 2022-2023 school year and the median value of the SWEMWBS score.

To further understand the relationship between stress and mental health, this study plotted the SWEMWBS score against the students' self-reported stress status in Figure 10. The SWEMWBS in the "rarely stressed" group has the highest score, followed by the group reported as never stressed. This suggests that an appropriate amount of stress is beneficial and can increase students' mental health, while too much stress is detrimental. This follows Selye's theory²⁷ that academic, intellectual, social, and personal challenges are considered a domain of eustress, or beneficial stress, for high school youth. However, as the stress level increases, the median score of SWEMWBS decreases, indicating that while some stress is conducive to mental health, excessive stress harms mental health. Eustress is also suggested for undergraduates by O'Sullivan's research.²⁹ The SWEMWBS score of students is the most varied, according to the IQR, in the distributions of "rarely stressed" and "never stressed," while the distribution of "often stressed" and "always stressed" is relatively concentrated. In addition, from the median and the distance between the upper and lower four digits of each box chart, it can be seen that the distribution of the scores of "sometimes stressed" and "often stressed" is relatively symmetrical, while the scores of "never stressed" and "always stressed" are skewed right, with more scores concentrated in the lower half. Most SWEMWBS scores are between 15 and 20 for constantly stressed students. This result is unrelated to AP classes as all students are included in this graph, regardless of their AP enrollments.

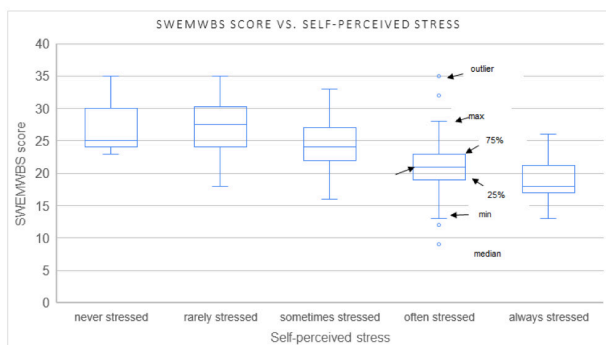


Figure 10: The relationship between self-perceived stress and SWEMWBS score.

Given the fast changes in the number of students' AP exams in the last 20 years (Figure 1 to Figure 6), student stress may have increased, but more research is needed to reach this

conclusion. If student stress increases among students taking AP classes, their mental health may be at risk, but it may also be true that stress may be beneficial eustress. However, as the demand for university admissions, student expectations, and academic pressures increase, it may be beneficial for educators and school districts to adjust and design a better academic plan for high school education and accelerated curriculums in the future.

Discussion

The results show that the median self-perceived stress increases as students take more AP classes. This may be due to the general trend of increased academic pressure from more time doing homework, as shown in Figure 5. This increasing amount of academics and homework, the possible subsequent lower amount of sleep due to increased time spent on homework, and the potential sacrifice of students' other activities may be contributors to student stress when the number of AP classes taken increases.

The relationship between the number of AP courses students take per year and the median value of the SWEMWBS score (Figure 9) suggests an optimum number of AP courses that can benefit mental health to be around 2. The study further examined this number by looking at another health indicator – sleep hours. The results of the one-tailed t-test of sleep hours in the subsection "sleep habits" could mean that besides the higher academic pressures (i.e., homework), students with high self-expectations, those who plan to enroll in many AP classes, may be working more to receive multiple AP credits, thus increasing their workload and leading to a habit of sleeping late. This result also supports Foust *et al.*'s conclusion that students who take accelerated classes tend to sacrifice sleep.¹⁷

Based on sleep habits, students who planned to take 0 to 12 APs throughout high school slept more than those who planned to take 12+ APs. This equates to an average of 3 APs per school year, suggesting students' sleep can be significantly reduced when their AP classes per year are greater than 3. Based on mental health and sleep results, the best number of AP classes for the general student is likely 1 to 3 AP classes per year. However, there is significant variability for each of the stress variables like mental health and sleep hours. This means that the number of AP classes each student takes mainly depends on their study habits, a prominent factor in student stress,³⁰ stress management, sleep habits, etc. For the general student, these results suggest that 1 to 3 APs is an optimal number per year in relation to stress variables.

The results of Table 2 suggest that students' coping ability also plays a role in how AP classes affect their well-being. This coping ability can include better study habits, good management of multiple tasks, and stress control strategies. Therefore, it is estimated that the optimal number of AP courses may be one to three per year for general students to maintain optimal mental status. However, this number could be higher for students with higher academic expectations or better stress control strategies.

Another possible interpretation of the results is that the students with the best mental well-being and higher median sleep

hours tend to select one to three AP classes per year. Further research will be needed to identify which is the case.

The research results are preliminary and need to be further developed. There are several limitations: 1) Only one school participated in the survey. Thus, the survey results may only be generalized to other public schools in the United States. Buchholz is also different from the average school in the United States as there are 29 AP classes offered, with multiple classes having pass rates above the national average. As a result, the survey results may not be generalizable to the typical high school in the United States. 2) The 176 valid responses received constitute only 7.4% of the school's population. Future researchers should expand the study to include more public and private high school students. 3) Since the survey was sent out through email and students answered voluntarily, the sample was not random and thus may have an under coverage of certain groups. 4) this study adopted a short SWEMWBS questionnaire with seven questions to obtain more students' participation in the questionnaire. Future research should address these limitations and obtain a broad, random sample using WEMWBS and other metrics, to obtain clearer mental health feedback. Furthermore, future research could also stratify students based on their scores on AP exams and grade point averages (GPA) to see whether students experience higher stress when they are higher achieving students. Obtaining information on how stress has changed for AP students could also be a topic of inquiry. Another limitation of the study was the factor of one-semester AP classes. Though few, semester classes like AP Government, AP Physics C, and AP Economics may need to be weighted differently from the regular two-semester AP classes. Freshmen's schedules at Buchholz High School are also slightly more limited than those of later graders. For example, math and reading are required for freshmen, but no AP classes are available for them at the 9th-grade level, meaning that the most AP classes freshmen can take is around 4 to 5. Thus, the amount of AP classes that a freshman can take is slightly fewer than the amount of AP classes a senior can. Moreover, in Figure 7, the median self-perceived stress no longer increases after two AP classes. This may be because the self-perceived stress levels were off after two AP classes, or it could be caused by the limitation of the 1-5 scale of self-perceived stress used, which did not include enough scores to see a significant change after two AP classes. To more accurately determine the relationship between self-perceived stress and the number of AP classes taken, scales like the Perceived Stress Scale (PSS) could be used.

Despite the limitations, the fact that most of the results align with previous research suggests that the results are still valuable. For example, based on the results of higher academic pressure for high achieving students and lower grades, parents, and teachers could better identify students who are at increasing risk of stress and when students experience elevated risk of stress so that teachers and parents can stop harmful coping behavior, such as drug and alcohol abuse. Moreover, students can develop a reasonable AP course plan based on their goals, expectations, study habits, coping capacity, and other factors. Through these implications, the results may provide useful

information to students and education institutes, such as the College Board, to develop an effective and balanced AP strategy by considering students' academic achievement and mental well-being.

■ Conclusion

The AP program has developed rapidly in the past two decades, and the AP course for high school students has become increasingly popular. From 2002 to 2021, the AP program was quickly promoted and popularized in the United States, and the average number of AP exams per student showed an increasing trend, with significant increases for 9th and 10th graders and faster growth for top percentiles. The results of self-perceived stress suggest that pressure on high school students is a common phenomenon in high school, which needs to be paid enough attention to. The survey indicates that AP enrollment is prominent at this North Central Florida public high school. Nearly 60% of students chose the first AP course in the 9th grade, with College/GPA being the main reason to enroll in AP classes. As students take more APs, students tend to spend more time on homework, which is also the primary reason for sleeping less. Students that took two AP classes had the best median SWEMWBS mental health, which is also consistent with self-perceived stress. This likely means that some stress and AP classes benefit students' well-being. Due to the heavy workload, students need to spend a lot of homework time to complete. Based on mental health, though stress levels increased when students took AP classes, some stress was favorable for higher median mental health: students who took one to three AP classes had the highest mental health.

■ Acknowledgment

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■ References

1. Moksnes, U. K., Espnes, G. A., & Haugan, G. Stress, sense of coherence and emotional symptoms in adolescents. *Psychology & Health*, **2014**; 29(1):32-49. <https://doi.org/10.1080/08870446.213.822868>.
2. OECD. PISA 2015 Results (Volume III). Paris, France. 2017.
3. Carter, J. S., Garber, J., Ciesla, J. A., and Cole, D. A. Modeling relations between hassles and internalizing and externalizing symptoms in adolescents: a four-year prospective study. *Journal of Abnormal Psychology*, **2006**; 115: 428-442. <https://doi.org/10.1037/0021-843x.115.3.428>.
4. Leonard, N. R., Gwadz, M. V., Ritchie, A., Linick, J. L., Cleland, C. M., Elliott, L., & Grethel, M. A multi-method exploratory study of stress, coping, and substance use among high school youth in private schools. *Frontiers in Psychology*, **2015**; 6: 1028. <https://doi.org/10.3389/fpsyg.2015.01028>.
5. Anne K. AP's equity face-plant. Washington Monthly, 8/29/2021. <https://washingtonmonthly.com/2021/08/29/aps-equity-face-plant/>. (Accessed by April 2023).
6. College board. AP program results: class of 2021. <https://reports.collegeboard.org/ap-program-results/class-of-2021> (Accessed by April 2023).

7. Adelman, C. *The toolbox revisited: Paths to degree completion from high school through college*. US Department of Education. 2006.
8. Zinth, J. D. *50-State Comparison: Advanced Placement Policies*. Education Commission of the States. 2016.
9. Conger, D., Kennedy, A. I., Long, M. C., & McGhee, R. The effect of Advanced Placement science on students' skills, confidence, and stress. *Journal of Human Resources*, **2021**, 56(1): 93-124. <https://doi.org/10.3368/jhr.56.1.0118-9298R3>.
10. Ackerman, P. L., Kanfer, R., & Calderwood, C. High school advanced placement and student performance in college: STEM majors, non-STEM majors, and gender differences. *Teachers College Record*, **2013**; 115(10): 1-43. <https://doi.org/10.1177/016146811311501003>.
11. Gall, M., & Stixrd, W. The 4S's of adolescent success. *Independent School*, **2008**; 67(4): 54-65.
12. Steinberg, J. *Many teachers in Advanced Placement voice concern at its rapid growth*. The New York Times, A16. 2009.
13. Suldo, S. M., Shaunessy-Dedrick, E., Ferron, J., & Dedrick, R. F. Predictors of success among high school students in advanced placement and international baccalaureate programs. *Gifted Child Quarterly*, **2018**; 62(4): 350-373. <https://doi.org/10.1177/0016986218758443>.
14. Taylor, L., Pogrebin, M., & Dodge, M. Advanced placement-advanced pressures: Academic dishonesty among elite high school students. *Educational Studies: Journal of the American educational studies association*, **2002**; 33(4), 403-421.
15. Blazer, C. Student Stress. *Information Capsule*. Volume 1006. Research Services, Miami-Dade County Public Schools. 2010.
16. Pope, D. *Beyond "Doing School." From "Stressed-Out" To "Engaged In Learning."* *Canadian Education Association*, **2010**; 50(1): 5-8.
17. Foust, R. C., Hertberg-Davis, H., & Callahan, C. M. "Having it all" at sleep's expense: the forced choice of participants in advanced placement courses and International Baccalaureate programs. *Research Review*, **2008**; 30(2):121-129. <https://doi.org/10.1080/02783190801955293>.
18. <https://reports.collegeboard.org/ap-program-results/data-archive>.
19. Stewart-Brown, S. & Janmohamed, K. Warwick-Edinburgh mental well-being scale. NHS Health Scotland. 2008. <http://www.mentalhealthpromotion.net/resources/user-guide.pdf>.
20. Hunter, S. C., Houghton, S., & Wood, L. Positive mental well-being in Australian adolescents: Evaluating the Warwick-Edinburgh mental well-being scale. *The Educational and Developmental Psychologist*, **2015**; 32(2), 93-104. <https://doi.org/10.1017/edp.2015.12>.
21. Melendez-Torres, G. J., et al. Measurement invariance properties and external construct validity of the short Warwick-Edinburgh mental wellbeing scale in a large national sample of secondary school students in Wales. *Health and Quality of Life Outcomes*, **2019**; 17: 1-9. <https://doi.org/10.1186/s12955-019-1204-zs>.
22. Suldo, S. M., & Shaunessy-Dedrick, E. Changes in stress and psychological adjustment during the transition to high school among freshmen in an accelerated curriculum. *Journal of Advanced Academics*, **2013**; 24(3): 195-218. <http://dx.doi.org/10.1177/1932202X13496090>.
23. Buchholz High School Data, personal communication.
24. Explore F.W. Buchholz high school. Niche. (2023, June 27). <http://www.niche.com/k12/fw-buchholz-high-school-gainesville-fl/>.
25. Vaccaro, A., Dor, Y. K., Nambara, K., Pollina, E. A., Lin, C., Greenberg, M. E., & Rogulja, D. Sleep loss can cause death through accumulation of reactive oxygen species in the gut. *Cell*, **2020**;181(6): 1307-1328. <https://doi.org/10.1016/j.cell.2020.04.049>.
26. Gariépy, G., Doré, I., Whitehead, R. D., & Elgar, F. J. More than just sleeping in: a late timing of sleep is associated with health problems and unhealthy behaviours in adolescents. *Sleep medicine*, **2011**; 9: 66-72. <https://doi.org/10.1016/j.sleep.2018.10.029>.
27. Selye, H. (1974). *Stress without distress*. New York: Signet. 1974.
28. Suldo, S. M., Shaunessy, E., Thalji, A., Michalowski, J., & Shaffer, E. Sources of stress for students in high school college preparatory and general education programs: group differences and associations with adjustment. *Adolescence*, **2009**; 44(176): 925-48.
29. O'Sullivan, G. The relationship between hope, eustress, self-efficacy, and life satisfaction among undergraduates. *Social Indicators Research*, **2011**; 101: 155-172. <https://doi.org/10.1007/s11205-010-9662-z>.
30. Kadapatti, M. G., & Vijayalaxmi, A. H. M. Stressors of academic stress—a study on pre-university students. *Indian Journal of Scientific Research*, **2012**; 3(1): 171-175.
31. Lazarus, R.S., Folkman, S. *Stress, appraisal, and coping*. New York, NY: Springer. 1984.

■ Author

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■ Appendix A

4/26/23, 12:37 AM

AP Classes and Stress Questionnaire

AP Classes and Stress Questionnaire

Hello! My Name is [REDACTED] and thank you for taking the time to complete this survey! The purpose of this survey is to identify how enrollment in AP (advanced placement) classes relates to student stress. All participants are anonymous and emails are not used. This quiz allows you to evaluate your stress level, so please answer the questions honestly. Most of the questions could be answered very quickly. Similarly, the best way to answer stress-related questions is to answer relatively quickly, so this survey should take about 3 - 5 minutes to complete. Thank you!

My email is [REDACTED] please contact me if you have any questions!

* Required

1. Please answer one of the following *

Mark only one oval.

- I am over 18
 I am under 18 and have a parent/guardian with me

2. 1) What grade are you in? *

Mark only one oval.

- 9th
 10th
 11th
 12th
 Other: _____

3. 2) What time to you usually go to bed? *

Mark only one oval.

- earlier than 7pm
 7 - 8pm
 8 - 9pm
 9 - 10pm
 10 - 11pm
 11pm - 12am
 12 - 1am
 1am - 2am
 later than 2am

4. 3) How many hours do you sleep on average per night? *

8

9

10

10 AP classes

5. 4) If you sleep late, why? *

Check all that apply.

- I do not sleep late
- Poor study habits
- Homework/ academics
- Social reasons
- Family reasons
- Entertainment (ex: games)
- Social Media
- Other: _____

6. 5) How often do you get stressed? *

Mark only one oval.

- never stressed
- rarely stressed
- sometimes stressed
- often stressed
- always stressed

7. 6) Please fill out the following scale *

Below are some statements about feelings and thoughts.
Please select the answer that best describes your experience of each over the last 2 weeks.

	None of the time Rarely	Some of the time Often	All of the time		
A. I've been feeling optimistic about the future	1	2	3	4	5
B. I've been feeling useful	1	2	3	4	5
C. I've been feeling relaxed	1	2	3	4	5
D. I've been dealing with problems well	1	2	3	4	5
E. I've been thinking clearly	1	2	3	4	5
F. I've been feeling close to other people	1	2	3	4	5
G. I've been able to make up my own mind about things	1	2	3	4	5

Mark only one oval per row.

	None	Rarely	Sometimes	Often	All of the time
A.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
B.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
C.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
D.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
E.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
F.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
G.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

8. 7) How many hours do you usually allocate to academics and studying on the weekend? *

9. 8) How many hours on average do you spend doing homework/academics outside of school * per day?

AP Questions

Just 5 more questions about AP classes.

10. 9) How many AP classes are you currently taking this year (if you are not taking any, put 0)

Mark only one oval.

0 AP classes

0

1

2

3

4

5

6

7

11. 10) How many AP classes have you taken in high school in total (including this year)?

12. 11) What grade were you when you took your first AP class?

Mark only one oval.

- I have not taken an AP class
- 9th
- 10th
- 11th
- 12th
- Other: _____

13. 12) How many total AP classes do you **plan** on taking (or have taken if you are a senior) throughout high school?

Mark only one oval.

- 0
- 1-3
- 4-6
- 7-9
- 10-12
- 12-14
- 15-17
- 18+

14. 13) If you take AP classes, why do you take AP classes?

Check all that apply.

- I do not take AP classes
- Parental influence
- Interests/ learning more
- High self-expectation
- Peer pressure
- College/ GPA
- Saves money for College
- Other: _____

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Google Forms

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Rise of the Internet and Evolution of Startup Style Norms

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ABSTRACT: This research paper investigates the transformation of startup-style norms due to the expansion of the internet, contrasting these with traditional business norms like etiquette, professional appearance, and punctuality. The paper argues that the emergence of the internet fostered a shift towards an inclusive, value-centric, empathetic, and productivity-focused startup culture, largely supplanting profit-driven objectives. Silicon Valley, as the epicenter of startup culture and the birthplace of influential startups, is analyzed. The paper recounts how business norms evolved from stringent practices in rigid workspaces to more relaxed, effective ones after the internet's rise, propelled by pioneering tech entrepreneurs. The key conclusion is that the internet has enabled entrepreneurs to access information, connect with diverse audiences, and scale businesses more efficiently, cultivating a risk-tolerant, innovative startup culture that prizes rapid prototyping and experimentation over conventional practices. This shift has disrupted industries and spawned new markets. The paper is of particular importance for tech startups, tracing the evolution of startup-style norms and emphasizing the role of Silicon Valley in sparking this change. It adds to the sociological understanding of how technological shifts shape social structures and norms.

KEYWORDS: Sociology; Silicon Valley; Internet bubble; Style norms; Culture; Startup casual; Primping.

■ Introduction

This paper delves into the pivotal role of the internet's expansion in the evolution of startup-style norms over time. It explores how 'style norms' - a term used to describe the accepted behaviors, practices, and procedures within a business environment - have morphed with the rise of the internet. By contrasting traditional business norms like etiquette, punctuality, and professional appearance with contemporary startup norms, such as value definition, inclusivity, cultural assessment, empathy, and productivity, we understand how the business mindset has shifted. The focus has moved from a sole profit-centric perspective to a more employee-friendly and wellbeing-oriented approach. Silicon Valley, the heart of the startup community and the birthplace of numerous successful startups, is presented as the locus of this transformation.

The paper begins by discussing business norms before the internet's rise, characterizing them as stringent and confined within rigid workspaces. The arrival of the internet heralded a change brought forth by tech-focused entrepreneurs who revolutionized business practices, making them less strict and more effective. This research aims to decipher why and how these business practices changed and identify the key individuals involved in this transition. We conclude that the Internet has simplified information access for entrepreneurs, eased connections with potential investors and customers, and facilitated business scaling. This has ushered in a fast-paced, innovative, and agile startup culture emphasizing risk-taking, experimentation, and rapid prototyping over traditional practices. The research contributes significantly to the startup sector, particularly tech startups, by providing a clear perspective on the evolutionary trajectory of style norms. It also underscores Silicon Valley's importance in this transformation, a point of-

ten overlooked as changes are usually attributed to individual workspaces. The research offers a valuable contribution to sociology by examining the changing norms within the startup ecosystem, shedding light on how technological changes shape social structures and norms and, in turn, our behavior and interactions. A qualitative research methodology underpins this paper, incorporating evidence-based discussions. The paper's qualitative methodology incorporates a discussion substantiated by evidence.

■ Discussion

The center of the startup community:

The rise of the startup community propelled the world of business, trade, and commerce. It gave a direct leap into the growth of newly founded companies. The startup community was the most significant catalyst; for those organizations considered the market movers in today's world. A "cowboy capitalism" system surfaced in its genesis form.² The center of the startup community, as comprehended, is widely famed to be Silicon Valley. A region where high technological innovation arose. Silicon Valley was initially coined in 1971 in a magazine called 'Electronic News.'³ While there is no concise data that pins it down to the genesis of Silicon Valley, an astute assumption can be made that speaks about how the foundation of the Shockley semiconductor, which attracted many companies to the Valley, made under the partnership agreement of William Hewlett and David Packard, fuelled what came to be later called the Silicon Valley.⁴ The Santa Clara Valley only became known to the general public after the name changed. The rise of the startup community took place with the advent of the dot com boom, also called the dot com bubble, in the 1990s. Only then was the valley dormant, with little development in the startup sector: the only development was in the automobile

and electronic industries. Then came the idea of the internet. The internet expanded in its use and became the new medium of trade. The internet thus helped establish a totally new system of work, conduct, and style norms, all combined with changing the business culture.

The era of style norms before the rise of the internet:

Culture is “the whole complex of traditional behavior which has been developed by the human race and is successively learned by each generation”.⁵ Historically speaking, the style norms, particularly the pre-establishment of Silicon Valley in the 1950s time period, were extremely strict. The business and office norms that had to be followed were rigid. Primarily a “business formal” dress code was observed in all companies. In addition, there were stern rules that prohibited all casual dressing styles.⁶ Office spaces, too, were constricted and orthodox. The open office system prevailed, and cubicles were not yet introduced into offices. Furthermore, the spaces were not conducive to any form of expression or creativity. Style norms are the norms that have been followed (particularly in the business community). Business and its style norms in the 50s, before the rise of the internet, were conservative and traditional. Clothing was seen as an article used to advocate one's social position. A suit was regarded as a garment upon which a person's status, character, and ability were determined. It projected the superiority of an individual in any business position.⁷ A suit, tie, and vest were all regarded as accessories that elevated a person and their character. Therefore, you would always find businessmen and women dressed in the best suits and garments. The charismatic quality that a person possessed was regarded as gentlemanly. Businessmen and women were highly formal in their presentation and conduct. Punctuality was given utmost importance, and being present on time was one of the norms. Focusing on the appearance aspect of the topic, hair was treated with paramount care; you would seldom see a strand of hair out of place, and nails were always cut and filed to perfection.



1958 men's sport coats- mix and match trousers and jackets

Figure 2: The image above accurately portrays how business people dressed in the 1950s before the dot com bubble.⁹

The rise of the internet:

The internet initially came to exist in the early 1960s when the cold war began. The United States and the Soviet Union were in competition in all elements and were also in a race to expand their global influence.¹⁰ It was during this time that the Internet started actually to come into play. There were rapid advancements in technology and infrastructure to use the Internet. Based on common facts, the internet was initially just a fad that no one believed would take off. However, some entrepreneurs used it to their advantage. During the 1980s, the internet was finally commercialized, and a myriad of people had email addresses and websites. The internet became a global phenomenon, and soon, a host of countries joined in the revolution. The exponential growth of the internet led to the formation of many internet-based companies.¹¹ This was when there was an increased capital investment into the equity and financial markets, pushing the National Association of Securities Dealers Automated Quotations (NASDAQ) to reach its highest highs. It was also a phase where many companies suffixed the ‘.com’ to their names to publish web addresses through which their operations could be run. Most of these startups were incorporated into Silicon Valley. DeLong and Magin (2006) speak about how the dot com bubble was short-lived.¹² However, the short period of the bubble was enough to revolutionize a cultural change that is bound to last for generations.

A series of successful companies survived the internet bubble, influencing a generation of culture and style norms. A list of a few of the companies that survived the bubble includes - Amazon (Nasdaq - AMZN), eBay (Nasdaq - EBAY), Qualcomm (Nasdaq - QCOM), and Cisco (Nasdaq - CSCO). Some other multi-national organizations that were also prevalent and in their genesis stage during the bubble include;



Figure 1: The picture above represents the office space environment in the 1950s.⁸

and Google (Nasdaq - GOOGL) under the parent company Alphabet Inc. One thing in common in all these companies is that extremely focus-driven and task-efficient entrepreneurs started them. They were the "college geeks" who capitalized on the emergence of the internet. What initially may have started as an idea of a student came to grow as the market movers we know them to be. The series of entrepreneurs who were a part of the startup stage of the companies contributed to the evolution of the style norms that initially persisted in the marketplace.

The era of style norms after the rise of the internet:

These style norms started to evolve and drastically changed over the next few decades. The way of conducting business changed to a more casual mannerism. It evolved into what is known as "startup casual."¹³ Startup casual is defined as "work attire that is even less formal than business casual"¹⁴ The startup casual does not necessarily pertain to work attire but also to work norms and office working environments. The central ideology reflects the office mentality and brings a newer modern approach to the company. The startup casual ecosystem has been mainly inspired largely by Silicon Valley, which saw the rise of nascent technological startups founded by young entrepreneurs. The key aspects of startup casual are; flexible and more efficient working techniques, changes in the landscape of office norms, newer adaptations to clothing and appearance, and a newer mentality of conducting business on a larger scale. Recently, we have noticed stark differences in how a company works. Today, business organizations adapt and move forward more relaxed and task efficiently. There has been a change in how we think, operate, and conduct ourselves. Office spaces that used to be constricted and dull are now open spaces with abstract designs that favor coherent working conditions. Progression has also occurred in the way people dress to work nowadays. What used to earlier consist of stuffy and expensive suits is now replaced with casual wear that embodies a contemporary style of dressing. In any startup, you will only find Gen - Z with the funkiest dressing styles, new hairstyles, comfortable apparel, and a classic pair of headphones.

The goal of any startup is to develop a self-sustaining network of talent and resources that seek to solve issues affecting a larger community.¹⁵ What this translates to, in simple terms, is the goal of efficiency. Any startup aims to attain maximum efficiency, which all new-age entrepreneurs have practiced. For example, Mark Zuckerberg, the founder of Facebook, now called Meta, is always dressed in a grey t-shirt with denim pants. Alternatively, Steve Jobs, who was known to be dressed in his classic black turtleneck, all have a similar ideology. They perceive ease of attire as a factor that reduces decision fatigue, which is - not letting your decision act as a causative factor for fatigue apparel leads to more choice, which in turn leads to a waste of time and exhaustion.¹⁶ Zuckerborg and Steve Jobs, like myriad other entrepreneurs, believe that startup casual workwear has more to do than just a casual approach; they perceive it to be a message of their nature of work. It facilitates independent thinking for individuals as they are comfortable in their attire.

There are no set rules that dictate how one must dress; thus, the startup casual dressing sense was adopted by all businesses, let alone startups. In another psychological aspect, entrepreneurs adopted startup casual dress because it makes them more approachable; employees often find it comforting to redress problems with an authoritative figure if they are seen in casual clothing.¹⁷ Finally, re-iterating a key aspect; how a person dresses in the business/startup domain are solely concerned with their priorities. Efficiency being the number one goal today amidst alarming competition, enables people to adopt the casual way of conducting themselves for maximum output. The next section of the literature review discusses how influential entrepreneurs can be on their employees/work members to disseminate a new style norm.



Figure 3: The image above represents the newer way business is conducted and gives insight into the startup casual system.¹⁸

Role of Entrepreneurs in Disseminating Norms:

An entrepreneur is a rational decision-maker who assumes the risk and provides management for the firm.¹⁹ They are directly responsible for all activities in a business and spearhead decision-making. Entrepreneurs, as leaders of the organization, have a unique identity. They carry a particular style that they disseminate amongst their employees and, on a larger scale, to the entire community linked to them. A successful and charismatic entrepreneur is defined by characteristics that shape them. These are - a high need for achievement, independence, imagination, persistence, competitiveness, and, most importantly, risk-taking abilities.²⁰ After the dot com bubble, when the internet was rampantly growing, there was a growth in the entrepreneurial culture worldwide. Most entrepreneurs were college students or individuals relatively younger than the average business community. This youthful leadership was peremptory in disseminating the norms: the youth entrepreneurs were the ones who capitalized on the dot com bubble and the rise of the internet. Their optimism and understanding of the exact trends that the newer generation wants is the reason for their success. Further, their technological knowledge was a boosting factor too. Young entrepreneurs were kept from traditional business norms and practices that were prevalent at the time. They had grown up in an era of social and cultural change, where informal and casual styles were becoming more prevalent in society. As a result, they were more open to experimenting with new styles and approaches to doing business.

The rise of the internet and social media during this period enabled young entrepreneurs to connect and share ideas more easily. This led to the emergence of a new startup culture that was more informal and collaborative, which also extended to the way people dressed and presented themselves. Overall, young entrepreneurs were able to spearhead the change of style norms for startups and businesses in the 1990s and 2000s because they were more open to experimentation, more innovative in their approach to business, and more connected with each other through technology and social media. As a result, they were able to break free from traditional business norms and create a new startup culture that was more informal and casual, both in terms of work norms and fashion and appearance.

The competition back then was at an all-time high; getting your service or product in the market before someone else did was the biggest challenge. During the lift-off stages of a startup, an entrepreneur's role is to achieve maximum growth potential for their company. Thus, efficiency and efficacy were the only goals. It was during this phase that they adopted the startup casual. The focus changed from a good impression to a qualifiable result. You could now see the entrepreneurs adopt casual and comfortable working conditions and employ only the most efficient labor forces. Hoodies, t-shirts, denim accessories, and the latest gizmos were all seen in the style norms of the entrepreneurs. Considering that the biggest companies today originated in Silicon Valley, the culture started to disseminate from here. Entrepreneurs with this casual approach spread their way of work to their employees and business partners: the company and workforce they envisioned held good in the office. This was only possible because of the influence a person in authority (the entrepreneur) has over those working under them. Seeing the "boss" dress casually and conduct themselves casually changed the mindset of all employees who later went on to create their own companies sometimes and do the same. This is called role model perception, which means learning by copying other persons' actions by observing them doing them.²¹ This certainly increased the equitability and inclusivity in organizations. Especially in the modern day and age, people are free to choose to dress in what they are most comfortable in and are allowed to express their personality without fearing penalization for it. Modern workspaces and startups are promoters of just that. They allow employees to be who they are in the most comfortable attire. Another term that could be used to describe what caused this evolution is 'primping.' It means to dress or groom oneself carefully.²² Primping and fitting into the crowd to replicate what all others do, is one of the other leading causes that affected the evolution of the style norms.

Role of the COVID-19 Pandemic in changing office norms:

The COVID-19 pandemic, too, has been pivotal in changing the style norms that persist in the present-day business community. The onset of the work-from-home change has defined these changing norms. Hybrid work models have become the next best alternative with the ongoing pandemic, which has subsided drastically in most nations. However, they

have been a preferred working model for several employees, as they believe it eliminates unnecessary inefficiency and bureaucracy in a firm. Style norms got even more relaxed as we saw work being done from the comfort of someone's bedroom or lounge. Clothing was also relaxed to the most basic clothing any individual would wear at home. As per reports, people in India embraced comfort over style in the last few years. Formal outfits were totally replaced by athleisure, sandals, and shorts, even for business meetings.²³ Similarly, in Italy, many working women found solace in knitwear during the pandemic.²⁴

Thus, this gradual shift in style norms has shaped a very individual-friendly way of work environments and apparel alike. Employees today are much more comfortable in their personal styles, which goes a long way in boosting the productivity of any person and the organization they are employed in.

■ Conclusion

In conclusion, this research paper delved into the evolutionary causes of style norms and their progression with the advent of the internet. The paper was structured into two eras, the pre-internet and post-internet eras, to explore the factors contributing to the evolution of style norms. Through this analysis, several key factors emerged as influential drivers of change.

Strict and formal style norms in the business community characterized the pre-internet era. However, the rise of the internet and the emergence of young entrepreneurs during the dot com boom brought about a cultural shift. Unencumbered by traditional norms, entrepreneurs embraced a more casual and innovative approach to conducting business. The startup casual style, characterized by relaxed work attire, flexible working techniques, and a modern mentality, became a defining feature of the post-internet era.

The role of entrepreneurs in disseminating these newer norms must be considered. Their leadership, characterized by risk-taking, independence, and a focus on efficiency, influenced their own companies and the broader startup community. Entrepreneurs acted as role models, shaping their employees' and partners' mindsets and style norms.

Additionally, the COVID-19 pandemic further accelerated the transformation of office norms. The shift to remote work and hybrid models allowed for even more relaxed style norms, emphasizing comfort and individual expression. This change in work dynamics and attire reflected the growing importance of employee well-being and productivity.

Looking ahead, the future of business style norms remains uncertain. The possibility of an artificial intelligence takeover or the emergence of digital style norms raises intriguing questions about the evolving nature of work and fashion. Exploring these potential developments and their implications will continue to be an interesting and fascinating area of research.

In conclusion, understanding the evolutionary causes of style norms in the startup community provides valuable insights for entrepreneurs, organizations, and the broader business landscape. By recognizing the influential factors and staying attuned to changing trends, businesses can adapt and foster a dynamic and inclusive work environment. The

ongoing evolution of style norms reflects the ever-changing dynamics of technology, culture, and societal values, shaping the future of work in remarkable ways.

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■ References

1. (1) Brennan, G.; Eriksson, L.; Goodin, R. E.; Southwood, N. Explaining Norms; Oxford University Press, 2016.
2. Sturgeon, T. How Silicon Valley Came to be. 2000, 46.
3. *Startup history*. MinnaLearn. <https://courses.minnalearn.com/en/courses/startup/introduction/startup-history/>
4. Kenney, M. *UNDERSTANDING SILICON VALLEY - THE ANATOMY OF AN ENTREPRENEURIAL REGION*; Stanford University Press: California, 2000.
5. (1) Birokou, A.; Blanzieri, Giorgini; Giunchiglia. A Formal Definition of Culture of a Set of Agents. *PSU* 2009.
6. (1) Reynolds, J. How the Typical Office Looked in the 1950s <http://www.tinypulse.com/blog/how-the-typical-office-looked-in-the-1950s>.
7. (1) Molloy, J. *Dress for Success*; 1975.
8. (1) TINYpulse. *Office Spaces in the 1950s*; Reynolds, J., Ed.; 2016.
9. (1) Debbie; Oscar. *1950s Men's Fashion History for Business Attire*; 2016.
10. (1) Almagor, R. Moral, Ethical, and Social Dilemmas in the Age of Technology: Theories and Practice. *IGI Global* 2013.
11. Fan, Y. Dissecting the Dot-Com Bubble in the 1990s NASDAQ. Arxiv 2022.
12. DeLong, B.; Magin, K. A SHORT NOTE ON THE SIZE OF THE DOT-COM BUBBLE. *NATIONAL BUREAU OF ECONOMIC RESEARCH* 2006.
13. (1) Erin, A. Startup Casual: A New Dress Code for a New Generation <https://apuedge.com/startup-casual-a-new-dress-code-for-a-new-generation/>
14. (1) Erin, A. Startup Casual: A New Dress Code for a New Generation <https://apuedge.com/startup-casual-a-new-dress-code-for-a-new-generation/>
15. (1) Aleisa, E. *Startup Ecosystems*. 2013
16. (1) Move Over, "Business Casual." There's Another Kid on the Block: "Startup Casual." <https://www.entrepreneur.com/article/313647> (accessed 2022 -09 -04).
17. (1) Move Over, "Business Casual." There's Another Kid on the Block: "Startup Casual." <https://www.entrepreneur.com/article/313647> (accessed 2022 -09 -04).
18. (1) Empathy First, Then Fun: Avoiding a Disengaged Workplace <https://www.liveplan.com/blog/empathy-first-then-fun-avoiding-a-disengaged-workplace/>
19. (1) Carland, J.; Hoy, F.; Carland, J. "Who Is an Entrepreneur?" *Is a Question Worth Asking*; AJSB, 1988.
20. (1) Dvir, D.; Sadeh, A.; Pines, A.; Shenhar, A. Key Entrepreneurial Traits and Their Relationship to Venture Uncertainty and Venture Success. *ResearchGate* 2009.
21. (1) Liñán, F.; Santos, F. J.; Fernández, J. The Influence of Perceptions on Potential Entrepreneurs. *International Entrepreneurship and Management Journal* 2011, 7 (3), 373–390. <https://doi.org/10.1007/s11365-011-0199-7>.
22. (1) Definition of PRIMP <https://www.merriam-webster.com/dictionary/primp>
23. (1) How Working from Home Changed Wardrobes around the

World. *The New York Times*. April 15, 2021.

24. (1) How Working from Home Changed Wardrobes around the World. *The New York Times*. April 15, 2021.

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Pride Comes Before a Risky Decision: An Analysis on How Pride Affects Financial and Social Risk Preference

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ABSTRACT: Research on the impact of discrete emotions on risk preference has revealed the importance of studying these emotions in different risk-taking domains, such as financial risk-taking, recreational risk-taking, social risk-taking, health risk-taking, and ethical risk-taking. This study investigates the effects of the understudied emotion of pride on financial and social risk preference for oneself and the impact of pride on surrogate decisions in the financial and social domains. Two experimental studies examined whether pride would increase financial risk preference for oneself, increase social risk preference for oneself, increase social other-interested risk preference, and decrease financial other-interested risk preference. A one-way ANOVA revealed that pride increased risk preference in all four domains. In addition, two mediating variables, prosociality, and locus of control, were investigated for causation to understand this phenomenon further. Prosociality partially mediates the results for social self-interested risk preference and financial other-interested risk preference.

In contrast, locus of control did not mediate any of the relationships. This suggests that prosocial emotions may have unique effects on risk preference that should be further explored. In addition, this study has practical implications for understanding how individuals make risky decisions when in a prideful state, such as investing in the stock market.

KEYWORDS: Behavioral and Social Sciences; Sociology and Social Psychology; Risk Preference; Discrete Emotions; Pride.

■ Introduction

In recent years, researchers have increasingly recognized that discrete emotions- a set of universally recognized distinct emotional states- play a large role in influencing risk preference; one might even go so far as to say it is the death of the homo economicus. In the traditional economic sense, risk preference is considered a stable trait that refers to an individual's propensity to take risks based on the uncertainty of relevant outcomes, which widely impacts human decision-making. Nevertheless, recent research on the effect of violence, financial shocks, emotions, and life events such as parenthood on risk behavior indicates that risk preference is at least partially deterministic.¹⁻⁴

With the seminal paper of Smith and Ellsworth proposing an appraisal model of emotion, research on the role of emotions has expanded beyond considerations of their emotional valence to the effect of other cognitive dimensions, such as perceptions of control and certainty.⁵ The appraisal tendency framework builds upon this stream of research, suggesting that emotions affect risky judgments through situational vs. self vs. other control, where emotions that lead to higher perceptions of self-control result in increased risk tolerance.⁶ For example, emotions linked to higher internal control (locus of control), such as guilt, have positively influenced risk preference.⁷ In multiple studies, emotions like happiness and anger, characterized as high-control emotions, have been associated with increased risk preference.^{4,8} Conversely, fear, a low-control emotion, has been linked to lower levels of risk preference.⁸

However, the effect of these emotions is more nuanced than a simple "increase" or "decrease" in general risk preference- one must also consider domain-specific risk preferences. Emotions fundamentally serve as responses to help individuals deal with the events that triggered them.⁹ Thus, specific emotions are associated with actions intended to address the situations that elicited those emotions. For instance, anger is associated with a defensive response when feeling threatened,¹⁰ while happiness is frequently associated with the desire to broaden one's experiences and play.¹¹ Consequently, different emotions may lead to different domain-specific risk preferences. For example, an angry person may be more willing to take ethical risks,¹² while a happy person may be more inclined to take recreational risks (e.g., mountain climbing).¹³

One emotion that has been especially understudied regarding risk preference, even in a general (not domain-specific) context, is the emotion of pride. As conceptualized in this study, pride refers to a high locus of control, positive emotion typically stemming from a sense of accomplishment. Despite new research suggesting that pride may be one of our primal emotions,¹⁴ has received less attention than other emotions associated with a high locus of control. Pride is unique in that it stems from a sense of accomplishment, motivates individuals to act to improve their status in a hierarchy, and is linked to prosocial behavior. As I discuss in the literature review, these distinctive appraisals and characteristics of pride may significantly influence risk preferences in the financial and social domain.⁵ Therefore, the primary aim of this study is to determine the impact of pride in these domains and whether this

effect may be mediated through the locus of control and prosociality.

Since emotions impact real-life risky decision-making, this study has practical implications in people's daily lives. Situations involving feelings of pride and risky decisions can carry significant weight, such as when a health campaign aims to use high-control emotions such as pride or guilt to convince their audience to take specific actions.^{15,16} Economists have long since been interested in what makes investors more risk-taking, and the impact of emotional states is a crucial part of understanding investor decisions.³ Moreover, domain-specific risk preference predicts human behavior more accurately than general risk preference. Understanding the factors influencing an individual's monetary and interpersonal decisions is key to understanding human risk.¹⁷

Literature review:

Considering the different responses different emotions elicit, emotions that may appear similar at first glance can be quite distinct, such as pride and happiness. Pride is both a high locus of control and positive emotion. However, it is linked to higher levels of internal control than happiness.⁵ Unlike happiness, pride is primarily based on achievement, and it motivates one to accomplish goals that increase one's position in a hierarchy.¹⁸ Indeed, pride has been found to lead to greater perseverance even in tasks with negative initial costs.¹⁹ Moreover, pride has been linked to actions associated with an upwards change in a given hierarchical structure. For example, in a study that looked at how proud vs. happy participants responded to loyalty programs utilized by firms to encourage repeat purchases, only pride differentially increased repurchase intentions in customer tier programs (programs that rank customers into different tiers).²⁰ Interestingly, the desire for more power is linked to taking more extreme risks, which carries implications for the domains in which pride may lead to more risk-taking, such as financial risks.²¹ Both monetary gains and social status are often viewed as markers for where one is in a hierarchy, suggesting that through a high locus of control, emotional pride will increase willingness to take risks to obtain these hierarchy markers.²² Therefore, it is hypothesized that pride will increase both financial risk preference (H1) and social risk preference for oneself (H2).

A mediating variable, prosociality, is explored to investigate further the effect between pride and social and financial risk preference. Prosociality is a broad construct encompassing behaviors and propensities characterized by the intention of benefiting others. For example, one study found that organizational leaders who experienced pride were likelier to engage in prosocial behaviors through social justice and altruism.²³ Others have found that authentic pride from confidence and accomplishment signal prosociality.^{24,25} In *The Nature of Pride*, J.L. Tracy states that "pride in one's successes and relationships...[can] promote future positive behaviors in the achievement domain and to contribute to further prosocial investments such as relationship maintenance and altruism."²⁶ From a theoretical perspective, this may affect risk-taking because prosociality often stems from higher perceptions of self-efficacy (an individual's belief in their capacity to achieve a

specific goal).^{27,28} As previously discussed, more robust levels of internal control, which seems to have a positive relationship with self-efficacy, are associated with higher risk tolerance. Indeed, one study demonstrated that when young preschool children were given more agency (when they could choose between a selfish option or a kind one), they were more likely to engage in prosocial behaviors in the long run than if forced to choose the kind option.²⁹ This implies that prosociality stems from a sense of control and higher risk tolerance.

Additionally, people evaluate prosocial initiatives more optimistically,³⁰ and optimism have been linked to increased risk preference.³¹ Optimism is also linked to increased wishful thinking, a possible proxy for risk perception.^{32,33} It is thus hypothesized that prosociality may mediate the relationship between pride and both financial and social risk preferences for oneself (H5).

The question arises about how one makes decisions for others when in a prosocial state. According to the social values theory, participants make riskier decisions for others in domains where risk-taking is valued and make fewer risky decisions in situations where risk-taking is not socially valued.³⁴ This suggests that prosocial individuals are more likely to follow socially-valued risk-taking behaviors when making decisions for others. For example, in a meta-analysis conducted by Batteaux *et al.*, the researchers found that decisions in the interpersonal (social) domain were more risk-averse for oneself than others but that decisions in the medical domain were more risk-seeking than for others. This is consistent with the notion that when making surrogate decisions (other-interested risk preference), people are more likely to follow socially prescribed norms because medical risk-taking is less socially accepted than interpersonal/social risk-taking.³⁵ In the financial domain, research has found that financial professionals tend to be less prosocial and more risk-taking,³⁶ and with limited accountability, surrogate financial decisions tend to involve increased risk preference.³⁷ As such, it is predicted that pride (a prosocial emotion) will decrease other-interested financial risk preference (H3) but increase other-interested social risk preference (H4) since interpersonal risk preference is socially valued. Still, financial risk on behalf of others is not, as per social values theory.

The hypotheses investigated to explore the relationship between pride and risk preference are as follows:

H1: Pride increases financial risk preference for oneself.

H2: Pride increases social risk preference for oneself.

H3: Pride decreases financial other-interested risk preference.

H4: Pride increases social other-interested risk preference.

H5: Prosociality mediates the effect of pride on risk preferences.

H6: Locus of control mediates the effect of pride on risk preferences.

■ Methods

To examine whether pride was linked to increased risk preference, a pride condition survey and a neutral condition survey were distributed on Amazon Mechanical Turk to a random sample of predominantly middle-aged participants

aged 19 to 71+. About half of the participants in both conditions identified themselves as female and half as male. Initially, there were 100 participants in each group. Still, to ensure that participants in the pride condition were truly prideful and participants in the neutral condition were genuinely neutral, only those who scored five or above in the manipulation check question (“How prideful are you feeling right now, on a scale of 1-10?”) were kept for the pride condition. Likewise, only those who scored five or below were held for the neutral condition. The final sample size was 66 in the neutral condition and 77 in the pride condition.

Procedure:

The surveys were distributed on Amazon Mechanical Turk, and participants were told that they were participating in a study regarding how various factors influence decision-making. In this experimental design, there was one pride condition and one neutral condition. First, participants in the pride condition were primed by a question that asked them to “Please think about and list three to five things that make you most proud,” followed by another question that asked them to “Please describe in detail one situation that makes, or has made you feel most proud.” Next, participants were asked to list three to five things in their typical day in the neutral condition. The manipulation check question, which asked how prideful participants were feeling on a Likert scale, was used to verify the success of the manipulation of the two conditions, but this confirmation measure was not used for subsequent analysis.

Participants were then asked the General Risk Question (GRQ),¹⁷ which states, “Are you generally a person who is willing to take risks or do you try to avoid taking risks” on a scale of 0-10. This same question was also repeated, except “risks” was replaced with “financial risks” and “social risks,” respectively (GRQ-financial and GRQ-social). The GRQ is a reliable predictor of risky behavior based on paid lottery experiments.¹⁷

As part of some exploratory data, a general trust question asked, “Generally speaking, would you say that most people can be trusted?”³⁸ The Prosociality Scale and Rotter’s Locus of Control Scales were adapted to measure prosociality and locus of control.^{39,40}

After that, participants were asked domain-specific questions. The Domain-Specific Risk-Taking Scale (DOSPERT)¹⁷ was adapted to measure social and financial risk preference. Finally, participants were asked age and gender demographic questions.

Scales:

Rotter’s Locus of Control (LCS):

The LCS was adapted to measure an individual’s internal and external control.⁴¹ A higher internal locus of control indicates that an individual feels they can influence the events in their life. Previous research demonstrates satisfactory test-retest reliability ($r_{tt} = .71$) for internal locus of control. Considering the small number of items and broad construct, the internal consistency estimates are also satisfactory for research because the broad constructs come at the expense of internal consistency (McDonald’s $\omega = 0.63$).⁴¹

Domain-Specific Risk-Taking Scale (DOSPERT):

The Domain-Specific Risk-Taking Scale is a self-report scale that measures risk-taking across five different domains: ethical, financial, health and safety, recreational, and social (from which three domain-specific representative items were chosen).⁴² Research has found relatively high internal consistency for the financial risk preference questions (Cronbach’s $\alpha = 0.76-0.80$) and adequate internal consistency for the social risk preference questions (Cronbach’s $\alpha = 0.66-0.70$).⁴²

The Prosociality Scale:

The Prosociality Scale was adapted from Caprara *et al.*⁴³ to measure an individual’s tendency to engage in prosocial behavior and empathetic reactions. Prior research has supported the construct validity of this scale, with prosociality scores matching expected correlations for agreeableness and empathetic self-efficacy. In the most recent sample using this scale, the Cronbach alpha for the entire scale was 0.94.⁴³

■ Results and Discussion

One-Way ANOVA:

A one-way ANOVA (Analysis of Variances) was conducted to determine whether increased pride resulted in increased risk preference for self-interested financial decisions (hereafter financial-self) and self-interested social decisions (hereafter social-self). As both ANOVA and t-tests yield equivalent results when there are two conditions, ANOVA was chosen as a framework for future studies that may compare the effect of multiple emotional states on risk preference. The ANOVA revealed that pride was associated with higher risk preferences. There was a statistically significant difference in financial-self, $F(1, 141) = 9.179$, $p = 0.003$, as well as in social-self, $F(1, 141) = 6.260$, $p = 0.013$. Consistent with what was hypothesized, pride was linked to an increased risk preference for self-interested decisions. The means of these groups are presented in Table 1. As discussed in the literature review section, positive valence, high locus of control, and prosociality are the probable causes of these observed differences. The mediation analysis section discusses the effects of prosociality and locus of control.

Table 1: Means for financial and social risk preferences (r.p.).

	Financial Self-Interested R.P.		Financial Other-Interested R.P.		Social Self-Interested R.P.		Social Other-Interested R.P.	
Neutral	1.727	Supported	1.439	Significant in Opposite direction	3.147	Supported	2.944	Supported
Pride	2.204		1.831		3.550		3.476	

In addition, a one-way ANOVA was also conducted to determine the effects of pride on surrogate risky decision-making. As was hypothesized, there was a statistically significant difference in social risk-taking for others (hereafter social-other); $F(1,141) = 10.609$, $p = .001$ between the pride and neutral condition. The results (pride increased social-other risk preference) in this domain are consistent with social values theory because taking risks in the interpersonal (social) domain is socially valued. This also points to the role of prosociality, which is discussed in the mediation analysis section. Unexpectedly, the effect of pride on financial other-interested risk preference (hereafter financial-other) was significant in the opposite direction than was hypothesized, $F(1,141) = 9.210$, $p = .003$. According to social values theory and the relationship between

pride and prosociality, one would expect financial surrogate risk-taking to decrease with increased pride. However, the positive effect of pride on financial-other suggests that some other mediating factor explains the impact, especially since the ANOVA revealed a significant positive relationship between pride and prosociality $F(1, 141)=4.605, p=0.034$.

Aside from the scenario-based questions taken from the DOSPERT scale analyzed above, I also asked the GRQ, GRQ-financial, GRQ-social, and trust questions (described in the procedure) as an additional measure of self-reported risk preference in groups. Pride was related to significant differences for the GRQ [$F(1, 141)=6.737, p=.010$], GRQ-financial [$F(1, 141)=8.241, p=.005$], and GRQ-social [$F(1, 141)=3.981, p=.048$]. These results are expected because participants answered similarly about specific risky behaviors. While the trust question was not statistically significant, there was directional support in favor of pride increasing trust and, therefore, risk preference [$F(1, 141)=3.204, p=.076$]. One could consider trusting a proxy for social risk preference because of previous research on how risk preference negatively affects trust and how trust can mediate one's willingness to join a sharing community.^{44,45} Not only would this be consistent with predictions made about pride and social risk preferences, but this could be an interesting avenue for future research. For example, researchers could study how emotion affects the social domain by exploiting questions about trust from pre-existing data. The means for GRQ, GRQ-financial, GRQ-social, and trust are shown in Table 2.

Table 2: Means for GRQ, GRQ-fin, GRQ-social, and trust (* significant to $p<0.05$).

	GRQ*	GRQ-fin*	GRQ-social*	Trust
Neutral	3.36	2.74	3.38	4.98
Pride	4.44	4.01	4.29	5.77

Mediation Analysis:

To test whether the predictive effect of pride on financial-self, financial-other, social-self, and social-other is mediated by prosociality and locus of control, I utilize an SPSS macro developed by Andrew Hayes, Model 4.⁴⁶ The results show that there is a positive total effect (Figure 1) between pride and financial-self ($b=0.467, SE=0.157, p=0.003$). Analyzing the mediating effects (Figure 2), the results reveal that condition (pride/neutral) significantly affects prosociality ($b=0.2439, SE=0.1136, p=0.0336$), but that prosociality does not, in turn, significantly affect financial-self ($b=-0.0856, SE=0.1203, p=0.4779$). Similarly, while pride significantly affects the locus of control ($b=0.2576, SE=0.1091, p=0.0196$), the locus of control does not significantly affect financial-self ($b=0.1502, SE=0.1253, p=0.2372$). Therefore, neither of the mediating variables appears to mediate this relationship. It appears that path c' (direct effect; the effect of IV on DV with mediating variables in the model) has a positive, significant impact on risk preference ($b=0.4584, SE=0.1619, p=0.0053$) but is less significant than path c (total effect; the effect of IV on DV without mediating variables in the model), indicating a partial mediation. Surprisingly, locus of control does not appear to affect risk preference even though it is higher in the pride

condition. This suggests that pride has specific characteristics and unique appraisals that differentiate it from other emotions showing increased risk preference through the increased locus of control.

Moreover, the beta coefficient for path b was negative (non-significant), indicating that prosociality might have a negative effect on risk preference for financial-self. While this result was expected for financial risk preference for others, this negative direction for financial-self was unexpected. It may indicate that pride leads to making more socially valued risky decisions for oneself and others. This would have large implications for how prosociality affects risk preference, but further research would be necessary to understand this relationship.

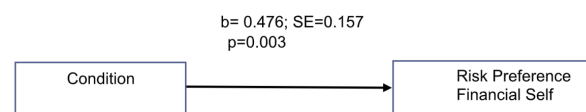


Figure 1: Total effects of pride on financial-self.

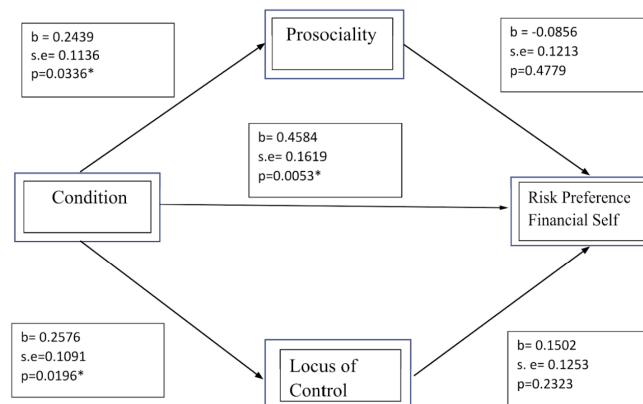


Figure 2: Mediation model for pride and financial-self.

Furthermore, I test whether prosociality and locus of control mediate the effect of pride on financial-other. The total impact (path c) is positive and significant (Figure 3) between pride and financial-other ($b=0.392, SE=0.129, p=.003$). As to the mediating effects (Figure 4), the pride condition significantly affects prosociality ($b=0.2439, SE=0.1136, p=.0336$) which in turn significantly affects financial-other ($b=-0.1967, SE=0.0978, p=.0484$). Interestingly, prosociality has a significant, negative effect on financial-other risk preference (as was hypothesized) even though the total effect was positive. This result lends credence to the argument that prosociality causes individuals to subscribe to social values theory as financial-other is not socially-valued. However, the locus of control did not significantly affect financial-other ($b=0.1220, SE=0.1019, p=.2332$), even though pride significantly affected the locus of control ($b=0.2576, SE=0.1091, p=.0196$). Again, this suggests that other specific characteristics of pride likely drive the effect of pride on risk preference. The significance of the direct path suggests a partial mediation ($b=0.4078, SE=0.1316, p=.0024$).

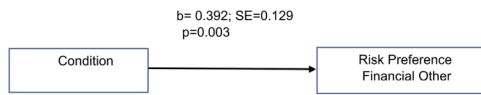


Figure 3: Total effects of pride on financial other.

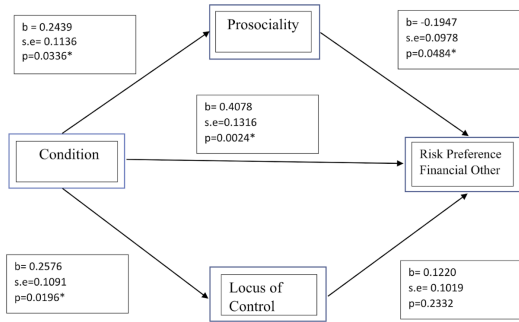


Figure 4: Mediation model for pride and financial-other.

I also consider whether prosociality and locus of control mediate the effect of pride on the social-self. Path c is positive and significant (Figure 5) between pride and social-self ($b=0.413$, $SE=0.161$, $p=0.013$). Prosociality appears to mediate this relationship (Figure 6) because pride significantly affects prosociality ($b=0.2439$, $SE=0.1136$, $p=0.03$), and prosociality significantly affects social-self ($b=0.3100$, $SE=0.1206$, $p=0.0112$). This supports the idea that prosociality positively affects risk preference in specific domains based on high self-efficacy and optimism characteristics. Moreover, social values theory could also drive this effect when making decisions for oneself, which the results for financial-self also suggest. However, further research is necessary to determine the relationship between prosociality and social values theory and how prosociality affects risk preference in specific domains. As with the financial-self and financial-other, the locus of control did not mediate the relationship (Figure 6) because the locus of control did not significantly affect social-self ($b=0.2576$, $SE=0.1091$, $p=0.02$) even though pride did significantly affect locus of control ($b=0.0724$, $SE=0.1256$, $p=0.5652$). Moreover, path c' ($b=0.3091$, $SE=0.1623$, $p=0.0589$) is less significant than path c , consistent with mediation.

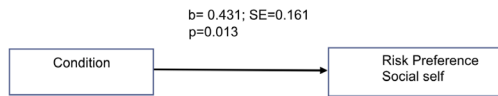


Figure 5: Total effects of pride on social-self.

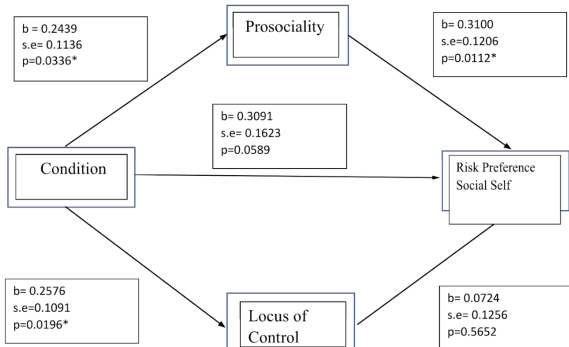


Figure 6: Mediation for pride on social-self.

Testing whether the effect of pride on social-other is mediated by prosociality and locus of control, I find that pride positively and significantly affects prosociality ($b=0.2439$, $SE=0.1136$, $p=0.0336$) and that the effect of prosociality on social-other is marginally significant ($b=0.2288$, $SE=0.1225$, $p=0.0638$). Thus, there is strong directional support for the effect of prosociality on social-other, as expected based on the social-values theory. In both “other-interested” domains, prosociality acts as either a significant or marginally significant mediator, demonstrating a link between prosociality and other-interested risk preference. Locus of control was not found to significantly affect social other ($b=0.1849$, $SE=0.1276$, $p=0.1495$), even though pride positively, significantly affected locus of control ($b=0.2576$, $SE=0.1091$, $p=0.0196$). The total effect ($b=0.532$, $SE=0.163$, $p=.001$) was, as would be expected, positively significant (Figure 7). However, since the direct effect is also positively significant ($b=0.4288$, $SE=0.1648$, $p=.0104$), this suggests partial mediation through other factors not considered since neither prosociality nor locus of control significantly mediated the relationship. The mediation model between pride and social-other is shown in Figure 8.

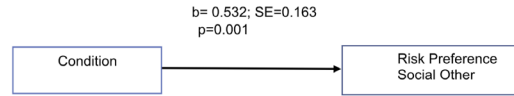


Figure 7: Total effects of pride on social-other.

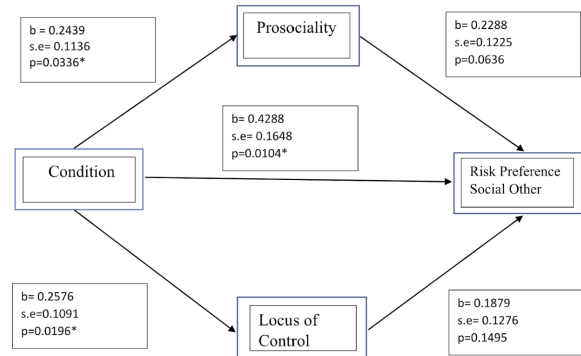


Figure 8: Mediation for pride on social-other.

Conclusion

Pride appears to have a significantly positive impact on risk preference for all four domains (as was hypothesized except for financial-other): financial-self, financial-other, social-self, and social-other. This supports previous research suggesting that pride has differentiated effects from other emotions and has distinct appraisals and dimensions. In addition, pride was also mediated by prosociality for financial-other and social-self, and prosociality was a marginally significant mediator between pride and social-other. Prosociality is a unique dimension that impacts risk preference. It may have effects outside the scope of social values theory because it was found to affect the social-self domain. Therefore, the distinct dimensions of pride appear to be more nuanced than previously suggested.

Further exploration of the relationship between pride and prosociality would be necessary to understand this effect better. Future research may center on the prosocial component of emotions and, through the use of a longitudinal study, examine whether certain emotions have a stable and positive correlation with prosocial behavior over time or if they show fluctuations in prosocial tendencies. This, in turn, can help researchers better understand the nuanced effect of emotions of risk preference in different domains. It allows for exploration based on relevant prosocial considerations applicable to each domain. In this study, prosociality affected financial-other risk preference negatively, as was expected, even though the total results of pride on financial-other was positive. Surprisingly, locus of control did not mediate the relationship between pride and risk preference in any of these domains. However, pride did increase locus of control significantly in all domains, indicating that the effects of internal control on risk preference may be emotion-specific or have differentiated effects based on hitherto unstudied factors. A summary of these effects is provided in Table 3.

Table 3: Support for hypotheses.

Hypothesis Number	Proposed Relationship	Supported
H1	Positive effect of pride on financial-self	Supported
H2	Positive effect of pride on social-self	Supported
H3	Negative effect of pride on financial-other	Not Supported; significant in the opposite direction
H4	Positive effect of pride on social-other	Supported
H5	Prosociality as a mediator between pride/neutral condition and risk preference	Partially supported; 1. Prosociality significantly mediates the relationship between pride and financial-other and social-self 2. Prosociality is a marginally significant mediator between pride and social-other 3. Pride significantly affects prosociality for financial-self, but prosociality does not in turn affect risk preference
H6	Locus of control as a mediator between condition and risk preference	Not Supported 1. Pride significantly affected locus of control in all domains, but this did not in turn significantly affect risk preference

Considering the large role specific emotions play in financial decisions and interpersonal relationships, understanding the role of emotions like pride is essential to understanding human decision-making.⁴⁷ Different emotions have distinct traits that help people reach specific goals, whether to defend, engage in play, etc. In some cases, emotions hinder rather than help an individual reach a specific goal, particularly regarding pathological risk-taking. The quintessential example of this is gambling, and subsequent studies may research the relationship, if any, between pride and gambling to better understand this phenomenon. Indeed, while many studies have examined the relationship between gambling and lack of emotional regulation,⁴⁸ few have studied the impact of specific, discrete emotions. Other areas for further research include the particular characteristics of understudied emotions, such as pride and compassion; an understanding of these emotions is necessary if we wish to understand which traits associated

with particular emotions motivate individuals to behave in specific ways.

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■ References

1. Callen, M.; Isaqzadeh, M.; Long, J. D.; Sprenger, C. Violence and Risk Preference: Experimental Evidence from Afghanistan. *American Economic Review* **2014**, *104* (1), 123–148. DOI: <http://dx.doi.org/10.1257/aer.104.1.123>
2. Kettlewell, N. Risk Preference Dynamics around Life Events. *Journal of Economic Behavior & Organization* **2019**, *162*, 66–84. DOI: <https://doi.org/10.1016/j.jebo.2019.04.018>
3. Loewenstein, G. F.; Weber, E. U.; Hsee, C. K.; Welch, N. Risk as Feelings. *Psychological Bulletin* **2001**, *127* (2), 267–286. DOI: 10.1037/0033-2909.127.2.267
4. Meier, A. N. Emotions and Risk Attitudes. *American Economic Journal: Applied Economics* **2022**, *14* (3), 527–558. DOI: 10.1257/app.20200164
5. Smith, C.; Ellsworth, P. Patterns of Cognitive Appraisal in Emotion. *J Pers Soc Psychol.* **1985**, *48* (4).
6. Lerner, J. S.; Han, S.; Keltner, D. Feelings and Consumer Decision Making: Extending the Appraisal-Tendency Framework. *Journal of Consumer Psychology* **2007**, *17* (3), 181–187. DOI: 10.1016/S1057-7408(07)70027-X
7. Kouchaki, M.; Oveis, C.; Gino, F. Guilt Enhances the Sense of Control and Drives Risky Judgments. *Journal of Experimental Psychology: General* **2014**, *143* (6), 2103–2110. DOI: 10.1037/a0037932
8. Lerner, J. S.; Keltner, D. Fear, Anger, and Risk. *Journal of Personality and Social Psychology* **2001**, *81* (1), 146–159. DOI: 10.1037//0022-3514.81.1.146
9. Frijda, N. H. The Laws of Emotion. *American Psychologist* **1988**, *43* (5), 349–358. DOI: <https://doi.org/10.1037/0003-066X.43.5.349>
10. Di Giuseppe, M.; Perry, J. C. The Hierarchy of Defense Mechanisms: Assessing Defensive Functioning with the Defense Mechanisms Rating Scales Q-Sort. *Frontiers in Psychology* **2021**, *12*. DOI: <https://doi.org/10.3389/fpsyg.2021.718440>
11. Fredrickson, B. L. The Broaden-and-Build Theory of Positive Emotions. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **2004**, *359* (1449), 1367–1377. DOI: 10.1098/rstb.2004.1512
12. Singh, J. J.; Garg, N.; Govind, R.; Vitell, S. J. Anger Strays, Fear Refrains: The Differential Effect of Negative Emotions on Consumers' Ethical Judgments. *Journal of Business Ethics* **2016**, *151* (1), 235–248. DOI: <https://doi.org/10.1007/s10551-016-3248-x>
13. Hareli, S.; Elkabetz, S.; Hanoch, Y.; Hess, U. Social Perception of Risk-Taking Willingness as a Function of Expressions of Emotions. *Frontiers in Psychology* **2021**, *12*. DOI: <https://doi.org/10.3389/fpsyg.2021.655314>
14. Tracy, J. L.; Robins, R. W.; Lagattuta, K. H. Can Children Recognize Pride? *Emotion* **2005**, *5* (3), 251–257. DOI: <https://doi.org/10.1037/1528-3542.5.3.251>
15. Kazancoglu İpek; Aydın, H.; Mishra, A. The Effect of Guilt on Post-Purchase Regret: Attitudes and Repurchase Intentions towards Smoking. *Ege Akademik Bakis (Ege Academic Review)* **2021**, *59*–79. DOI: <https://doi.org/10.21121/eab.874032>
16. Coleman, J. T.; Royne (Stafford), M. B.; Pounders, K. R. Pride, Guilt, and Self-Regulation in Cause-Related Marketing Advertisements. *Journal of Advertising* **2019**, *49* (1), 34–60. DOI: <https://doi.org/10.1080/00913370.2019.1611111>

- oi.org/10.1080/00913367.2019.1689871
17. Dohmen, T.; Falk, A.; Huffman, D.; Sunde, U.; Schupp, J.; Wagner, G. G. Individual Risk Attitudes: New Evidence from a Large, Representative, Experimentally-Validated Survey. *SSRN Electronic Journal* **2005**. DOI: <http://dx.doi.org/10.2139/ssrn.807408>
 18. Weidman, A. C.; Tracy, J. L.; Elliot, A. J. The Benefits of Following Your PRIDE: Authentic Pride Promotes Achievement. *Journal of Personality* **2015**, *84* (5), 607–622. DOI: 10.1111/jopy.12184
 19. Williams, L. A.; DeSteno, D. Pride and Perseverance: The Motivational Role of Pride. *Journal of Personality and Social Psychology* **2008**, *94* (6), 1007–1017. DOI: 10.1037/0022-3514.94.6.1007
 20. Septianto, F.; An, J.; Chiew, T. M.; Paramita, W.; Tanudharma, I. The Similar versus Divergent Effects of Pride and Happiness on the Effectiveness of Loyalty Programs. *Journal of Business Research* **2019**, *99*, 12–22. DOI: <https://doi.org/10.1016/j.jbusres.2019.02.021>
 21. Chen, S.; Su, X.; Wu, S. Need for Achievement, Education, and Entrepreneurial Risk-Taking Behavior. *Social Behavior and Personality: an international journal* **2012**, *40* (8), 1311–1318. DOI: <https://doi.org/10.2224/sbp.2012.40.8.1311>
 22. Dubois, D.; Ordabayeva, N. Social Hierarchy, Social Status, and Status Consumption. *The Cambridge Handbook of Consumer Psychology* **2015**, 332–367. DOI: <https://doi.org/10.1017/CBO9781107706552.013>
 23. Michie, S. Pride and Gratitude. *Journal of Leadership & Organizational Studies* **2009**, *15* (4), 393–403. DOI: <https://doi.org/10.1177/1548051809333338>
 24. Wubben, M. J.; De Cremer, D.; van Dijk, E. Is Pride a Prosocial Emotion? Interpersonal Effects of Authentic and Hubristic Pride. *Cognition & Emotion* **2012**, *26* (6), 1084–1097. DOI: <https://doi.org/10.1080/02699931.2011.646956>
 25. Nakamura, J. Pride and the Experience of Meaning in Daily Life. *The Journal of Positive Psychology* **2013**, *8* (6), 555–567. DOI: 10.1080/02699931.2011.646956
 26. Tracy, J. L. Nature of Pride. *PsyEXTRA Dataset* **2007**.
 27. Patrick, R. B.; Bodine, A. J.; Gibbs, J. C.; Basinger, K. S. What Accounts for Prosocial Behavior? Roles of Moral Identity, Moral Judgment, and Self-Efficacy Beliefs. *The Journal of Genetic Psychology* **2018**, *179* (5), 231–245. DOI: <https://doi.org/10.1080/00221325.2018.1491472>
 28. Caprara, G. V.; Steca, P. Prosocial Agency: The Contribution of Values and Self-Efficacy Beliefs to Prosocial Behavior across Ages. *Journal of Social and Clinical Psychology* **2007**, *26* (2), 218–239. DOI: <https://doi.org/10.1521/jscp.2007.26.2.218>
 29. Chernyak, N.; Kushnir, T. The Influence of Understanding and Having Choice on Children's Prosocial Behavior. *Current Opinion in Psychology* **2018**, *20*, 107–110. DOI: <http://dx.doi.org/doi:10.1016/j.copsyc.2017.07.043>
 30. Child, C.; Witesman, E. M. Optimism and Bias When Evaluating a Prosocial Initiative*. *Social Science Quarterly* **2019**, *100* (3), 666–677. DOI: <https://doi.org/10.1111/ssqu.12585>
 31. Dohmen, T.; Quercia, S.; Willrodt, J. Willingness to Take Risk: The Role of Risk Conception and Optimism. *SOEPpapers on Multidisciplinary Panel Data Research, No. 1026* **2019**, No. 1026.
 32. Heger, S. A.; Papageorge, N. W. We Should Totally Open a Restaurant: How Optimism and Overconfidence Affect Beliefs. *Journal of Economic Psychology* **2018**, *67*, 177–190. DOI: <https://doi.org/10.1016/j.joep.2018.06.006>
 33. Mayraz, G. Wishful Thinking. *SSRN Electronic Journal* **2011**. DOI: <http://dx.doi.org/10.2139/ssrn.1955644>
 34. Stone, E. R.; Allgaier, L. A Social Values Analysis of Self-Other Differences in Decision Making Involving Risk. *Basic and Applied Social Psychology* **2008**, *30* (2), 114–129. DOI: <https://doi.org/10.1080/01973530802208832>
 35. Batteux, E.; Ferguson, E.; Tunney, R. J. Do Our Risk Preferences Change When We Make Decisions for Others? A Meta-Analysis of Self-Other Differences in Decisions Involving Risk. *PLOS ONE* **2019**, *14* (5). DOI: <https://doi.org/10.1371/journal.pone.0216566>
 36. Deter, M. Prosociality and Risk Preferences in the Financial Sector. *SOEPpapers on Multidisciplinary Panel Data Research* **2020**, No. 1075.
 37. Pollmann, M. M. H.; Potters, J.; Trautmann, S. T. Risk Taking by Agents: The Role of Ex-Ante and Ex-Post Accountability. *Economics Letters* **2014**, *123* (3), 387–390. DOI: 10.1016/j.econlet.2014.04.004
 38. Inglehart, R. World Values Surveys and European Values Surveys, 1981–1984, 1990–1993, and 1995–1997. *ICPSR Data Holdings* **2000**.
 39. Caprara, G. V.; Steca, P.; Zelli, A.; Capanna, C. A New Scale for Measuring Adults' Prosocialness. *European Journal of Psychological Assessment* **2005**, *21* (2), 77–89. DOI: <https://doi.org/10.1027/1015-5759.21.2.77>
 40. Rotter, J. B. Generalized Expectancies for Internal versus External Control of Reinforcement. *Psychological Monographs: General and Applied* **1966**, *80* (1), 1–28. DOI: <https://doi.org/10.1037/h0092976>
 41. Nießen, D.; Schmidt, I.; Groskurth, K.; Rammstedt, B.; Lechner, C. M. The Internal-External Locus of Control Short Scale-4 (IE-4): A Comprehensive Validation of the English-Language Adaptation. *PLOS ONE* **2022**, *17* (7). DOI: 10.1371/journal.pone.0271289
 42. Shou, Y.; Olney, J. Assessing a Domain-Specific Risk-Taking Construct: A Meta-Analysis of Reliability of the DOSPERS Scale. *Judgment and Decision Making* **2020**, *15* (1), 112–134. DOI: 10.1017/S193029750000694X
 43. Luengo Kanacri, B. P.; Eisenberg, N.; Tramontano, C.; Zuffiano, A.; Caprara, M. G.; Regner, E.; Zhu, L.; Pastorelli, C.; Caprara, G. V. Measuring Prosocial Behaviors: Psychometric Properties and Cross-National Validation of the Prosociality Scale in Five Countries. *Frontiers in Psychology* **2021**, *12*. DOI: <https://doi.org/10.3389/fpsyg.2021.693174>
 44. Albanese, G.; de Blasio, G.; Sestito, P. Trust, Risk and Time Preferences: Evidence from Survey Data. *International Review of Economics* **2017**, *64* (4), 367–388. DOI: <https://doi.org/10.1007/s12232-017-0282-7>
 45. Marth, S.; Sabitzer, T.; Hofmann, E.; Hartl, B.; Penz, E. The Influence of Regulation on Trust and Risk Preference in Sharing Communities. *Frontiers in Psychology* **2020**, *11*. DOI: <https://doi.org/10.3389/fpsyg.2020.01369>
 46. Hayes, A. F. PROCESS: A Versatile Computational Tool for Observed Variable Mediation, Moderation, and Conditional Process Modeling **2012**.
 47. Zaleskiewicz, T.; Traczyk, J. Emotions and Financial Decision Making. *Psychological Perspectives on Financial Decision Making* **2020**, 107–133. DOI: 10.1007/978-3-030-45500-2_6
 48. Neophytou, K.; Vujanovic, A.; O'Connell, K. P.; Barrault, K. M.; Bonn-Miller, M. O. Gambling to Escape: A Systematic Review of the Relationship Between Avoidant Emotion Regulation/Coping Strategies and Gambling Severity. *Journal of Contextual Behavioral Science* **2023**.

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The Effect of Atmospheric Conditions on the Voltage of Solar Panels

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ABSTRACT: This study aimed to analyze the effect of different atmospheric conditions on the voltage output of a solar panel. The temperature, humidity, amount of dust, and light intensity were varied by using a heat fan, hair drier, humidifier, flour, and incense and by changing the height of the light bulb. Statistical analysis was done using linear regression and Pearson's correlation. Several machine learning methods were conducted for higher accuracy using Python. The linear regression produced an equation of the voltage output as a function of the amount of dust, humidity, temperature, and light intensity: $\text{Voltage} = 2.801 - 0.105 \times \text{Dust} + 0.746 \times \text{Light} - 0.207 \times \text{Humidity} + 0.335 \times \text{Temperature}$ ($R^2 = 0.78$, $P < 0.001$). However, machine learning showed better performance than logistic regression. The Random Forest algorithm outputted the lowest RMSE (0.02196) and MAE score (0.0240), proving it is the best predictive model. The light intensity was the most important factor in determining the voltage output and temperature, humidity, and dust in descending order. This study suggests the possibility of predicting solar power voltage at various atmospheric conditions through a machine learning model, especially random forest. Further investigation to validate this model in real fields will be needed.

KEYWORDS: Energy; Physical; Solar; Voltage; Machine learning; Prediction.

■ Introduction

Solar energy is widely used as a new technology to reduce greenhouse gas emissions, mitigate climate change, and reduce the negative impacts of global warming.¹ With the increasing interest in solar power, it is a challenge to use its full potential.² Solar panels are usually rated based on their maximum power output under standard test conditions. However, the power output of solar panels highly depends on environmental situations; energy losses result from soil, snow, dust, and other contaminants that block the sun's irradiance by covering the photovoltaic (PV) panel's interface.³ Accurate voltage predictions contribute to better estimations of the energy yield of a solar power system in the real world.

The amount of power a solar panel can produce is directly proportional to the amount of light on it. As the intensity of light increases, so does the solar panel's power output up to a certain threshold.⁴ This relationship is not necessarily linear, and the efficiency of a solar panel may decrease at very high levels of irradiance due to the effects of temperature.⁵

Factors such as temperature, humidity, and shading can also affect the performance of solar panels and may reduce their efficiency. An experiment investigated a photovoltaic module's performance under high temperature and humidity conditions. When humidity increases by 50%, the power output decrease by 34%.⁶ Under partially shadowed conditions, the PV system suffers from a nonlinearity problem between voltage and current. The loss of power might range from 10-70%.⁷ The PV performance parameters were computed by measuring its output voltage and current, the amount of solar radiation in-

cident on the panel's surface, and its surface temperature by varying humidity levels.⁸ Using multiple regression analysis, solar power generation is affected in the order of sun altitude angle, temperature, humidity, and fine dust.⁹

There are two types of dust shading on the PV unit. Hard shading exists as solids accumulate on the panel's surface and obstruct sun irradiance in a transparent and definable way. Soft shading is caused by particles in the air, such as smog or dust, that decrease the solar cells' total power by decreasing solar irradiance.¹⁰ While various experiments on the effects of hard shading were conducted, few experiments were conducted concerning soft shading. Shading on a solar panel can cause a reduction in transmission of up to 17%, with the degree of reduction depending on the type of solar cell material used.¹¹ The impact of a 5-day Saharan dust outbreak on solar power was quantified to 40-50% for PV. The daily impact of atmospheric aerosols on a PV module was 14 % during clear-sky days and up to 48 % during a dust outbreak.¹² Careful consideration about designing and operating PV systems in regions with frequent dust outbreaks can be needed. The results of the indoor experiments reveal a linear relationship between the dust density and the normalized PV power, with a drop of 1.7% per g/m^2 . Periodic cleaning of PV modules may be necessary to maintain their performance in dusty environments.¹³

Multiple studies analyzed the effects of various factors on solar energy individually, but understanding how they interact in a controlled environment comprehensively is essential for accurate predictions and efficient system design. Therefore,

predictive model of the voltage output of solar panels. This research can be utilized as a baseline for predicting accurate voltage for maximum efficacy.

Methods

A laboratory-oriented experimental study compared the solar panel's voltage output with four-atmosphere factors: temperature, light, dust, and humidity. The schematic views of the experimental setup while working are shown in Figure 1. The experiment was conducted in the physics office at Choate Rosemary Hall. The experimental set-up consists of an acrylic tank with 20x40x15 cm to place the solar panel into and control the atmosphere's conditions. Directly above the acrylic tank is a light source- a 60 W incandescent bulb- used to model the sun in real life. The height of the light source was 57cm from the flat frame. The polycrystalline solar panels (5x11 cm) were used and placed on the bottom of the acrylic tank. A Vernier logger pro and Pasco 550 Universal interface were connected to the solar panel to collect voltage output. A SHARP GP2Y1014AUOF sensor, DHT11 sensor, and LDR resistor were connected to the Arduino Mega and were used to measure the temperature, humidity, dust density, and light intensity. The Arduino was connected to the laptop and was coded to collect data twice every second. The Logger Pro and Pasco Capstone were set so that data was collected twice each second, depending on which device was used for data collection.

The light in the room was turned off, and every light source was blocked. Next, the incandescent bulb was turned on, and data was collected on the Arduino and Vernier LabQuest2 and PASCO 550 Universal Interface. Afterward, different conditions were applied individually to the air inside the acrylic tank, as stated in the paragraph below. The experimental datasets were created from thirty-six conditions mentioned in Table 1.

First, incense sticks and the humidifier were turned on and placed into the acrylic box. The number of incense sticks and the amount of water emitted from the humidifier were changed. Data were collected after the incense sticks and humidifier was turned off and when the acrylic box was full of smoke and mist. Then, the humidifier was turned on and placed into the acrylic box. The amount of water emitted from the humidifier was changed in several data sets. Afterward, incense sticks were turned on and put into the acrylic box. The number of incense sticks was changed in different data sets, and data was taken after the incense sticks were removed, yet when the acrylic box was still full of smoke. Subsequently, a heat fan was turned on. The strength of the heat fan and the height of the heat fan were changed. After that, the height of the light bulb was increased by 7 cm three times. Then, flour was added to the acrylic box and blown away using a lawn blower. The amount of flour was changed in each data set. Finally, a hair drier was turned on, and the hot air was put into the box through a small hole on the side of it.

Table 1: Experimental conditions.

	Incense 1 Humidifier level 2
	Incense 1 Humidifier level 2 off
	Incense 3 Humidifier level 1
	Incense 3 Humidifier level 1 off
	Incense 3 Humidifier level 2
	Incense 3 Humidifier level 2 off
Incense	control
	1 incense
	1 incense off
	3 incense
	3 incense off
Humidity (humidifier only)	control
	Humidity interval
	Humidity continuous
	Unlimited
Heat fan	Setting test
	weak heat fan
	strong heat fan
	weak heat fan 20cm elevated
distance	strong heat fan 20cm elevated
	0cm elevation (control)
	7cm elevation
	14cm elevation
flour	21cm elevation
	control
	0.1g flour
	0.3g flour
	0.5g flour
Hair drier	1g flour
	Hair drier level 1
	Hair drier level 1 after turning off
	Hair drier level 2
	Hair drier level 2 after turning off

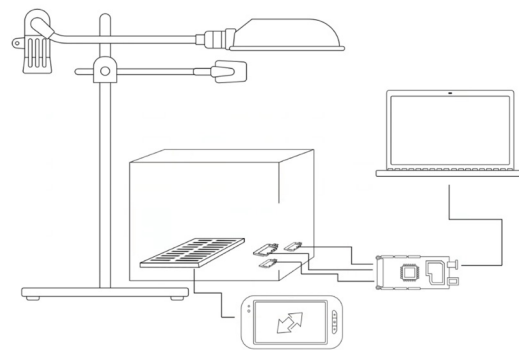


Figure 1: Schematic view of the experiment.

Each dataset contained about 300 observations. The power output was the target feature, and other features were inputs to the models, and those were divided into four sections: dust, humidity, temperature, and light. Groups were made based on similar experimental conditions. After combining the separated datasets, obtaining appropriate features to predict the voltage output of solar panels was done. The relationship between the voltage output of the solar panel and experimental groups was subsequently analyzed using Pearson's correlation and multiple linear regression using SPSS (IBM Corporation, Armonk, NY, USA). Voltage output prediction models were built using Python v 3.9.7 (<https://python.org>), which in

cludes Linear Regression, Ridge regression, Lasso regression, Random Forest Regressor (RF), Gradient Boosting Regressor (GBM), XGBOOST regression, Light GBM, Simple RNN, LSTM, and GRU. Specifically, the Scikit-learn library¹⁴, the XGBoost package (<https://xgboost.readthedocs.io>), and the statsmodel api (<https://statsmodel.org>) were utilized to build these models. The dataset was randomly divided into training and test sets in a ratio of 7:3. In addition, to deal with the multi-collinearity issue, z-scaling was conducted on the data. Finally, the importance of the four variables (light, dust, humidity, and temperature) was calculated for each model from Scikit learn's Gridsearch CV. Models were evaluated using popular error rates methods, such as Mean Absolute Error (MAE) and Root Mean Squared Error (RMSE). Plots were created using the matplotlib python library (<https://matplotlib.org>)

■ Results and Discussion

Figure 2 shows the box-and-whiskers plot of dust, humidity, temperature, light, and voltage among seven groups of experiments. For the average data throughout this experiment, the mean dust value was 340 ug/cm^3 , with the 25th and 75th percentile as 100 ug/cm^3 and 740 ug/cm^3 . The mean light value is 120 ug/cm^3 , with the 25th and 75th percentile as 10 ug/cm^3 and 900 ug/cm^3 . The mean light value was 160 lux, with the 25th and 75th percentile as 100 lux and 280 lux. The mean humidity was 49%, with the 25th and 75th percentile at 27% and 91%. The mean temperature was 21C, with the 25th and 75th percentile at 18°C and 23°C. The voltage value was 4.0V and 4.6V throughout the whole experiment.

The incense + humidifier group had the highest mean humidity, and the heat fan group had the lowest humidity. The heat fan group, however, showed the highest temperature. The humidity group led the highest light, and the flour and incense + humidifier group showed the most increased dust. The incense-only group and humidity group showed the highest voltage.

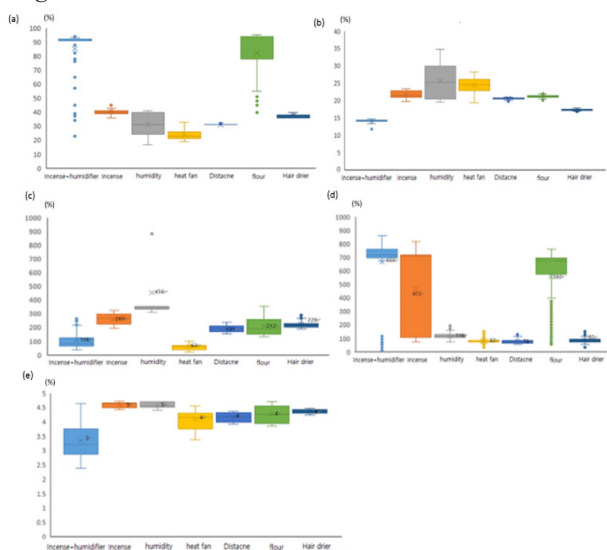


Figure 2: Comparisons of the humidity (a), temperature (b), light (c), dust (d), and voltage (e) by seven experimental conditions. The Incense + humidifier group had the highest humidity, and the heat fan group had the highest temperature. The humidity group led the highest light, and flour and

incense + humidifier showed the most increased dust. Incense only group and humidity group showed the highest voltage.

Table 2 shows the Pearson correlation coefficient and R^2 of multiple linear regression analysis among seven experimental groups. Light, dust, temperature, and humidity significantly correlated with voltage output. Figure 3 shows a heat map of the Pearson correlation coefficient between voltage and another parameter, including dust, humidity, temperature, and light. Light and temperature showed a positive relationship with voltage output, and Dust and humidity showed an inversely proportional relationship with the voltage output of the solar panel. Pearson's R coefficient showed that light intensity was the most important factor in determining the voltage output, with humidity, dust, and temperature in descending order. Each experimental group except the flour group was explained using linear regression. For example, as for the Incense + humidifier group, the voltage was significantly correlated with dust (Pearson's R coefficient -0.709), light (0.962), humidity (-0.750), and temperature (-0.787) ($p < 0.001$). Thus, the order of strength of relationships in these variables are as follows; Light > Temperature > Humidity > Dust. The R^2 value of linear regression conducted on this group was 0.981, suggesting a high accuracy.

Table 2: The Pearson correlation coefficient and R^2 of multiple linear regression analysis among seven experimental groups.

	Dust	Light	Humidity	Temp	R^2
Incense + humidifier	-0.709	0.962	-0.750	-0.787	0.981
Incense	-0.587	0.791	-0.884	-0.941	0.960
humidity	-0.716	0.930	-0.751	-0.172	0.928
Heat fan	0.197	0.910	-0.299	0.351	0.856
Distance	-0.001	0.978	0.350	-0.509	0.961
Flour	-0.362	0.284	0.148	0.244	0.196
Hair drier	-0.325	0.769	0.932	-0.908	0.952

All $p < 0.05$ or 0.001

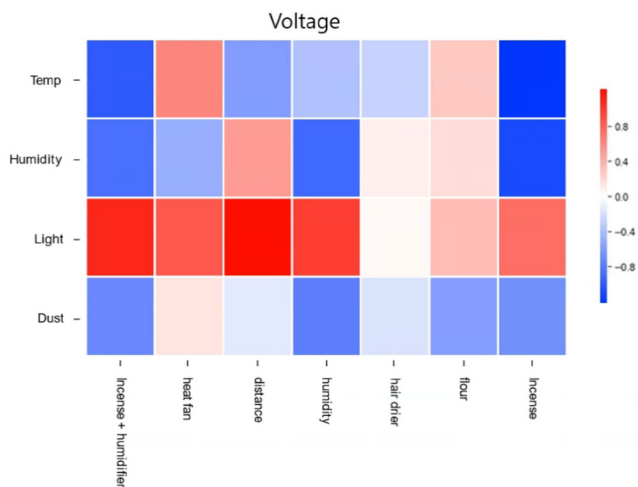


Figure 3: Heat map of Pearson correlation. Light and temperature showed a positive relationship, and Dust and humidity showed a negative association with the voltage output of the solar panel.

The linear regression analysis was used to produce a final equation, which was [Voltage= $2.801 - 0.105 \times \text{Dust (ug/m}^3) + 0.746 \times \text{light (lux)} - 0.207 \times \text{Humidity (\%)} + 0.335 \times \text{Temperature (}^\circ\text{C)}$]. The equation had a 79% accuracy (R^2 value 0.788, P -value < 0.001). Pearson's coefficient test showed that the light value had the strongest relationship with the voltage

(coefficient 0.681). humidity (-0.544), dust (-0.423), and temperature(0.389) followed in that order. When removing the group simultaneously, the accuracy increased; the single experiment group showed the best accuracy using linear regression modeling.

Table 3: Multiple linear regression modeling for seeking the final model.

	R ²	Equation
Total	0.788	$2.80-0.11(\text{dust})+0.75(\text{light})-0.21(\text{humidity})+0.36(\text{temp})$
Remove hair drier	0.819	$2.26-0.10(\text{dust})+0.77(\text{light})-0.18(\text{humidity})+0.42(\text{temp})$
Remove hair drier, flour	0.810	$2.55-0.13(\text{dust})+0.71(\text{light})-0.17(\text{humidity})+0.41(\text{temp})$
Remove hair drier, flour, distance	0.826	$3.54-0.01(\text{dust})+0.60(\text{light})-0.53(\text{humidity})+0.17(\text{temp})$
Incense/humidifier + heat fan	0.857	$3.63-0.14(\text{dust})+0.65(\text{light})-0.48(\text{humidity})+0.13(\text{temp})$
Incense/humidifier + Incense	0.936	$2.18+0.03(\text{dust})+1.08(\text{light})+0.09(\text{humidity})-0.03(\text{temp})$
Incense/humidifier	0.981	$2.08+0.22(\text{dust})+1.37(\text{light})+0.39(\text{humidity})-0.14(\text{temp})$

All $p < 0.001$

The RMSE and MAE using a decision tree, random forest, XGboost, Light GBM, GBM, linear regression, ridge regression, and lasso regression are shown in Figure 4. The RF model is clearly the most accurate model for voltage output. The machine learning algorithm showed better performance than linear regression.

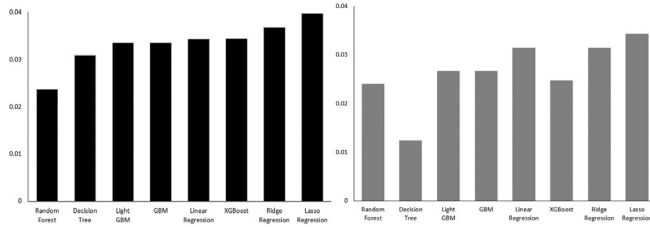


Figure 4: Comparisons of RMSE (a) and MAE (b) among various methods. The RF model showed the lowest RMSE, showing the most accurate model for voltage output.

Figure 5 shows the feature importance of each variable. It is clear that for all models, light intensity is the most important, and temperature, humidity, and dust are important in that order. However, the absolute importance of each variable is what differs; for the Random Forest and Decision tree models, the humidity and dust seem to be significantly less important than the light and temperature, while for the linear regression model, the two variables seem more comparable. In addition, the linear regression model's temperature variable appears less important than the temperature in the random forest and decision tree models.

Figure 6 shows the actual voltage value vs. the predicted voltage value of the model given the four variables. The linear regression has the highest deviation between the actual and predicted values, while the random forest and the decision tree don't have that large of a deviation. This reflects the MAE value difference between these models. The Random Forest algorithm has the lowest RMSE of 0.02196, which suggests it is the most effective modeling method with this data. It is because the relationship between the data is not a linear function. And, as the random forest can average error in every tree, it would be more resistant to noise in the data.

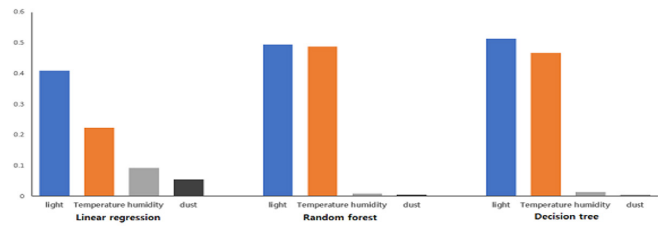


Figure 5: Comparison of feature importance among linear regression, random forest, and decision tree. The light intensity is the most important, and temperature, humidity, and dust are important in that order. However, the importance of each variable is different.

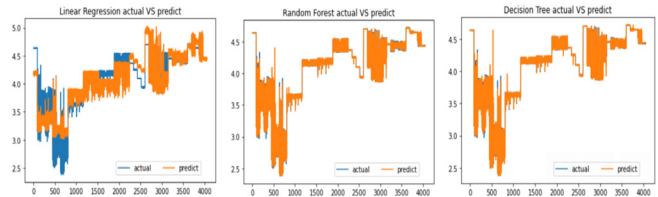


Figure 6: Comparison of predicted performance among linear regression, random forest, and decision tree. The linear regression has the highest deviation between the actual and predicted values. In contrast, the random forest and the decision tree don't have that large of a deviation.

Discussion

This study confirmed that atmospheric conditions such as dust, light, temperature, and humidity nonlinearly affect voltage output. Furthermore, the dust and humidity were found to have an inversely proportional relationship with the voltage output, while the light and temperature had a directly proportional relationship. Therefore, this random forest algorithm can be used to estimate the amount of voltage a solar panel will generate given certain conditions of the atmosphere.

One study that aimed to analyze the influence of dirt accumulation on panels used 100W spotlights to simulate solar radiation and vary the number of spotlights to change the intensity of solar radiation. The radiation energy from the light was measured using a photo-radiometer, and output voltage and current from the solar PV panel were measured using a digital multimeter.¹⁵ In this study, 60 W incandescent bulbs changed the distance between the solar panel and the incandescent bulb. The radiation energy and the output current were not addressed, while the output voltage was measured using a Vernier LabQuest 2 and a PASCO 550 Universal Interface. In addition, the other study utilized dust, sand, moss, and water droplets to change the dust amount.¹⁶ However, this study used flour and incense sticks to change the amount of dust.

Another study that was conducted outdoors showed that there is a direct proportionality between current, solar flux, and efficiency. The ambient temperature, humidity, current and voltage output of the PV panel, and the panel's temperature were measured. In this study, the current output and temperature of the panel still need to be addressed. However, the ambient temperature and humidity were measured using an Arduino and a DHT11 sensor, and the voltage output was measured using a Vernier LabQuest2 and a PASCO 550 Universal Interface. In contrast to the outdoor study, this study controlled the humidity using a humidifier and the temperature using a hair drier and a heat fan. Dust, soiling, and pollutants can significantly influence the performance of the

PV system. Using natural dust composition in a polluted city, deposited dust-related efficiency loss gradually increased with the mass and was about 2.1%, which follows the exponential.¹⁷ In our study, dust showed an inversely proportional relationship with the voltage output of the solar panel. However, it is not from natural dust but from artificial laboratory conditions.

The Pearson's R coefficient test shows that the light intensity variable in this study has a coefficient of 0.681. Since the coefficient is positive and over 0.5, the results show that the light intensity strongly correlates positively with the voltage output. A previous study showed that the amount of power a solar panel was directly proportional to the amount of light, validating the results of this study.⁴ Another study showed that Pearson's R coefficient of the light intensity was 0.62, further validating the result of this study.⁹

Using linear regression analysis, six groups were very reliable in linear modeling, all with an R^2 value over 0.9. In addition, the heat fan group showed relatively high performance with an R^2 value of 0.856. However, the Flour group showed an extremely low R^2 value of 0.196 and extremely low Pearson's r coefficients for all four variables. These low values suggest that a linear model could not be the exact model the study sought. This study's final linear regression analysis model was as follows: [Voltage= 2.801-0.105x Dust+0.746xlight-0.207xHumidity +0.335xTemperature]. The equation had a 79% accuracy (R^2 value 0.788, P-value < 0.001). Another study showed that using multiple regression analysis, solar power generation is affected in the order of sun altitude angle, temperature, humidity, and fine dust.¹⁸

The study attempted to find the most accurate model by excluding some groups and adding others. When some groups were excluded, all groups, excluding the hair drier group, showed 81% accuracy (R^2 value 0.806, P-value < 0.001), and adding the incense and humidifier and the incense-only group showed 94% accuracy (R^2 value 0.936, P-value < 0.001) due to similar conditions. This result indicates that if different conditions are added to the linear model, the accuracy drops and therefore becomes less explainable through a linear model. While the Linear Regression provided some excellent results with a relatively high value, some data sets have a very low R^2 -value. This model performs well when the data are highly correlated. However, this data set has a VIF factor of almost 10. This shows that there is a multicollinearity issue in the data set. To be multicollinear means that two or more variables in a regression depend on each other. In general, if the VIF factor of one of the variables goes over 10, the data is considered to have a significant collinearity issue. This explains why the linear regression model did not fit in well for some results. The humidity VIF factor was almost ten in the data before the Z-scaling occurred. This could lead to issues in terms of overfitting the model. Therefore, a technique called "Z-scaling" was conducted, a method of normalizing data meant to reduce the multicollinearity. This is shown in the data after the Z-scaling; a significant decrease in the VIF factor for humidity was shown, which decreases the probability of overfitting, thus creating a more accurate model. This is very much anticipated, as Z-scaling is a method to achieve precisely this.¹⁵

The RMSE represents the deviation of the predicted value from the actual value in the Euclidian distance, which describes the accuracy of the data. If the RMSE is closer to 0, the prediction is more accurate.¹⁸

The Random Forest algorithm has the lowest RMSE of 0.02196, which suggests that the random forest algorithm is the most effective in modeling this data. The reasoning behind this may be quite simple; if the relationship between the data is not a linear function, the linear regression model will not work as well as the random forest algorithm. In addition, as the random forest can average error in every tree, it would be more resistant to noise in the data. Because of these two characteristics, the Random Forest algorithm likely became more successful. In addition, random forest is inherently a more comprehensive version of decision trees. Therefore, it would have a lower RMSE, thus an increased accuracy. The Random Forest algorithm has the lowest RMSE of 0.02196 and MAE of 0.0106, which suggests it is the most effective modeling method in this data. In another study, the RMSE and MAE value of the random forest algorithm was 0.9 and 0.29, respectively. Therefore, the study stated that the random forest algorithm was the best method. Since the values in this study are lower than those in the other study, it seems reasonable to conclude that the data was more precise and better aligned with the random forest algorithm.

The strength of this study is that multiple variables could be controlled. While other studies have either been outdoors or only controlled one variable at a time, this study comprehensively controls multiple variables at once, which allowed for a more thorough analysis and, therefore, results.

The study had several limitations, including the inability to block out every light source and the human error of slight movement of measuring equipment, solar panels, and light source during each run. The inability to block out every light source can lead to errors of different voltage values based on the experiment's time, creating an unaccounted variable. The human error of components of the investigation shifting slightly can lead to slight drops/increases in the voltage measurement and inaccurate sensor data. However, this was minimized by placing the tape on each component's location and matching the components to those locations. Furthermore, as this study was conducted as an indoor experimental study, it can be differentiated from real environmental features like wind, temperature, sunlight, and others. The voltage was measured instead of the power, which can also lead to a limitation in generalizing to real-life situation analysis. Also, due to irregular solar panels operation, there can be the possibility of outliers in the voltage output data points.

■ Conclusion

This study showed the effect of various atmospheric conditions on the voltage output of solar panels comprehensively. The voltage output is related to all four variables, including the amount of light, temperature, humidity, and the amount of dust, with its importance in the order of the amount of light, temperature, humidity, and amount of dust. The voltage can be modeled in an equation stated: voltage= 2.801-0.105xDust (ug/m3)+0.746xlight (lux)-0.207x Hu

midity (%) + 0.335 × Temperature (°C). Using machine learning models, the Random Forest algorithm was confirmed as the most accurate method for predicting the voltage output of solar panels. In addition, the amount of light, temperature, humidity, and dust was important in that order. For future work, validating this algorithm in outdoor experimental conditions will be necessary to predict the accurate performance of solar energy systems. It can have widespread applications in various settings, including residential, outdoor, and more expansive spaces.

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■ References

1. Agency, I. E. Global Energy Review 2020. <https://www.iea.org/reports/global-energy-review-2020/renewables> (accessed May 10).
2. Breyer C, Bösch D, Gulagi A, Aghahosseini A, Barbosa LSNS, Koskinen O, Barasa M, Caldera U, Afanasyeva S, Child M, Farfan J, Vainikka P. On the role of solar photovoltaics in global energy transition scenarios. *Progress in Photovoltaics: Research and Applications*. **2017**;28(3):237-247.
3. Abanda, F. H.; Aganaba, A. G.; Nkwetta, D. N. Analysis of Photovoltaic Performance Degradation Factors: A Review. *Sustain. Energy Technol. Assess.* **2020**, *40*, 100656.
4. Solanki, C. S. *Solar Photovoltaic Technology and Systems: A Manual for Technicians, Trainers and Engineers*; PHI Learning Pvt. Ltd.: New Delhi, 2013.
5. Al-Sulaiman, F. A. Review of Photovoltaic Technologies. *Renew. Energy* **2007**, *32*, 2350-2364.
6. Ullah MHH, Hasan MH, Rahim NA. Performance analysis of a photovoltaic module under high temperature and humidity. *Journal of Cleaner Production*. **2019**;221:222-229.
7. Said, S.A.K.; Mekhilef, S. Partial Shading of Photovoltaic Arrays. *Renew. Energy* **2008**, *33*, 1973-1987.
8. Shen, H.; Jiang, L. Impact of Dust on the Efficiency of a Photovoltaic System in a Desert Region. *Sustainability* **2019**, *11*, 2662.
9. Song, H.; Jin, C. Performance Analysis of Photovoltaic Systems Considering Meteorological Factors. *Energies* **2021**, *14*, 1043-1056.
10. Al-Ammar, E.A.A.-S.; Farhan, A. Investigating Shading Effects on Photovoltaic Panel Performance. *Energies* **2018**, *11*, 1785-1799.
11. Mekhilef, S.D.; Rahim, N.A.; Mokhlis, H.; Mustafa, M.W. Effect of Shading on Photovoltaic Modules. *Renew. Sustain. Energy Rev.* **2011**, *15*, 67-73.
12. Jaidi, N.B.; Kahloun, M.S.; Bergès, L.K. Impact of a 5-Day Saharan Dust Outbreak on High Photovoltaic (PV) Power Production. *Renew. Energy* **2020**, *159*, 1017-1026.
13. Muneer, T.A.; Asif, M.; Alghoul, M.A.-N. Effect of Dust Accumulation on Photovoltaic Performance. *Renew. Energy* **2004**, *29*, 1834-1839.
14. Pedregosa, F.; Varoquaux, G.; Gramfort, A.; Michel, V.; Thirion, B.; Grisel, O.; Blondel, M.; Prettenhofer, P.; Weiss, R.; Dubourg, V.; Vanderplas, J.; Passos, A.; Cournapeau, D.; Brucher, M.; Perrot, M.; Duchesnay, É. Scikit-Learn: Machine Learning in Python. *J. Mach. Learn. Res.* **2011**, *12*, 2825-2830.
15. Khalil, A.S.; Zaidi, Z.H.; Rahim, R.A.; Kadhum, A.A.H. Effect of Dust on the Performance of PV Panels. *Energy Procedia* **2012**, *14*, 1834-1839.
16. Zhou, W.; Lou, L.; Yang, H. Dust Effect on the Performance of Solar Photovoltaic Systems. *Renew. Sustain. Energy Rev.* **2016**, *62*, 97-106.
17. Jaszczur, M.; Teneta, J.; Styszko, K.; Hassan, Q.; Burzyńska P.; Marcinek, E.; Łopian, N. The field experiments and model of the natural dust deposition effects on photovoltaic module efficiency. *Environ Sci Pollut Res.* **2019**, *26*, 8402-8417.
18. Kaldellis, J.K.; Zafirakis, D. The Impact of Meteorological Parameters and Dust on the Performance of Photovoltaic Systems. *Renew. Energy* **2011**, *36*, 665-670.

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Examining the Effects of Positive and Negative Messaging of Red Meat Consumption on Dietary Choices

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ABSTRACT: As environmental and public health become a global concern, the need to reduce emissions, pollution, and disease risk is becoming more evident. A common denominator is red meat consumption, especially with 18% of total greenhouse gas emissions coming from the livestock industry. This paper will examine whether discouraging red meat (negative messaging) or encouraging a green diet (positive messaging) impacts viewers more significantly, which can help shape future actions to reduce overall consumption. A survey was implemented that collected demographic factors and split participants into two groups, who were exposed to information either pertaining to the consequences of red meat or the benefits of a green diet. Before and after this treatment, participants answered the same questions relating to their desire to reduce red meat consumption. The quantitative change and their qualitative reasoning were analyzed by calculating and comparing the raw difference, mean, and standard deviation, as well as categorizing the reasons behind the qualitative answers, showing that both treatments changed respondents' opinions on dietary habits, with the negative treatment being more effective. These findings could prove useful as policymakers work to define effective interventions involving red meat consumption to ultimately improve the global environment. However, these changes were not statistically significant in the context of this study, meaning that in the future, applied treatments should be stronger to result in a larger shift in opinion. Demographics, specifically age, race, and gender, did not significantly impact the likelihood of a respondent changing their dietary habits.

KEYWORDS: Behavioral and Social Sciences; Sociology and Social Psychology; Communication; Positive and Negative Messaging; Red Meat Consumption.

■ Introduction

Over the last few decades, the dramatic shift in the United States' agricultural industry from purely agrarian to highly industrial in cultivating crops and animal husbandry has placed significant pressure on public and environmental health. On any given day, between 63% and 74% of individuals consume red or processed meat in North America.¹ The presence of heme, nitrates, nitrites, heterocyclic amines, and polycyclic amines in red meat increases the risk for bowel cancer and high blood pressure,² two significant causes of fatality worldwide. In addition, raising cattle and swine, processing meat, and transporting it to distributors releases large amounts of greenhouse gases, significantly contributing to climate change, with the supply chains of just ruminant animal products (cattle, sheep, and goats) contributing 16% of current levels of greenhouse gas emissions.³ Growing feed for livestock requires significant amounts of water and land, as does providing water to the animals, using up considerable amounts of natural resources. Encouraging a reduction in red meat consumption is therefore critical to decreasing detrimental impacts on public and environmental health.

Direct behavioral change will only be possible via the education of individuals, following the Health Belief Model, which states that an individual's beliefs, perceived benefits, and self-efficacy provide reasoning for behavior related to personal health. While many previous studies have examined individuals' responses to messaging about the impacts of red

meat consumption via subsequent decreases in red meat consumption, little data exists comparing whether encouraging greener diets, defined as predominantly plant-based diets, or discouraging red meat consumption is more effective.⁴⁻⁶ Most existing studies look at either the positive or negative effects of red meat consumption rather than comparing its effectiveness to other dietary options.⁷⁻¹⁰ Furthermore, there is a lack of research regarding the socio-demographic factors associated with responsiveness to different messaging schemes (i.e., gender, age, race).

This paper addresses these gaps by assessing how positive versus negative messaging about the impacts of red meat consumption on public and environmental health affects dietary choices. Information focusing on the benefits of a greener diet (positive messaging) and the consequences of eating red meat (negative messaging) will be used to see which is more effective in changing public opinion. This study also addresses the need for localized data collection, given the nuances in cultural differences among various regions in the United States. Instead of focusing on entire countries such as the United States¹¹ or Italy,¹² this study is confined to residents of Georgia. A Georgia-centered analysis may provide a ground for hypotheses regarding the broader South. By optimizing messaging through either discouraging the consumption of red meat or encouraging the inclusion of more green foods, strategies can appropriately be adjusted for other areas to reduce detrimental impacts on public and environmental health.

■ Methods

To assess the effects of dietary choice messaging on an individual, researchers employed a pre-experimental design and recruited participants through convenience sampling in Georgia. Descriptive data, including age, race, and gender as part of the cross-sectional study, were collected via survey using quantitative and qualitative methods. Participants were recruited through the social media platforms Instagram and Facebook. Before taking the survey, participants were notified that the data they provided would not be associated with their name or other identifying information, no risks were apparent, participation was voluntary, and they were able to withdraw at any time or refrain from answering a question. The typical time to fill out this survey was between five to ten minutes, suggesting this was a relatively short survey that would not discourage one from responding. As a pre-experimental design, the population was separated into two distinct sample sizes exposed to one of two treatments: one detailed the benefits of switching to a greener diet, and one presented the consequences of high levels of red-meat consumption. The benefits and implications regarding the impacts on personal health and the environment were framed. To begin the survey, respondents provided their demographic information and answered questions about their current red-meat consumption and desire to change their diet. The questions were adapted from Szczebylo's 2022 study titled "Is Eating Less Meat Possible? Exploring the Willingness to Reduce Meat Consumption among Millennials Working in Polish Cities"¹³ as follows:

Question 1: "At this time, how much do you want to limit your red meat consumption, 1 being definitely not and 5 being definitely yes?"

Question 2: "At this time, I can picture myself not eating red meat regularly, 1 being definitely not and 5 being definitely yes."

Question 3: "At this time, I foresee red meat being an important part of the majority of my meals for the next month, 1 being definitely not and 5 being definitely yes."

Upon completion of the survey, participants were organized into two different treatment groups: the positive-messaging group that received messaging detailing the benefits of a green diet and the negative-messaging group that received messaging detailing the negative impacts of red meat consumption (shown below). They were again asked to answer the same questions as before pertaining to their new desire to reduce red-meat consumption. The difference in their initial responses to their subsequent ones represented the specific effect of the treatment. The treatments were as follows:

Negative Treatment:

Recently, research about the impacts of red meat consumption has expanded. Some of the novel findings are:

- Frequent meat consumption is linked to an increased colorectal (colon) cancer risk.¹⁴

- Red meat consumption is directly associated with high blood pressure; 102.6 g (approximately a steak the size of one's palm, as thick as a deck of cards) of higher meat consumption is associated with a higher systolic blood pressure of 1.25 mm Hg.¹⁵

- 18% of total greenhouse gas emissions are from the livestock industry, which is also the largest source of methane.¹⁶

- Meat produces more emissions per unit of energy compared to plant-based foods.¹⁷

Positive Treatment:

Recently, research about the impacts of red meat consumption has expanded. Some of the novel findings are:

- 400-600 grams of fruits and vegetables per day is associated with a 50% decrease in many common forms of cancer.¹⁸

- Diets abundant in fruits and vegetables are associated with a reduced risk of heart disease and many chronic diseases.¹⁹

- Plant-based diets have the potential to reduce diet-related greenhouse gas emissions by 49%.²⁰

- Plant-based diets could reduce diet-related land use by 76%, eutrophication (pollution of bodies of water that significantly harms ecosystems) by 49%, and green and blue water use by 21% and 14%.²⁰

Following the treatment received, participants were then asked an open-ended question about their reported desire to reduce (or not) their red-meat consumption. Based on the responses, I analyzed data using the tendency of people to reduce their red meat consumption according to the treatment they received based on calculating and comparing the raw difference, mean, and standard deviation for each question. While this survey is not fully randomized and has respondents centralized around the city of Atlanta, valuable information is still provided that can inform future research and approaches to dietary communication in states similar to Georgia.

■ Results

There were 258 respondents across the two weeks the survey was open, significantly exceeding the target of 100 respondents; however, 23 were removed for insufficient data or not living in Georgia, as the study required a comparison of answers before and after the treatment and focused on Georgia. Most responses originated from the Atlanta area; therefore, the municipal location did not play a role in the analysis. The first question asking how many days a week the respondent ate red meat was answered by 234 people. Of these, 55 already did not eat red meat, and 204 ate red meat three days a week or less.

By separating the numerical results, ranked 1-5 by the Likert scale, by treatment for each question both before and after the treatment, and subtracting the initial from the final, the raw difference, mean, and standard deviation were calculated and compared. The positive treatment was defined as the information pertaining to the benefits of increasing plant-based consumption. In contrast, the negative treatment was defined as the information related to the drawbacks of red meat consumption.

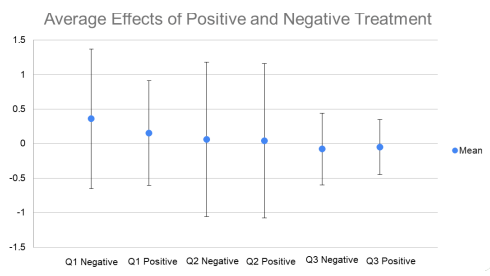


Figure 1: Mean and standard deviation from the Likert scale across both treatments for each question.

For question 1 concerning the desire to limit red meat consumption, the means were 0.15 and 0.36 for the positive and negative treatments, respectively, and the standard deviations were 0.76 and 1.01, respectively (Figure 1).

Following the same format, the means for question 2 on being able to picture oneself not eating red meat regularly were 0.04 (positive treatment) and 0.06 (negative treatment), while the standard deviations were both 1.12 (Figure 1).

For question 3, relating to the importance of red meat in future meals, the means were -0.05 (positive treatment) and -0.08 (negative treatment), and the standard deviations were 0.40 and 0.52 (Figure 1). In question 3, the means are negative because choices 1-5 were flipped, in that people preferring to reduce red meat consumption would have chosen 1 rather than 5 as in the previous two questions.

Based on the participants' responses to the demographic questions, this table was created to display the spread of data across age, race, and gender, followed by their willingness to change by question, which was calculated by taking the number of people willing to change after the treatment and dividing by the total number surveyed in a particular group, to calculate the percent willing to change.

Table 1: Age, race, and gender, and their association to a willingness to change by question.

QUESTION 1				QUESTION 2				QUESTION 3			
POSITIVE TREATMENT	Willing to Change	No Change	Percent Willing to Change	POSITIVE TREATMENT	Willing to Change	No Change	Percent Willing to Change	POSITIVE TREATMENT	Willing to Change	No Change	Percent Willing to Change
18-24	0	2	0	18-24	0	2	0	18-24	0	2	0
25-34	0	2	0	25-34	1	4	20	25-34	0	29	0
35-44	2	27	6.9	35-44	2	27	6.9	35-44	0	29	0
45-54	1	39	2.6	45-54	1	39	2.6	45-54	0	41	0
55-64	0	11	0	55-64	0	11	0	55-64	0	11	0
65+	0	19	0	65+	2	12	14.3	65+	3	19	15.8
White	12	67	15.19	White	13	66	19.69	White	3	36	7.9
Asian	1	29	3.45	Asian	2	12	14.29	Asian	3	16	18.75
Black or African American	0	0	0	Black or African American	2	4	50	Black or African American	1	5	20
Hispanic, Latino, or Spanish	0	7	0	Hispanic, Latino, or Spanish	1	6	16.67	Hispanic, Latino, or Spanish	1	6	16.67
Middle Eastern or North African	0	2	0	Middle Eastern or North African	1	2	50	Middle Eastern or North African	0	2	0
Native Hawaiian or Other Pacific Islander	0	0	0	Native Hawaiian or Other Pacific Islander	0	0	0	Native Hawaiian or Other Pacific Islander	0	0	0
American Indian or Alaska Native	0	1	0	American Indian or Alaska Native	0	1	0	American Indian or Alaska Native	0	1	0
Female	12	103	10.62	Female	16	112	14.29	Female	9	104	7.24
Male	0	2	0	Male	2	2	100	Male	0	2	0
Non-binary/gender	0	1	0	Non-binary/gender	0	1	0	Non-binary/gender	0	1	0

Reasons for Changing Amount of Red Meat Consumption

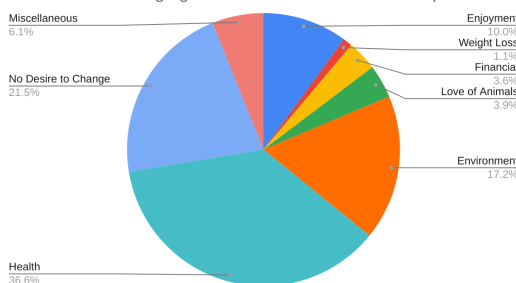


Figure 2: Percentage of reasons to either increase or decrease red meat consumption by theme.

The last question in the survey, “In the future, why or why not would you want to reduce your red meat consumption?” was an optional open-ended question for gathering qualitative data. Although 258 respondents participated, 183 responses were received for the open-ended question. The resulting statements and reasons were qualitatively coded for the common themes (Figure 2). Some respondents included multiple reasons in their responses. In addition, each unique theme was coded, thereby resulting in more themes than total respondents. The analysis resulted in the themes of enjoyment, weight loss, finances, love of animals, environment, health, no desire to change, and miscellaneous. In total, there were 279 statements after responses were separated into categories. The most prevalent responses were related to health (36.56%), with responses going as far in depth as “[t]hree months ago I learned I have a genetic heart defect and keeping low cholesterol can reduce the chance of complications from this. It also has a noticeable impact on inflammation in my hips. Red meat increases cholesterol and inflammation,” and the environment (21.51%), with responses as detailed as “I know that beef production is resource intensive and it is harmful to the environment.” In comparison, the least referenced categories were finances (3.58%) and weight loss (1.08%).

Discussion

The average treatment across all questions shows that focusing on the negative impacts of red meat consumption was more persuasive than the positive impacts. This provides notable information for the kind of framing that environmental communicators and policymakers should focus on when trying to reduce red meat consumption. However, the standard deviation across all three questions was large, likely due to the relatively small sample size and lack of time to implement a long-term treatment. While the treatments did result in a change in mindset toward reducing meat consumption, this treatment effect could not be distinguished from zero due to the large standard deviation. Across the questions, the negative treatment, providing the harmful effects of red meat, resulted in the greatest change in opinion toward consuming less red meat; however, neither is statistically significant. The greater change resulting from the negative treatment rather than the positive treatment is likely because people are more motivated by fear as opposed to rewards. Notably, these results highlight the difficulties of changing red meat consumption behavior. More than simply providing education, as many environmental campaigns do, is needed to shift attitudes significantly. Long-term, in-depth education could be more persuasive. Based on these results, future research should explore the impacts of long-term education about the negative effects of red meat consumption. Furthermore, many respondents already did not eat red meat or ate it infrequently, thus introducing a critical confounding variable into decisions to change current red meat consumption patterns. For future research, the chosen population should consist of a larger percentage of red meat eaters so that the full potential of the treatments can be observed.

Age played a negligible role in the results. While specific trends could be seen across individual treatments (Table 1), they varied so much that age was not a significant factor overall. Because a large percentage of respondents were white (Table 1), being white correlated with a higher likelihood of willingness to change behavior after exposure to the treatment. This was also seen with gender: women were more likely to change their behavior, but most respondents were women (Table 1), suggesting that further exploration of differences across gender is necessary for future research.

The qualitative results provide insight into the reasons behind the lack of behavior change. The two categories targeted explicitly in the treatments were health and environment, possibly explaining why those two categories are the most frequently mentioned in the open-ended response question, 36.56%, and 21.51%, respectively. While the majority of respondents cited these as reasons to change, with examples such as “I would like to keep working on making it a smaller portion of my consumption to improve my health and decrease risk to health” and “[t]o reduce environmental impacts of red meat production/consumption,” some respondents also used the health category as a reason not to change red-meat consumption habits, providing statements such as “[i]t has not caused my family or me any health issues, and we have eaten it our entire lives. My parents lived to 82 and 92 and had none of the illnesses mentioned.” Most respondents used the environment to limit red-meat consumption, citing reasons such as “but also to limit accessory pollution that is associated with the production of red meat in general.” However, it was also said that “[m]ost plant-based foods are brought to us via mono-cropping, which is MUCH worse for the environment.”

The third most common category was “no change” (17.20%), meaning many people were not influenced enough to change their dietary habits. The qualitative results also provide insight into additional motivators that should be explored further in future research to decrease the number of people in the “no change” category. Enjoyment of red-meat products was mentioned in approximately 10% of responses, with most respondents producing statements such as “I would not reduce my consumption because I like the taste.” However, a small number also provided reasons such as “I also don’t really like the way red meat taste[s] in general.” Communication strategies directly addressing the enjoyment of red-meat alternatives could assuage these concerns. Additionally, though much less common, a love of animals, financial concerns, and a desire to lose weight was also noted as reasons for changing (or not) red-meat consumption patterns. These smaller factors could also be targeted while creating messaging regarding reducing red meat consumption. All of the factors mentioned in the qualitative responses align with pre-existing research on reasons for vegetarianism or veganism.^{21, 22}

■ Limitations

Although this study provides critical insights into the difficulties of changing red-meat consumption and potential avenues for future communication strategies, there are some limitations that future studies should address. First, the survey was distributed through social media, resulting in a biased

sample since respondents must have had social media to participate. In addition, because it was disseminated via social networks, the survey needed to be more randomized. Finally, the sample size only partially represents all of Georgia, as the majority of respondents were from Atlanta; however, the high number of responses helps to increase the power of the results.

Time was also a limitation in the design of the entire methodology, as a short window to complete this study meant there was not sufficient time to implement a long-term treatment, such as a course on the impacts of red-meat consumption, which may have had a more substantial effect on desires to change behavior. This resulted in statistically insignificant differences in the data across varying treatments, as stronger treatments would have likely resulted in more significant changes in behavior.

■ Conclusion

Consuming red meat has negative implications for both public and environmental health. Inspiring behavior change is critical to reducing these detrimental impacts, and understanding whether a frame that focuses on the positive health effects or one that centers on the negative health effects is more effective is critical to reducing the health risks and harmful emissions that impact the environment. This study found that providing data on either the impact of continuing or reducing red meat consumption motivated some respondents to change their dietary habits. However, this change was not statistically significant across the sample as a whole. This suggests that future studies should aim to increase the effect of the treatment, such as by providing more data or implementing a more extended educational campaign, to see a bigger change in behavior. The negative treatment certainly had a much larger impact on the respondents than the positive treatment. Though this difference was not large enough to definitively conclude that providing negative framing around continuing red meat consumption is the best way to modify dietary habits, it points towards an important future direction for investigation by researchers, environmental educators, and policymakers alike.

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I express my extreme gratitude to my mentor Gabriela Nagle-Alverio, a J.D. - Ph.D. student at Duke University’s University Program in Environmental Policy. Her guidance made this study possible and helped me tremendously in writing this paper. I would also like to thank Nandini Meenakshi and Tyler Moulton for their assistance in the writing and editing process. Finally, I would like to thank my family for their support and encouragement.

■ References

1. Frank, S. M.; Jaacks, L. M.; Batis, C.; Vanderlee, L.; Taillie, L. S. P. Patterns of Red and Processed Meat Consumption across North America: A Nationally Representative Cross-Sectional Comparison of Dietary Recalls from Canada, Mexico, and the United States. *International Journal of Environmental Research and Public Health* **2021**, *18* (1), 357.
2. Patrick, K. How does processed meat cause cancer and how much matters? https://news.cancerresearchuk.org/2021/03/17/bacon-salami-and-sausages-how-does-processed-meat-cause-cancer-and-how-much-matters/?_gl=1%2Axtao15%2A_ga%2ANjAxjAx

- NjUwMTY0LjE2NzQ5NjM0MTg.%2A_ga_58736Z2GNN%2AMTY4MDQOTA1MC44LjAuMTY4MDQOTA1Mi41OC4wLjA.&_ga=2.33150910.1037225888.1680449050-601650164.1674963418 (accessed Mar 1, 2023).
3. Davison, T. M.; Black, J. L.; Moss, J. F. Red Meat—an Essential Partner to Reduce Global Greenhouse Gas Emissions. *Animal Frontiers* **2020**, *10* (4), 14–21.
 4. Stea, S.; Pickering, G. J. Optimizing Messaging to Reduce Red Meat Consumption. *Environmental Communication* **2018**, *13* (5), 633–648.
 5. Wistar, A.; Hall, M. G.; Bercholz, M.; Taillie, L. S. Designing Environmental Messages to Discourage Red Meat Consumption: An Online Experiment. *International Journal of Environmental Research and Public Health* **2022**, *19* (5), 2919.
 6. Taillie, L. S.; Prestemon, C. E.; Hall, M. G.; Grummon, A. H.; Vesely, A.; Jaacks, L. M. Developing Health and Environmental Warning Messages about Red Meat: An Online Experiment. *PLOS ONE* **2022**, *17* (6).
 7. McAfee, A. J.; McSorley, E. M.; Cuskelly, G. J.; Moss, B. W.; Wallace, J. M. W.; Bonham, M. P.; Fearon, A. M. Red Meat Consumption: An Overview of the Risks and Benefits. *Meat Science* **2010**, *84* (1), 1–13.
 8. González, N.; Marquès, M.; Nadal, M.; Domingo, J. L. Meat Consumption: Which Are the Current Global Risks? A Review of Recent (2010–2020) Evidences. *Food Research International* **2020**, *137*, 109341.
 9. Kouvari, M.; Notara, V.; Kalogeropoulos, N.; Panagiotakos, D. B. Diabetes Mellitus Associated with Processed and Unprocessed Red Meat: An Overview. *International Journal of Food Sciences and Nutrition* **2016**, *67* (7), 735–743.
 10. Wang, X.; Lin, X.; Ouyang, Y. Y.; Liu, J.; Zhao, G.; Pan, A.; Hu, F. B. Red and Processed Meat Consumption and Mortality: Dose–Response Meta–Analysis of Prospective Cohort Studies. *Public Health Nutrition* **2015**, *19* (5), 893–905.
 11. Taillie, L. S.; Prestemon, C. E.; Hall, M. G.; Grummon, A. H.; Vesely, A.; Jaacks, L. M. Developing Health and Environmental Warning Messages about Red Meat: An Online Experiment. *PLoS ONE* **2022**, *17* (6).
 12. Catellani, P.; Carfora, V.; Piastra, M. Framing and Tailoring Prefactual Messages to Reduce Red Meat Consumption: Predicting Effects through a Psychology-Based Graphical Causal Model. *Frontiers in Psychology* **2022**, *13*.
 13. Szczybyło, A.; Halicka, E.; Rejman, K.; Kaczorowska, J. Is Eating Less Meat Possible? Exploring the Willingness to Reduce Meat Consumption among Millennials Working in Polish Cities. *Foods* **2022**, *11* (3), 358.
 14. Corpet, D. E. Red Meat and Colon Cancer: Should We Become Vegetarians, or Can We Make Meat Safer? *Meat Science* **2011**, *89* (3), 310–316.
 15. Tzoulaki, I.; Brown, I. J.; Chan, Q.; Van Horn, L.; Ueshima, H.; Zhao, L.; Stamler, J.; Elliott, P. Relation of Iron and Red Meat Intake to Blood Pressure: Cross Sectional Epidemiological Study. *BMJ* **2008**, *337* (jul15 1).
 16. Gerber, P. J. *Mitigation of greenhouse gas emissions in livestock production: A review of technical options for non-CO₂ emissions*; Food and Agriculture Organization of the United Nations: Rome, 2013.
 17. Park, G.-woo; Kim, J.-yung; Lee, M. H.; Yun, J. I.; Park, K.-H. Comparing Greenhouse Gas Emissions and Nutritional Values Based on Korean Suggested Meal Plans and Modified Vegan Meal Plans. *Journal of Animal Science and Technology* **2020**, *62* (1), 64–73.
 18. Heber, D.; Bowerman, S. Applying Science to Changing Dietary Patterns. *The Journal of Nutrition* **2001**, *131* (11).
 19. Boeing, H.; Bechthold, A.; Bub, A.; Ellinger, S.; Haller, D.; Kroke, A.; Leschik-Bonnet, E.; Müller, M. J.; Oberritter, H.; Schulze, M.; Stehle, P.; Watzl, B. Critical Review: Vegetables and Fruit in the Prevention of Chronic Diseases. *European Journal of Nutrition* **2012**, *51* (6), 637–663.
 20. Gibbs, J.; Cappuccio, F. P. Plant-Based Dietary Patterns for Human and Planetary Health. *Nutrients* **2022**, *14* (8), 1614.
 21. Pribis, P.; Pencak, R. C.; Grajales, T. Beliefs and Attitudes toward Vegetarian Lifestyle across Generations. *Nutrients* **2010**, *2* (5), 523–531.
 22. Jabs, J.; Devine, C. M.; Sobal, J. Model of the Process of Adopting Vegetarian Diets: Health Vegetarians and Ethical Vegetarians. *Journal of Nutrition Education* **1998**, *30* (4), 196–202.

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Machine Learning and Applications in Argumentation Mining

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ABSTRACT: Argumentation Mining (AM) is a field in Natural Language Processing that aims to extract arguments from text and determine their relationships. Current research has explored a multitude of application domains and a wide range of machine-learning models/methods used to support AM. However, there needs to be more synthesis of the existing literature and mapping of common correlations between machine learning and applications. Through a literature review spanning 52 papers, this paper finds that AM has been applied to user-generated texts, non-English texts, speeches/debates, legal texts, scientific/medical texts, and more, with user-generated texts (such as online comments and forum posts), being the most frequent application. Most papers surveyed use traditional machine learning. The most frequent methods/models used for AM are SVM, BERT, and BiLSTM. The most frequent association is between user-generated texts and SVM-based methods. Scientific/medical applications preferred BERT-based models, speech/debate used SVM-based methods, and non-English texts preferred BERT-based and LSTM-based models. Finally, the paper argues that future research should focus on researching less used applications such as scientific and medical text more and investigating how deep learning and traditional machine learning compare for user-generated texts and non-English texts.

KEYWORDS: Robotics and Intelligent Machines; Machine Learning; Natural Language Processing; Argumentation Mining.

■ Introduction

In a time where both informal and formal arguments and debates are present everywhere, from social media to scientific publications, argumentation mining, also referred to as argument mining, has become an increasingly expanding and necessary field. Argumentation mining is defined as “the automatic extraction of arguments from natural texts.”¹ It pertains to opinion mining or sentiment mining, which are utilized to understand the opinions of individuals on a subject.² Building upon this purpose, argumentation mining aims to extract arguments from text to understand why an opinion or viewpoint is held.²

The current research on argumentation mining for particular applications includes legal texts,³ user-generated texts⁴ (such as online comments and forum posts), and non-English text,⁵ along with many other domains. It uses various machine learning models and methods to perform this task. Much of the literature in argumentation mining uses traditional machine learning. Still, the development and progress in deep learning for natural language processing has caused deep learning, such as transformer-based models, to become more prominent in recent years. The current literature reviews that have been conducted on this topic explain the stages of argumentation mining, machine learning features used in existing research,² techniques for argument mining,⁶ and how argumentation mining systems can obtain “common sense and world knowledge” to fulfill their purposes.⁷ However, more work is needed to investigate the relationship between applications and machine learning used for argumentation mining, including the most frequent machine learning models or methods used for each application.

To address this gap, this paper aims to determine the current scope of argumentation mining applications, what different machine learning techniques are being applied to argumentation mining, and whether there are any salient patterns or correlations between machine learning and application domains. The review synthesizes 52 papers in the field. It organizes information about applications and machine learning from each paper surveyed to analyze the correlations. This research contributes to the field by enumerating the relationships between applications and machine learning and suggesting topics for future research based on the findings. In the future, researchers in the field could use the results of this literature review to select models, contribute to the field, or enhance their research.

■ Discussion

Background: Argumentation Mining and Machine Learning Components:

Argumentation mining requires selecting an argumentation model, a system for organizing and classifying argument components and structure. One way to analyze the organization of arguments in a text is by categorizing them as “abstract” or “structured.” Abstract argumentation models advance the idea that an argument has no internal structure, which can be useful in analyzing if an argument refutes another argument.² Conversely, structured argumentation models propose that an argument has an internal structure. While there is no universal definition for structured argumentation,² it focuses on the argument’s components, such as claims and premises, and the support or attack relations between argument components and individual arguments.⁸ Structured argumentation is typically used for argumentation mining.²

Another classification scheme for argumentation models is to organize them into micro-level (monological), macro-level (dialogical), or rhetorical models. Micro-level models, which are used the most in argumentation mining, pinpoint an individual argument's components and internal organization. The macro-level model focuses on the relations between arguments and their external structure.⁹ The rhetorical model emphasizes the rhetorical organization of texts based on the audience, and the "argument is evaluated by judgments rather than the truth of a proposition."⁹

The most prominent micro-level models include the simple premise/claim, Toulmin, and Freeman models. The simple premise/claim model splits an argument into three components: a conclusion, some premises, and a link between the premises and the conclusion.² The widely-used Toulmin model divides an argument into six components: a claim, data, warrant, backing, qualifier, and a rebuttal.⁴ The Freeman model, which builds on and modifies Toulmin's model, includes premise, conclusion, modality, rebuttal, and counter-rebuttal.¹⁰

Beyond classification components, a corpus of sample texts or arguments is needed to train a machine-learning method or model and implement an argumentation mining system. The corpus must then be annotated according to the selected argumentation model to provide training data for the machine learning method or model, including labeling the components of and relations between arguments.² Due to the different argumentation models used to determine the components and structure of the argument and the absence of a standardized guide or method to annotate documents, creating and comparing corpora and argumentation mining techniques in this field has been challenging.²

The process of argumentation mining can be sectioned into subtasks. Some papers divide this process into two parts: argument component extraction and argument relation prediction.^{9,11} Argument component extraction refers to the process of identifying arguments in the text. This process can be divided into a stage when the argument components are identified, and another for the "identification of their textual boundaries".¹¹ Argument relation prediction consists of predicting the relation between arguments in the text and between each of the arguments' claims and evidence. The relationships between the arguments or components can be classified as support, attack, or neutral. Other studies section argumentation mining into four subtasks, the first of which is classifying text as non-argumentative or argumentative.^{2,12} Typically, this is performed with a machine learning classifier.² The second subtask is to label each argument component as a claim, premise, etc., based on the argumentation model chosen. The third subtask is "determining which argumentation components are in a relationship," while classifying relationships as support or attack is the last subtask.²

Machine learning is an area of computer science that aims to use data and computer algorithms to emulate human learning.¹³ It can be classified into three categories: traditional machine learning, neural networks, and deep learning. Traditional machine learning includes machine learning techniques that have been used for many years, which is a basis for new

machine learning.¹⁴ Traditional machine learning comprises techniques such as clustering (grouping data), classification (predicting a class), and regression (predicting a number).¹⁴ Neural networks are a subset of machine learning that imitate the human brain with nodes modeled after neurons. There is one input layer of nodes, one output layer of nodes, and several hidden layers in between. The nodes are connected and have weights and thresholds. If the output of a node is above the threshold, then the node sends the data to the next layer.¹⁵ Once the hidden layers have produced outputs, these results are used by the output layer to obtain the final output.¹⁶ Deep learning is a subset of neural networks consisting of neural networks with more than three layers, including the input and output layers.¹⁶

Notable research in this field includes a literature review that describes argumentation mining from a machine learning perspective, including subtasks of computational argumentation mining, corpora, and challenges in the field.² Another notable application of argumentation mining was the IBM Debater, which competes with humans using information from newspaper articles and Wikipedia.¹⁷ Project Debater first breaks down the newspaper corpus into sentences and words and references the words on Wikipedia. Then, it uses neural and knowledge-based methods to rank each sentence by the probability that it represents an argument and classifies the relations between each argument and the topic. Another study developed the "first neural end-to-end solution for computational" argumentation mining.¹⁸ Finally, a noteworthy persuasive essays corpus consisting of 402 annotated persuasive essays was created in 2017 and has been used in many argumentation-mining research papers.¹⁹

■ Methodology

The papers reviewed were found through a search on Google Scholar for "argument mining machine learning" and "argumentation mining and machine learning." Papers that mentioned machine learning, deep learning, or neural networks, in addition to argumentation mining or argument mining, were selected. Of these, papers that tested the performance of at least one machine learning method or model in argumentation mining and papers that used machine learning and argumentation mining to analyze text were included in the literature review. Research that used machine learning in argumentation mining to investigate and make conclusions about other aspects of argumentation mining was also included. Research that used argumentation mining for non-text applications, such as images, was excluded from the review. Research that did not use machine learning was also excluded because this paper's focuses on machine learning in argumentation mining, so research using only rule-based argumentation mining, or argumentation mining without machine learning, and research in which the model used was unclear or not stated out of the scope of this paper. Other research on machine learning models and literature reviews on argumentation mining were included for background information on the field.

The current literature on argumentation mining spans the applications of user-generated texts, non-English texts, contr-

Contributions to argumentation mining, persuasive essays, speeches or debates, legal texts, scientific or medical texts, philosophical texts, and multiple applications. For each of the 52 papers reviewed, the application was determined by analyzing the title of the paper and the introduction and determining which application was the most prominent or the intended contribution. For example, Suhartono *et al.*²⁰ translate persuasive essays into Bahasa Indonesia for their research. The application of this paper was determined to be “Non-English Text,” as the title mentioned Bahasa Indonesia, implying that the article was focused on the effectiveness of argumentation mining in a non-English language. For papers that did not apply argumentation mining to a specific purpose but instead intended to improve or experiment with features, classifiers, or other aspects of argumentation mining, the application was determined by the training corpora used. If multiple types of training corpora were used or the training corpora encompassed more than one application, all the relevant applications were counted. However, papers that specifically attempted to use transfer learning or cross-domain argumentation mining and those that aimed to use multiple domains or make a context-independent system were counted as “Multiple Applications.” Papers that used the AraucariaDB corpus were marked as “Multiple Applications” as well due to the wide variety of sources and domains in this database. To determine the machine learning model or method used, all models or methods were counted if the paper used one or multiple machine learning models together in their approach or framework for argumentation mining. If the paper tested different models against each other, only the most successful model or method was counted.

Findings: Argumentation Mining Applications:

Each of the 52 papers found through Google Scholar was categorized into one or more of the following applications: User-Generated Text, Multiple Applications, Persuasive Essays, Speech/Debate, Non-English Text, Legal Text, Scientific/Medical Text, and Philosophical Text. Figure 1 displays the frequency of each application in the papers reviewed.

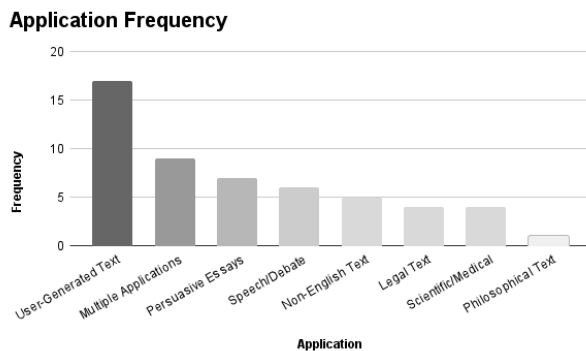


Figure 1: Frequency of argumentation mining applications in the papers reviewed.

Seventeen papers focus on identifying arguments in user-generated texts, which include comments, blog posts, articles, and forum posts taken from the Web. In Habernal and Gurevych's 2015 paper²¹, the authors apply argumentation mining to posts on debate portals, which are websites

that allow users to post on controversial topics. Subsequently, in 2017, Habernal and Gurevych used the Toulmin model to apply argumentation mining to a corpus of comments, discussion forums, blog posts, and articles.⁴ Passon *et al.*²² employ an off-the-shelf argumentation mining system to identify arguments in Amazon product reviews. Basile *et al.*²³ use the CorEA corpus from online comments on Italian news blogs to classify the comments as neutral, support, or attack. Park and Cardie created an annotated dataset of user comments from an eRulemaking website, where users discuss regulations from the government to apply argumentation mining with a machine learning algorithm and specific features.²⁴ Swanson *et al.*²⁵ combine the previously made Internet Argument Corpus with other data from an online debate network, which allows users to discuss different issues and viewpoints. The researchers then perform the argument extraction. Dusmanu *et al.*²⁶ perform argumentation mining on Twitter, while Chakrabarty *et al.*²⁷ use argumentation mining on the “change my view” subreddit. Tran and Litman²⁸ use the same data as Chakrabarty *et al.*²⁷ and attempt to improve the precision scores of Chakrabarty *et al.* Aker *et al.*¹ investigate which features and machine learning classifiers perform best in argumentation mining, and one of their corpora used to test the data was composed of Wikipedia articles. Sobhani *et al.*²⁹ perform stance classification on online news comments. Rocha *et al.*³⁰ create the ArgMine Framework, which aims to automate parts of argumentation mining and use a corpus made of Portuguese news articles. Rocha and Cardoso³¹ also use the same corpus for a relation-based implementation of argument mining, which identifies the relationship between premises and conclusions. Conceição *et al.*³² perform argumentation mining with data from online debates. Cocarascu and Toni³³ use argumentation mining to detect misleading product reviews. Sardanios *et al.*³⁴ identify argument components in Greek news articles. Lastly, Lytos *et al.* use data from Twitter to perform argument detection for short texts.

Nine papers use argumentation mining for multiple applications. Lippi and Torroni³⁵ created MARGOT, a web server that a more comprehensive community can use to perform argumentation mining. MARGOT was designed to perform argumentation mining on various files and topics for multiple applications. Lawrence and Reed⁶ combine different strategies for argumentation mining, including topical similarity, discourse indicators, and supervised machine learning. They use the Araucaria corpus, which encompasses data from multiple domains and applications. Schiller *et al.*³⁶ investigate how dataset sizes affect the performance of argumentation mining and discover that a large dataset is unnecessary if the suitable dataset composition and models are chosen. They use data from the UKP Corpus, which has diverse data sources. Wambsganss *et al.*³⁷ use Transfer Learning and multiple corpora to create a domain-independent implementation of argumentation mining. Schulz *et al.*³⁸ use multi-task learning for argumentation mining and data from multiple domains, including persuasive essays and news comments, to improve argumentation mining with smaller datasets. Rooney *et al.*³⁹ use kernel methods for argumentation mining and the AraucariaDB.

Furthermore, Bouslama *et al.*⁴⁰ test argumentation mining for cross-domain applications. Lippi and Torroni⁴¹ implement context-independent argumentation mining, which can detect claims without background information on the topic. Lastly, Mochales and Moens⁴² use the Araucaria corpus to investigate argumentation mining's applications and challenges and how machine learning can improve argumentation mining.

Seven papers use the application of persuasive essays. Sazid and Mercer¹² use machine learning methods to apply argumentation mining to the persuasive essays corpus created by Stab and Gurevyich.¹⁹ Kusmantini *et al.*⁴³ use another corpus of 90 persuasive essays to perform argumentation mining, while Wang *et al.*⁴⁴ execute a series of experiments on the persuasive essays corpus. Aker *et al.*¹ also use a corpus of persuasive essays, categorized into the persuasive essays application and user-generated texts. Using the persuasive essays corpus, Eger *et al.*¹⁸ build the first neural end-to-end solution for all argumentation mining subtasks. Persing and Ng⁴⁵ also use the persuasive essays corpus for unsupervised argumentation mining. Lastly, Wambsganss *et al.*⁴⁶ created an app using argumentation mining to give feedback on students' persuasive writing.

Six papers use argumentation mining in the field of speech or debate. Slonim *et al.* create an autonomous debating system that can compete with humans.¹⁷ They used a corpus of 400 million newspaper articles, broken into sentences and words, and related Wikipedia concepts. Then, the system uses neural and knowledge-based methods to rank the sentences according to whether they are likely to represent arguments and "classify the stance of each argument towards the motion".¹⁷ Lippi and Torroni further train a machine learning classifier on the 2015 UK political election debates to determine if claim detection in argument mining can be improved by using features from speech.⁴⁷ They build a pipeline such that audio is inputted into a speech recognition system. The feature extraction model processes it and produces the features needed for a machine learning model. They find that vocal cues increase the accuracy of argumentation mining. Menini *et al.*⁴⁸ use argumentation mining on speeches and declarations delivered during the 1960 presidential campaign in the United States. Tang uses argumentation mining to extract claims from TED talk subtitles.⁴⁹ Naderi and Hirst⁵⁰ test argumentation mining on debate speeches from the Canadian Parliament. Mestre *et al.*⁵¹ uses both text and audio from US political debates to perform argumentation mining.

Papers related to non-English texts often translated existing corpora from English to the intended language or created a new dataset to determine the efficacy of argumentation mining on the text of that language. As a note, only papers that specifically intended to study the application of argumentation mining in another language or had a contribution related to a non-English language were included in this category. Papers such as Rocha *et al.*³⁰, which performed argumentation mining on a Portuguese corpus of news articles, were not included because they emphasized another application.⁵ papers used argumentation mining for the non-English text application. Suhartono *et al.*²⁰ translate persuasive essays into Bahasa

Indonesian and use machine learning techniques to apply argumentation mining. Fishcheva *et al.*⁵ translate the persuasive essays corpus into Russian to assess the performance of specific machine learning models. Kumilachew *et al.*⁹ attempt to perform argument relation prediction, classifying arguments as support, attack, or neutral, on texts in the Amharic language. Toledo-Ronen *et al.*⁵² use a multilingual machine learning model to perform argumentation mining on English data translated into German, Dutch, Spanish, French, and Italian. Lastly, Eger *et al.*⁵³ 2018 translated persuasive essays into German, French, Spanish, and Chinese to implement cross-lingual argumentation mining.

Argumentation mining for legal texts is analyzed by Xu *et al.*,³ Poudyal,⁵⁴ Zhang *et al.*,⁸ and Lippi *et al.*⁵⁵ Xu *et al.*³ tests multiple machine learning models to identify argument components to create case summaries that may aid legal professionals. Using argumentation mining, Poudyal aims to classify sentences as argumentative or non-argumentative in legal texts.⁵⁴ Zhang *et al.*⁸ use machine learning models pre-trained with legal text to improve legal argument mining. Finally, Lippi *et al.*⁵⁵ create a new corpus and perform argumentation mining on European Court of Justice decisions.

Mayer *et al.*,¹¹ Accuosto *et al.*,⁵⁶ and Fergadis *et al.*,⁵⁷ and Binder *et al.*⁵⁸ investigate the application of argumentation mining in medical and scientific fields. Mayer *et al.*¹¹ use argumentation mining in clinical trials by using MARGOT to extract the components of arguments in clinical trials. They conclude that argumentation mining has potential in the medical field. Accuosto *et al.*⁵⁶ annotated abstracts of papers in computational linguistics and biomedicine to create a new corpus for argumentation mining and evaluate a machine learning model's performance. Lastly, Fergadis *et al.*⁵⁷ collect scientific literature on specific policy targets, annotate the data, and assess the dataset using argumentation mining and machine learning. Binder *et al.*⁵⁸ perform argument detection and argument relation on scientific publications.

Lawrence *et al.*⁵⁹ first manually analyzed data from 19th-century philosophical texts to train the machine learning model for argumentation mining. Then, they applied the automatic analysis with the machine learning model. They concluded that the automatic identification of some features was strong, but much more training data is needed.

Machine Learning Models/Methods Used for Argumentation Mining in the Literature:

Like the categorization of applications, each paper's machine learning models/methods were collected and categorized into one or more of the following categories: traditional machine learning, neural networks, and deep learning. Figure 2 shows the frequency of the type of machine learning used for argumentation mining in the papers reviewed. The machine learning methods and models used in these papers include SVM, BERT, SVM-HMM, Random Forest, BiLSTM, XGBoost, Naive Bayes, LSTM, RuBERT, HAN, CNN, FastText, SciBERT, Logistic Regression, and Ernie 2.0.

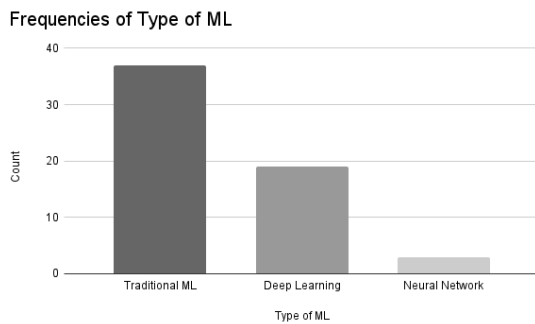


Figure 2: Frequency of type of ML used in the argumentation mining papers reviewed.

Figure 3 depicts the frequency of each model used or chosen as best in the reviewed papers

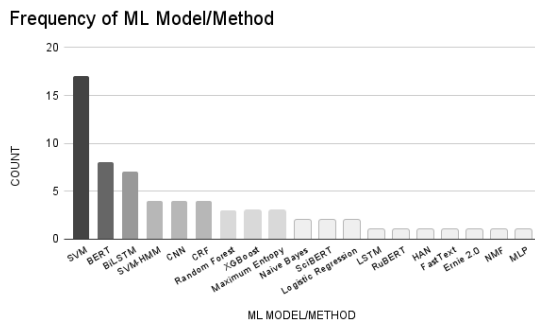


Figure 3: The frequency of machine learning models/methods used in the surveyed argumentation mining papers.

The traditional machine learning methods and models include SVM, SVM-HMM, CRF, Random Forest, XGBoost, Maximum Entropy Models, Naive Bayes, Logistic Regression, and NMF. Support Vector Machines (SVM) are supervised learning methods that can be used for classification and regression. SVM can also perform non-linear classification by using kernels, such as linear, polynomial, Sigmoid, and radial basis function (RBF) kernels.⁶⁰ SVM-HMM is a combination of Support Vector Machines and Hidden Markov Models. Conditional Random Field, or CRF, is a probabilistic method that has been implemented in many domains, such as natural language processing and bioinformatics.⁶¹ Random Forest is a model composed of multiple decision trees, and “the final result depends on majority vote.”³ XGBoost implements gradient boosting.⁵ A Maximum Entropy model is based on the maximum entropy principle: inferences should be made with “the probability distribution that has the maximum entropy” from the information possessed.⁴² A Naive Bayes model assumes that features are unrelated and is used for clustering and classification.⁶⁰ A Logistic Regression model is a linear model that uses a function to classify data.⁶² Non-Negative Matrix Factorization (NMF) is an unsupervised method and data decomposition method. It can increase the comprehensibility of the output data.⁶³

The neural network used by *Xu et al.* is FastText.³ It was created by Facebook workers in 2016 and is an extension of word2vec. FastText is an unsupervised model that creates vector representations for words.⁶⁴ Multi-layer Perceptron, used

by Lytos *et al.*,⁶⁵ is a type of Artificial Neural Network that transforms input to output with an activation function.⁶⁵

The deep machine learning models include BERT, BiLSTM, LSTM, RuBERT, HAN, CNN, SciBERT, and Ernie 2.0. BERT, Bidirectional Encoder Representations from Transformers, is based on the Transformer architecture and is pre-trained, then fine-tuned for a specific natural language processing task.⁵ RuBERT is a multilingual version of BERT pre-trained on Russian Wikipedia pages and news. At the same time, SciBERT is a BERT-based model pre-trained by Beltagy *et al.*⁶⁶ on 1.14 million papers in computer science and the biomedical field. LSTM (Long Short-Term Memory) is based on RNN (Recurrent Neural Network). It is said to be the best RNN and handles sequential data well.²⁰ BiLSTM is a bidirectional version of LSTM. HAN (Hierarchical Attention Network) performs well when the data is a hierarchy.²⁰ BERT allows users to solve tasks in a context-dependent way.⁵ CNN (Convolutional Neural Network) performs well in classification tasks and does not need sequential data.²⁰ Lastly, Ernie 2.0 is a pre-trained model that uses continual multi-task learning.⁶⁷

Temporal Analysis of Collected Research:

To further analyze argumentation mining applications and machine learning, the year of publication was collected for each paper to investigate how time has influenced applications and machine learning. Figure 4 shows the prevalence of application types in the collected research. Every year, an application was used in the 52 papers collected, and a dot was marked for that application. The lines in the figure show the range of years that the application was used.

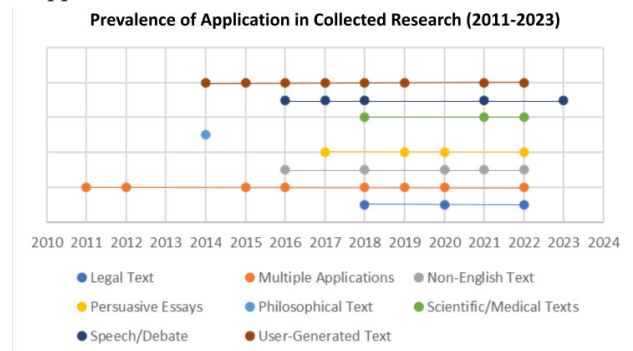


Figure 4: Prevalence of application in the collected research from 2011 to 2023.

Most of the applications were used in research from 2018 to 2022, possibly due to the expanding nature of the argumentation mining field. While applications such as scientific and medical texts and Legal texts seem relatively newer, User-Generated texts and multiple applications have been used for longer. However, most of the applications, except for philosophical texts, have been continued in recent research.

Figure 5 shows the prevalence of machine learning models and methods in the collected research. For every year that a model or method was used in the papers collected, a dot was marked for the application, and the lines through the dots portray the range of years that the model was used.

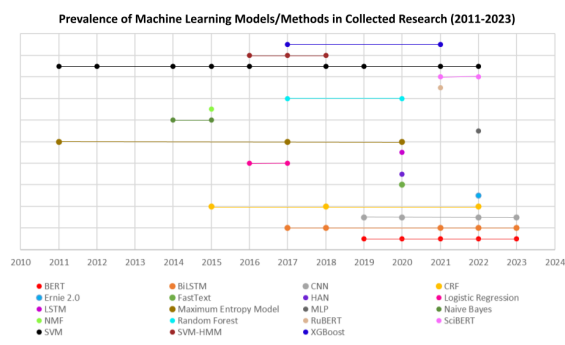


Figure 5: Prevalence of machine learning models and methods in the collected research from 2011 to 2023.

As the figure shows, models like BERT, BiLSTM, and CNN have become more prevalent in recent years, reflecting recent developments in deep learning. Specifically, transformer-based models, like BERT, have been used more over recent years, which suggests that as deep learning models improve, trends in the field of argumentation mining change. While deep learning models are prevalent throughout recent research, the graph demonstrates that the use of some traditional machine learning models such as Logistic Regression, Maximum Entropy, and Random Forest seem to have been used less in recent research. Although the use of deep learning models has increased over time and most of the machine learning techniques used in 2022 were deep learning, the data suggests that the traditional machine learning method SVM is still being used consistently, in contrast to other traditional machine learning models. This may be due to its past effectiveness in argumentation mining.

Correlations between Argumentation Mining Applications and Machine Learning Models/Methods:

To organize the information collected about application and machine learning, Figure 6 shows the relations between the various applications of argumentation mining and their use of neural networks, traditional machine learning, and deep learning. The thickness of each arrow in the diagram represents the number of papers that use the indicated type of machine learning for that application. Figure 7 shows the relations between the applications and the specific machine learning algorithms or models used.

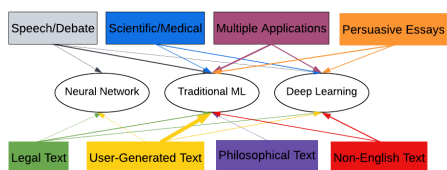


Figure 6: Relationships between the type of machine learning and argumentation mining applications.

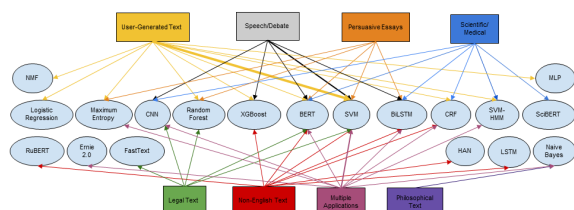


Figure 7: Relationships between machine learning models/methods and applications of argumentation mining.

For the application of debate, one paper uses neural networks, one paper uses deep learning, and four use traditional machine learning. Slonim used neural networks for Project Debater¹⁷, while Lippi and Torroni⁴⁷ used a traditional machine learning method, SVM, to perform argumentation mining on political debates. The paper did not mention the specific neural network model that Project Debater used. Mestre *et al.*⁵¹ use the deep learning models BERT, BiLSTM, and CNN. Menini *et al.*⁴⁸ use SVM, while Tang⁴⁹ utilizes XGBoost. Naderi and Hirst⁵⁰ use SVM, as well.

Of the four papers that performed argumentation mining on medical or scientific texts, two used traditional machine learning, and three used deep learning. Mayer *et al.*¹¹ used MARGOT. This was classified as using the traditional machine learning algorithm of SVM-HMM. Accuosto *et al.*⁵⁶ used BERT, while Fergadis *et al.*⁵⁷ utilized SciBERT and BiLSTM together. Binder *et al.*⁵⁸ used the deep learning models BiLSTM, SciBERT, and CNN and also used CRF.

Four papers analyzing persuasive essays used traditional machine learning, and the other three used deep learning. Kusmantini *et al.*⁴³ employed SVM. Aker *et al.*¹ used Random forest, Persing and Ng⁴⁵ used the Maximum Entropy model, and Wambsganns *et al.*⁴⁶ used SVM. Sazid and Mercer¹² used BiLSTM, and Wang *et al.*⁴⁴ chose BERT, and Eger *et al.*¹⁸ used BiLSTM.

Three papers from the non-English text application used traditional machine learning, while four used deep learning. Suhartono *et al.*²⁰ used LSTM and HAN, and Toledo-Ronen *et al.*⁵² used BERT, all deep learning models. SVM was used in Kumilachew *et al.*⁹ In Fishcheva *et al.*⁵ both traditional machine learning (SVM) and a deep learning model (RuBERT) were used. Eger *et al.*⁵³ use BiLSTM and CRF.

For legal text, one paper used a neural network, three used traditional machine learning, and two used deep learning. Xu *et al.*³ used CNN, Random Forest, and FastText, which include all three categories of traditional machine learning, deep learning, and neural networks. Poudyal⁵⁴ and Lippi *et al.*⁵⁵ use SVM, while Zhang *et al.*⁸ utilize the deep learning model BERT.

Fifteen user-generated text papers reviewed used traditional machine learning, but two used deep learning, and one used neural networks. In 2017, Habernal and Gurevych⁴ used SVM-HMM for user-generated texts; in 2015, they used SVM for debate portals.²¹ Passon *et al.*²² use MARGOT, which utilizes SVM-HMM, while Basile *et al.*²³ use SVM and Random Forest. Aker *et al.*¹ also use Random Forest. Park and Cardie²⁴, Rocha *et al.*³⁰, Conceição *et al.*³² and Swanson *et al.*²⁵ employ SVM. Dusmanu *et al.*²⁶ and Cocarascu and Toni³³ use logistic regression. Sobhani *et al.*²⁹ use NMF, Rocha, and Cardoso³¹ use the Maximum Entropy Model, and Sardianos *et al.*³⁴ use CRF. Chakrabarty *et al.*²⁷ and Tran and Litman²⁸ both use BERT, but Tran and Litman additionally utilize XGBoost. Lastly, Lytos *et al.*⁶⁵ use MLP.

The paper applying argumentation mining to philosophical texts, created by Lawrence *et al.*⁵⁹ used Naive Bayes, a traditional machine learning model.

For the papers that used argumentation mining for multiple applications, six used traditional machine learning, and four

used deep learning. Lawrence and Reed⁶ use Naïve Bayes, Lippi, and Torroni³⁵ use SVM-HMM, Rooney *et al.*³⁹ use SVM, and Lippi and Torroni⁴¹ use SVM. Mochales and Moens⁴² use SVM and the Maximum Entropy Model. Schiller *et al.*³⁶ use Ernie 2.0, Wambsgans *et al.*³⁷ use BERT, and Bouslama *et al.*⁴⁰ use CNN. Schulz *et al.*³⁸ use BiLSTM and CRF.

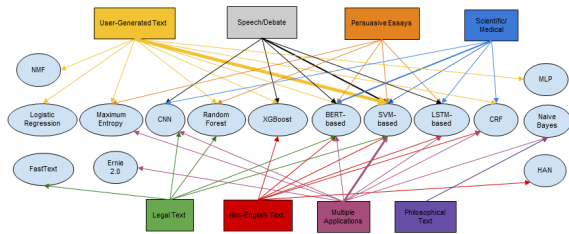


Figure 8: Simplified relationship chart of machine learning models/methods and argumentation mining applications.

Figure 8 is a simplified version of Figure 7. SVM and SVM-HMM were combined into the category of SVM-based methods. BERT, SciBERT, and RuBERT have also been incorporated into the category of BERT-based models. Similarly, LSTM and BiLSTM combined to form LSTM-based models. As a result, the user-generated text application used SVM-based methods eight times and a BERT-based model twice. The non-English text application used a BERT-based model twice, an LSTM-based model twice, and an SVM-based method once. The Scientific/Medical application used a BERT-based model thrice, an SVM-based method once, and an LSTM-based model twice. Multiple applications used an SVM-based method four times, an LSTM-based model once, and a BERT-based model once. Persuasive Essays had one use of the BERT-based models, two uses of LSTM-based models, and two uses of SVM-based methods. The legal text had one BERT-based model and two SVM-based methods. The application of speech and debate had one BERT-based model, three SVM-based methods, and one LSTM-based model.

Synthesis, Future Work, and Limitations:

As the results from the information collected show, user-generated text was the most frequent application for argumentation mining, with seventeen of the surveyed papers. Many researchers applied argumentation mining to online forums, articles, and comments from social media sites. Furthermore, several data and corpora have been collected and annotated in this field. The interest in argumentation mining for user-generated texts may be prevalent because platforms such as social media and Web forums provide an insightful opportunity to attain information about the public's viewpoint using argumentation mining and using argumentation mining to determine not only what but how large numbers of people on the Internet think can have many possible benefits, such as determining the political views of a large group or improving businesses and products based on online reviews. The second most frequent application was multiple applications, and the third was persuasive essays. There may be more research on multiple applications and cross-domain argumentation mining due to the potential for broader argumentation mining systems that can detect arguments in texts of multiple different

domains. The focus on multiple applications is important and justified because such cross-domain implementations could reduce the need for different argumentation mining systems for different applications and could have lasting effects on how argumentation mining is applied. Furthermore, the application of persuasive essays may have been popular due to the argumentative nature of such essays, and the Persuasive Essays corpus may have facilitated the research process.

The least common applications include philosophical texts, scientific and medical texts, and legal texts. The lack of research on literary or philosophical texts may be because many of these texts may need to provide a clear argument or take too long to annotate. Yet, there is more potential for research in scientific texts because detecting arguments could help summarize papers and simplify research for a more general audience. Compared to other applications, the research in this domain is not sufficient. Because of the relative lack of research in this application compared to others, there is an opening in the field to further test and improve argumentation mining for scientific or medical texts.

SVM, a traditional machine learning method, was the most frequent method used throughout all applications, followed by the deep learning model BERT and then by BiLSTM. Overall, SVM-based models were the most popular in argumentation mining and were also used, as shown in the temporal analysis, throughout many years. BERT may have also been widely used because of its previous efficacy in some natural language processing tasks⁶⁸ and because it can perform well with less annotated data.⁸ Other BERT-based models, like SciBERT and RuBERT, which are pre-trained on data for a specific domain, can also perform well in that specific domain. Further usage of BERT-based models and other transformer-based models can improve results in multiple domains while also reducing the amount of data and annotation necessary. SVM may have surpassed other deep learning models overall due to its consistent use throughout the years. Still, there has been an overall shift to deep learning in argumentation mining, as evidenced by the temporal graphs (Figure 5). Despite this shift, future research should still experiment with traditional machine learning methods that have succeeded in this field, such as SVM. It should evaluate the training, implementation, and results differences between traditional machine learning and deep learning for argumentation mining.

The strongest correlation between applications and machine learning models was between user-generated texts and SVM-based methods. This could indicate that SVM works particularly well for this application. Future research in user-generated text applications may still want to continue to use SVM due to its proven effectiveness from past research. However, the difference between the number of papers using traditional machine learning and the number using deep learning for user-generated texts is large; further work is necessary to investigate if deep learning is a good fit for user-generated texts. Speech and debate, legal text, and multiple applications all also preferred SVM-based methods.

On the other hand, scientific and medical texts preferred BERT-based models, which is unsurprising because it is a

more recent application. Non-English text preferred BERT-based models and LSTM-based models. Along with scientific and medical text, this application was one of the only ones not to prefer an SVM-based model. Non-English text's preference for BERT and LSTM-based models is worth further research, considering that it is one of the older applications.

Furthermore, more popular applications, such as user-generated texts and multiple applications, had a wide spread of machine learning models or methods used in the research. In contrast, applications such as speech and debate or scientific or medical texts used fewer models overall. This reflects the results from the application graph (Figure 1) and reveals a correlation between the number of papers that used an application and how large the spread of tested machine learning models or methods for that application was. This reinforces the idea that further research in less-used applications is necessary because it allows researchers to experiment with more machine learning models and introduce findings that can inform further investigation for that application. Because of this, future research can focus on using domains such as scientific and medical text, philosophical text, and legal text while also broadening the types and amounts of machine learning models or methods used for these applications.

The limitations of this study include that only 52 papers were included in the diagrams and figures. Future research could expand on this paper's findings by analyzing and adding more papers to the data collected in this literature review.

Conclusion

There have been many applications of argumentation mining in recent years, with researchers investigating its role in analyzing user-generated texts, non-English texts, contributions to the process of argumentation mining, persuasive essays, speeches or debates, legal texts, scientific or medical texts, philosophical texts, and systems for the general public's use. The most commonly used models are SVM, BERT, and BiLSTM, and the current literature has tested traditional machine learning more than deep learning. The main contribution of this paper was to determine any relationships between applications and machine learning models. The most evident relations are that studies on user-generated texts strongly tend to use SVM-based methods, while research on medical/scientific texts used BERT-based models. The application of speech/debate also tended to lean towards SVM-based methods, while non-English texts preferred BERT-based and LSTM-based models.

This literature review implies that future researchers may want to focus on researching less used applications such as scientific and medical text more while broadening the range of machine learning methods used for these applications. Furthermore, the field of argumentation mining could benefit immensely from continued research on transformer-based models like BERT. Still, argumentation mining systems may also want to continue to use SVM-based methods due to their proven effectiveness from past research. Finally, future research could analyze how deep learning and traditional machine learning compare for user-generated texts since most of the research collected on this application was for traditional

machine learning and non-English texts since it favors deep learning despite being used earlier.

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References

1. Aker, A.; Sliwa, A.; Ma, Y.; Lui, R.; Borad, N.; Ziyaci, S.; Ghobadi, M. What Works and What Does Not: Classifier and Feature Analysis for Argument Mining. *In Proceedings of the 4th Workshop on Argument Mining*; Association for Computational Linguistics: Copenhagen, Denmark, 2017; pp 91–96. <https://doi.org/10.18653/v1/W17-5112>.
2. Lippi, M.; Torroni, P. Argument Mining: A Machine Learning Perspective. *In Theory and Applications of Formal Argumentation*; Blazek, E., Modgil, S., Oren, N., Eds.; Lecture Notes in Computer Science; Springer International Publishing: Cham, 2015; Vol. 9524, pp 163–176. https://doi.org/10.1007/978-3-319-28460-6_10.
3. Xu, H.; Šavelka, J.; Ashley, K. D. Using Argument Mining for Legal Text Summarization. *In Frontiers in Artificial Intelligence and Applications*; Villata, S., Harašta, J., Krěmen, P., Eds.; IOS Press, 2020. <https://doi.org/10.3233/FAIA200862>.
4. Habernal, I.; Gurevych, I. Argumentation Mining in User-Generated Web Discourse. *Computational Linguistics* **2017**, *43* (1), 125–179. https://doi.org/10.1162/COLI_a_00276.
5. Fishcheva, I. N.; Goloviznina, V. S.; Kotelnikov, E. V. Traditional Machine Learning and Deep Learning Models for Argumentation Mining in Russian Texts.
6. Lawrence, J.; Reed, C. Combining Argument Mining Techniques. *In Proceedings of the 2nd Workshop on Argumentation Mining*; Association for Computational Linguistics: Denver, CO, 2015; pp 127–136. <https://doi.org/10.3115/v1/W15-0516>.
7. Moens, M.-F. Argumentation Mining: How Can a Machine Acquire Common Sense and World Knowledge? *AAC* **2018**, *9* (1), 1–14. <https://doi.org/10.3233/AAC-170025>.
8. Zhang, G.; Nulty, P.; Lillis, D. Enhancing Legal Argument Mining with Domain Pre-Training and Neural Networks. *Journal of Data Mining & Digital Humanities* **2022**, NLP4DH, 9147. <https://doi.org/10.46298/jdmhdh.9147>.
9. Kumilachew, A.; Lake, M.; Tesfaye, D. Argument Mining from Amharic Argumentative Texts Using Machine Learning Approach.
10. Wang, J. On Freeman's Argument Structure Approach.
11. Mayer, T.; Cabrio, E.; Lippi, M.; Torroni, P.; Villata, S. Argument Mining on Clinical Trials. **2018**.
12. Sazid, M. T.; Mercer, R. E. A Unified Representation and Deep Learning Architecture for Argumentation Mining of Students' Persuasive Essays.
13. IBM. *What is Machine Learning?* <https://www.ibm.com/topics/machine-learning>.
14. Stellwall, M. *Overview of Traditional Machine Learning Techniques*. Oracle AI & Data Science Blog. <https://blogs.oracle.com/ai-and-datascience/post/overview-of-traditional-machine-learning-techniques>.
15. IBM. *What are neural networks?* <https://www.ibm.com/topics/neural-networks>.
16. Kavlakoglu, E. AI vs. Machine Learning vs. Deep Learning vs. Neural Networks: What's the Difference? IBM. <https://www.ibm.com/cloud/blog/ai-vs-machine-learning-vs-deep-learning-vs-neural-networks>.
17. Slonim, N.; Bilu, Y.; Alzate, C.; Bar-Haim, R.; Bogin, B.; Bonin, F.; Choshen, L.; Cohen-Karlik, E.; Dankin, L.; Edelstein, L.; Ein-Dor, L.; Friedman-Melamed, R.; Gavron, A.; Gera, A.; Gleize,

- M.; Gretz, S.; Gutfreund, D.; Halfon, A.; Hershovich, D.; Hoory, R.; Hou, Y.; Hummel, S.; Jacovi, M.; Jochim, C.; Kantor, Y.; Katz, Y.; Konopnicki, D.; Kons, Z.; Kotlerman, L.; Krieger, D.; Lahav, D.; Lavee, T.; Levy, R.; Liberman, N.; Mass, Y.; Menczel, A.; Mirkin, S.; Moshkovich, G.; Ofek-Koifman, S.; Orbach, M.; Rabino vich, E.; Rinott, R.; Shechtman, S.; Sheinwald, D.; Shnarch, E.; Shnayderman, I.; Soffer, A.; Spector, A.; Sznajder, B.; Toledo, A.; Toledo-Ronen, O.; Venezian, E.; Aharonov, R. An Autonomous Debating System. *Nature* **2021**, 591 (7850), 379–384. <https://doi.org/10.1038/s41586-021-03215-w>.
18. Eger, S.; Daxenberger, J.; Gurevych, I. Neural End-to-End Learning for Computational Argumentation Mining. In *Proceedings of the 55th Annual Meeting of the Association for Computational Linguistics (Volume 1: Long Papers)*; Association for Computational Linguistics: Vancouver, Canada, 2017; pp 11–22. <https://doi.org/10.18653/v1/P17-1002>.
19. Stab, C.; Gurevych, I. Parsing Argumentation Structures in Persuasive Essays. *Computational Linguistics* **2017**, 43 (3), 619–659. https://doi.org/10.1162/COLI_a_00295.
20. Suhartono, D.; Gema, A. P.; Winton, S.; David, T.; Fanany, M. I.; Arymurthy, A. M. Argument Annotation and Analysis Using Deep Learning with Attention Mechanism in Bahasa Indonesia. *J Big Data* **2020**, 7 (1), 90. <https://doi.org/10.1186/s40537-020-00364-z>.
21. Habernal, I.; Gurevych, I. Exploiting Debate Portals for Semi-Supervised Argumentation Mining in User-Generated Web Discourse. In *Proceedings of the 2015 Conference on Empirical Methods in Natural Language Processing*; Association for Computational Linguistics: Lisbon, Portugal, 2015; pp 2127–2137. <https://doi.org/10.18653/v1/D15-1255>.
22. Passon, M.; Lippi, M.; Serra, G.; Tasso, C. Predicting the Usefulness of Amazon Reviews Using Off-The-Shelf Argumentation Mining. In *Proceedings of the 5th Workshop on Argument Mining*; Association for Computational Linguistics: Brussels, Belgium, 2018; pp 35–39. <https://doi.org/10.18653/v1/W18-5205>.
23. Basile, P.; Basile, V.; Cabrio, E.; Villata, S. Argument Mining on Italian News Blogs. In *Proceedings of the Third Italian Conference on Computational Linguistics CLiC-it 2016*; Corazza, A., Montemagni, S., Semeraro, G., Eds.; Accademia University Press, 2016. <https://doi.org/10.4000/books.aaccademia.1706>.
24. Park, J.; Cardie, C. Identifying Appropriate Support for Propositions in Online User Comments. In *Proceedings of the First Workshop on Argumentation Mining*; Association for Computational Linguistics: Baltimore, Maryland, 2014; pp 29–38. <https://doi.org/10.3115/v1/W14-2105>.
25. Swanson, R.; Ecker, B.; Walker, M. Argument Mining: Extracting Arguments from Online Dialogue. In *Proceedings of the 16th Annual Meeting of the Special Interest Group on Discourse and Dialogue*; Association for Computational Linguistics: Prague, Czech Republic, 2015; pp 217–226. <https://doi.org/10.18653/v1/W15-4631>.
26. Dusmanu, M.; Cabrio, E.; Villata, S. Argument Mining on Twitter: Arguments, Facts and Sources. In *Proceedings of the 2017 Conference on Empirical Methods in Natural Language Processing*; Association for Computational Linguistics: Copenhagen, Denmark, 2017; pp 2317–2322. <https://doi.org/10.18653/v1/D17-1245>.
27. Chakrabarty, T.; Hidey, C.; Muresan, S.; McKeown, K.; Hwang, A. AMPERSAND: Argument Mining for PERSuasive ONline Discussions. In *Proceedings of the 2019 Conference on Empirical Methods in Natural Language Processing and the 9th International Joint Conference on Natural Language Processing (EMNLP-IJCNLP)*; Association for Computational Linguistics: Hong Kong, China, 2019; pp 2933–2943. <https://doi.org/10.18653/v1/D19-1291>.
28. Tran, N.; Litman, D. Multi-Task Learning in Argument Mining for Persuasive Online Discussions. In *Proceedings of the 8th Workshop on Argument Mining*; Association for Computational Linguistics: Punta Cana, Dominican Republic, 2021; pp 148–153. <https://doi.org/10.18653/v1/2021.argmining-1.15>.
29. Sobhani, P.; Inkpen, D.; Matwin, S. From Argumentation Mining to Stance Classification. In *Proceedings of the 2nd Workshop on Argumentation Mining*; Association for Computational Linguistics: Denver, CO, 2015; pp 67–77. <https://doi.org/10.3115/v1/W15-0509>.
30. Rocha, G.; Teixeira, J.; Lopez Cardoso, H. ArgMine: A Framework for Argumentation Mining; 2016.
31. Rocha, G.; Lopes Cardoso, H. Towards a Relation-Based Argument Extraction Model for Argumentation Mining. In *Statistical Language and Speech Processing*; Camelin, N., Estève, Y., Martín-Vide, C., Eds.; Lecture Notes in Computer Science; Springer International Publishing: Cham, 2017; Vol. 10583, pp 94–105. https://doi.org/10.1007/978-3-319-68456-7_8.
32. Conceição, L.; Carneiro, J.; Marreiros, G.; Novais, P. Applying Machine Learning Classifiers in Argumentation Context. In *Distributed Computing and Artificial Intelligence, 17th International Conference*; Dong, Y., Herrera-Viedma, E., Matsui, K., Omatsu, S., González Briones, A., Rodríguez González, S., Eds.; Advances in Intelligent Systems and Computing; Springer International Publishing: Cham, 2021; Vol. 1237, pp 314–320. https://doi.org/10.1007/978-3-030-53036-5_34.
33. Cocarascu, O.; Toni, F. Detecting Deceptive Reviews Using Argumentation. In *Proceedings of the 1st International Workshop on AI for Privacy and Security*; ACM: The Hague Netherlands, 2016; pp 1–8. <https://doi.org/10.1145/2970030.2970031>.
34. Sardianos, C.; Katakis, I. M.; Petasis, G.; Karkaletsis, V. Argument Extraction from News. In *Proceedings of the 2nd Workshop on Argumentation Mining*; Association for Computational Linguistics: Denver, CO, 2015; pp 56–66. <https://doi.org/10.3115/v1/W15-0508>.
35. Lippi, M.; Torroni, P. MARGOT: A Web Server for Argumentation Mining. *Expert Systems with Applications* **2016**, 65, 292–303. <https://doi.org/10.1016/j.eswa.2016.08.050>.
36. Schiller, B.; Daxenberger, J.; Gurevych, I. On the Effect of Sample and Topic Sizes for Argument Mining Datasets.
37. University of St.Gallen (HSG), Institute of Information Management, St.Gallen, Switzerland; Wambsgans, T.; Molyndris, N.; Söllner, M.; University of Kassel, Information Systems and Systems Engineering, Kassel, Germany. Unlocking Transfer Learning in Argumentation Mining: A Domain-Independent Modelling Approach. In *WI2020 Zentrale Tracks*; GITO Verlag, 2020; pp 341–356. https://doi.org/10.30844/wi_2020_c9-wambsgans.
38. Schulz, C.; Eger, S.; Daxenberger, J.; Kahse, T.; Gurevych, I. Multi-Task Learning for Argumentation Mining in Low-Resource Settings. arXiv May 4, 2018. <http://arxiv.org/abs/1804.04083> (accessed 2023-08-22).
39. Rooney, N.; Wang, H.; Browne, F. Applying Kernel Methods to Argumentation Mining; The Florida AI Research Society, 2012.
40. Bouslama, R.; Ayachi, R.; Amor, N. B. Using Convolutional Neural Network in Cross-Domain Argumentation Mining Framework. In *Scalable Uncertainty Management*; Ben Amor, N., Quost, B., Theobald, M., Eds.; Lecture Notes in Computer Science; Springer International Publishing: Cham, 2019; Vol. 11940, pp 355–367. https://doi.org/10.1007/978-3-030-35514-2_26.
41. Lippi, M.; Torroni, P. Context-Independent Claim Detection for Argument Mining; 2015.
42. Mochales, R.; Moens, M.-F. Argumentation Mining. *Artif Intell Law* **2011**, 19 (1), 1–22. <https://doi.org/10.1007/s10506-010-9104-x>.
43. Kusmantini, H. A.; Asror, I.; Bijaksana, M. A. Argumentation Mining: Classifying Argumentation Components with Partial Tree

- Kernel and Support Vector Machine for Constituent Trees on Imbalanced Persuasive Essay. *J. Phys.: Conf. Ser.* **2019**, *1192*, 012009. <https://doi.org/10.1088/1742-6596/1192/1/012009>.
44. Wang, H.; Huang, Z.; Dou, Y.; Hong, Y. Argumentation Mining on Essays at Multi Scales. In *Proceedings of the 28th International Conference on Computational Linguistics*; International Committee on Computational Linguistics: Barcelona, Spain (Online), 2020; pp 5480–5493. <https://doi.org/10.18653/v1/2020.coling-main.478>.
45. Persing, I.; Ng, V. Unsupervised Argumentation Mining in Student Essays. In *Proceedings of the Twelfth Language Resources and Evaluation Conference*; European Language Resources Association, 2020.
46. Wambsganss, T.; Caines, A.; Buttery, P. ALLEN App: Argumentative Writing Support To Foster English Language Learning. In *Proceedings of the 17th Workshop on Innovative Use of NLP for Building Educational Applications (BEA 2022)*; Association for Computational Linguistics: Seattle, Washington, 2022; pp 134–140. <https://doi.org/10.18653/v1/2022.bea-1.18>.
47. Lippi, M.; Torroni, P. Argument Mining from Speech: Detecting Claims in Political Debates. *AAAI* **2016**, *30* (1). <https://doi.org/10.1609/aaai.v30i1.10384>.
48. Menini, S.; Cabrio, E.; Tonelli, S.; Villata, S. Never Retreat, Never Retract: Argumentation Analysis for Political Speeches. *AAAI* **2018**, *32* (1). <https://doi.org/10.1609/aaai.v32i1.11920>.
49. Tang, B. Adopting Argumentation Mining for Claim Extraction from TED Talks.
50. Naderi, N.; Hirst, G. Argumentation Mining in Parliamentary Discourse. In *Principles and Practice of Multi-Agent Systems*; Baldoni, M., Baroglio, C., Bex, F., Grasso, F., Green, N., Namazi-Rad, M.-R., Numao, M., Suarez, M. T., Eds.; Lecture Notes in Computer Science; Springer International Publishing: Cham, 2016; Vol. 9935, pp 16–25. https://doi.org/10.1007/978-3-319-46218-9_2.
51. Mestre, R.; Middleton, S. E.; Ryan, M.; Gheasi, M.; Norman, T.; Zhu, J. Augmenting Pre-Trained Language Models with Audio Feature Embedding for Argumentation Mining in Political Debates; Association for Computational Linguistics, 2023; pp 274–288.
52. Toledo-Ronen, O.; Orbach, M.; Bilu, Y.; Spector, A.; Slonim, N. Multilingual Argument Mining: Datasets and Analysis. In *Findings of the Association for Computational Linguistics: EMNLP 2020*; Association for Computational Linguistics: Online, 2020; pp 303–317. <https://doi.org/10.18653/v1/2020.findings-emnlp.29>.
53. Eger, S.; Daxenberger, J.; Stab, C.; Gurevych, I. Cross-Lingual Argumentation Mining: Machine Translation (and a Bit of Projection) Is All You Need! arXiv July 24, 2018. <http://arxiv.org/abs/1807.08998> (accessed 2023-08-22).
54. Poudyal, P. A Machine Learning Approach to Argument Mining in Legal Documents. In *AI Approaches to the Complexity of Legal Systems*; Pagallo, U., Palmirani, M., Casanovas, P., Sartor, G., Villata, S., Eds.; Lecture Notes in Computer Science; Springer International Publishing: Cham, 2018; Vol. 10791, pp 443–450. https://doi.org/10.1007/978-3-030-00178-0_30.
55. Lippi, M.; Lagioia, F.; Contissa, G.; Sartor, G.; Torroni, P. Claim Detection in Judgments of the EU Court of Justice. In *AI Approaches to the Complexity of Legal Systems*; Pagallo, U., Palmirani, M., Casanovas, P., Sartor, G., Villata, S., Eds.; Lecture Notes in Computer Science; Springer International Publishing: Cham, 2018; Vol. 10791, pp 513–527. https://doi.org/10.1007/978-3-030-00178-0_35.
56. Accuosto, P.; Neves, M.; Saggion, H. Argumentation Mining in Scientific Literature: From Computational Linguistics to Biomedicine. **2021**.
57. Fergadis, A.; Pappas, D.; Karamolegkou, A.; Papageorgiou, H. Argumentation Mining in Scientific Literature for Sustainable Development. In *Proceedings of the 8th Workshop on Argument Mining*; Association for Computational Linguistics: Punta Cana, Dominican Republic, 2021; pp 100–111. <https://doi.org/10.18653/v1/2021.argmining-1.10>.
58. Binder, A.; Verma, B.; Hennig, L. Full-Text Argumentation Mining on Scientific Publications. arXiv October 24, 2022. <http://arxiv.org/abs/2210.13084> (accessed 2023-08-22).
59. Lawrence, J.; Reed, C.; Allen, C.; McAlister, S.; Ravenscroft, A. Mining Arguments From 19th Century Philosophical Texts Using Topic Based Modelling. In *Proceedings of the First Workshop on Argumentation Mining*; Association for Computational Linguistics: Baltimore, Maryland, 2014; pp 79–87. <https://doi.org/10.3115/v1/W14-2111>.
60. Mahesh, B. Machine Learning Algorithms - A Review. **2018**, *9* (1).
61. Sutton, C.; McCallum, A. An Introduction to Conditional Random Fields. arXiv November 17, 2010. <http://arxiv.org/abs/1011.4088> (accessed 2023-08-23).
62. Qu, Y.; Quan, P.; Lei, M.; Shi, Y. Review of Bankruptcy Prediction Using Machine Learning and Deep Learning Techniques. *Procedia Computer Science* **2019**, *162*, 895–899. <https://doi.org/10.1016/j.procs.2019.12.065>.
63. Hafshejani, S. F.; Moaberfard, Z. Initialization for Nonnegative Matrix Factorization: A Comprehensive Review. *Int J Data Sci Anal* **2023**, *16* (1), 119–134. <https://doi.org/10.1007/s41060-022-00370-9>.
64. Alharbi, N. M.; Alghamdi, N. S.; Alkhamash, E. H.; Al Amri, J. F. Evaluation of Sentiment Analysis via Word Embedding and RNN Variants for Amazon Online Reviews. *Mathematical Problems in Engineering* **2021**, *2021*, 1–10. <https://doi.org/10.1155/2055/2021/5536560>.
65. Lytos, A.; Lagkas, T.; Sarigiannidis, P.; Argyriou, V.; Eleftherakis, G. Modelling Argumentation in Short Text: A Case of Social Media Debate. *Simulation Modelling Practice and Theory* **2022**, *115*, 102446. <https://doi.org/10.1016/j.simpat.2021.102446>.
66. Beltagy, I.; Lo, K.; Cohan, A. SciBERT: A Pretrained Language Model for Scientific Text. In *Proceedings of the 2019 Conference on Empirical Methods in Natural Language Processing and the 9th International Joint Conference on Natural Language Processing (EMNLP-IJCNLP)*; Association for Computational Linguistics: Hong Kong, China, 2019; pp 3613–3618. <https://doi.org/10.18653/v1/D19-1371>.
67. Sun, Y.; Wang, S.; Li, Y.; Feng, S.; Tian, H.; Wu, H.; Wang, H. ERNIE 2.0: A Continual Pre-Training Framework for Language Understanding. *AAAI* **2020**, *34* (05), 8968–8975. <https://doi.org/10.1609/aaai.v34i05.6428>.
68. Gonzalez-Carvajal, S.; Garrido-Merchan, E. C. Comparing BERT against Traditional Machine Learning Text Classification.

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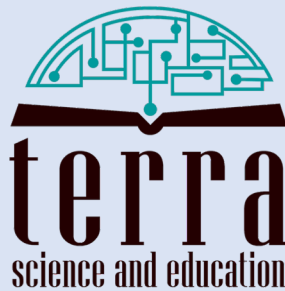
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