Platelets

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1. Introduction

General information about platelets, origin of plateletes and granule contents of platelets were summarized.

2. Platelets

These cell fragments are morphologically small scale but functionally vital under life threatening conditions (1). They originate from megakaryocytes located mainly in the bone marrow, found in circulating blood and stored in spleen (2). Platelets don't contain a nuclei and during their inactive state they have a discoid morphology with a diameter of 2-4 micrometer (3, 4). But whenever they are active they can change their morphology very rapidly to an irregular branched spread form (5). Currently platelets are being used at wide spread clinic treatments from cosmetic needs to supporting insufficient heart (6, 7).

2.1. Development of Platelets

It is not exactly explained how platelets originate from megakaryocytes. There are several models to explain formation of platelets.

Megakaryocytes seem to locate as triple form. With their VEGF secretion capacity they hold vessel endothelial cells close to themselves (8). The most scientifically accepted three models are mentioned as,

1. Simply blebbing from the cell membrane of megakaryocytes (1).
2. In megakaryocytes there are special cell fields defined as "Demarcation Membrane System" where granules of platelets condense and fragments break away (9).
3. The most popular theory seems to be "Proplatelet Formation". Here megakaryocytes have long thin branch like extensions at the blood circulating site of blood vessels of

bone marrow and on these branches there are uprising small bodies where by the help of blood shear force platelets enter directly to circulating blood stream. It was suggested that the concept of platelet like bodies arise from pseudopods of Megakaryocytes, the forming platelets were named as "proplatelet" (10).

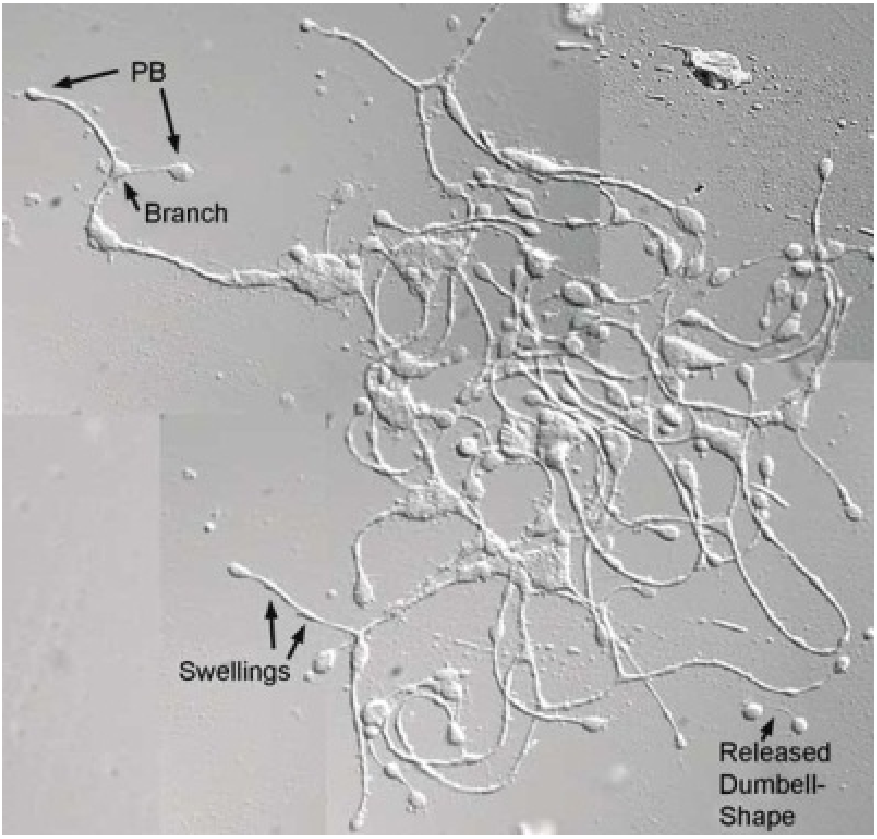

Figure 1. Megakaryocyte branches with Platelet Buds (PB) are seen. Proplatelets are released as Dumbell shaped bodies. This image is referenced from Hartwig and Italiano 2003 (Thanks for the kind permission of John Wiley and Sons to use this image) (11) .

Kinetics of platelets; they have a life span as 7-10 days and in 1 liter human blood it is estimated that there are 150-400X10^9 platelets so for a balanced number they are formed 15X10^9 -40X10^9 daily. Megakaryocytes located in the bone marrow sinusoids form a barrier to other bone marrow cells, it forms a physical barrier preventing direct contact to blood circulation. But there are canallicular openings in megakaryocyte membrane which permits cell migration to other cells to enter blood stream; this is named as "Emperipolesis" (8).

These small cell fragments have complex properties; 2 cytoplasmic regions can be seen in platelets

1. **Hyalomere:** The light blue homogeneous region of the peripheral cytoplasm is called Hyalomere. Hyalomere includes cytoplasmic filaments and circumferential microtubule bundle under the cell membrane. These elements of the cytoskeleton provide the movement and the protection of the platelets' shapes.
2. **Granulomere (Chromomere):** This is the central region and tight area. It is ranging in color from blue to purple-staining. Granulomere includes small Golgi complex, smooth endoplasmic reticulum, lysosome, scattered granules surrounded by a membrane and a variety of mitochondria (4).

Platelets have a simple appearance but carry very complex functional properties. By dividing this simple cell fragment to four regions helps for a better understanding of the functions of platelets.

1. Peripheral Zone:
   This region is composed from unit membrane with open canalicular system. Three parts are defined as;

a. Exterior outer layer:
   This is a glycocalix membrane with 10-20 nm thickness and thicker than the other blood cells, rich from glycoproteins that are mainly receptors for cell-cell and cell-vessel interactions(1, 8).

b. Platelet Unit Membrane:
   Platelet unit membrane has some similarities and appearance with other unit membranes of cells, it is composed from bilipid layer rich of phospholipids (12), it can distribute molecules according to phsico-chemical properties for passing the membrane. The membrane has anionic and cationic pumps. Platelet unit membrane is an important catalyst for liquid phase coagulation.

c. Submembrane Zone:
   Just located under the unit membrane a layer composed of microflament network. This network is anatomically and functionally related to membrane glycoproteins and cytoplasmic filament system.

2. Sol-Jel Zone:
   This is cytoplasm corresponding part of the cellular fragment, platelet. It is in soluble or gel phase according to changes of polymerization of the filaments; actin and microtubules(1).
   Just under the submembrane zone there are microtubules forming a peripheral ring which helps platelet to maintain its discoid shape in inactive form. When activated, the microtubules surround the organelles and with the contribution of other filaments (13), the organelles are tightly contracted. During silent form only 30-40 % of actin filaments are polymerized, when platelets are activated the polymerized amount increases(1).

3. Organel Zone:
   This is the zone where granule's, peroxisome's, lysosome's and mitochondria's are localized. There are enzymes, adenine nucleotids, calcium, serotonin and many other proteins in this region (1).

4. Membrane Zone
   There is a distinguishing feature of platelets that their plasma membrane contains wide spread invaginations that forms a network inside platelet. Finally with pore openings the inner network is directly in contact with outer zone. This system is named as "open canallicular system" (OCS) and with this system an extensive amount of surface area stays as potential in silent state. With this system also platelet gains a large area for molecular trafficking. A second canal system is composed from endoplasmic reticulum networks and named as "Dense Tubular System" (DTS). Here in DTS many enzymes and calcium ions that are important for activation are located. DTS is not directly connected to outer membrane (1, 14) but has close connections with OCS. These two systems actively exchange molecules (1).
   The granulles have diameters ranging between 200 to 500 nm and they are found as spherical or oval structures (15). There are 3 types of granules in platelets, Alfa Granules, Dense granules, lysosomes. Alpha granules are most prominent in terms of material content and majority. These granules include inflammatory molecules, cytokines, cell-activating molecules, proteins, Growth Factors, adhesion molecules, integrins and other proteins These granules are filled by megakaryocytes (3).

3. Alpha granules

It is widely accepted that these granules come from the budding of trans golgi apparatus organel of megakaryocytes (16, 17).

These are 200-400 nm diameter granules widespread in the cytoplasm (16) which gives the granular appearance in Romanoski stained smear preparations, each platelet contains around 50-80 of these granules. The content of granules is very diverse; a brief list is given in table 1 (14, 18, 19, 20, 21).

When platelets are activated these alpha granules fuse with each other, OCS and plasma membrane. The secretion of alpha granules is mediated by some proteins (such as SNARE) and membrane lipids (19).

The secretions effect platelet and cells in the environment (such as endothelial, leukocytes) for migration, adhesion and proliferation(14).

A rare syndrome named as Gray Thrombocyte Syndrome (GTS) is both involved with the quantity and quality of platelets which cases susceptibility for bleeding. In GTS the proteins synthesized by megakaryocytes are abnormal and don't enter platelets as they do in normal individuals and additionally the endocytotic mechanisms don't work properly. As a result the secretions spread to bone marrow and a fibrosis forms (miyelofibrozis)(22, 23).

Thrombospondin
P-selectin
platelet factor 4
beta thromboglobulinler
Factors V, XI, XIII fibrinogen
von Willebrand factor
fibronectin
vitronectin
high molecular weight complexes kininogen
chemokines
mitogenic growth factors (platelet-derived growth factor)
vascular endothelial growth factor
TGF-beta

Table 1. Some main components of alpha granules.

4. Dense granules

These are smaller granules with 150 nm diameter (24), because of the calcium and phosphate content there image seems dense under electron microscopic (EM) observation (21, 25). Each platelet contains 3-8 of these granules (14). The components of dense granules are briefly given in Table 2 (10, 14, 19, 20).

Ca
Mg
P
pyrophosphate Nucleotides ATP, GTP, ADP, GDP
Membrane proteins
CD63 (granulophysin)
LAMP 2
Serotonin
GPIb, GPIIb/IIIa
P-Selectin
Histamine
Epinephrine

Table 2. Some main components of dense granules.

In activated platelet these granules fuse with plasma membrane and expel their ingredients to their environment which causes other platelets to aggregate and a local vasoconstriction (especially by serotonin) in the involved vessels. Also the ADP content is a very important participant for homeostasis (14).

The importance of the components of dense granules for homeostasis is recognized when the diseases of the deficiency of dense granules was defined as Hermansky-Pudlak

Syndrome (26, 27, 28) and Chediak Higashi Syndrome. In both syndromes stoppage of bleeding is defective based on the impairment in dense granules (14).

5. Lysosomes

They have a diameter of 200-250 nm which places them to middle size granule (14). They can't be distinguished from alpha granules under EM observation because of the similarities in dense electron appearance. By the content of acid phosphates and arylsulphates cytochemical staining techniques can effectively distinguish lysosomes from alpha granules. In an activated platelet they expel their contents to environment as the other two granules by membrane fusing mechanisms. The difference for lysosomes to be involved in activation is that they need a more potent stimulus. The role of lysosomal components in homeostasis is not well understood as the other granules contribution. They are involved in thrombus formation and extracellular matrix remodeling (8).

It seems that lysosomes in platelets don't have any distinguished features, they share the common features with other cells lysosomes (29).

The components of dense granules are briefly given in Table 3 (8, 18, 30, 31, 32).

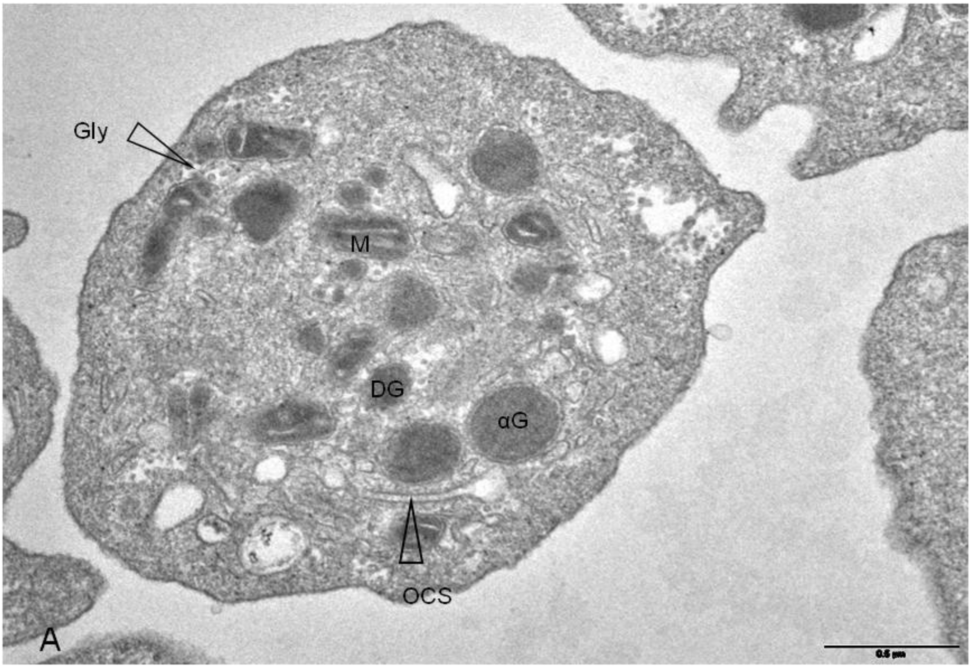

Figure 2. M: Mitochondria, αG: alfa-granules, DG: dense granules, Gly: glycogen particles and OCS: open canalicular system. The morphology can be seen in equatorial section of a human platelet. This image is referenced from Zufferey 2011 (Thanks for the kind permission of John Wiley and Sons to use this image)(33).

PF3
Acid phosphatase
Glucose-6 phosphatase
Arabinosidase
N-Acetyl-galactosominidase
ATP = adenosine triphosphate
TGF
CD63
Cathepsin
lysosomal membrane proteins (LAMP-1, LAMP-2)
acid hydrolases
cathepsins

Table 3. Some main components of platelet lysosomes

6. Autologous platelet rich plasma (PRP)

The application of growth factors in medical practice is one of the areas where basic clinical research has focused its attention but there are many problems associated with their local administration. For example, recombinant human growth factors are not cost effective, they have limited shelf life, and ineffectively delivered to target cells and in addition, to get efficient therapy, large doses are needed. The use of autologous platelets concentrates for tissue regeneration and wound healing has now become an alternative easy and cheap way to obtain high concentrations of these growth factors (34).

The autologous blood collected from a patient just before surgery can be prepared as platelet concentrates, platelet-rich plasma (PRP) and platelet gel for the treatment the patient specifically needs (35). These forms are prepared by gradient density centrifugation techniques to obtain high (x5) concentration of platelets (36). This autologous concentration includes a large amount of growth factors, especially PRP is an easy and inexpensive technique to accelerate the wound healing (37).

This quite new field is open for research, there are a lot of techniques still under development stage such as platelet gels can be obtained by adding thrombin to autologous platelet-rich plasma. The initiation of fibrin polymerization and the release of platelets factors and cytokines can be achieved by the specific activators such as thrombin, glass, freeze-thaw cycle to platelet-rich plasma depending on what is required during the surgery (35).

In spite of the distinct features of platelet-rich plasma (PRP) and its use by different fields of medicine, no adverse reactions were documented until now(38, 39, 40, 41).

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