REVIEW

Platelets

Gökhan Cüce and Tahsin Murad Aktan

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/50969

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 in widespread clinical treatments from cosmetic needs, to supporting insufficient heart function, and maintaining hemostasis. (6, 7).

2.1. Development of Platelets

It is not explained exactly how platelets originate from megakaryocytes, but there are several models to help explain the formation of platelets.

The most scientifically accepted models mentioned are:

1. Simply blebbing from the cell membrane of megakaryocytes (1).
2. Megakaryocytes have special cell fields defined as a "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 located at the blood circulating site of blood

vessels near the bone marrow. On these branches there are small uprising bodies, where by the help of blood's shear force, platelets break off and enter directly into the circulating blood stream. It is also suggested that platelet-like bodies arise from pseudopods of Megakaryocytes, where the formed platelets are known as "proplatelets" (10).

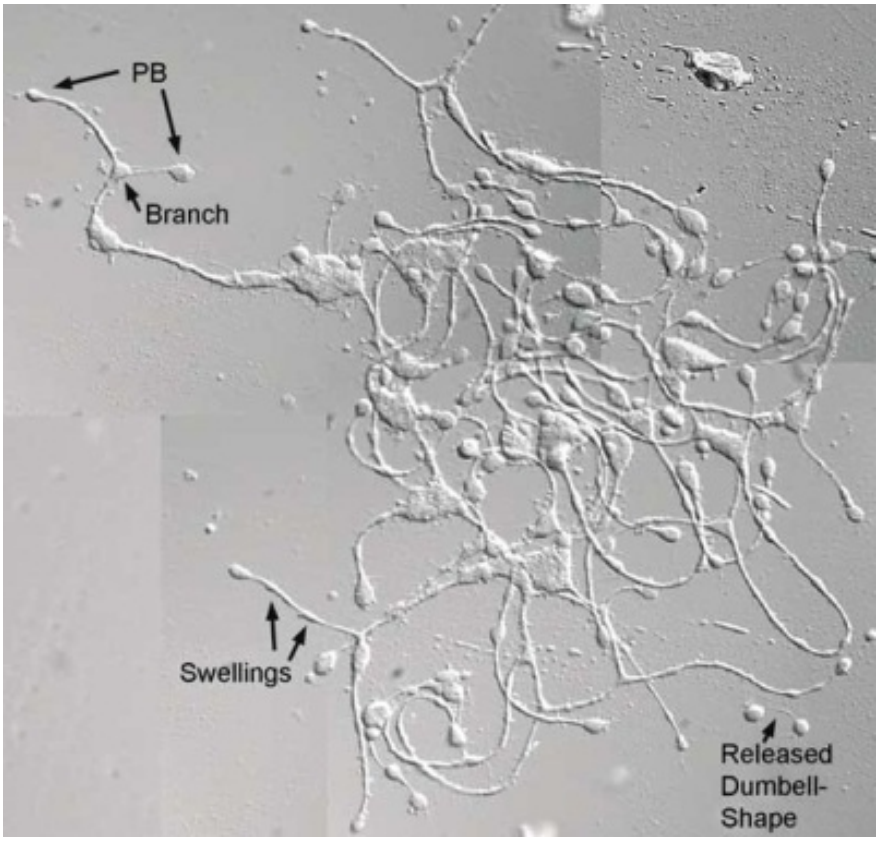

Figure 1. Megakaryocyte branches with Platelet Buds (PB) are seen. Proplatelets are released as Dumbell shaped bodies. This image is referenced from Hartwig and Italiano 2003 (Thanks for the kind permission of John Wiley and Sons to use this image) (11) .

Kinetics of platelets: they have a life span of 7-10 days and in 1 liter human blood it is estimated that there are 150-400X10^9 platelets; so to maintain a consistent count, there are ~15X10^9 - 40X10^9 new platelets formed daily. Megakaryocytes located in the bone marrow sinusoids, form a physical barrier to other bone marrow cells, preventing direct contact to them and the blood circulation. There are canalicular openings in the megakaryocyte membrane, which permits cell migration to other cells, thereby allowing entry to the 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 the Hyalomere. The Hyalomere includes cytoplasmic filaments and circumferential microtubule bundles 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 ranges in color from blue to purple-staining. The Granulomere includes a small Golgi complex, smooth endoplasmic reticulum, lysosomes, 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 it helps to better understand the function of platelets.

1. Peripheral Zone:
   This region is composed of a unit membrane with an open canalicular system. The three parts are defined as:

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

b. Platelet Unit Membrane:
   The Platelet unit membrane has some similarities and appearances of other unit membranes of cells. It is composed of a bilipid layer rich with phospholipids (12), it can distribute molecules past the membrane, has anionic and cationic pumps, and is an important catalyst for liquid phase coagulation.

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

2. Sol-Gel Zone:
   Just under the submembrane zone there are microtubules forming a peripheral ring which helps platelets to maintain their discoid shape while in an inactive form. When activated, the microtubules surround the organelles and with the contribution of other filaments (13), the organelles are tightly contracted. During the inactive form only 30-40% of actin filaments are polymerized, while in activated platelets the polymerized amount increases(1).

3. Organelle 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 folds that form a network inside platelet, and coupled with pore openings, the inner network has direct contact with the outer zone. This system is known as "open canallicular system" (OCS). This system allows for an extensive amount of surface area when platelets are in an inactive state, allowing for a large area for molecular trafficking. A second canal system is composed from endoplasmic reticulum networks and named "Dense Tubular System" (DTS). The DTS has many enzymes and calcium ions that are important for activation. The DTS is not directly connected to the outer membrane (1, 14) but has close connections with the OCS. These two systems actively exchange molecules (1).

   The granules 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 organelle of megakaryocytes (16, 17).

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

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

The secretions effect platelets 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 causes susceptibility for bleeding. In GTS the proteins synthesized by megakaryocytes are abnormal and don't enter platelets as they do in normal individuals. Additionally the endocytotic mechanisms don't work properly and as a result the secretions spread to bone marrow and create fibrosis forms (miyelofibrozis).

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

Dense Granules are smaller granules with a 150 nm diameter (24) and because of the calcium and phosphate content their image appears 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 below 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 platelets these granules fuse with the plasma membrane and expel their contents into their environment, causing other platelets to aggregate and a local vasoconstriction (especially by serotonin) to occur in the involved vessels. It should be noted, the ADP contained in the granules is very important for homeostasis (14).

The importance of the components of the dense granules for homeostasis is recognized in diseases of deficiency of these granules including in, Hermansky-Pudlak Syndrome

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

5. Lysosomes

Lysosomes have a diameter of 200-250 nm which places them as a middle size granule (14). They can't be distinguished from alpha granules under EM observation because of the similarities in their dense electron appearance. But with 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 into their environment, while the other two granule types do so by membrane fusing mechanisms. Another difference is for lysosomes to be involved in activation they need a more potent stimulus. The role of lysosomal components in homeostasis is not as well understood as with the other granules. They are, however, involved in thrombus formation and extracellular matrix remodeling (8).

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

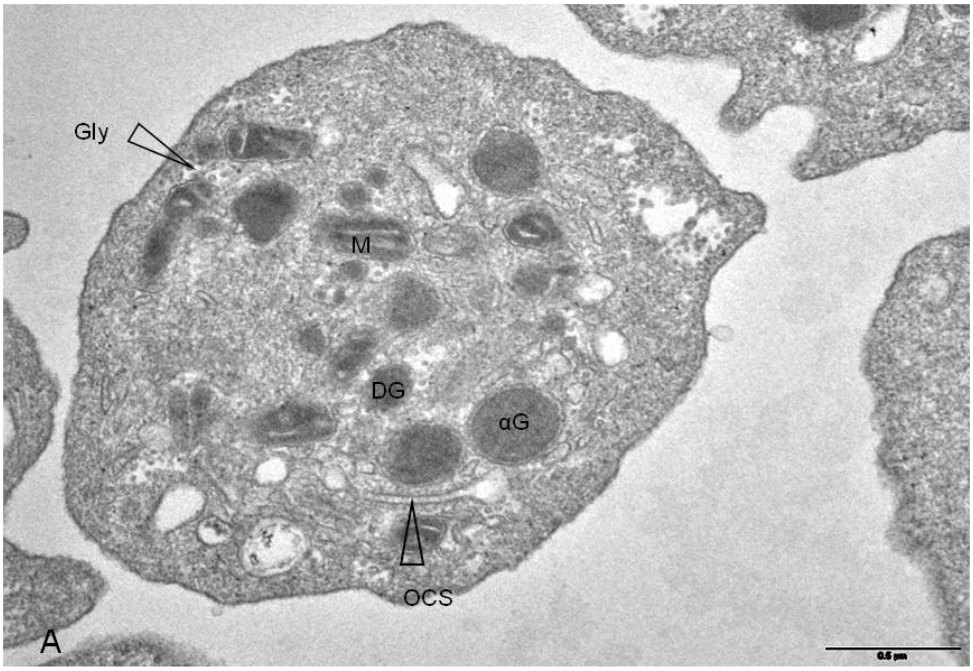

Figure 2. M: Mitochondria, αG: alfa-granules, DG: dense granules, Gly: glycogen particles and OCS: open canalicular system. The morphology can be seen in equatorial section of a human platelet. This image is referenced from Zufferey 2011 (Thanks for the kind permission of John Wiley and Sons to use this 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 can be many issues associated with their local administration. For example, recombinant human growth factors are not cost effective, have a limited shelf life, and ineffectively get delivered to target cells. 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 alternate, easy, and inexpensive way to obtain high concentrations of these growth factors (34).

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

This newer field is still open for additional research, as there are a lot of techniques still in the development stage, such as platelet gels that can be created 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 its use in different fields of medicine, no adverse reactions have been documented to date in the use of platelet-rich plasma (PRP)(38, 39, 40, 41).

Author details

Gökhan Cüce* and Tahsin Murad Aktan
Deparment of Histology and Embryology, Faculty of Meram Medicine University of Konya Necmettin Erbakan, Turkey

7. References

[1] Becker RC. Platelet Biology: The Role of Platelets in Hemostasis, Thrombosis and Inflammation. Platelets in Cardiovascular Disease. In:Bhatt DL. Imperial College Press. London, 2008:1-3.
[2] Mason KD, Carpinelli MR, Fletcher JI, Collinge JE, Hilton AA, Ellis S, Kelly PN, Ekert PG, Metcalf D, Roberts AW, Huang DC, Kile BT. Programmed anuclear cell death delimits platelet life span. Cell. 2007;128(6):1173-86.
[3] Rozman P, Bolta Z. Use of platelet growth factors in treating wounds and soft-tissue injuries. Acta Dermatovenerol Alp Panonica Adriat. 2007;16(4):156-65.
[4] Ovalle WK, Nahirney PC. Netter Essential Histology. Saunders; 2007;166.
[5] Klages B, Brandt U, Simon MI, Schultz G, Offermanns S. Activation of G12/G13 results in shape change and Rho/Rho- kinase-mediated myosin light chainphosphorylation in mouse platelets. J Cell Biol. 1999;144(4):745-54.
[6] Anitua E, Sánchez M, Nurden AT, Nurden P, Orive G, Andía I. New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol. 2006(5):227-34.
[7] Mishra A, Velotta J, Brinton TJ, Wang X, Chang S, Palmer O, Sheikh A, Chung J, Yang PC, Robbins R, Fischbein M. RevaTen platelet rich plasma improves cardiac function after myocardial injury. Cardiovasc Revasc Med. 2011;12(3):158-63.
[8] Drouin A, Cramer EM. Production of Platelets. Editor: Gresele P, Page CP, Fuster V, Vermylen J. Platelets in Thrombotic and Non-Thrombotic Disorders: Pathophysiology, Pharmacology and Therapeutics. Cambridge University Press; 2002;25.USA.
[9] Schulze H, Korpal M, Hurov J, Kim SW, Zhang J, Cantley LC, Graf T, Shivdasani RA. Characterization of the megakaryocyte demarcation membrane system and its role inthrombopoiesis. Blood. 2006;107(10):3868-75.
[10] Italiano JE Jr, Shivdasani RA. Megakaryocytes and beyond: the birth of platelets. J Thromb Haemost. 2003;1(6):1174-82.
[11] Hartwig J, Italiano J Jr. The birth of the platelet. J Thromb Haemost. 2003;1(7):1580-6.
[12] White JG. Platelet Structure. Editor:Michelson AD. Platelets. Elsevier: USA, Second Edition, 2007;45
[13] White JG. Views of the platelet cytoskeleton at rest and at work. Ann N Y Acad Sci. 1987;509:156-76.
[14] Rumbaut RE, Thiagarajan P. Platelet-Vessel Wall Interactions in Hemostasis and Thrombosis. Editör: Granger DN, Granger JP. Colloquium Series on Integrated Systems

* Corresponding Author

Physiology: From Molecule to Function to Disease. Morgan & Claypool Life Sciences; 2009-2011:5.
[15] Gassling VL, Açil Y, Springer IN, Hubert N, Wiltfang J. Platelet-rich plasma and platelet-rich fibrin in human cell culture. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009 ;108(1):48-55.
[16] King SM, Reed GL. Development of platelet secretory granules. Semin Cell Dev Biol. 2002(4):293-302.
[17] Blair P, Flaumenhaft R. Platelet alpha-granules: basic biology and clinical correlates. Blood Rev. 2009(4):177-89.
[18] McNicol A, Israels SJ. Platelet dense granules: structure, function and implications for haemostasis. Thromb Res. 1999;95(1):1-18.
[19] Reed GL. Platelet secretory mechanisms. Semin Thromb Hemost. 2004;30(4):441-50.
[20] Askari AT, Messerli AW, Lincoff M. Thrombosis and Antithrombotics in Vascular Disease. Management Strategies in Antithrombotic Therapy. Editör: Askari AT, Messerli AW.Wiley; USA. 2008:3.
[21] Ma AD, Key NS. Molecüler Basis of Hemostatic and thrombotic Diseases. Editör: Coleman WB, Tsongalis GJ, London. Molecular Pathology: The Molecular Basis of Human Disease. Academic Press; 1 edition, 2009:258.
[22] Di Paola J, Johnson J. Thrombocytopenias due to gray platelet syndrome or THC2 mutations. Semin Thromb Hemost. 2011(6):690-7.
[23] Nurden AT, Nurden P. The gray platelet syndrome: clinical spectrum of the disease. Blood Rev. 2007(1):21-36.
[24] Rendu F, Brohard-Bohn B. The platelet release reaction:granules' constituents, secretion secretion and functions. Platelets. 2001;12(5):261-73.
[25] Ruiz FA, Lea CR, Oldfield E, Docampo R. Human platelet dense granules contain polyphosphate and are similar to acidocalcisomes ofbacteria and unicellular eukaryotes. J Biol Chem. 2004;279(43):44250-7.
[26] King SM, McNamee RA, Houng AK, Patel R, Brands M, Reed GL. Platelet dense-granule secretion plays a critical role in thrombosis and subsequent vascularremodeling in atherosclerotic mice. Circulation. 2009;120(9):785-91.
[27] Nisal M, Pavord S, Oppenheimer CA, Francis S, Khare M. Hermansky-Pudlak syndrome: management of a rare bleeding disorder in a twin pregnancy. J Obstet Gynaecol. 2012 ;32(2):185-6.
[28] Saftig P, Klumperman J. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. Nat Rev Mol Cell Biol. 2009;10(9):623-35.
[29] Skoglund C. Platelets in inflammation. Linköping University Medical Dissertations. 2010-Sweden;14.
[30] Gerrard JM, Phillips DR, Rao GH, Plow EF, Walz DA, Ross R, Harker LA, White JG. Biochemical studies of two patients with the gray platelet syndrome. Selective deficiency of platelet alpha granules. J Clin Invest. 1980;66(1):102-9.
[31] Nishibori M, Cham B, McNicol A, Shalev A, Jain N, Gerrard JM. The protein CD63 is in platelet dense granules, is deficient in a patient with Hermansky-Pudlaksyndrome, and appears identical to granulophysin. J Clin Invest. 1993;91(4):1775-82.

[32] Grau AJ, Reiners S, Lichy C, Buggle F, Ruf A. Platelet function under aspirin, clopidogrel, and both after ischemic stroke: a case-crossoverstudy. Stroke. 2003;34(4):849-54.

[33] Zufferey A, Fontana P, Reny JL, Nolli S, Sanchez JC. Platelet proteomics. Mass Spectrometry Reviews, 2011; 31, 331–351.

[34] Nikolidakis D, Jansen JA. The biology of platelet-rich plasma and its application in oral surgery: literature review. Tissue Eng Part B Rev. 2008 Sep;14(3):249-58.

[35] Soffer E, Ouhayoun JP, Anagnostou F. Fibrin sealants and platelet preparations in bone and periodontal healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;95(5):521-8.

[36] Huang Q, Wang YD, Wu T, Jiang S, Hu YL, Pei GX. Preliminary separation of the growth factors in platelet-rich plasma: effects on the proliferation of human marrow-derived mesenchymal stem cells. Chin Med J (Engl). 2009;122(1):83-7.

[37] Napolitano M, Matera S, Bossio M, Crescibene A, Costabile E, Almolla J, Almolla H, Togo F, Giannuzzi C, Guido G. Autologous platelet gel for tissue regeneration in degenerative disorders of the knee. Blood Transfus. 2011;25:1-6.

[38] Edwards SG, Calandruccio JH. Autologous blood injection for refractory lateral epicondylitis. *J Hand Surg [Am].* 2003;28(2):272-278.

[39] Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. *Am J Sports Med.* 2006;34(11):1774-1778.

[40] Kajikawa Y, Morihara T, Sakamoto H, et al. Platelet-rich plasma enhances the initial mobilization of circulation-derived cells for tendon healing. C*ell Physiol.* 2008;215(3):837-845.

[41] Sánchez M, Anitua E, Azofra J, Aguirre JJ, Andia I. Intra-articular injection of an autologous preparation rich in growth factors for the treatment of knee OA: a retrospective cohort study. *Clin Exp Rheumatol.* 2008;26(5):910-913.