# PLATELET CONCENTRATES FROM PLATELET-RICH PLASMA AND APHERESIS MACHINES: BIOCHEMICAL PARAMETERS, FUNCTIONAL ASSAYS AND CLINICAL OUTCOMES

**BY**

**MAZURA BAHARI**

**Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy in Transfusion Medicine**

**JULY 2013**

## DECLARATION

Here, I declare that this research work is forwarded to Universiti Sains Malaysia (USM) for the degree of Doctor of philosophy in Transfusion Medicine. It has not been sent to any other university. With that, this research may be used for consultation purpose and photocopied as reference.

**MAZURA BAHARI**

**JULY 2012**

## ACKNOWLEDGMENTS

In the name of Allah, the Most Beneficent, the Most Merciful.

Alhamdulillah, this project was finally completed. This project would not have been initiated and completed without the persistent help of many people. I owe my greatest debt of gratitude to my supervisor, Associate Professor Dr. Narazah Mohd Yusoff, from the Advanced Medical and Dental Institute, Universiti Sains Malaysia for her guidance, support and tremendous flexibility. Her patience and countless assistance had led to the completion of this study.

I would like to address my sincere thanks to my advisor, Dato Dr. Faraizah Dato Abd Karim from the National Blood Center, Kuala Lumpur, for her ideas, advice, help, supervision, support and trust throughout my Ph.D. journey. I would also like to acknowledge Dr. Nor Asiah Muhammad from the Institute For Medical Research and Dr. Hasnah Hashim from the Advanced Medical and Dental Institute, Universiti Sains Malaysia for their skillful statistical assistance.

I wish to thank the staff of the Hematology, Component Unit and Bleeding Room of the National Blood Center, Kuala Lumpur for providing samples and facilities. I would also like to acknowledge our collaborators and staff from the Kuala Lumpur Pediatrics Institute, Ampang Hospital and Hospital Kuala Lumpur for their excellent and overall support. I wish to thank all my colleagues from the Faculty of Health Sciences, UiTM Puncak Alam for their assistance and overall support.

Undoubtedly, I am blessed and strengthened by my beloved husband, Mr. Azli Ismail and children, Mohd Ammar, Mohd Najmi, Ainul Nabihah, Ainul Najwa and Mohd Hurairah, for their love, aspiration, countless sacrifices and unconditional support and tolerance.

Finally, I dedicate this thesis to my beloved mother who passed away two years ago. May Allah bless you. For any unintended offences, I offer my apologies.

## TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

## ABBREVIATION

| | |
|---|---|
| PCs | Platelet concentrates |
| DIVC | Disseminated intravascular coagulation |
| CCL5 or RANTES | Regulated upon Activation, Normal T-cell Expressed, and presumably Secreted |
| TGF-ß1 | Transforming Growth Factor-ß1 |
| TRALI | Transfusion-Related Acute Lung Injury |
| NBC | National Blood Center |
| TNF-α | Tumor Necrosis Factor-α |
| PRP | Platelet-rich plasma |
| GP | Glycoprotein |
| LRS | Leukocyte Reduction System |
| FITC | Fluorescein isothiocyanate |
| PE | Phycoerythrin |
| FSC | Forward Light Scatter |
| SSC | Side Scatter |
| PerCP | Peridinin Chlorophyll Protein |
| BD | Becton Dickinson |
| AABB | American Association of Blood Banks |

**SEL PEMBEKU DARIPADA PLASMA YANG DIPERKAYA DENGAN SEL PEMBEKU DAN MESIN AFERESIS: PARAMETER BIOKIMIA, FUNGSI ASAI DAN HASIL KLINIKAL**

## ABSTRAK

Fungsi sel pembeku ialah untuk membentuk mekanikal plak untuk mengelak pendarahan akibat salur vaskular yang cedera hasil daripada tindakbalas normal homeostatis. Secara amnya, pemindahan sel pembeku boleh mengurangkan risiko kematian akibat pendarahan. Disamping kondisi pesakit, kejayaan rawatan adalah bergantung kepada kualiti sel pembeku yang dipindahkan. Membekalkan pesakit dengan sel pembeku yang befungsi secara optimum adalah menjadi kebimbangan kepada Pusat Darah Negara (PDN). Pada masa ini di PDN, sel pembeku yang disediakan oleh mesin apheresis dan kaedah manual digunakan dengan meluas secara klinikal kerana permintaan sel pembeku yang semakin meningkat di Malaysia. Walau bagaimanapun, kualiti sel pembeku yang dihasilkan melalui kaedah manual dan melalui mesin apheresis mestilah disiasat dan dibandingkan kualitinya. Kualiti sel pembeku yang dihasilkan dipengaruhi oleh kaedah penyediaan, jenis bekas penyimpanan, larutan penyimpanan dan kondisi penyimpanan. Kajian ini dijalankan untuk membandingkan kualiti sel pembeku yang disediakan melalui dua kaedah penyediaan sel pembeku yang berbeza; kaedah manual berbanding kaedah mesin automasi apheresis; “W”, “X”, “Y” and “Z” dengan menentukan pH, sterility, bacaan sel pembeku, tahap sitokin dan pengaktifan pembeku dan setrusnya mengkaji kesan pemindahan sel pembeku seteleh dipindahkan kepada pesakit. Kualiti sel pembeku yang diperolehi boleh dipengaruhi oleh beberapa keadaan, seperti pengemparan pada kelajuan yang tinggi, pendedahan kepada permukaan tiruan,

penyimpanan dalam pelbagai jenis beg dan kaedah penyediaan sel pembeku yang berbeza yang mungkin bukan sahaja mempengaruhi tahap lesi penyimpanan sel pembeku tetapi juga boleh menyumbang kepada kurangnya keberkesanan dan tindak balas transfusi klinikal. Pengaktifan sel pembeku boleh menyumbang dilesi penyimpanan sel pembeku. Akibat lesi, sel pembeku akan memberikan kesan pada struktur dan fungsinya dalam proses hemostasis biasa.

Parameter biokimia yang diuji termasuk pngiraan sel pembeku, pH, steril dan tahap cytokines (RANTES dan TGF-β1) menggunakan ELISA , fungsi asai yang dikaji ialah pengaktifan sel pembeku (CD62) menggunakan flow sitometer dan mengkaji kesan klinikal pencapaian *in vivo* selepas pemindahan sel pembeku ke dalam aliran darah pesakit melalui bacaan sel pembeku pada pra dan pos pemindahan. Kajian ini menunjukkan tiada perbezaan yang signifikan diperhatikan pada tahap sitokin (RANTES dan TGF-β1), pengiraan sel pembeku dan steriliti sel pembeku dalam kedua-dua kaedah penyediaan. Tiada perbezaan yang signifikan dalam pengaktifan sel pembeku antara kedua-dua kaedah, manual dan mesin apheresis. Kedua-dua kaedah menunjukkan peningkatan pengaktifan sel pembeku dari hari ke sehari semasa dalam penyimpanan. Namun kajian ini menunjukkan bahawa sel pembeku yang disediakan oleh mesin apheresis "Y" dan "Z" menunjukkan sedikit kelebihan iaitu dengan pengaktifan sel pembekunya yang paling minima. Mesin apheresis paling tinggi sel pembeku teraktif ialah mesin "X". Berdasarkan keputusan kajian ini, mesin apheresis yang menghasilkan sel pembeku teraktif yang paling minima ialah mesin "Y"dan"Z". Dengan itu adalah disyorkan kedua-dua mesin tersebut digunapakai sebagai kaedah penyediaan sel pmbeku mengatasi kaedah-kaedah yang lain di PDN, Malaysia.

**PLATELET CONCENTRATES FROM PLATELET-RICH PLASMA AND APHERESIS MACHINES: BIOCHEMICAL PARAMETERS, FUNCTIONAL ASSAYS AND CLINICAL OUTCOMES**

## ABSTRACT

The function of platelets is to form a mechanical plug to seal the site of vascular injury during hemostatic. Generally, platelet transfusion can reduce the risk of mortality. Aside from the general condition of patients, the quality of platelets to be transfused is highly important for a successful treatment. Providing patients with optimally functioning platelet concentrates (PCs) is the major concern of National Blood Center (NBC). For more than three decades, the manual method of platelet preparation has been the standard procedure in the National Blood Center (NBC) of Malaysia. However, the quality of PCs prepared through the manual and apheresis methods has yet to be investigated and compared. The quality of PCs is affected by preparation methods, types of storage bags, storage solution and storage conditions.

This study aims to determine the quality and outcomes of the PCs obtained using the manual method and the automated apheresis machines; "W", "X", "Y" and "Z" by detecting the pH, sterility, platelet count, cytokine and platelet activation levels as well as determining the efficacy of platelet after being transfused to the patients. The quality of platelet concentrates obtained is affected by several factors such as high centrifugation forces, exposure to artificial surfaces, storage in different types of bags and different platelet preparation methods. These factors may affect the extent of platelet storage lesions and contribute to the poor clinical effectiveness and transfusion reactions of platelet concentrates. Platelet activation in PCs may contribute to platelet storage lesions. These lesions influence the structures and

functions of platelets during normal hemostatic processes. The biochemical parameters that were tested included platelet count, pH, sterility and cytokine levels (RANTES and TGF-β1) using ELISA. The functional assay that was analyzed included platelet activation (CD62) using flow cytometry. The outcomes of platelet transfusion were also evaluated through the pre- and post-platelet counts. Any adverse effect of platelet transfusion on the patients was also determined. This study showed that there was no significant differences in cytokine levels (RANTES and TGF-β1) found between the PCs obtained using manual methods and PCs obtained from different types of apheresis machines. No significant difference was observed in PCs obtained using manual method and PCs obtained from different types of apheresis machines in platelet activation level. Significant differences in platelet activation were noted between groups across time in both methods of platelet preparation. However, this study shows that the PCs with the least activated platelets were those collected using machines "Y" and "Z", although there was no significant differences were observed. Among the PCs obtained using the other apheresis machines, those obtained using machine "X" showed the highest platelet activation. Based on the results of this study, machines that produced the least activated platelets were machines "Y" and "Z". Hence, these two machines, was suggested serving as compared to the others methods, as the standard of platelet collection in the NBC of Malaysia.

# CHAPTER 1

# INTRODUCTION

## 1.1 Back ground of the study

The global demand for platelet transfusion is continuously increasing because of therapies that use platelet concentrates (PCs). The best and effective treatment for patients with bleeding problems and other diseases, such as severe thrombocytopenia, DIVC (disseminated intravascular coagulation), acute leukemia and aplastic anemia is transfusion. Generally, platelet transfusion can reduce the risk of mortality due to hemorrhage.

The function of platelets is to form a mechanical plug to seal the site of vascular injury during homeostasis. Platelets promote the regeneration of injured tissues in surgical, oncological and transplant patients. Patients with low platelet counts (5,000/μL to 10,000/μL) use PCs as prophylaxis. Patients with thrombocytopenia caused by diseases or chemotherapy need high-quality platelets to overcome their haemostatic problems. Therefore, the platelets to be transfused must possess effective haemostatic functions. Aside from the general condition of patients, the quality of platelets to be transfused is highly important for successful treatment.

The manual method for platelet collection is through manual centrifugation of whole blood and obtaining platelet-rich plasma (PRP). This method involves the separation of platelets from multiple bags of whole blood collected from several donors. Several units of PCs are pooled because a single unit of donated whole blood contains only a small amount of PCs.

This manual method involves several processing steps that may cause platelet surface changes (morphological changes), thereby affecting the quality of platelet product. The risk of alloimmunization and virus transmission to a recipient is higher in single unit of PCs because of the number of blood donation from several donors. Moreover, the manual method is more time consuming and labor intensive compared with the automated apheresis machines. Automated apheresis machines are widely used not only because of the lower risk of alloimmunization and viruses transmission but these machines can extract as many platelets as possible from a single donation (1). Hence, the quality and quantity of platelets yield are improved and the time required to prepare the PCs is reduced. All the procedures for platelet preparation were under standardized protocols. The equipment, materials, reagents and staff involved in the preparation of PCs using apheresis method meet the quality system requirements based on international guidelines from American Association of Blood Banks, (AABB). However, the quality of PCs prepared via the automated apheresis method should be considered and compared with that of PCs prepared using the manual method.

The quality of the PCs obtained from the apheresis method can be influenced by several factors, such as high centrifugation forces, exposure to artificial surfaces, storage in different types of bags and different platelet preparation methods. These factors may influence the extent of platelet storage lesions and contribute to the poor clinical effectiveness and transfusion reactions of PCs (2–3). PCs undergo a number of changes and alteration during collection, processing and storage, which are classified as platelet storage lesions (4, 5). It has been demonstrated that platelet activation plays a major role in the generation of platelet storage lesions in PCs.

These lesions affect the structures and functions of platelets during normal haemostatic processes, leading to loss of integrity of platelet function, changes in aggregation and release, rearrangement of platelet cytoskeleton, exposure of phosphatidyl serine on the outer membrane surface and microvesiculation (5,6).

Alterations in shear stress and the biocompatibility of platelet surfaces may provide variable platelet activation, thereby affecting the quality of PCs (7–10).

Studies showed that a reduced response to agonist stimulation (8–11), low survival of transfused platelets and prolonged *in vitro* bleeding time are associated with high degree of platelet activation in PCs (12, 13).

The initial lesion involved during the isolation process is essential for the quality of PCs during storage. It is proposed that deterioration of PCs during storage might be related to platelet activation occurring during preparation (14–18).

It has been shown that platelet activation leads to the degranulation and release of platelet-derived cytokines include CCL5 or Regulated upon Activation, Normal T-cell Expressed and presumably Secreted (RANTES). This cytokine has been suggested to be implicated in non-hemolytic transfusion reactions together with Transforming Growth Factor-ß1, (TGF-ß1) (19–22). Incidences of non-hemolytic transfusion reaction are recognized as adverse events after PCs transfusion.

Common reactions usually involve chills, nausea and fever, whereas more serious reactions include anaphylaxis and TRALI (transfusion-related acute lung injury). Non-hemolytic transfusion reactions following platelet transfusion does not involve immune-mediated events but appear to be caused by cytokines (22–25) and administration of plasma containing large amounts of cytokines (26).

Platelet-derived cytokines may contribute towards the events of non-hemolytic transfusion reactions following high levels of RANTES in PCs that can cause allergic reaction (27). Studies have shown that a large part of febrile transfusion reactions results from transfusion of stored PCs which contains higher levels of cytokines and not from antigen-antibody reactions (28). The release of platelet chemokines into the suspension medium of stored PCs may be mediated by active secretion due to platelet activation during storage or it may be related to the lyses of some platelets. Increased supernatant levels of these cytokines possibly indicate platelet activation or destruction. Although very few data are available on the *in vivo* effects of transfused cytokines, it has been suggested that the high incidence of adverse reactions is often observed after platelet transfusion (29).

For more than three decades, the manual method of platelet preparation has been the standard procedure in the National Blood Center (NBC) of Malaysia. In the 1980s, automated apheresis machines were introduced. The use of automated apheresis machines can reduce the risk of alloimmunization and virus transmission to patients afforded by reduced donor exposure (1).

Since then, manual and apheresis methods have been the standard of platelet component preparation in the NBC of Malaysia. Currently, PCs prepared via the apheresis and manual methods in NBC are in widespread clinical use because of the continuously increasing demand for PCs in Malaysia. Providing patients with optimally functioning PCs is the major concern of NBC. However, the quality of PCs prepared through the manual and apheresis methods has yet to be investigated and compared. The quality of PCs is affected by preparation methods, storage bags types, storage solution and storage conditions.

The proportion of activated platelets has been suggested to be a predictor of platelet quality. Previous studies also have suggested that cytokines, such as IL-1, IL-6, IL-8, TNF-α (tumor necrosis factor-α) and RANTES, which are released by leucocytes and/or other cell types during platelet component storage, may play causative roles in the origin of adverse reactions in some recipients (22–27, 29). The quality of PCs can be evaluated *in vitro* by analyzing the following parameters: pH, bacterial contamination, platelet activation, cytokine level, white blood cell count, platelet swirling, clotting time and coagulum elasticity.

Platelet quality is related to the preparation methods for PCs. The quality of platelets to be transfused to patients is the priority of the NBC. Accordingly, this study compares the quality of the platelet product obtained using the manual method and the automated apheresis machines by detecting the platelet activation and cytokine levels after PCs preparations. The efficacy of the PCs *in vivo* was evaluated by detecting the pre- and post-platelet counts after PCs transfusion. Thus so far, the efficacy of PCs prepared using the manual method and the apheresis method has never been officially reported in whole blood center of Malaysia.

## 1.2 Objectives

### 1.2.1 General objective

This research aim to compare the quality of PCs and outcome of the platelet count in patient after being transfused with the PCs obtained using the manual method and PCs obtained from four types of automated apheresis machines ("W", "X", "Y" and "Z"), by detecting the cytokines levels and platelet activation after platelet preparation.

### 1.2.2 Specific objectives

1. To determine and compare platelet activation in PCs prepared using the manual method and four types of apheresis machines through flow cytometry.
2. To determine the levels of cytokines (RANTES) in PCs prepared using the manual method and four types of apheresis machines using enzyme-linked immunosorbent assay (ELISA).
3. To determine the levels of cytokines (TGF-β1) in PCs prepared using the manual method and four types of apheresis machines using enzyme-linked immunosorbent assay (ELISA).
4. To determine the pre- and post-transfusion platelet counts in patients transfused with PCs prepared using the apheresis methods from patient's record.
5. To determine the adverse effects of transfusion in patients as a result of apheresis platelet transfusion from patient's record.

## 1.3 Significance of the study

Methods used for the preparation of PCs are important in influencing the platelet activation process and cytokine levels in PCs. This study embarked on finding the best method to achieve high-quality PCs and improve the function of platelets *in vivo*, potentially leading to the improved clinical effectiveness of platelet transfusion by the NBC of Malaysia as well as the whole blood center in Malaysia.

## 1.4 Hypothesis

### 1.4.1 Null Hypothesis (Ho)

Ho**:** There is no differences exist in the quality of PCs prepared using the manual method and different types of apheresis machines.

### 1.4.2 Study Hypothesis (Ha)

$H_1$: There is differences exist in the quality of PCs prepared using the manual method and different types of apheresis machines.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Platelets

### 2.1.1 Platelet production

Platelets bud off from large bone marrow cells called megakaryocytes through cytoplasm fragmentation (Fig. 2.1.1). Megakaryocytes are derived from hemopoietic stem cells, which are stimulated to differentiate to mature megakaryocytes. Megakaryoblasts are the precursors of megakaryocytes; they arise through differentiation from hemopoietic stem cells.

Megakaryocytes mature via endomitotic synchronous replication, enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two (30). Thrombopoietin is a humoral agent that controls platelet production. The interval time for human stem cell differentiation to produce platelets is approximately 10 days. After their release from the bone marrow, young platelets are trapped in the spleen for up 36 hours before entering the blood circulation. The normal platelet lifespan is approximately 10 days in the blood and the normal platelet count is approximately $250 \times 10^9$/L (range $150–400 \times 10^9$/L).

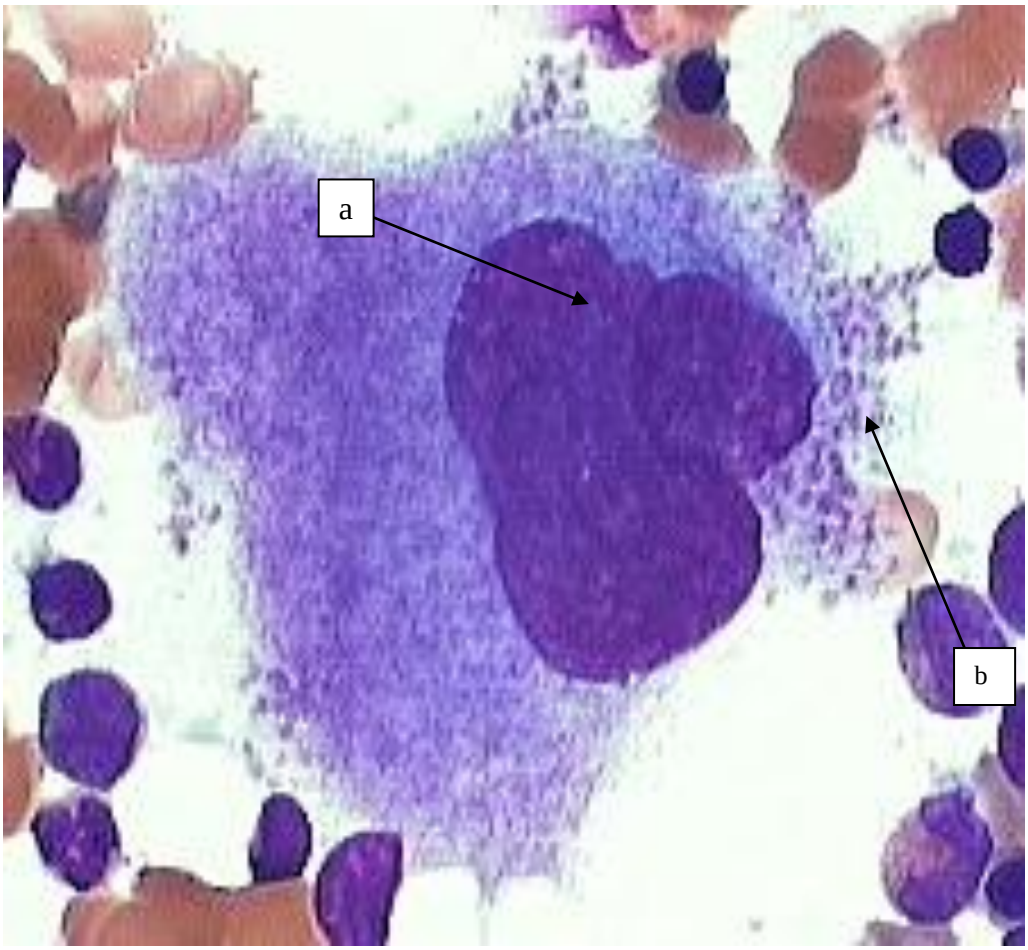



**Figure 2.1.1 Platelets budding off from a megakaryocyte.**
**Megakaryocyte with lobulated nucleus (a) and platelets (b).**
**(Adopted from Ann Bell, The morphology of Human Blood Cells, 2005)**

### 2.1.2 Platelet structure

Platelets are small and discoid enucleated blood cells with a diameter of 3.0 × 0.5 μm. Platelets play important roles in hemostasis and thrombosis. The small size and shape of platelets allow them to be pushed to the edge of vessels, placing them next to endothelial cells and enabling them to respond to vascular damage. During adhesion and aggregation, the GP glycoprotein of the surface coat plays an important role in the formation of plugs during hemostasis. Platelets contain three main types of storage granules: dense-granules, α-granules and lysosomes. Each type rapidly releases its contents upon activation (30). The ultra structure of platelets is shown in Fig. 2.1.2 (a).

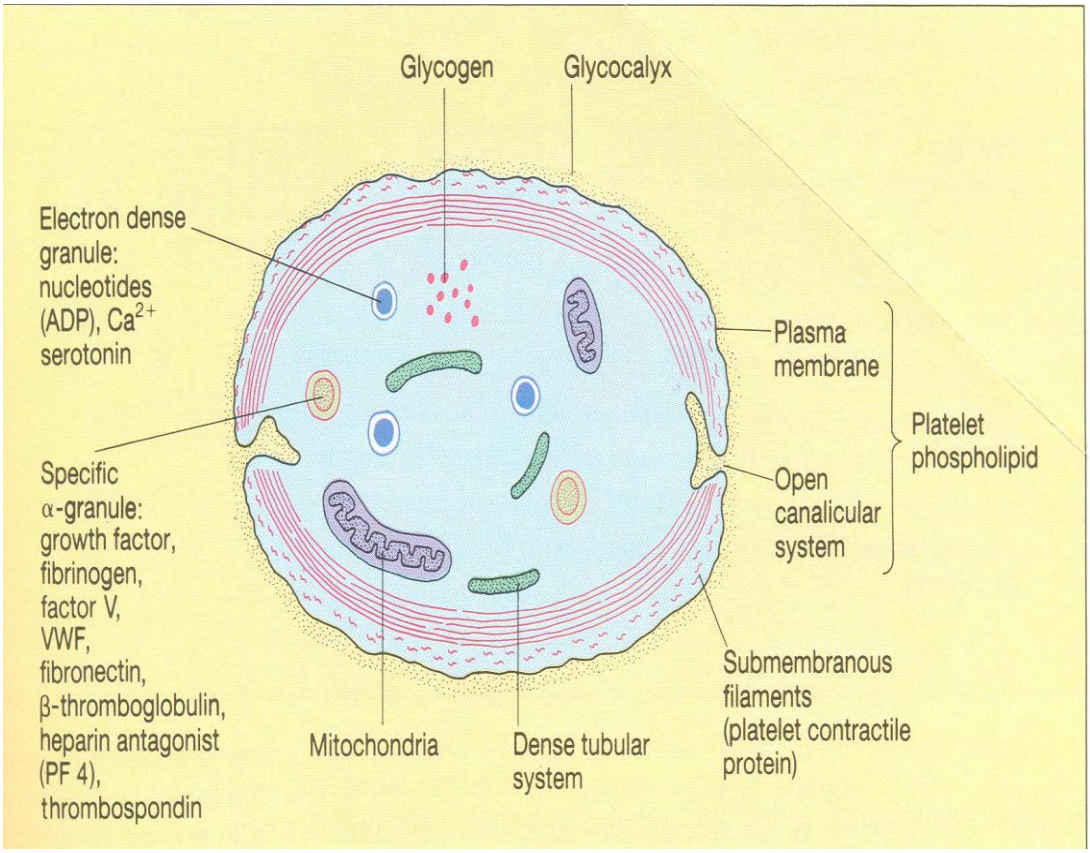
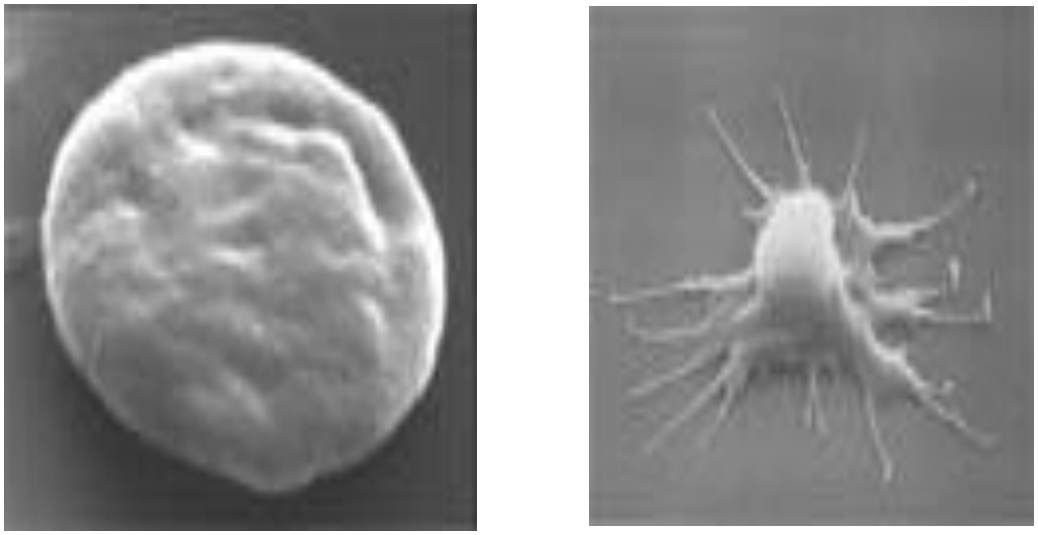



**Figure 2.1.2 (a) Ultrastucture of platelets. ADP, adenosine diphosphate; PDGF, platelet-derived growth factor; PF, platelet factor; and vWF, von Williebrand factor.**

**(Adopted from A.V.Hoffbrand, Essential Hematology, 1993)**

Although platelets are fragments of cells, they contain most cellular organelles, including microtubules, mitochondria, peroxisomes and specific granules. Resting platelets are discoid and have smooth surfaces, whereas activated platelets are spherical in shape with pseudopod extrusion [Fig. 2.1.2 (b)]. Platelets are surrounded by a bilamellar plasma membrane that extends into the channels of the surface connected to the canalicular system. Through this surface membrane, intrinsic GPs extrude to serve various functions in homeostasis (30). Platelets are enriched in signaling proteins and surface receptors, enabling them to respond immediately and effectively during homeostasis.

The outer layer of a platelet is coated with numerous GPs, whose surfaces contain several receptors called platelet-independent surface markers. These markers take part in binding with various aggregating and adhesive agents in normal platelet adhesion or aggregation, leading to the formation of platelet plugs during homeostasis. Platelet membrane GPs serve various functions, including receptor binding of ligands necessary for the adhesion of platelets to leucocytes, fibrin, endothelial cells and extracellular matrix (31).



(i) (ii)

**Figure 2.1.2 (b) Electron micrographs of resting (i) and activated (ii) platelets. Resting platelets are smooth and discoid (left). Activated platelets have an irregular shape with several protruding pseudopodia (right). Source: http://www.platelet-research.org/l/function_hemo.htm##**

The expression of GPs on the surface of platelets reflects the activation status and the extent of secretion. The surface expression of p-selectin (CD62P) can be assessed when platelets become activated, reflecting the translocation of α-granule membrane protein (α-GMP) on the platelet surface and the conformational change of the GPIIb-IIIa complex (32). The presence of GP platelet membrane on the platelet surface has become useful in fundamental research towards the development of new drugs and anti-thrombin. Previous studies reported that thrombus or hemostatic plugs are poorly formed or absent in patients with congenital defect of GPIb-IX (Bernard-Soulier syndrome) or GPIIb-IIIa (Glanzman thrombasthenia) (33 and 34).

### 2.1.3 Platelet functions

Platelets are essential in hemostasis to prevent bleeding, secrete growth factors to stimulate healing after injury and maintain normal vascular integrity. In the absence of platelets, spontaneous leakage of blood may occur through small vessels (30). Platelet functions fall into four categories: adhesion, aggregation, secretion and activation. These processes occur on the extracellular matrix components of the exposed sub endothelium of injured vessels (35) as represented in Fig. 2.1.3.

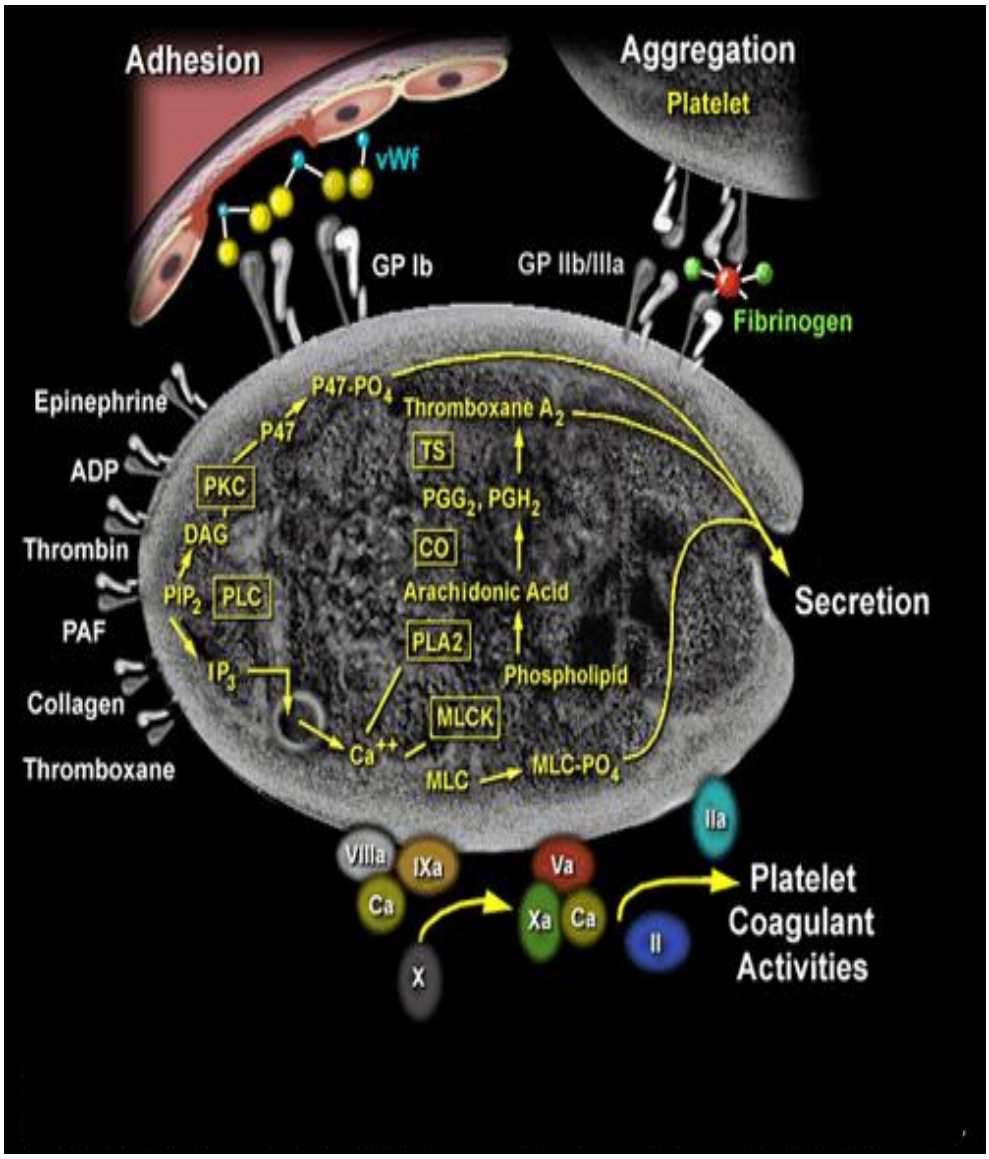



**Figure 2.1.3 Simplified diagram of platelet adhesion, activation, aggregation, and secretion in primary hemostasis.**
**Source: http://www.strokecenter,org/professionals/brain-anatomy/platelet-activation**

### 2.1.4 Platelet activation

Platelet activation is a process in which platelets change shape, aggregate, adhere to vascular walls and secrete cytokines during homeostasis. Platelet activation involves a series of changes in platelet metabolic biochemistry, morphology and functions. This process is controlled by complex networks of activating and inhibiting intracellular signaling pathways. Platelet activation occurs in both *in vivo* and *in vitro* stages. The extent of platelet activation *in vitro* depends on various circumstances, such as the PCs preparation method (2), storage medium of PCs (i.e., in plasma or in additive solution) and duration of storage.

P-selectin (CD62), a protein stored in the alpha granule membrane protein (α-GMP) of activated platelets, may be used as an index to determine the activation status of PCs *in vitro*. When platelets are activated, platelet surface molecules will changes, which can be detected by flow cytometry using fluorescent-conjugated antibodies to fibrinogen, p-selectin (CD62P), GPIIb-IIIa (CD41), GPIb alpha (CD42b), and GPV (CD42d) (32, 40). These seen as a useful approach to determine the platelet quality in platelets concentrate in various platelet preparation method.

### 2.1.5 Platelet storage lesions

Platelet storage lesions are characterized by the following phenomena. First is the change in platelet shape from discoid to spherical. Second is the generation of lactic acid from glycolysis, with an associated decrease in pH. Third is the release of cytoplasmic and granule contents. Fourth is the decrease in various *in vitro* measurements of platelet function, particularly osmotic challenge. Fifth is the adenosine di-phosphate (ADP) induced change in shape. These lesions may have an impact on platelet viability and haemostatic function. The storage lesion leads to structural, biochemical and functional alterations that are hallmarks of the process of activation, a multifaceted phenomenon in which substances are released from granules, membrane constituents are modified and functional responses are altered (43). Last is the reduction of *in vivo* recovery and survival (41 and 42). Platelets become spherical in shape, reducing their viability and ability to perform well in homeostasis once activated. Changes in platelet function and structure in PCs can be explained in terms of sequelae of activation (11). Platelet activation plays major role in storage lesions in PCs (12).

Platelet activation can be detected by flow cytometry. As shown in Fig. 2.1.5 (a, b and c), platelet activation has three phases: activation of GPIIb/IIIa, release reaction with the exposure of p-selectin (CD62p), and platelet procoagulant activity (44).

Tomer et al., revealed that the first event that can be detected in this process is the activation of the membrane integrin $\alpha_{2\beta}\beta_3$ (GPIIb/IIIa, CD41a).

The activated GPIIb/IIIa complex creates a functional receptor for fibrinogen, which provides a link between adjacent platelets to form aggregates. The activation of this receptor can be detected by specific monoclonal antibodies such as PAC-1 and anti-ligand-induced binding site 1 (anti-LIBS1). The former antibody competes with fibrinogen, whereas the latter antibody interacts with a ligand-induced binding site and a receptor-induced binding site (RIBS) on the fibrinogen molecule [Fig 2.1.5 (a)].

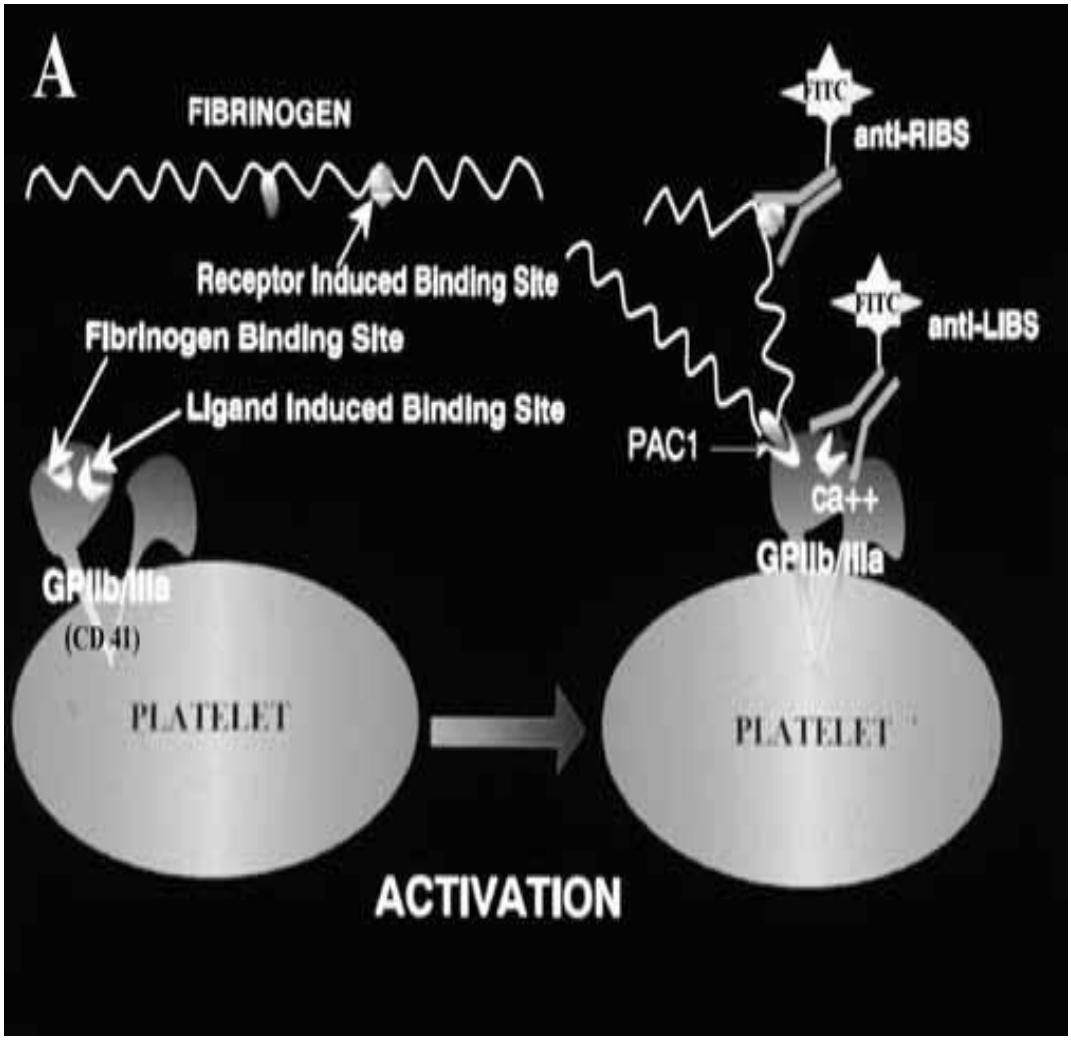



**Figure 2.1.5 (a) Activation of GPIIb/IIIa. The first event is the activation of the membrane integrin $\alpha_{2\beta}\beta_3$ (GPIIb/IIIa, CD41a).**
**(Adopted from Tomer et al. 2004).**

The second event in platelet activation process is the secretion of active mediators, such as ADP, serotonin, and thromboxane, resulting in further recruitment of platelets at the injury site. The secretion process is accompanied by the translocation to the outer membrane of the α-GMP p-selectin (CD62P), which can be detected by a specific monoclonal antibody [Fig. 2.1.5 (b)].

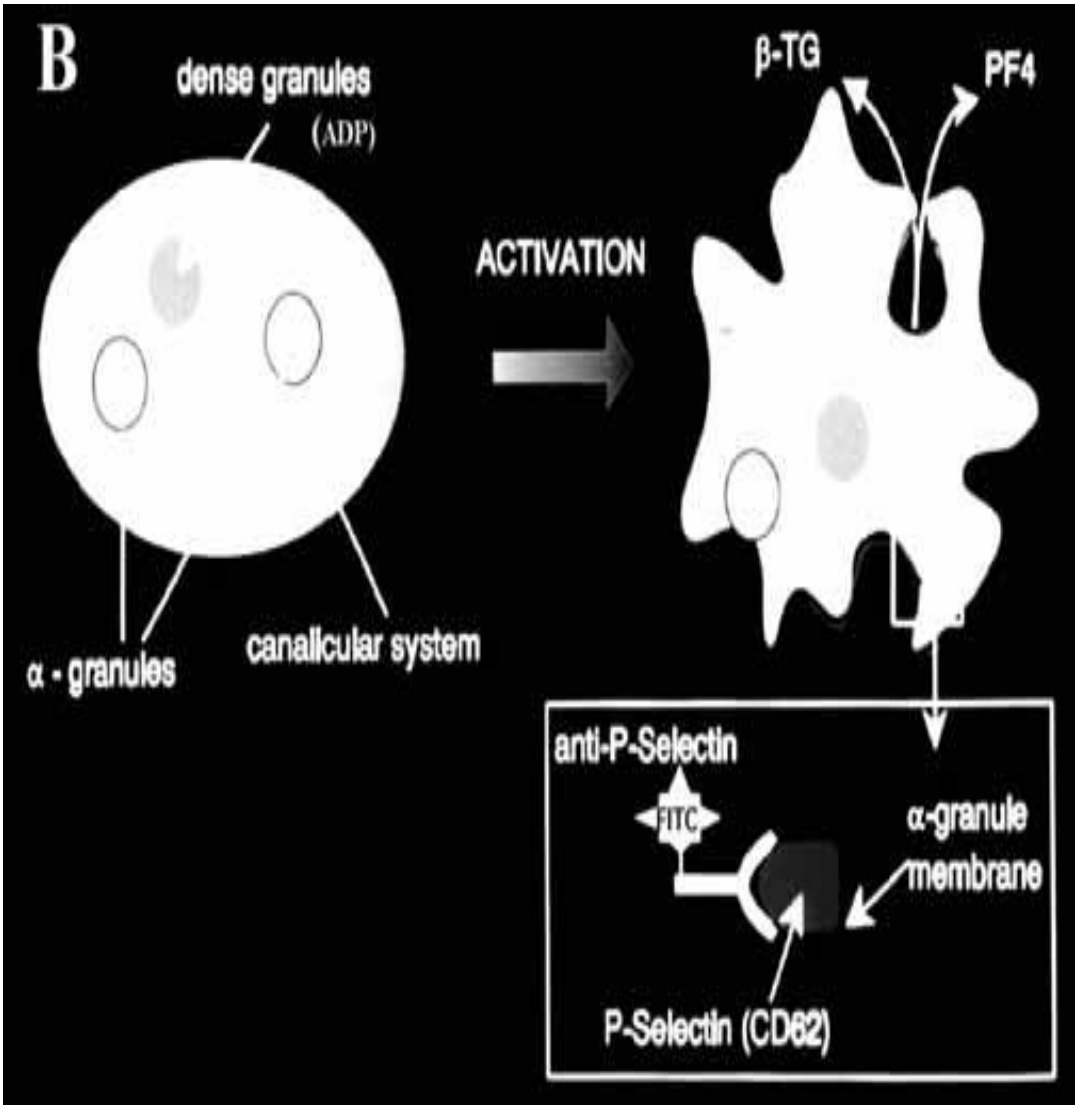



**Figure 2.1.5 (b) Release reactions with the exposure of p-selectin. The second event is the secretion of active mediators such as ADP, serotonin, and thromboxane.**
**(Adopted from Tomer et al. 2004).**

The final event in the platelet activation process is the expression of procoagulant activity via the exposure of membrane anionic phospholipids. The exposed determinants serve as binding sites for the prothrombinase complex (including prothrombin and coagulation factors Xa and Va). This assembly markedly enhances the generation of thrombin, which is a key factor in hemostasis. The membrane procoagulant activity can be detected by the binding of the placental anticoagulant protein annexin V to the anionic phospholipids or of monoclonal antibodies directed against factor Xa or Va, such as V261, on the platelet surface [Fig. 2.1.5 (c)].

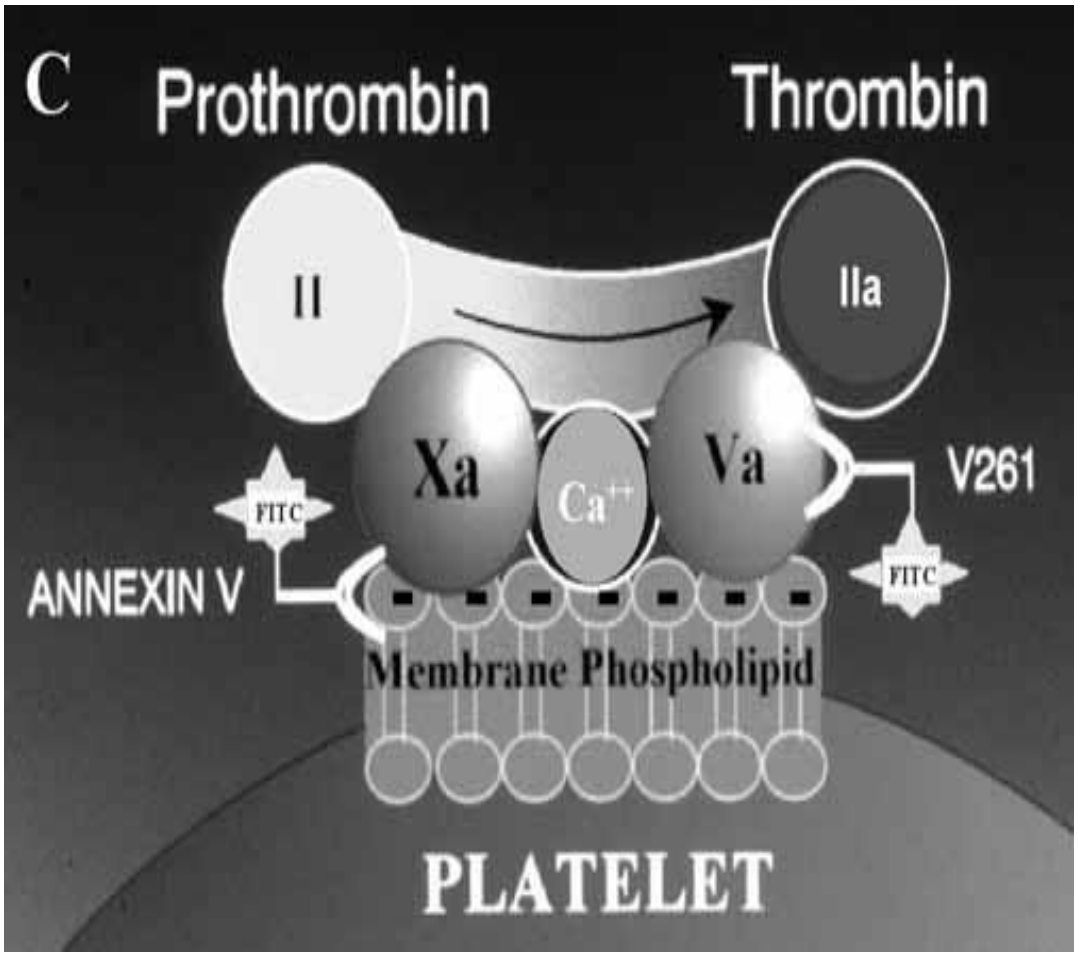



**Figure 2.1.5 (c) Platelet procoagulant activities. The final event is the expression of procoagulant activity via the exposure of membrane anionic phospholipids. (Adopted from Tomer et al. 2004).**

## 2.2 Flow cytometry

### 2.2.1 Introduction

Flow cytometry is a valuable tool in medical research because of its ability and competency to analyze several thousand particles with the multiple physical characteristics of a single cell as they flow in a fluid stream through a beam of light in a short period without physical damage to the cells. Flow cytometry is also a reliable and useful method for the detection of platelet activation markers [Figure 2.2.1 (a)]. Flow cytometric characterization of platelet membrane glycoprotein (GP) expression and procoagulant surface is a sensitive technique for the identification of platelet activation in PCs (53). Monoclonal antibodies against platelet surface GP is use to identify activated platelets and the markers as well as platelet interactions with other cell were detected through flow cytometry. Activated platelets can be reliably detected in whole blood using activation-dependent monoclonal antibodies. Flow cytometry is time-consuming and expensive but it's a valuable research tool (35). Flow cytometry, is a useful technique for the assessment of the degree of platelet activation and the efficacy of antiplatelet therapy in clinical disorders (38 and 54). Moreover, flow cytometry is a useful tool for studying the mechanisms that induce *in vivo* and *in vitro* platelet activation and for detecting ongoing *in vivo* prothrombotic activity (28).

**Figure 2.2.1 (a) Becton Dickinson (BD) FACSCalibur.**
**Flow cytometry is a technology that simultaneously measures and then analyzes multiple physical characteristics of single particles (cells or beads), as they flow in a fluid stream through a beam of light.**

Flow cytometers consist of three important components: fluid system, an optical system, and an electronic system. These three systems work together to give information on relative cell size, internal complexity and fluorescence [Fig. 2.2.1(b)]. All flow cytometers consist of a fluidics system to introduce the cells for interrogation. The role of the fluidics system is to carry the suspended cells out of the sample test tube and into the sensing region of the flow cell. Cells are carried in the sample core stream in single file and measured individually. The function of an optical system is to collects light signals generated as cells pass through the laser and the role of an electronic system is to converts light signals to proportional electronic signals that are then digitized for computer analysis (45).

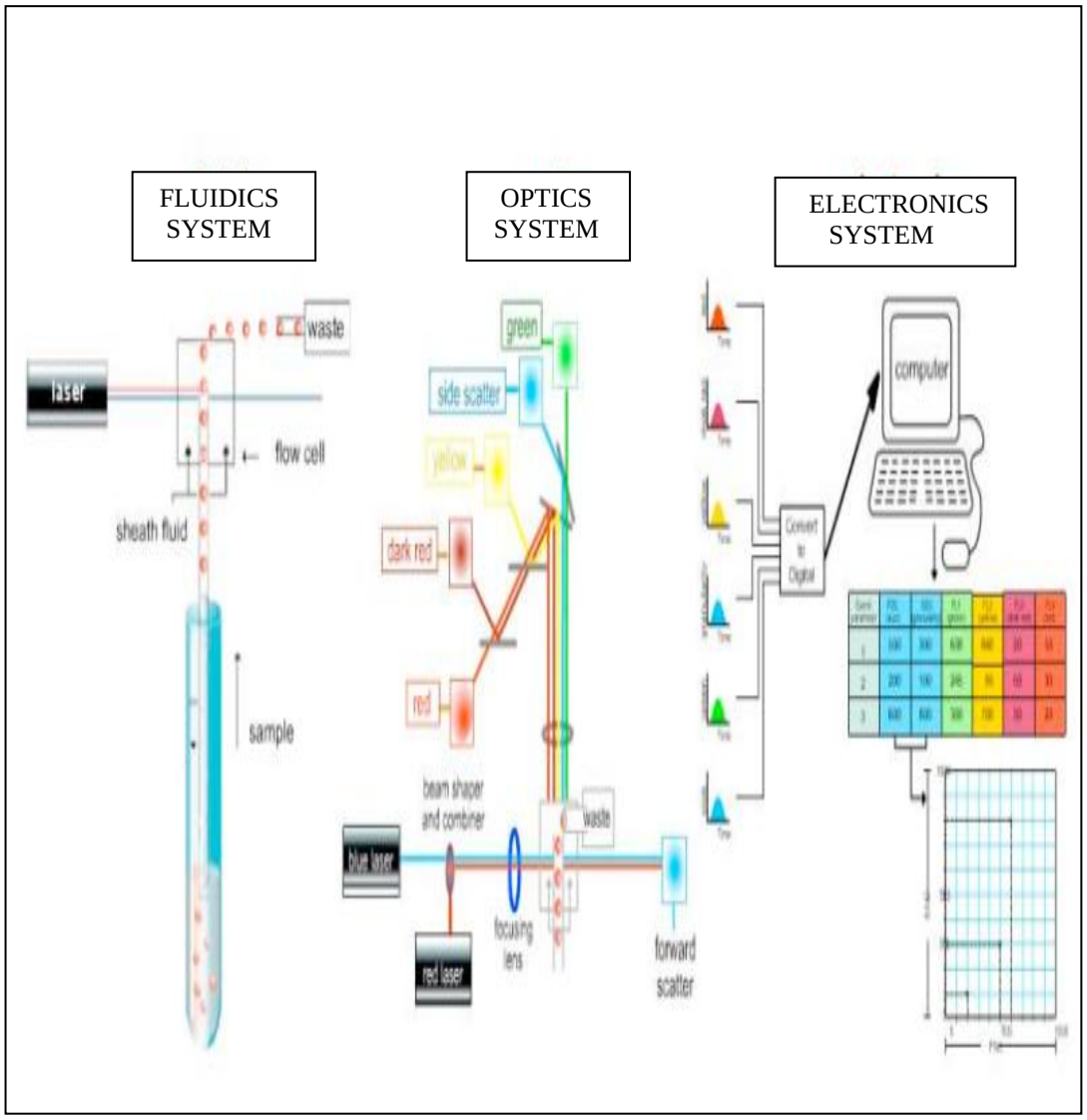



**Figure 2.2.1(b) Diagram showing the fluidic, optical and electronic systems of a flow cytometer. These three systems work together to give information on relative cell size, internal complexity and fluorescence**
**(Adopted from Introduction to Flow cytometry, Becton Dickinson, BD 2008)**