Signaling Pathways of Receptors Involved in Platelet Activation and Shedding of These Receptors in Stored Platelets

Asra Amelirad, Karim Shamsasanjan, Parvin Akbarzadehlahaleh, Davod Pashoutan Sarvari

Abstract
All cells encounter various signals coming from the surrounding environment and they need to receive and respond to these signals in order to perform their functions. Cell surface receptors are responsible for signal transduction. Platelets are blood cells which perform several functions using diverse receptors. Platelet concentrate is one of the most consumed blood products. However, due to the short lifespan of the platelets and platelet damage during storage, we face shortage of platelet products. One of the damages that platelets undergo during storage is the loss of surface receptors. Since cell surface receptors are responsible for all cell functions, the loss of platelet receptors reduces the quality of platelet products. In this study, we reviewed the important receptors involved in platelet activation and their associated signaling pathways. We also looked at the platelet receptors that shed during storage and the causes of this incident. We found that GPIbα, P-selectin, CD40 and GPVI are platelet receptors that fall during platelet storage at room temperature. Considering that GPVI and GPIbα are the most important receptors which involve in platelet activation, their shedding can cause decrease in platelet activation after transfusion and decrease thrombus consistence. Shear stress and platelet contact with the container wall are among the mechanisms discussed in this process, but studies in this area have to be continued.

Introduction
Platelets are the smallest blood cells (~2.5 μm) and human adults approximately have 1 trillion platelets in circulation that are turned over every 8–10 days. They are metabolic active cells, and are seen in numerous functional organelles, highly organized cytoskeleton, vast array of receptors, and many secretory granules. Platelets are formed from mature megakaryocytes and arisen from the long tube-like developed cytoplasmic extension called proplatelets in particular platelets develop process. After release of platelets, the megakaryocyte nucleus, its envelope, and its neighbor cytoplasm, usually remains in the marrow, and finally, phagocytized by macrophages. Megakaryocytes are derived from hematopoietic stem cells (HSCs) in bone marrow. Bone marrow microenvironment contains cellular and acellular compartments. Cellular compartment contains HSCs, mesenchymal stem cells (MSCs), and some other kinds of stromal cells. On the other hand, acellular compartment includes scaffold proteins known as extra cellular matrix. HSCs are able to produce various blood cells. MSCs which recognized as main components of stromal cell niches support HSCs homing, proliferation, self-renewal, and differentiation in the bone marrow. Cheng et al demonstrated under co-culture conditions, MSCs are able to support megakaryocyte differentiation and platelet formation from CD34b HSC. Although platelets are well-known because of their essential role in homeostasis and thrombus formation, they have many different functions. Platelets release pro-inflammatory and anti-inflammatory, angiogenic factors, and microparticles into the circulation and play serious roles in the host defense, inflammation, angiogenesis, tumor growth and metastasis. Platelet receptors are responsible for all these functions and their density and affinity controls the cell function directly. Platelets are unable to perform these functions in the absence of their receptors. Disorder of platelet receptors were first described by Glanzmann in 1918 and Bernard & Soulier in 1948. After the recognition of these disorders, structure and functions of platelet receptors were extensively studied. In the loss of ligand binding and in shear stress status, cell surface receptors are down regulated. One of the receptors down regulation mechanisms is ectodomain shedding. In this mechanism, protein will break in a near location to external surface of membrane layer. One of the conditions in which platelets experience shear stresses in storage conditions. In such situation, some of the platelet...
Platelet receptor signaling and shedding

Table 1. Platelet receptor signaling with a focus on signaling pathways associated with platelet activation

<table>
<thead>
<tr>
<th>Receptor family</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integrins</td>
<td>α5β11, α5β111, αIIbβ312</td>
</tr>
<tr>
<td>Lucine rich repeat family</td>
<td>GP Ib-IX-V2</td>
</tr>
<tr>
<td>Selectins</td>
<td>CD62P1, CELEC212</td>
</tr>
<tr>
<td>Tetraspanins</td>
<td>CD6314</td>
</tr>
<tr>
<td>Transmembrane receptors</td>
<td>P2Y1, and P2Y12</td>
</tr>
<tr>
<td>Prostaglandin receptors</td>
<td>Prostacyclin receptors, thromboxane receptors</td>
</tr>
<tr>
<td>Lipid receptors</td>
<td>PAF receptors40</td>
</tr>
<tr>
<td>Immunoglobulin superfamily receptors</td>
<td>GPVI, CD3260</td>
</tr>
<tr>
<td>Tyrosine kinase receptors</td>
<td>Thrombopeitin receptors41</td>
</tr>
<tr>
<td>Miscellaneous platelet membrane receptors</td>
<td>Serotonin receptors42</td>
</tr>
</tbody>
</table>

Platelet receptor signaling

Platelets perform their functions inside the vessels by using 3 types of signals: inhibitory, activation and negative feedback.23

Inhibitory signals

Inhibitory signals allow platelets to circulate in a resting state. Platelets are activated even in the absence of activation signals. In healthy vessels, endothelium expresses fundamental forms of nitric oxide synthase (NOSIII) and cyclo-oxygenase-1 (COX-1), which produce the vasoactive hormones NO and prostacyclin (PGI2), respectively. Both NO and PGI2 are co-released by endothelial cells and act in synergy to inhibit platelet activation, thereby limiting thrombosis. Nitric oxide activates soluble guanylyl cyclase, present in the cytosol, causing an increase in intracellular cGMP from GTP. An immediate consequence of increasing cGMP is direct activation of protein kinase g (PKg). The activated PKg reduces the intracellular calcium and cell activation by phosphorylation of several targets. PGI2 is able to bind with the prostacyclin receptor (IP) on the surface of platelets. Activation of the IP receptor on the surface of platelets induces production of cAMP, causing activation of PKa, the subsequent inhibition of several pathways including PKc activation, calcium release, and platelet inhibition. In addition to the NO and PGI2, CD39 is another mechanism of inhibitory function in the vascular endothelium. In platelet membrane, CD39 hydrolyze endothelium and red cells secreted ADP to AMP and adenosine. Adenosine activates the Gs-coupled adenosine receptor, and leads to inhibition of platelet through elevation of cAMP.23-28

Activatory signals

Among the various platelet receptors that are known, 2 groups of platelets receptors are involved in platelets activation: adhesion receptors and G protein-coupled receptors. Glycoprotein (GP) Iб-IX-V, GP Ia/IIa and GPV1 are 3 important adhesion receptors, which play a role in platelet activation.29 In the following, we explain the pathways for platelet activation (Table 1).

VWF/GP Ib-IX mediated platelet activation

Von will brand factor is a multi-subunit glycoprotein that circulates in plasma. It is synthesized by endothelial cells and megakaryocytes, that released through a regulated pathway after storage in endothelial Weibel-Palade bodies and platelet α granules.43-44 The mature von Willebrand factor (VWF) subunit has 2,050 residues with multiple A-, B-, C-, and D-type domains. The A1 domain contains binding site for platelet GPIba,45-46 GPIb-IX-V is composed by 4 distinct trans membrane proteins: 2 chains of GPIba (135 kDa), 2 GPIbβ (26 kDa), 2 GPIX (20 kDa) and 1 GPV (82 kDa). These proteins are encoded by 4 different genes and belong to lucine rich family that map to chromosomes 17q12 (GPIba), 22q11.2 (GPIbβ), 3q29 (GP5) and 3q21 (GP9), respectively. GPIb-IX-V expresses on the platelet membrane exclusively. There are approximately, 25,000 copies of this receptor per platelet. When blood vessels are disrupted, circulating platelets adhere to exposed subendothelial surfaces through interactions of platelet GPIba with VWF, which is immobilized on collagen fibers. This is the first step in a cascade of adhesion and signaling events that produce a hemostatic plug at the injured site. Platelets tether to and roll on immobilized VWF, but do not adhere firmly.37-50 The interaction between VWF and GPIb-IX-V not only mediates transient platelet adhesion but also initiates a signaling cascade result in platelet integrin αIIbβ3 activation and outcome stable platelet adhesion, spreading, and aggregation.51,52 Platelet activation via VWF/GPIb-IX occurs only in high flow rates. Several intracellular signaling molecules and pathways in GPIb-IX-mediated platelet activation have been included: the phosphatidyl inositol 3-kinase (PI3-kinase) protein kinase b (AKT) pathway, the mitogen-activated protein kinase (MAPK) pathways, and the FCRγ-SYK/PLCγ2 pathway. Nevertheless, the detailed mechanism of this process remains unclear. There have been conflicted reports regarding to the role of spleen tyrosine kinase (SYK) in GPIb signaling.53,54

Collagen/GPVI mediated platelet activation

Collagens are the most numerous proteins in the subendothelial extracellular matrix and in addition are essential in platelet adherence and platelet plug...
CLEC-2/podoplanin mediate platelet activation

CLEC-2 is a C-type lectin-like type ii transmembrane receptor, in platelet binding of rhodocytin (exogenous ligand) and podoplanin (endogenous ligand) to CLEC-2 in platelets surface triggers a novel platelet-signaling pathway. Similar to collagen receptor glycoprotein (GP) VI/FCRγ-chain complex, CLEC2 ligand binding leads to tyrosine phosphorylation in the cytoplasmic tail of CLEC-2, which promotes the binding of SYK, subsequent activation of PLCγ2, and platelet activation and aggregation. Podoplanin is expressed on the surface of defined types of cancer cells and various normal cells such as kidney podocytes, type I lung alveolar cells, fibroblastic reticular cells in lymph nodes, and lymphatic endothelial cells. CLEC-2-deficient platelets displayed normal adhesion under flow conditions, but further thrombus formation was severely impaired in vitro and in vivo. Considering that, Podoplanin is expressed on the surface of tumor cells. Therefore, platelet activation by CLEC-2/podoplanin interaction facilitates tumor metastasis.

Platelet activation and signaling mediated by G protein-coupled receptors

Platelet signaling begins with activation of platelet receptors by agonists such as PAF, collagen, thrombin, ADP, TxA2 and epinephrine. Except collagen, which is described and acts as the first line of hemostasis in platelets, other agonists work through one or more members of G-coupled receptor superfamilies. Through the activation of G protein–mediated signaling pathways, they can further increase their own formation and release; thus they acting as positive-feedback mediators that amplify the initial signals to ensure the rapid activation and recruitment of platelets into a growing plaque. G protein–coupled receptors (GPCRs) compose one of the largest families of membrane proteins involved in intracellular signaling. All GPCRs share a common serpentine structure of seven transmembrane-spanning domains, with an extracellular N-terminus and an intracellular C-terminus. GPCRs are so-called because they are physically associated with heterotrimeric G proteins. Each G protein is composed of α, β, and γ subunit. After receptor ligation α subunit dissociates from the βγ subunits, which allows exposure of surfaces on both α and βγ subunits for interaction with effector proteins. G proteins are generally classified into 4 families: Gq, Gi/Go/Gz, Gq/G11, and G12/13. Each of them is coupled to selective receptors and downstream effectors. Platelet activation via G protein-coupled receptors involves 3 major G protein-mediated signaling pathways that are initiated by the activation of the G proteins, Gq, Gi, and G. Although, in the absence of Gq-, Gi-, or G,-mediated signaling, some platelet activation can occur, efficient activation of platelets in vitro and in vivo requires all 3 G protein–mediated signaling pathways. TP (thromboxane a2 receptor), PAR3, PAR4 and PAR1 (thrombin receptors) which are coupled to Gq, and G12/13, P2Y1 (ADP receptor) are coupled to Gq, and P2Y12 (ADP receptor) are coupled to G, Gq transmit cellular signals commonly through its interaction and activation of PLCβ. Gq signaling is necessary for GPCR-stimulated platelet granule secretion, integrin activation, and consequent platelet aggregation. Gq signaling is necessary but insufficient for platelet aggregation and induced by ADP and optimal platelet response induced by TXA, or low dose thrombin. Gq also needs Gi-coupled to carry out these activities. Ga13 knockout platelets show reduction in granule secretion and unstable platelet aggregation induced by TxA2 analog U46619. In addition, platelet aggregation induced by low dose thrombin in Ga13 knockout platelets is decrease. All GPCR activity in the platelets depends on the G protein, as the knock-out of individual G proteins has been sufficient to disrupt platelet responses to receptor agonists (Table 2).

Negative feedback signals

Maintenance of the proper balance between platelet activation and platelet inhibition is critical because disruption of this balance can cause thrombotic or bleeding disorders, respectively. Following initiation of platelet
activation and shedding, to control excess clot production, a number of negative regulator signals prevent the activation of more platelets. A number of endogenous inhibitory mechanisms are inhibitory receptors on the surface of platelets e.g., platelet endothelial cell adhesion molecule-1. Intracellular inhibitory receptors e.g., Liver X Receptor α and β and Emerging inhibitory pathways e.g., semaphorin 3A and junctional adhesion molecule-a.85-89

Key events in platelet activation
Platelet activation through agonists-GPCRs Signaling pathways induces platelet-shape change, degranulation, and integrin αIIbβ3-mediated aggregation.27,90 In this section, we describe the Integrin activation.

Integrin activation
Integrins are a widely family of heterodimeric transmembrane receptors that are connecting extracellular ligands to mediate cell adhesion αβ1 and intracellular signaling pathways.91 Integrins composed by α- and β-subunits, which are non-covalently linked to each other. Both subunits traverse the plasma membrane and terminate short cytoplasmic domains.92 αIIbβ3 (fibrinogen receptor), α5β1 (vitronectin receptor), (collagen receptor), α6β1 (fibrinogen receptor), and α9β1 (laminin receptor) are expressed in platelet. Among these integrins, αIIbβ3 is the most abundant integrin in the platelets. αIIbβ3 is normally kept in a low affinity state in circulating platelets, but transforms into a high affinity state following platelet activation.93 This transformation allows αIIbβ3 to bind Arg-Gly-Asp (RGD) sequence in their ligands. Activation of αIIbβ3 is tightly regulated through a process termed ‘inside-out signaling’. It has been shown that inside-out signaling requires the binding of talin and kindlins to the cytoplasmic domain of β3. In addition, recent studies suggest that CalDAG-GEF1 and its downstream target, Rap1, plays an important role in inside-out signaling.46,91 Conversely, the interaction between integrins and their various ligands (fibrinogen, VWF, vitronectin and fibronectin) induces outside-in signals across the membrane that allows αIIbβ3 clustering, e.g. during platelet aggregation. One of the earliest events occurring during integrin outside-in signaling is the tyrosine phosphorylation of specific substrates. The Src family of kinases (SKFs) has a dominant effect in these phosphorylation events. Src was originally proposed to be constitutively associated with the β3 integrin C-terminal tail in an inactive conformation of resting platelets via its SH3 domain. Upon ligand binding to αIIbβ3 and integrin clustering, protein phosphatases relieve the inhibitory Src phosphorylation with dissociation of C terminal Src kinase from β3, permitting Src activation. Src activation results in activation of the tyrosine kinase Syk. Syk substrates contain important outside-in effectors, including the RhoGEFs Vav1 and Vav3, and the SH2- containing leukocyte protein of 76 kDa (SLP-76). In addition, SKFs phosphorylate a host of signaling and cytoskeletal-associated proteins in platelets, including phospholipase Cγ2 (PLCγ2), focal adhesion kinase (FAK), and degranulation promoting adapter protein (ADAP), resulting recruitment and/or activation of these proteins.94 Activated FAK modulates the activity of a broad range of downstream signaling proteins, including PI3- Kinases, PLCγ as well as a number of small GTPases such as Ras, Rac, and Rho.95 ADAP interactions with talin and kindlin promote platelet integrin αIIbβ3 activation and stable fibrinogen binding.96 In general, inside-out signaling activates the ligand binding function of integrins and outside-in signaling mediates cellular responses induced by ligand binding to integrins leading to cell spreading, granule secretion, retraction, migration, and proliferation.97

Platelet receptor shedding
Role of shedding in platelet function
Cells membrane receptors are common beginners in cell signaling. Therefore, receptors density and affinity controls the cell function.14 However, there are extensive information in literature about activation of platelet receptor.19 Nevertheless, downregulation of these receptors have not been recognized properly. One of the external cells receptors downregulation mechanisms is ectodomain shedding. In this mechanism, protein breaks near the external surface of membrane layer and the isolated ectodomain will release into the plasma.19,98,99 Disjunction part can be operational or use as a private biomarker of platelet.100 Ectodomain shedding can be

---

Table 2. G protein-coupled receptors on human platelets

<table>
<thead>
<tr>
<th>G protein</th>
<th>Agonist</th>
<th>Receptor</th>
<th>Effector/signaling</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gq</td>
<td>Thrombin</td>
<td>PAR1</td>
<td>Phospholipase C/Ca++ release, PKC activation</td>
<td>73,72</td>
</tr>
<tr>
<td>Gq</td>
<td>Thrombin</td>
<td>PAR4</td>
<td>Phospholipase C/Ca++ release, PKC activation</td>
<td>73-75</td>
</tr>
<tr>
<td>Gq</td>
<td>ADP</td>
<td>P2Y1</td>
<td>Phospholipase C Ca++ release, PKC activation</td>
<td>76,77</td>
</tr>
<tr>
<td>Gq</td>
<td>TxA2</td>
<td>TP</td>
<td>Phospholipase C, Ca++ release, PKC activation</td>
<td>49</td>
</tr>
<tr>
<td>G13</td>
<td>Thrombin</td>
<td>PAR1</td>
<td>Rho activation, actin remodeling</td>
<td>74</td>
</tr>
<tr>
<td>G13</td>
<td>TxA2</td>
<td>TP</td>
<td>Rho activation, actin remodeling</td>
<td>78-79</td>
</tr>
<tr>
<td>G2</td>
<td>ADP</td>
<td>P2Y12</td>
<td>↓ cAMP, PI3Kγ activation</td>
<td>80-81</td>
</tr>
<tr>
<td>Gs</td>
<td>PGI2</td>
<td>IP</td>
<td>↑ cAMP</td>
<td>82</td>
</tr>
<tr>
<td>Gs</td>
<td>Epinephrine</td>
<td>α2, adrenergic</td>
<td>↓ cAMP</td>
<td>75-81</td>
</tr>
</tbody>
</table>

PAR1, Protease activated receptor; TP, Thromboxane receptor; IP, Prostacyclin receptor; PI3, Phosphoinositide 3-kinase
a useful mechanism in abnucleus cells such as platelets because in these cells control of receptor surface through regulation of gene expression has an inconspicuous role.\textsuperscript{18,100} Ectodomain shedding can have other roles in addition to controlling levels of superficial proteins. For instance, the capacity of platelets to form filopodia and lamellipodia and spread on a VWF and/or collagen matrix requires the dynamic breaking of existing receptor/matrix ligand bonds and formation of new receptor/matrix ligand interactions at the tips of filopodia or the spreading lamellipodial edge. One mechanism for how this could occur is through receptor ectodomain shedding.\textsuperscript{19} According to the several investigations, 69 platelet membrane proteins have been identified in activated protein supernatant which are prone for shedding. It has been observed that shedding occurs in 12 membrane proteins out of these 69 proteins including semaphorin7a, CD84, GPV, amyloid beta A4, GPIba, TLT-1, P-selectin, JAMA-1, CD40-L, semaphorin 4D, PECAM and GPVI. There are limited surveys on the remaining 57 membrane proteins.\textsuperscript{101-111}

**Shedding in stored platelet Receptor**

Platelet concentrates (PCs) are the most vulnerable blood products with the shortest shelf life.\textsuperscript{111,112} In recent years, requirement to platelet products is being increased due to increment of the patients who are being treated in bone marrow suppression. At the moment platelet are not only being used to control or prevent bleeding but also being increasingly used as a source of growth factors in tissue repair, wound redressing, and skin rejuvenation.\textsuperscript{113} Using autologous platelet rich products including platelet lysate, platelet rich plasma and platelet rich fibrin for MSCs expansion become more general employing autologous platelet rich products for MSCs expansion is a convenient, non-toxic, safe and cheap therapeutic method that promotes using MSCs for cell therapy. These 3 products contain a variety of growth factors including platelet-derived growth factor, fibroblast growth factor, insulin-like growth factor, transforming growth factor, platelet factor 4, and platelet-derived epidermal growth factor. These growth factors enhance and accelerate MSC.\textsuperscript{114} however, the short half-life of platelets has caused these products have the most wasting amounts among blood products; for example, in Canada platelet’s half-life is about 5 days and 30% of them will be out of reach.\textsuperscript{115} PSL (platelet storage lesion) is one of the main reasons of platelets short half-life. PSL explains structural and functional changes in platelets from bloodletting until the platelet transfusion.\textsuperscript{115} Shedding of platelets surface receptor during storage is one of the PSL. The receptor shedding has an obvious difference with other processes such as the losing of receptor surface through internationalization, release of micro particles, and secretion process. In the secretion process, proteins from platelet storage granules releases.\textsuperscript{110,118} PSL accelerates clearance of platelet after transfusion and has connections with various elements including media, agitation method, bag materials, storage temperature, and so on. Unlike the erythrocyte, platelets are kept between 20-24°C.\textsuperscript{117,119-123} However, keeping the platelets in this range of temperature increases the percentage of bacterial infection and decreases hemostatic activity. Refrigerated platelets do not have these problems, however they eliminate from blood circulation shortly after injection. Desialylation of platelets is considered as one of the mechanisms for rapid elimination of refrigerated platelets that purge them by liver macrophage or hepatocyte through recognition of exposed glycan. Notably, desialylated GPIba also shows increased susceptibility toward ADAM17-mediated metalloproteolysis.\textsuperscript{113,124}

Beside the platelet activation, there are some other materials such as NEM (N-ethylmaleimide), W7 (N-6-
Platelet receptor signaling and shedding

Aminoheptyl-5-chloro-1-naphthalenesulphonamide), PMA (PKc activator phorbol-12-myristate-13-acetate), ASA (acetylsalicylic acid), and CCCP (mitochondrial-targeting compound carboxyl cyanide 3-chlorophenylhydrazone) which can induce GPIba shedding. This shedding cause production of a part called glycolacin, a soluble n-terminal 130 kDa ectodomain fragment, which is further processed in the plasma by proteases. Glycolacin is a private biomarker at PSL and refrigerated platelets.

Since shedding is a proteolytic reaction that is dependent on enzyme, controlling of enzyme or substrate is inevitable for restraining shedding. In the case of controlling enzymes for GPIba shedding, injection of GM6001 or MAPK p38 (which control ADAM17 enzyme) in the storage platelets decreases shedding of this glycoprotein and improves the result of platelet transfusion. Moreover, monoclonal antibody 5G6, which acts as a substrate controller, connects to GPIba cleavage and prevents enzymes to connect to GPIba. Thus, there is a close connection between ectodomain shedding and clearance of injected platelets. The reason can be described as follows: decrease of GPIba surface affected by shedding in storage platelets is effective on adhesion strength of injected platelets under the increase of venous rate share. These platelets have lower functional power and will be eliminated from blood circulation rapidly. GPV is another glycoprotein that experiences shedding during refrigerating of platelets. It seems that GPV shedding is controlled by both ADAM17 and ADAM10, and it is a dependent mechanism on thrombin. On the other hand, GPIba shedding only depends on ADAM17. The other platelet surface glycoproteins including GPVI, GPIbβ, GPIX and glycoprotein IIb/IIIa are not affected by refrigerated storage.

GPIba shedding is also occurred in storage platelets in room temperature. In room temperature, P-selectin, CD40 and GPVI are also involved in surface deduction by shedding mechanism beside GPIba. The amount of expression and shedding GPIba and GPVI have a close correlation, and at the same time, there is a negative connection between these 2 glycoproteins with P-selectin and CD40 expression measure. However, all of their sheddings are increased during storage. Similar to GPIba, signaling depended on GPV has a significant role in cross-linking of receptor. This receptor shedding decreases cell signaling time and causes decreasing in platelet activation and secretion. In addition, these glycoproteins’ shedding decrease thrombus consistence and make an easy establishment of thromboembolus.

GPVI shedding is more seriously regulated than GPIba, and different shedding mechanisms may be involved GPVI shedding is induced by GPVI ligands such as collagen, convulxin and CRP. However, under conditions where GPVI is completely lost, GPIba is detected on platelets. This difference in regulation reflects the fact that GPVI directly binds to calmodulin at the cytoplasmic domain whereas the cytoplasmic domain of GPIba does not bind to calmodulin. Levels of GPVI shedding are higher in stored platelets in compared with non-stored samples activated under the effect of agonists such as Calcium Ionophore. Either interplaying of platelet surface with the container walls during storage or induced shear stress under long-period agitation might play role in the excessive shedding of GPVI during platelet storage. Shedding of 2 key platelet receptors, glycoprotein (GP) Ibα and GPVI, after exposed to the non-physiological high shear stress environment exists in blood contacting medical devices and stenotic blood vessels has also been reported.

Conclusion

All cells are constantly exposed to a variety of extracellular signals. The cells surface receptors are responsible for responding to these signals and, in this way, they control all cell functions. In this paper, we first describe those receptors and signaling pathways, which lead to the activation of platelets, and then explain the ectodomain shedding, which is one of the methods for controlling platelet surface receptors. Two main groups of receptors play roles in the activation of platelets: adhesion receptors and G protein-coupled receptors. However, the initial signaling mechanisms of these 2 receptor groups are different. They ultimately converge into common intracellular signaling events. In particular, almost all agonists induce activation of PLC. The cells use different mechanisms to control the level of their receptors. One of these mechanisms is ectodomain shedding which can be a useful mechanism in no-nucleus cells such as platelets because in these cells control of receptor surface through regulation of gene expression has an inconspicuous role. Regularly, shedding has been occurred after platelets activation, but it has been reported that it has also happened in circulating platelets. However, the platelet receptor shedding does not occur only in vivo. Several studies have suggested the presence of platelet receptors shedding in shear stress conditions, such as storage condition. GPIba, P-selectin, CD40 and GPVI are induced by ectodomain shedding mechanism in storage bag. As we know, GPVII and GPIba are the most important receptors, which involved in platelet activation. Therefore, their shedding can reduce cell signaling time and finally, cause decrease platelet activation and secretion. Moreover, these glycoproteins’ shedding decreases thrombus consistence and makes an easy establishment of thromboembolus. In addition to shear stress, the platelet contact with the container wall, as another possible cause of this mechanism, has been raised, but studies in this area have to be continued. Thus, finding the main reasons for platelet shedding, which is one of the PSL cases in platelet storage conditions, helps to find a solution to prevent this mechanism or reduce its rate and speed, and ultimately to improve the quality of the storage platelets. Based on the studies reviewed here, there are 2 main possibilities for improving shedding. First method is change the agitation method and its revolution speed.
and second is using different platelet storage bag. Thus, investigation the effects of these 2 mode on shedding decrease is an interesting subject for further studies.

**Ethical Issues**
Not applicable.

**Conflict of Interest**
The authors declare that they have no conflict of interest.

**Acknowledgments**
The authors would like to thank Mr. Omid Amelirad (Department of Mechanical Engineering, Sharif University of Technology, Tehran, Iran) and Ms. Sarah Aqmasheh (Department of Immunology, Tabriz University of Medical Science, Tabriz, Iran) for helping us in editing the draft article.

**References**


Platelet receptor signaling and shedding


characterization of the platelet ADP receptor targeted by thienopyridine antithrombotic drugs. J Clin Invest 2001;107(12):1591-8. doi: 10.1172/JCI12242
Platelet receptor signaling and shedding

10.1016/j.smedcb.2008.11.002


