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REVIEW

TRANSFUSION

# Platelet dysfunction after trauma: From mechanisms to targeted treatment

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## 1 | INTRODUCTION

Trauma-induced bleeding is a leading cause of preventable mortality worldwide.[1–3] Severely injured bleeding trauma patients frequently present with trauma-induced coagulopathy (TIC).[4,5] Platelets are important in hemostatic response but can rapidly become dysfunctional in these patients, which contributes to TIC, exsanguination, and early mortality.[5–13] Characteristically, circulating platelets display increased levels of surface activation markers and have a reduced ability to adhere and aggregate ex vivo, despite normal counts.[8,11–13] However, lack of a clear boundary between platelet function and dysfunction hampers development of specific diagnostic criteria.[14] Besides their role in hemostasis, platelets act as orchestrators of the initial immune response, which could contribute to immunothrombosis, organ dysfunction and late mortality.[11,15]

An appreciation of the "normal" adaptive response of platelets to local injury is necessary to understand the dysfunctional platelet response seen in TIC. Moreover, it forms the basis for potential targeted treatments to improve outcomes of severely injured trauma patients.

In this narrative review the aims are to: (1) describe "normal" platelet function following local tissue injury, (2) describe the characteristics of platelet dysfunction after trauma, (3) outline potential mechanisms, and (4) summarize current and novel treatment strategies for early and late trauma-induced platelet dysfunction.

## 2 | THE "RESTING" PLATELET AND THE VASCULAR WALL

Resting platelets are disc-shaped, anucleate blood cells, derived from megakaryocytes.[16] Platelets have a short lifespan (7–10 days), indicating a continuous production to maintain blood counts between 150–350 $\times 10^9$ per liter.[17,18] The exterior surface of resting platelets contains a layer of glycoproteins and lipids (platelet glycocalyx) (Table 1, Figure 1A).[19,20] The negative charge of the platelet glycocalyx prevents spontaneous aggregation with surrounding cells.[19,21] Furthermore, it facilitates endocytosis of plasma proteins, which are stored in platelet granules.[22] Platelet contain alpha granules, dense granules and lysosomes.[23–25] Alpha granules contain adhesion molecules (e.g., P-selectin, von Willebrand factor [VWF], fibrinogen), (anti)coagulation factors (e.g., factor V, antithrombin, protease-nexin-1), fibrinolytic factors (e.g., plasminogen activator inhibitor-1 [PAI-1]) and immune molecules (e.g., cytokines).[25,26] Dense granules contain polyphosphates (PolyP), amines (e.g., serotonin), nucleotides (e.g., ADP/ATP) and cations (e.g., $Ca^{2+}$, $K^+$, $Mg^{2+}$). Lysosomes contain protein degrading enzymes (e.g., collagenase), carbohydrate degrading enzymes and phosphatases.[25]

Endothelial cells also have a glycocalyx, which has important thromboresistant and anti-coagulant functions. Endothelial cells release ectonucleotidases, which break down ADP and ATP (CD39-CD73-adenosine pathway), preventing ADP-induced platelet activation via the $P2Y_1/P2Y_{12}$ receptor.[27] Endothelial cells also release prostacyclin ($PGI_2$) and nitric oxide (NO) which further inhibits platelet activation (Figure 1A).[28,29]

**TABLE 1** Platelet receptors and their ligand(s)

| Receptor | Main ligand(s) |
|---|---|
| Agonist receptors | |
| Protease activated receptor (PAR) | Thrombin |
| $\alpha_2$-adrenergic receptor ($\alpha_2$-AG) | Epinephrine |
| $5\text{-HT}_2$ | Serotonin |
| Thromboxane receptor (TP) | Thromboxane $A_2$ ($TXA_2$) |
| $P2Y_1/P2Y_{12}$ | ADP |
| GPVI | Collagen |
| Adhesion and aggregation receptors | |
| GPIb-IX-V | Von Willebrand factor (VWF) |
| GPIaIIa | Collagen |
| αVβ1 | Fibronectin |
| CD62P (P-selectin) | P-selectin glycoprotein ligand-1 (PSGL-1) |
| GPIIbIIIa | Fibrinogen |
| Pattern recognition receptors (PRR) | |
| Toll like receptors (TLRs) | DNA, histones, high-mobility group box 1 (HMGB1), S100-proteins |
| Receptor for advanced glycation end products (RAGE) | |

## 3 | LOCAL PLATELET RESPONSE TO VASCULAR INJURY

Inherent to local tissue injury is the disruption of the endothelial wall, exposing subendothelial structures to blood components (Figure 1B). Endothelial activation causes upregulation and release of adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1), VWF, platelet agonists and damage associated molecular patterns (DAMPs).[28] Under high shear conditions, platelets cannot readily bind to endothelial cells and subendothelial structures. Therefore, immobilized VWF on subendothelial collagen or VWF multimers on endothelial cells are needed to facilitate platelet binding (through GPIbα-IX-V).[30,31] More secure binding of platelets with endothelial cells occurs through GPIIbIIIa via fibrinogen which is bound to ICAM-1.[32] Platelets securely bind subendothelial structures through GPIaIIa (collagen) and αVβ1 (fibronectin) (Figure 1B).

Once activated, platelets transform from disc- to spherical shape, out of which extrusions grow (lamellipodia), increasing the surface area of the platelet membrane.[33] Intracellular calcium levels in platelets can rise up to 100-fold, causing activation.[34,35] An example is the calcium-dependent activation of the GPIIbIIIa complex, which has binding sites for fibrinogen and other extracellular proteins.[36] Upon activation, platelet granules fuse with the outer membrane, releasing their contents, amplifying coagulation and inflammation. Platelet–platelet interaction (i.e., aggregation) occurs primarily through GPIIbIIIa binding to fibrinogen.[36]

Platelets can be functionally classified into subpopulations, which differ in their contribution to clot formation.[37,38] In some platelets, cytoskeletal shape change is accompanied by phosphatidylserine (PS) mobilization to the outer membrane, which results in a procoagulant membrane surface.[39] Upon strong agonistic stimulation, PS-exposing platelets can transform into "coated" platelets, which express procoagulant proteins (e.g., VWF) with high fibrinogen-binding capacity.[40,41] The decrease in cytoskeletal proteins can also lead to transformation into PS-positive, balloon-like platelets as well as the formation of platelet extracellular vesicles (EVs).[42,43] Platelet

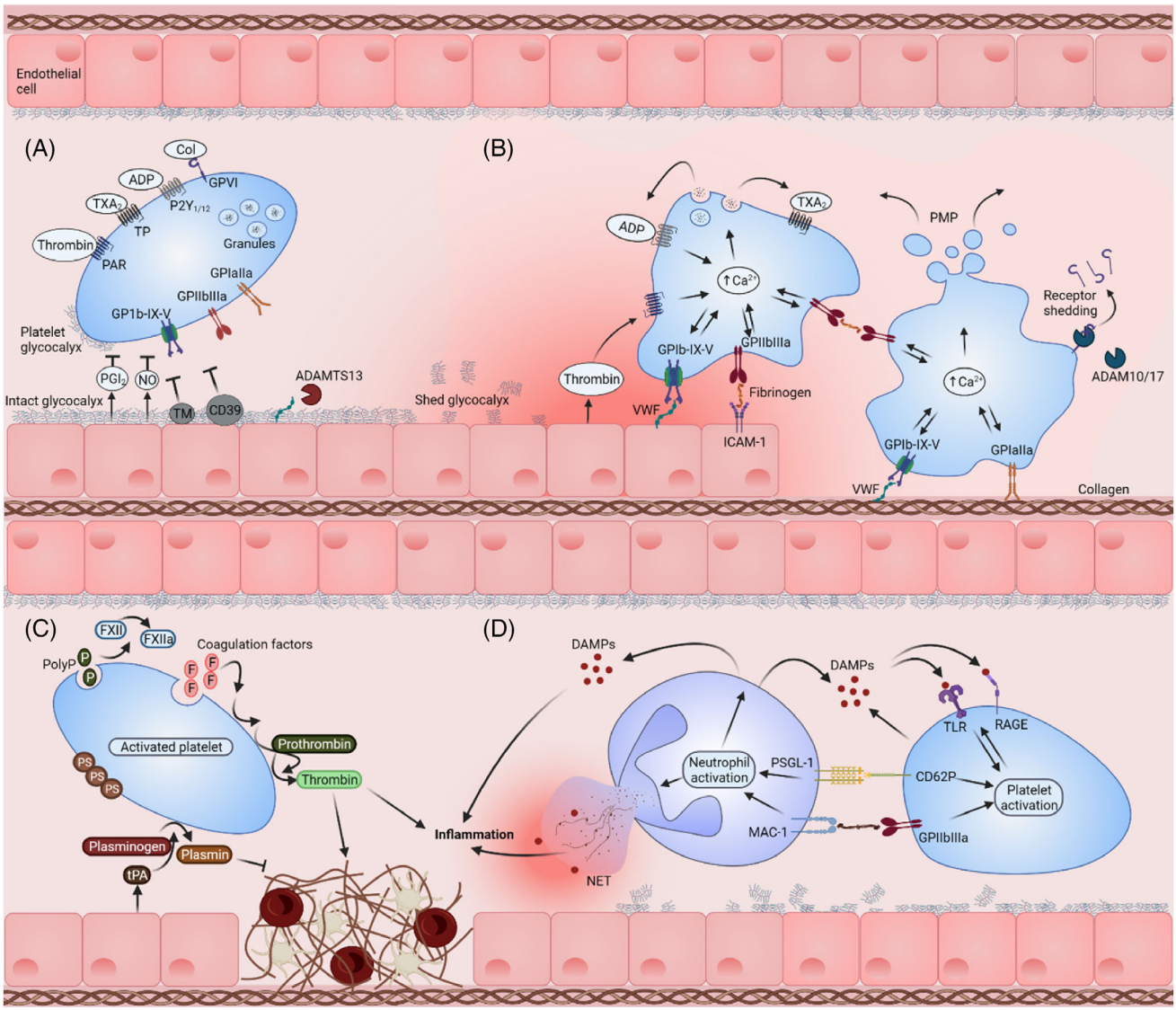


**FIGURE 1** Platelets at rest and the local response to vascular injury. (A) Platelets possess a large variety of membrane receptors (e.g., protease-activated receptors (PAR), thromboxane receptor (TP), P2Y1/P2Y12 receptors, and different glycoproteins (GP)) sensitive to agonists such as thrombin, thromboxane A2 ($TXA_2$), adenosine diphosphate (ADP), and collagen (Col). The surface of endothelial cells and platelets is lined with a glycocalyx, which has anticoagulant properties. Endothelial cells also express and release various molecules such as prostacyclin ($PGI_2$), nitric oxide (NO), thrombomodulin (TM), and CD39 which inhibit platelet function. A disintegrin and metalloproteinase with thrombospondin type 1 motifs, member 13 (ADAMTS13) cleaves Von Willebrand Factor (VWF)-multimers, inhibiting platelet adhesion to the vessel wall (B) After local tissue injury, endothelial cells get activated, glycocalyx is shed, and platelets come in contact with subendothelial structures such as collagen. Platelets are activated, resulting in a rise in intracellular calcium levels, causing structural and functional changes. Platelets secrete their granular content and agonists, initiating a feed-forward reaction which activates and binds nearby cells. In response to high intracellular calcium levels, platelet derived microparticles (PMP) are released. A disintegrin and metalloproteinase (ADAM)10/17 cleave platelet glycoproteins, thereby reducing reactivity (C) A subset of platelets express phosphatidylserine (PS), these promote coagulation by catalyzing the conversion of various coagulation factors, leading to thrombin generation. Polyphosphates (PolyP) also aid in the activation of various coagulation factors. Furthermore, platelets release pro- and anti-fibrinolytic proteins (e.g., PAI-1, tPA) and catalyze the conversion of plasminogen into plasmin. (D) Activated platelets bind to immune cells such as neutrophils, inducing inflammation and neutrophil extracellular trap (NET) formation. Additionally, platelets recognize damage-associated molecular patterns (DAMPs) by pattern recognition receptors.

EVs promote fibrin formation by tissue factor (TF)-bearing cells (e.g., monocytes, endothelial cells).[43] In this regard, platelets contain considerable amounts of tissue factor pathway inhibitor α (TFPIα) in the cytoplasm which is secreted upon strong stimulation and expressed on coated platelets.[44] Likewise, PS-positive platelets

promote fibrin formation by serving as assembly sites for intrinsic tenase (FIXa, FVIIIa, FX) and prothrombinase (FXa, FVa, FII) complexes (Figure 1C).[39] In addition, platelets can both inhibit and promote fibrinolysis, depending on their location within the thrombus architecture.[45] During thrombus formation, release of PAI-1 and α2-antiplasmin may inhibit unwanted fibrinolysis.[46,47] On the other hand, activated platelets also catalyze the conversion of plasminogen to plasmin on their surface membranes.[45,48]

The immunological roles of platelets are also important in the local response to tissue injury.[49] Activated platelets upregulate P-selectin, which interacts with P-selectin glycoprotein ligand (PSGL)-1, connecting platelets with different leukocytes (Figure 1D).[49] Furthermore, platelets produce leukocyte-stimulating molecules, promoting platelet-leukocyte interaction and leukocyte activation. Platelets further stimulate neutrophil extracellular trap (NET) formation, which is composed of DNA, histones and high-mobility group box 1 (HMGB1).[49,50] NETs further promote platelet PolyP release, amplifying fibrin formation.[51]

In all, platelets exert numerous functions following local tissue injury orchestrating hemostasis and the initial immune response.

## 4 | CHARACTERIZATION OF PLATELET DYSFUNCTION AFTER TRAUMA

Trauma-induced platelet dysfunction is poorly defined and not fully understood. Specific pre-existing factors (e.g., age, medical history, prior antiplatelet therapy), trauma-related factors (e.g., injury severity, shock, traumatic brain injury [TBI]) and prehospital resuscitation factors (e.g., use of crystalloids, blood products, calcium and tranexamic acid [TXA]) may affect diagnosis of platelet function. Currently, clinical diagnosis of platelet dysfunction after trauma relies on platelet count and viscoelastic hemostatic assays (VHAs).[52] For research purposes other tests are available (Table 2).

Decreased platelet counts after trauma and TBI have been associated with increased risk of mortality.[53,54] However, platelet counts remain relatively normal during bleeding (>100 × $10^9$ per liter) and only decrease 24-h post-injury.[11] Platelet dysfunction therefore often exists despite normal counts.[11,55] P-selectin[9,14,56] and GPIIbIIIa[56] expression on platelet membranes are increased after traumatic injury. However, after agonistic stimulation, further upregulation of these receptors (i.e., platelet reactivity) is impaired.[12,57] Additionally, platelets from trauma patients show reduced adhesion to collagen compared to healthy platelets.[7,10] Moreover, the platelet response to agonists in aggregation assays[9,11,12,58] and VHAs (e.g., platelet mapping) is impaired after trauma and is associated with injury severity, shock, transfusion requirements and mortality.[7,55,59]

In the most severely injured patients, approximately 10% of all platelets have balloon-like shapes and an increase in circulating platelet EVs is observed.[7] Platelet-leukocyte aggregate formation is also increased, which is associated with increased platelet activation and impaired function.[7,60]

In the post-resuscitation phase, there is an increased risk of thrombosis and organ dysfunction.[61,62] Shock, injury severity, TBI and early platelet dysfunction are all associated with these late complications.[15] Platelet counts drop during intensive care unit (ICU) stay, but rise 72-h post injury, resulting in a reactive thrombocytosis,[53] which is correlated with increased cloth strength.[63,64] This hypercoagulable profile is associated with (venous) thromboembolic events (VTE).[63,65] Post-injury arterial thrombosis is less frequently diagnosed and is associated with older age, indicating different underlying mechanisms.[66–69] Patients with VTE showed an increased maximum amplitude in VHA compared to patients without VTE.[62] Aggregation and platelet mapping remained impaired until day five of admission,[11,57,70] which is associated with development of VTE.[67] Platelets likely participate in the progression towards thrombosis and organ failure on the ICU.

## 5 | POTENTIAL MECHANISMS OF PLATELET DYSFUNCTION AFTER TRAUMA

The human body is well-adapted to deal with local tissue injury. However, humans likely have not been evolutionarily adapted to major traumatic injuries and shock. Nonetheless, modern resuscitation techniques allow many of these severely injured patients to survive, thus causing the original "adaptive" changes of the platelet to persists to later phases of the injury response where they may become "maladaptive."[14]

### 5.1 | Increased platelet activation: Platelet exhaustion

Impaired platelet reactivity after trauma could be due to early strong systemic activation of platelets, rendering platelets "exhausted" and dysfunctional. Systemically elevated levels of platelet agonists and DAMPs are thought to play an important role. Despite high intracellular

**TABLE 2** Diagnostics of platelet function

| Coagulation assay | Assay type | Description | Assay time | Pros | Cons |
|---|---|---|---|---|---|
| Conventional | | | | | |
| Platelet count | Whole blood | Measurement of number of platelets in whole blood | Fast (<15 min) | • Standardized<br>• Simple to perform | • Does not reveal functional defects |
| Bleeding time | Whole blood | Measurement of time until bleeding stops after making a small incision | Fast (<15 min) | • Standardized<br>• Simple to perform | • Limited accuracy to predict bleeding |
| Global coagulation | | | | | |
| Viscoelastic tests (thromboelastography (TEG) and rotational thromboelastometry (ROTEM) | Whole blood | Assessment of general coagulation potential by measuring viscoelasticity of whole blood | Fast, (<15 min first results) | • Standardized<br>• Simple to perform<br>• Whole blood assay<br>• Potential to differentiate (different reagents) | • Some expertise needed<br>• Limited sensitivity to detect mild platelet dysfunction |
| Platelet activation | | | | | |
| Flow cytometry | Whole blood or platelet-rich plasma | Quantitative measurement of platelet (activation) receptors and reactivity in whole blood or platelet-rich plasma | Relatively fast (30 min-1 h) | • Potential to differentiate (different antibodies)<br>• Abnormalities in quantity and function of receptors can be diagnosed | • Not standardized<br>• Expertise needed<br>• Prone to artifacts<br>• Unknown correlation with platelet dysfunction |
| Soluble activation markers | Plasma | Detection of platelet membrane protein shedding, most commonly measured by ELISA | Slow (>2 h) | • Simple to perform<br>• Low volume plasma needed | • Time-consuming (ELISA)<br>• Prone to artifacts<br>• Unknown correlation to platelet dysfunction |
| Platelet adhesion | | | | | |
| Microfluidic assays | Whole blood or platelet suspension | Platelet adhesion to extracellular matrix components under different shear conditions | Relatively fast (30 min-1 h), depending on methods | • Mimicking in vivo conditions | • Not standardized<br>• Expertise needed<br>• Unknown correlation with platelet dysfunction |

(Continues)

TABLE 2 (Continued)

| Coagulation assay | Assay type | Description | Assay time | Pros | Cons |
|---|---|---|---|---|---|
| Platelet aggregation | | | | | |
| Whole blood aggregometry (WBA) | Whole blood | Measures resistance in whole blood as platelets adhere/aggregate to electrodes in response to various agonists | Fast (<15 min) | • Standardized<br>• Whole blood assay<br>• Specific aggregation pathways can be studied in isolation | • Some expertise needed<br>• Sensitive to platelet count<br>• Limited accuracy to predict bleeding |
| Light transmission aggregometry (LTA) | Platelet-rich plasma | Platelet aggregation in platelet rich plasma, under low shear conditions, with the addition of various agonists | Relatively slow (1-2 h) | • Gold standard for platelet function<br>• Specific aggregation pathways can be studied in isolation | • Expertise needed |
| Platelet closure time (PFA100/200) | Whole blood | Formation of a platelet plug under high shear conditions in the presence of collagen or ADP | Fast (<15 min) | • Standardized<br>• Simple to perform | • Limited sensitivity to mild platelet dysfunction<br>• Affected by platelet count and or hematocrit |

calcium levels, calcium mobilization within platelets is impaired after trauma, which might explain the reduced reactivity.[57] Additionally, increased formation of platelet-leukocyte aggregates in response to agonists and DAMPs are thought to contribute to platelet exhaustion.[60]

## 5.2 | Reduced platelet adhesion: Receptor shedding

After trauma, the adhesion receptor GPIbα and collagen receptor GPVI are shed, which was associated with decreased platelet adhesion and aggregation.[8] Proteases such as plasmin and thrombin are elevated after trauma and can cleave platelet adhesion receptors.[71,72] The shedding of GPVI and GPIbα is mediated by a disintegrin and metalloproteinase (ADAM)10 and ADAM17, in response to elevated intracellular calcium.[73] Platelet binding to collagen and fibrin further contributes to GPVI shedding, resulting in desensitization to subsequent agonist stimulation.[8,74] During bleeding, increased ADAM10 and ADAM17 activity could be detrimental. Tissue inhibitors of metalloproteinases (TIMP) 1 and 3 inhibit ADAM10 and ADAM17 activity. These inhibitors may reduce platelet dysfunction after trauma.[75] However, due to the broad mechanisms of action of ADAM10 and ADAM17, they are a challenging target for treatment.

## 5.3 | Reduced platelet adhesion: The deranged VWF-ADAMTS13 axis

Plasma concentrations of VWF are increased after trauma and correlate with injury severity,[76–79] but lower plasma VWF can be associated with increased mortality and TIC.[77] Insufficient VWF could contribute to reduced platelet adhesion in trauma. VWF is regulated by its cleaving enzyme a disintegrin and metalloproteinase with thrombospondin type 1 motifs, member 13 (ADAMTS13), but possibly also by plasmin.[80] ADAMTS13 is, in general, decreased in concentration and activity after trauma.[76,81,82] However, increased ADAMTS13 activity can also exist in severely injured patients.[76] Under normal circumstances, ADAMTS13 circulates in an inactive form (its cleavage site is protected by CUB-domains), and becomes activated upon binding to unfolded VWF, underlining its specificity to VWF.[83–85] Several proteases that are elevated after traumatic injury (e.g., thrombin, plasmin) can degrade ADAMTS13, decreasing its activity.[86,87] However, specific proteolysis of CUB domains of ADAMTS13 can enhance ADAMTS13 activity and remove substrate specificity for VWF.[85,88] Hyperactive ADAMTS13 can cleave fibrinogen, potentially impairing platelet adhesion and

aggregation.[85,88] The CUB domains of ADAMTS13 also directly inhibit platelet adhesion to collagen under flow.[89] In all, various changes in the VWF-ADAMTS13 axis occur after traumatic injury, which could affect platelet function.

### 5.4 | Reduced platelet aggregation

Low fibrinogen levels in trauma are associated with poor outcomes and could contribute to the observed decrease in platelet aggregation after trauma.[90] Alternatively, GPIIbIIIa shedding, induced by high intracellular calcium concentration or by proteases such as plasmin may also contribute.[41] Acidosis and hypothermia could further worsen platelet aggregation.[91–93]

### 5.5 | DAMPs and platelet dysfunction

DAMPs are thought to play a key role in trauma-induced platelet dysfunction. DAMPs such as DNA, histones and HMGB1 are released in high concentration into the circulation after trauma.[94] Histones can activate platelets and facilitate platelet-dependent thrombin generation.[95] Histones can induce platelet dysfunction after trauma.[96] Particularly, histone H4 has been shown to induce platelet ballooning and contribute to the formation of platelet EVs.[7] Histones have also been linked to the development of thrombosis and organ dysfunction after traumatic injury.[97]

Likewise, nuclear protein HMGB1 is increased up to 300-fold within the first hours after injury.[94] Despite lacking a nucleus, platelets have been identified as a major source of HMGB1.[98] HMGB1 can induce platelet activation and inflammation via the toll-like receptors (TLRs) and receptor for advanced glycation end products (RAGE). In an experimental trauma model, platelet-specific HMGB1 knockout compared to wild type mice showed reduced inflammation and decreased platelet adhesion, highlighting the importance of HMGB1 in platelet function.[98,99] In line with this, high HMGB1 levels are associated with thrombosis and adverse outcomes.[94,98,100] Another DAMP activating TLR4 and RAGE that has recently gained interest with regards to trauma-induced platelet dysfunction is S100A8/A9.[96,101–103] S100A8/A9 is a heterodimeric, intracellular protein, especially abundant in neutrophils, where it comprises almost half of the cytoplasm proteins. Platelets may have some capacity for de novo synthesis of S100A8/A9, but it is unknown if they are the main source after trauma.[104] In vitro data shows that neutrophils are able to transfer S100A8/A9 to platelets upon activation, impairing platelet reactivity.[105]

In all, the precise mechanism by which DAMPs impair platelet function after trauma is unknown, but pattern-recognition receptors and their ligands are likely involved.

### 5.6 | Endothelial dysfunction, immunothrombosis, and organ dysfunction

Endothelial activation, glycocalyx shedding, and increased permeability are present early after trauma and are associated with the presence of shock.[106,107] Endothelial dysfunction can be worsened by crystalloid resuscitation.[108] Additionally, platelet-endothelial interactions distant from localized endothelial injury may aggravate endothelial dysfunction and contribute to thrombosis.[109] VWF multimers are continuously released, and ADAMTS13 is decreased, associated with microthrombi formation and organ dysfunction.[76,78] Moreover, histones and HMGB1 remain significantly elevated for multiple days following injury.[94,97] Furthermore, platelet TLR4 expression stimulates formation of platelet-leukocyte aggregates.[110] The extensive crosstalk between activated platelets, damaged endothelium and primed leukocytes could promote excessive release of platelet EVs,[111] platelet-leukocyte aggregates[7] and NETs.[112] The sustained inflammatory and procoagulant state which is aggravated by endothelial dysfunction may result in microvascular thrombosis and organ dysfunction.[113]

## 6 | TREATMENTS FOR TRAUMA-INDUCED PLATELET DYSFUNCTION

Targeted treatment of trauma-induced platelet dysfunction is time-dependent. The treatment priority switches from augmenting platelet hemostatic function during active bleeding, towards preventing thrombosis in the post-resuscitation phase. The exact timing of the shift from a hypocoagulable to a hypercoagulable state may be patient-, time-, injury- and/or shock dependent, but remains currently unclear. Figure 2 broadly summarizes (proposed) intervention times of current and experimental treatments.

## 7 | CURRENT EARLY TREATMENTS FOR PLATELET DYSFUNCTION

Current trauma resuscitation consists of early administration of TXA, permissive hypotension (i.e., limiting

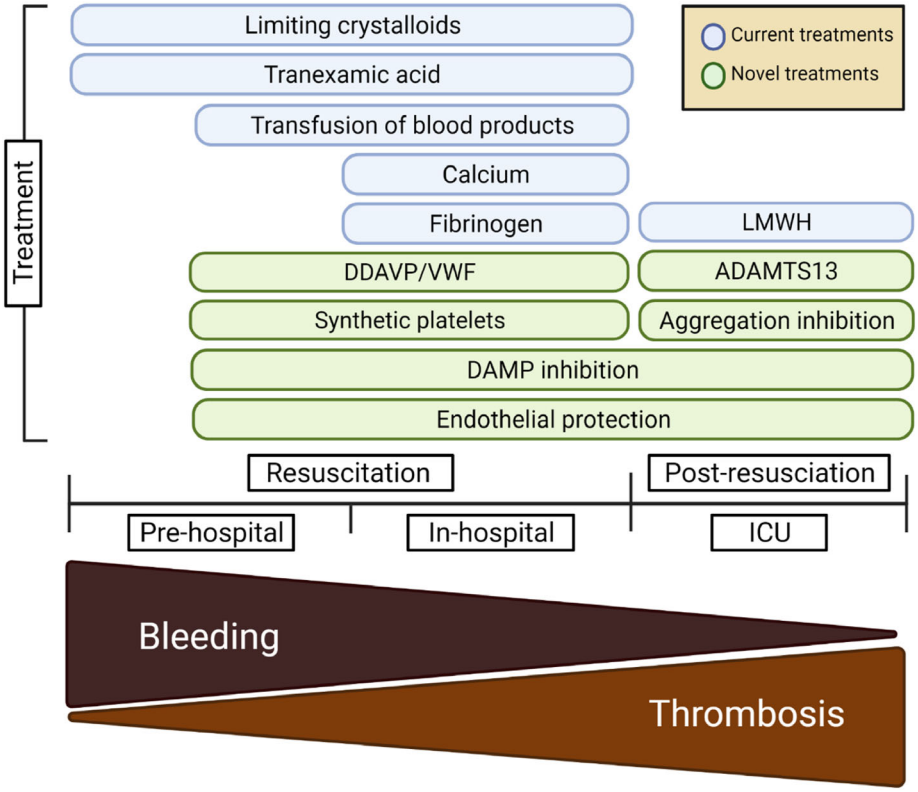


**FIGURE 2** Proposed timing of current and novel treatments for trauma-induced platelet dysfunction. Timing of treatment depends on bleeding and thrombosis risk. ADAMTS13, a disintegrin and metalloproteinase with thrombospondin type 1 motifs, member 13; DAMP, damage-associated molecular pattern; DDAVP, desmopressin; LMWH, low molecular weight heparin; VWF, von Willebrand factor.

crystalloid infusion), a balanced transfusion strategy, and fibrinogen and calcium supplementation. In addition, hypothermia and acidosis should be addressed early as it affects platelet dysfunction.[91–93] In some centers, treatment is guided by VHAs, which might be beneficial for patients with TIC.[114,115] Mechanisms of early treatments are shown in Figure 3.

## 7.1 | Tranexamic acid

Early use of TXA has been shown to significantly improve survival after trauma, including mild to moderate TBI.[116–118] TXA binds to plasminogen, preventing its conversion to plasmin, decreasing fibrinolysis. Moreover, in vitro, TXA improved clot strength, which could suggest improved platelet function.[119] Mechanistically, by plasmin inhibition, TXA could decrease proteolysis of platelet receptors. Plasmin also induces immune cell activation, which could explain part of the beneficial effects of TXA after trauma.[120]

## 7.2 | Platelet transfusion

Standard care is empiric transfusion of room temperature stored (RT) platelets in a balanced ratio with red blood cells and plasma.[121] Early and high-dosed platelet transfusion is associated with a significant reduction in mortality.[122] In contrast, in patients with mild TBI, platelet transfusion may be harmful.[123,124]

Despite the overall mortality benefits, it is unknown how platelet transfusion affects platelet dysfunction. Transfused platelets may adapt the same dysfunction as their endogenous counterparts and do not appear to improve platelet aggregation during active bleeding.[125,126] Interestingly, circulating PAI-1 concentration increases after platelet transfusion in trauma patients.[125] The clinical benefits of platelet transfusion could therefore be partly due to a reduction in hyperfibrinolysis rather than an improvement in platelet function.

The storage conditions and donor-related factors could affect the function of transfused platelets.[24] Cold-stored platelets showed hemostatic superiority over RT-stored platelets in vitro.[127,128] Clinical trials need to assess the hemostatic capacity of cold-stored platelets in trauma patients.

An alternative to platelet component transfusion is whole blood (WB), which has gained renewed interest in trauma resuscitation. Although WB may be clinical feasible and showed survival benefits over component therapy in an observational trial, randomized controlled trials (RCTs) are needed to evaluate its safety and effectiveness.[129–134]

## 7.3 | Fibrinogen

Fibrinogen can be supplemented as fibrinogen concentrate or as cryoprecipitate, the former of which has recently been

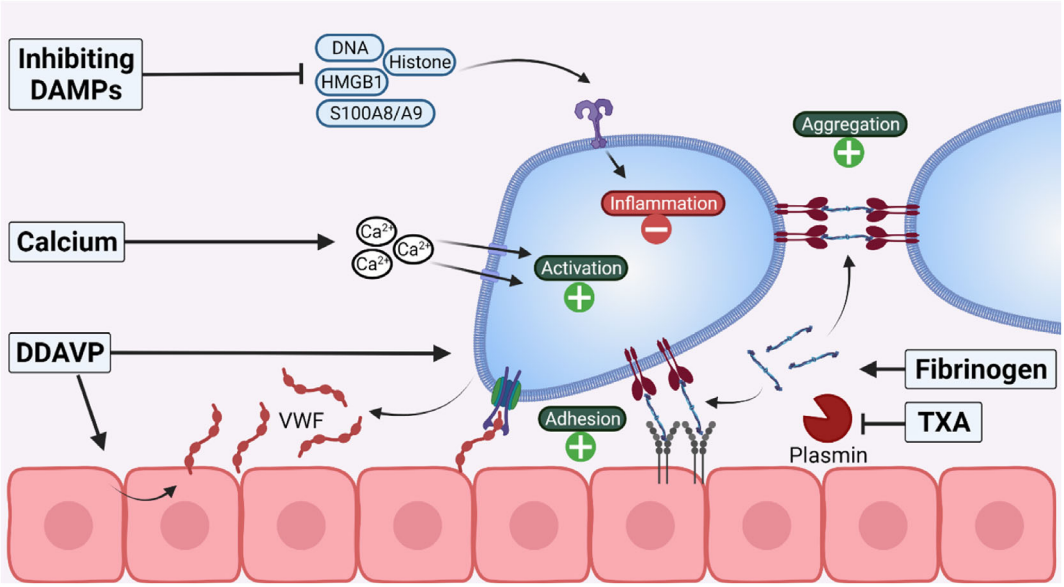


FIGURE 3 Mechanisms of early treatments for trauma-induced platelet dysfunction. The goal early after traumatic injury should be to increase platelet activation, adhesion and aggregation at the site of injury. Additionally, inhibiting DAMPs could decrease inflammation and improve platelet function. DDAVP, desmopressin; TXA, tranexamic acid; VWF, von Willebrand factor.

shown to improve survival after trauma.[135,136] The mechanism by which fibrinogen improves outcomes could be, in part, due to promoting platelet adhesion and aggregation. Cryoprecipitate also contains other pro-hemostatic factors, such as FXIII, FVIII and VWF, and is associated with superior clotting kinetics in vitro.[137,138] In vitro data suggest that cryoprecipitate increases thrombin generation.[137] More data is necessary to evaluate to role of fibrinogen concentrate and cryoprecipitate on platelet dysfunction.[139]

### 7.4 | Calcium

Hypocalcaemia is a common finding after trauma, associated with increased blood transfusion requirements, coagulopathy and mortality.[140] Ionized calcium is essential for platelet activation, adhesion and aggregation.[141] After trauma, hypocalcemia is independently associated with decreased platelet function and clot strength.[142] Hypocalcaemia cannot be detected in ROTEM, as calcium is added ex vivo. Although some in vitro data exist, the direct effect of supplementation of extracellular ionized calcium on platelet function needs further exploration.

## 8 | NOVEL EARLY TREATMENT STRATEGIES FOR PLATELET DYSFUNCTION

Despite decreased mortality with current resuscitation strategies, TIC and platelet dysfunction continue to be present in severely injured patients, highlighting the need for novel targeted treatments.

### 8.1 | Desmopressin

As mentioned, VWF is important for platelet adhesion. Increasing VWF through desmopressin (DDAVP) could therefore be beneficial in bleeding trauma patients. Besides promoting platelet adhesion to collagen and endothelium, DDAVP has various other pro-hemostatic effects on platelets.[143,144] A recent RCT showed that DDAVP compared to vehicle reduced the amount of blood product use during trauma rescusitation.[145] A retrospective study in TBI showed that platelet function after treatment with DDAVP was comparable to patients receiving platelet transfusion.[146]

### 8.2 | Potential role for (semi)synthetic platelets

In recent decades, synthetic nanoparticles that mimic the important hemostatic functions of platelets have gained interest.[147] These can be constructs derived and processed from natural platelets (Figure 4A,B), or be fully synthetic (Figure 4C,D). Ideally, such platelet-mimicking particles would adhere at the site of injury and interact with fibrinogen and locally activated platelets to form aggregates, without any significant immunogenic or thrombotic risks. Examples of semi-synthetic platelet products

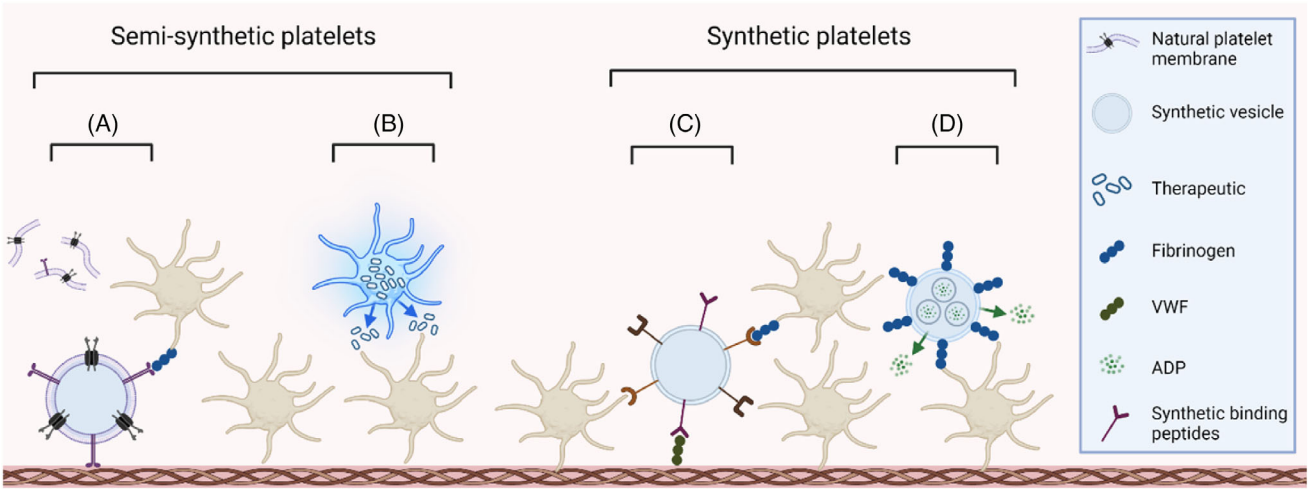


**FIGURE 4** Examples of (semi)synthetic platelet designs. (A) Extracted natural platelet membranes integrated into a synthetic vesicle (e.g., infusible platelet membrane, Thrombosome). (B) Natural platelets loaded with hemostasis-promoting agents to enhance platelet function or for targeted drug delivery. (C) Synthetic vesicle coated with VWF binding, collagen binding, and fibrinogen mimetic peptides (e.g., SynthoPlate design). (D) Latex beads, albumin or liposomes, coated with a fibrinogen mimetic such as HHLGGAKQAGDV (H12), which binds GPIIbIIIa. These vesicles can be loaded with agonists such as ADP.

that are derived from natural platelets include infusible platelet membranes[148,149] and infusion of platelet EVs.[150] Alternatively, loading platelets or fibrinogen coated-nanoparticles with hemostatic agents such as thrombin or TXA to more effectively reach the site of injury are promising novel treatment options for traumatic bleeding.[151,152]

Infusion of fully synthetic nanovesicles coated with VWF binding, collagen binding, and fibrinogen mimetic peptides (e.g., SynthoPlate design) was shown to be comparable to other platelet products in terms of safety and hemostatic efficacy in animal models of thrombocytopenia and traumatic bleeding.[153–155] Recently this design has been refined by incorporation of PS, which promotes thrombin generation.[156] Similarly, ADP containing vesicles coated with HHLGGAKQAGDV (H12), a fibrinogen mimetic, were effective in an animal model of thrombocytopenia and trauma.[157,158] Although promising, rigorous safety and efficacy clinical trials are needed to translate these technologies to trauma patients.

## 9 | REDUCING INFLAMMATION, IMMUNOTHROMBOSIS AND ORGAN DYSFUNCTION

Once the bleeding has stopped, treatment goals shift from promoting platelet hemostatic function to preventing and treating inflammation and thrombosis. As mentioned, activated endothelium, circulation of "exhausted platelets" and DAMPs maintain a vicious pro-inflammatory and procoagulant environment. These components have been proposed as targets for novel treatments. Additionally, and more directly, inhibiting platelet aggregation might be necessary to prevent thrombosis (Figure 5). However, individual bleeding risk assessment is needed.

### 9.1 | Targeting DAMPs: DNA, Histones, and HMGB1

Targeting circulating DNA and NETs has potential beneficial effects after trauma. DNase-1 is responsible for removing most of the cell free DNA in the circulation, making it a potentially promising treatment option. A recent observational study in trauma showed that DNase-1 is significantly decreased and coexisted with increased circulating DNA after traumatic injury.[159] In a rodent model of traumatic injury, scavenging free mitochondrial DNA decreased organ dysfunction.[160] Like circulating DNA, histones and HMGB1 are promising targets for treating platelet dysfunction after trauma. Several endogenous molecules, such as protein C and glycocalyx components are shown to inhibit histone-induced cytotoxicity.[161–163] In a swine model of trauma and shock, fresh frozen plasma (FFP) supplemented with histone deacetylase inhibitor valproic acid significantly increased plasma platelet activation markers P-selectin and sCD40L, and improved outcomes compared to FFP alone.[164] Therapeutic strategies to inhibit HMGB1 have been shown to reduce acute lung injury after trauma.[165] The effect of anti-HMGB1 on platelet dysfunction after trauma is currently unknown.

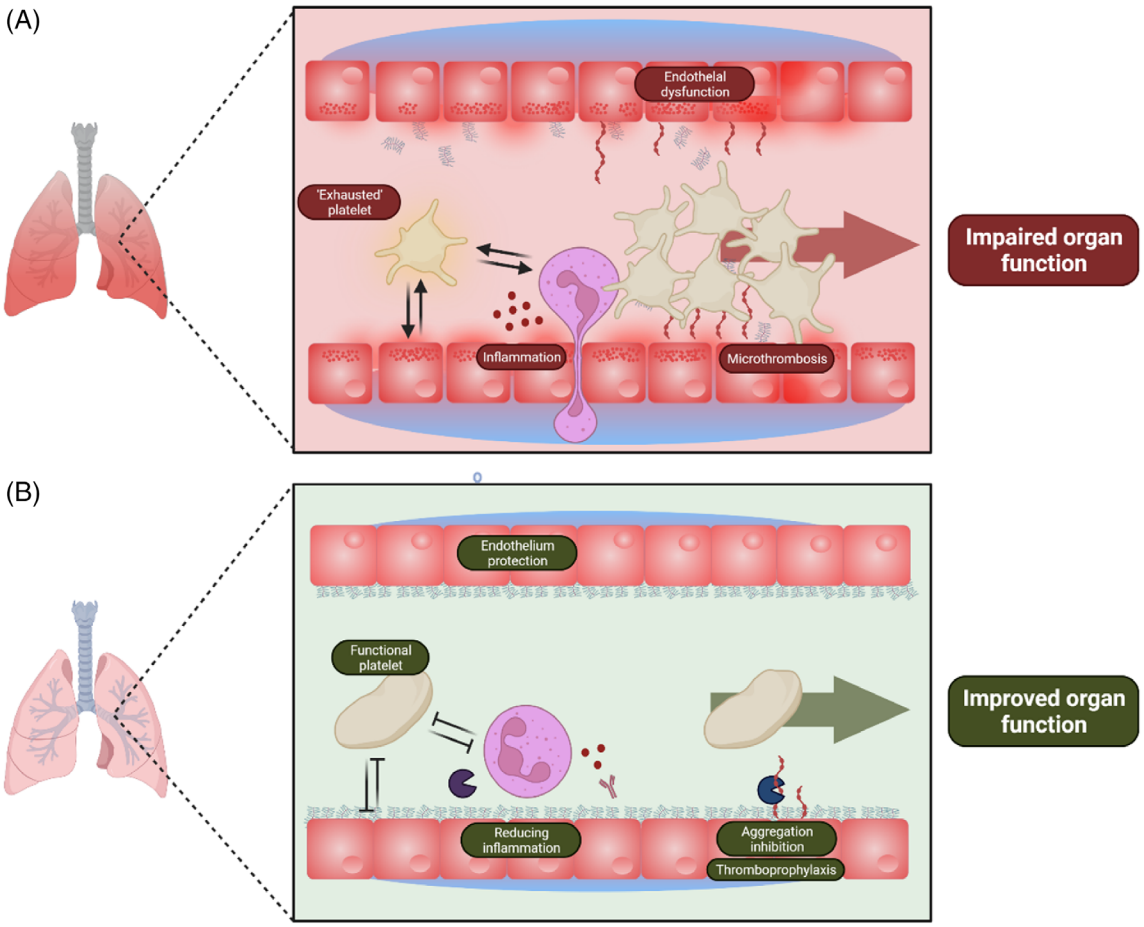


**FIGURE 5** Targets for late treatments to improve platelet dysfunction, reduce microthrombosis and organ dysfunction. (A) Platelets remain dysfunctional ("exhausted") during ICU stay, resulting in promiscuous platelet adhesion and aggregation and microthrombi formation. This platelet dysfunction is maintained due to endothelial activation and prolonged circulation of DAMPs. (B) To break the vicious cycle of late platelet dysfunction after trauma, various treatment strategies are possible.

Besides inhibiting their ligands, directly modulating or inhibiting immune receptors could be another target for trauma-induced platelet dysfunction. In experimental models of TBI, HMGB1 receptor antagonists reversed brain damage and decreased inflammation.[166–168] In addition, targeting platelet TLRs could be a promising target for thrombosis after traumatic injury.[169,170]

## 9.2 | Recombinant thrombomodulin

Recombinant thrombomodulin (rTM) inhibits DAMPs such as histones and HMGB1.[162,171,172] rTM reduces HMGB1 levels and improves inflammation and platelet function.[173] In a RCT in septic patients, rTM improved coagulopathy.[174] These results raise the question regarding the utility of rTM for TIC, which differs markedly from the coagulopathy in sepsis. In experimental models of coagulopathy, rTM inhibited histone-induced platelet aggregation.[175,176] The effects of rTM could be attributable to direct modulating effect on histone H4, or could be mediated by increased activated protein C.[162,175] In vitro data suggest that platelets incubated with rTM have normal aggregation, but reduced thrombin reactivity and tissue factor induced EV formation.[177] Besides the inhibitory effects of rTM on DAMPs, rTM has been shown to prevent thrombin induced degradation of ADAMTS13 in vitro, which could prevent low ADAMTS13 levels in trauma.[86] Dosage should depend on the phase after trauma (i.e., bleeding vs. thrombosis risk), because high concentration of rTM activates protein C, which could exacerbate early TIC.[178–180]

## 9.3 | Targeting endothelial dysfunction to improve platelet dysfunction

The endothelium can be targeted to potentially improve platelet dysfunction and reduce microthrombosis.[113] Firstly, the endothelial glycocalyx can be protected by

plasma transfusion, the benefits of which are shown in severely injured trauma patients and in animal trauma models.[76,181,182] Secondly, VWF release can be targeted by administration of recombinant ADAMTS13, associated with reduced endothelial permeability and organ failure.[76,79] Furthermore, the VWF-targeting thrombolytic agent Microlyse has been shown to reduce microvascular thrombosis, which may be beneficial after trauma.[183] Thirdly, prostaglandin receptor agonists (e.g., iloprost) can reduce endothelial activation, glycocalyx shedding, and platelet aggregation.[184,185] Fourthly, the CD39-CD72-adenosine pathway is a promising therapeutic target, as it inhibits ADP-induced platelet activation. In vitro, sCD39 inhibited platelet aggregation[27] and adenosine restored ADP induced platelet aggregation.[186] Lastly, NO inhalation during ventilation may reduce platelet activation and aggregation.[187–189] Together, targeting the endothelium may reduce organ dysfunction by improving late trauma-induced platelet dysfunction.

### 9.4 | Targeting thrombosis: LMWH and platelet aggregation inhibitors

Low-molecular weight heparin (LMWH) is the current pharmacological thromboprophylactic treatment after trauma and is associated with reduced VTE and mortality.[190] However, VTE was independently associated with acquired antithrombin deficiency 72 h post-injury, which raises a question on timing and dose of LMWH.[191] LMWH reduced platelet aggregation in vitro.[192] On contrary, administration of LMWH did not reduce the hypercoagulable profile and still showed patients developing VTEs, highlighting the need for additional therapies targeting late platelet dysfunction.[65] Patients on antiplatelet therapy showed a reduction in progression of organ dysfunction and late mortality.[193] The P-selectin-PSGL-1 interaction can be reduced by P-selectin antibodies (e.g., inclacumab, crizanlizumab). In a model of arterial injury, PSGL-1 inhibition compared to vehicle reduced thrombosis.[194] Similarly, inhibition of P-selectin prevented pulmonary arterial thrombosis in a murine model of traumatic chest injury.[109] Platelet aggregation reduction can be achieved by targeting GPIIbIIIa (e.g., tirofiban). In a model of systemic inflammation, induced by extracorporeal circulation, tirofiban protected platelets and decreased platelet-leukocyte binding.[195] Lastly, specific platelet-VWF interactions could be reduced by caplacizumab, which in patients with thrombotic thrombocytopenic purpura has been shown to reduce microthrombosis and organ failure.[196] The efficacy and optimal timing of these potential treatments require further study in trauma patients.

## 10 | CONCLUSION AND FUTURE DIRECTIONS

Platelets play an important role in the hemostatic response to local tissue injury, but can become dysfunctional in severely injured trauma patients. Trauma-induced platelet dysfunction is still poorly defined and additional characterization is needed to formulate accurate diagnostic criteria. Identification of potential mechanisms underlining platelet dysfunction after trauma has led to the emergence of novel targeted treatment options. Both early and late platelet dysfunction require different therapeutic interventions, which should be personalized based on the patient-specific coagulation defects. Early treatments should prioritize bleeding control, while late treatments should target thrombosis. To improve the care of severely injured trauma patients, safety, timing, and dose of proposed treatments should be further studied.

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## CONFLICT OF INTEREST

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