Platelet-mimicking procoagulant nanoparticles: potential strategies for mitigating blood shortages

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1.0 A critical need for platelets in transfusion medicine:

Bleeding can be a life-threating event following trauma or surgery. Many factors can increase a patient's likelihood of severe bleeding including the use of anti-coagulants, hereditary clotting disorders, or thrombocytopenia. In a hospital setting, transfusion of donor blood products, including red blood cells, platelets, and/or fibrinogen concentrates are used as a first line of defense to mitigate bleeding (1). Due to their critical roles in hemostasis, transfusion of platelets are particularly important for stopping uncontrolled bleeding. However, donor platelets have a short shelf life of approximately five to seven days under standard storage conditions. The use of cold stored platelets is gaining popularity as a means to enhance platelet shelf-life, but cold storage can cause platelet lesions and has not yet been widely adopted (2). Additionally, no matter the storage conditions used for platelets, because they are derived from human donors, platelet supply is dependent on donor availability and willingness, which often leads to shortages. This limitation has been particularly striking in the last year as the COVID-19 pandemic has led to an unprecedented blood product storage. In fact, in 2022 for the first time, the American Red Cross declared a "Blood Crisis" and implored eligible donors to give blood (3). Furthermore, while risk of disease transmission is low due to current screening standards for donor derived products, this risk is still non-zero (4). Finally, while platelets are a key therapeutic in the hospital setting, in emergency situations outside the hospital it is not practical to carry platelets or other blood products, therefore many patients suffering traumatic bleeding have delays in critical treatment that could prevent morbidity and mortality (5). Overall, these limitations highlight that a great need exists to identify an alternative to natural platelets to treat bleeding. Ideally, this alternative should be readily available, have a long shelf life, have storage conditions that are amenable to use in emergency medicine, and perform in an equivalent manner to natural platelets. The creation of artificial platelets to meet these design goals has been an active area of research in recent years and a new study by Sekhon et al. has demonstrated the creation of platelet-mimicking procoagulant nanoparticles (PPNs) that have advanced platelet function compared to prior designs (6). Here, we discuss the implications for these new findings in the field of synthetic platelet design.

2.0 Key developments in synthetic platelets

Artificial platelet design is focused on formulating biomaterials that can emulate key platelet functions including activation and accumulation at the injury site to create a platelet plug and/or amplification of the coagulation cascade to result in fibrin clot formation (7). One such early approach is the development of the "plateletsome" that contains various platelet derived glycoproteins incorporated into a liposomal membrane (8). More recently, other techniques have included decorating natural or synthetic microparticles with fibrinogen, platelet-binding peptides, or platelet relevant glycoproteins to enhance hemostasis (9, 10). Another approach is the use of deformable, synthetic microgels coupled to a fibrin-specific binding motif that can recapitulate platelet-fibrin interactions to promote hemostasis and clot retraction, which ultimately can improve wound healing after clotting (11). Previous research from the Sen Gupta lab constructed platelet

mimetic nanoparticles equipped with collagen and von Willebrand factor (VWF)-binding peptides and fibrinogen-mimetic peptides that can recapitulate native platelet adhesion and aggregation (12). Although this design was advantageous for mimicking primary hemostasis, it lacked the procoagulant action of native platelets that leads to amplified thrombin at the wound site. In the present study by Sekhon et al., the team validated a liposomal nanoparticle system that upon exposure to plasmin, exposes phosphatidylserine on the nanoparticle surface, triggering platelet-mimetic assembly of coagulation factors on the particle surface. Key findings from this new study and implications for the field of platelet mimetic therapeutics are described below. Modes of action of various platelet-mimetic nanoparticle therapeutics are illustrated in **Figure 1**.

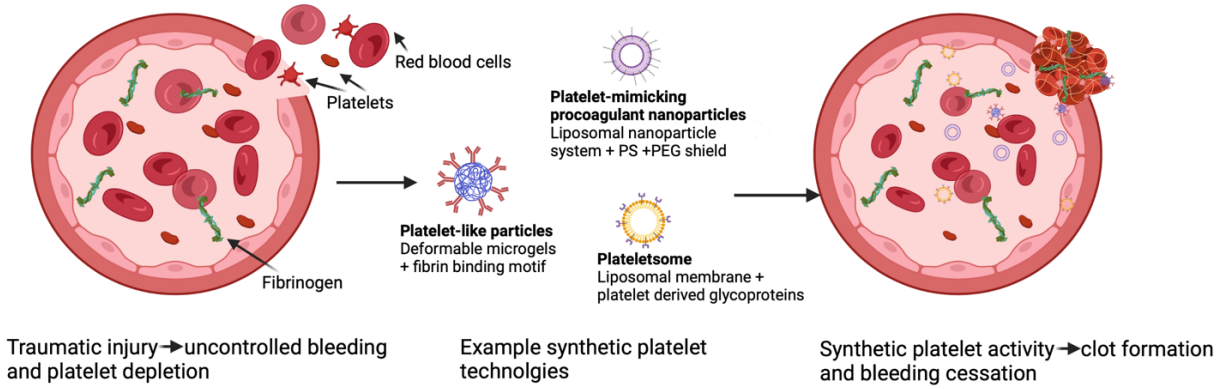

Figure 1: Examples of synthetic platelet designs and their action in hemostasis.

3.0 Platelet-mimicking procoagulant nanoparticles (PPNs)

The work by Sekhon et al. describes the characterization of injury-activated platelet-mimicking procoagulant nanoparticles (PPNs) formulated from a liposomal nanoparticle system containing distearoyl PS (DSPS) enclosed by polyethylene glycol (PEG). Upon injury, the PEG shield is cleaved by enzymatic action of endogenous plasmin, exposing PS, that can augment the coagulation cascade resulting in thrombin generation and fibrin formation. In vitro experiments revealed PPNs are injury-specific and were capable of generating thrombin and fibrin in platelet deficient human plasma that ultimately contributed to superior clot formation and resistance to fibrinolysis. In vivo, PPNs were demonstrated to reduce bleeding time and overall blood loss from a tail bleed when administered prophylactically in platelet deficient mice. Moreover, in a murine traumatic injury model, PPN administration led to greater fibrin formation at the wound site, thereby leading to a significant reduction in blood loss than controls and increased survival. An important finding from this study was that no off-target thrombotic risks were observed in any of the in vivo models. The authors noted several limitations of their studies including the necessity to examine efficacy and safety of various doses of PPNs and the shorter circulation time relative to native platelets (~1 day as opposed to multiple days). Additionally, while PPNs emulate natural platelet activity in primary hemostasis and thrombin amplification, they do not replicate all aspects

of native platelet functions. However, additional functions may not be necessary to facilitate hemostasis.

3.0 Broad impacts and future directions

A key need exists to develop synthetic replacements for platelet transfusion therapies. Many promising strategies have been described in the literature which primarily enhance hemostasis by either targeting constituents of primary or secondary hemostasis and amplifying platelet aggregation and/or fibrin generation. The approach described by Sekhon et al. represents a step towards a more "platelet-like" synthetic platelet design by demonstrating the ability to induce wound-triggered PS exposure and subsequent thrombin generation on the particle surface. While these particles do not recapitulate every aspect of platelet function, this added functionality certainly enhances the hemostatic function of the particles. As the authors point out in their manuscript, it is likely unnecessary to recapitulate every aspect of platelet function in synthetic platelet design. This point is very important to consider for all synthetic platelet designs. Particularly when thinking of actually translating these therapeutics into the clinic, simpler designs are preferable because they will likely have fewer hurdles during FDA approval. Additionally, simpler designs are also more likely to have lower price points. Cost will be an important consideration for translation of any synthetic platelet technology from a research setting to clinical practice. For all synthetic platelet technologies, it will be important to define what actions are necessary for the intended indication and to define what level of efficacy is required, while balancing cost of the final product.

Another key consideration for the PPNs described by Sekhon et al and other synthetic platelet technologies is how they perform in combination with other blood products, particularly platelet concentrates. While generating a complete platelet replacement might be necessary for use in emergency medicine situations, in the hospital setting, synthetic platelets could be used in conjunction with other blood products to decrease the total amount of blood products needed to achieve hemostasis. Even lessening some of the need for platelet transfusion would help address current and future blood shortages. While several synthetic platelet technologies have performed head to head comparisons with platelet transfusions, future studies should focus on synthetic platelets used in conjunction with natural platelets.

Overall, synthetic platelet therapies are an emerging approach to address blood product shortages and treat bleeding in both hospital and emergency medicine environments. Translation of these exciting technologies to clinical practice will require balancing design complexity, hemostatic efficacy, and overall product costs. While still in pre-clinical testing, the overall promise of synthetic platelet therapies to treat bleeding is high.

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