Modular Amplification of Hemostatic Output with Platelet-inspired Particles using Clot-augmenting Nanomaterials

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Introduction: In the cell-based model of hemostasis [1], activated platelets play a central role via multi-faceted mechanisms, including: (1) injury site-specific anchorage mechanisms where they adhere to and aggregate at the bleeding site via heteromultivalent ligand-receptor binding interactions, (2) membrane properties wherein the activated platelets expose negatively charged phosphatidyl serine (PS)-rich surface to enable co-localization of coagulation factors for propagation and amplification of coagulation outputs (thrombin and fibrin generation), and, (3) secretory mechanisms where the activated platelets release various hemostasis-augmenting factors (e.g. Adenosine di-phosphate or ADP, Polyphosphates or PolyP etc.) to further amplify clotting mechanisms as well as clot properties[3]. For these reasons, allogeneic platelet transfusions are routinely used in the clinic to treat bleeding disorders stemming from trauma, surgery, hematology/oncology and congenital coagulation defects. However, these natural platelet products have limited availability and portability, high risk of pathogenic contamination, very short shelf-life (~5 days), and several biologic side effects [2]. Especially, outside of large hospitals and trauma centers, platelets are not readily available for pre-hospital management of hemorrhagic complications, e.g. with civilian emergency response teams and battlefield medics for point-of-injury transfusion. To address these challenges, a significant interest has emerged in the development of synthetic platelet surrogates that can render efficient hemostasis via platelet-mimetic mechanisms, while allowing advantages of large-scale manufacture, sterilization to minimize contamination risks, longer shelf-life, and improved availability and portability. To this end, we are conducting research on bio-inspired design of platelet's hemostatically relevant mechanisms on a liposomal template, via utilization of nanomaterials that allow modular mimicry of each of the mechanistic components described above. By optimizing these modular capabilities independently yet synergistically, we envision to subsequently integrate them for a unique 'synthetic platelet' system for targeted modulation of hemostatic output (**Figure 1**).

Materials and Methods: Drawing inspiration from natural platelet's site-specific hemostatic mechanisms: (1) liposome surface was heteromultivalently modified with vWF-binding (VBP), collagen binding (CBP) and integrin GIIb-IIIa-binding fibrinogen-mimetic peptides (FMP), (2) liposome surface was modified with PS shielded with a plasmin-cleavable poly-ethylene glycol (PEG) motif that can be exposed in an injury site-specific stimuli-responsive manner to render amplification of coagulation outputs, and (3) liposome core was loaded with hemostasis-augmenting agents like tranexamic acid (TXA) and PolyP that can be released also in an injury site-specific stimuli-responsive manner to render local amplification of clot characteristics. The injury site-specific activities of these modular design components were evaluated in vitro in microfluidic chambers (e.g. with vWF and collagen coated channels), aggregometry, thrombin generation assays (e.g. with thrombin-specific fluorogenic substrate bis-(p-Tosyl-L-Glycyl-L-Prolyl-L-Arginine Amide) and fibrin generation and morphology assays (e.g. D-dimer assay and scanning electron microscopy). Effect of the constructs on clot characteristics was evaluated in whole blood rotational thromboelastometry (ROTEM). Subsequently the platelet function mimetic modularly integrated nanoconstructs were evaluated in mouse and rat models of bleeding.

Results and Discussion: In vitro microfluidic and aggregometry studies demonstrated that the platelet-inspired constructs could site-selectively enhance 'primary hemostasis' (platelet adhesion and aggregation) as well as 'secondary hemostasis' (thrombin amplification and fibrin generation). The secondary hemostatic outputs were substantially enhanced with PS-incorporated constructs (**Figure 1**). Furthermore, stimuli-triggered release of coagulation augmenting agents like TXA and PolyP demonstrated additional enhancement of coagulation output in terms of enhancing clot mechanics and morphology. In vivo studies in small animal bleeding models indicated substantial benefit of these constructs in mitigating bleeding defects, indicating potential applications in thrombocytopenia, surgery and trauma. Ongoing studies are focused on optimizing each of the platelet-inspired 'modular functional mimicry' components to render superior hemostatic action targeted at injury site for various bleeding dysfunctions.

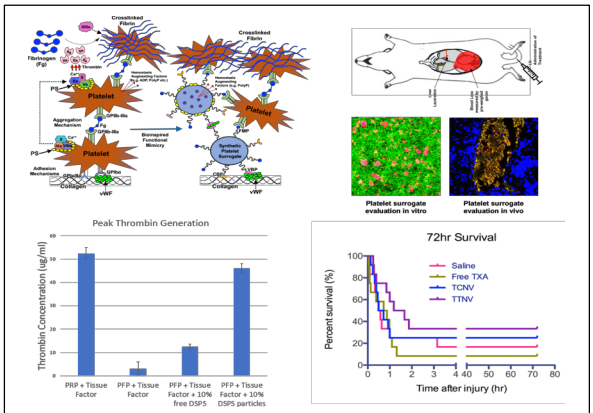

Figure 1. Schematic design and representative in vitro and in vivo study results on platelet-inspired particles

Conclusion: Modular nanoscale engineering of platelet-inspired hemostatic mechanisms on a liposomal template can allow site-specific enhancement of primary and secondary hemostatic mechanisms, thereby leading to the design of a superior synthetic platelet surrogate for applications in transfusion medicine.

References: [1] Versteeg HH, et al. ***Physiol Rev*** 93: 327–358, 2013; [2] Hickman et al., ***Advanced Materials***, 2018, https://doi.org/10.1002/adma.201700859