Platelet-inspired Biomaterials Engineering for Synthetic Intravenous Hemostat

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Statement of Purpose: Loss of platelets due to traumatic bleeding or surgery, as well as, drug-induced or congenital defects in platelet number and function, can lead to a variety of bleeding complications. For managing such scenarios, transfusion of natural platelet products is routinely used in the clinic. These transfusions primarily use allogeneic platelet concentrates (PCs) suspended in autologous plasma. PCs have a very short shelf-life (~3-5 days) and high risk of pathologic contamination [1]. Other platelet-derived products e.g. frozen (-80^{o}C), cold-stored (4^{o}C) or lyophilized platelets, platelet-derived microparticles and infusible platelet membranes (IPM) suffer from similar contamination risks [1]. Also, since all these products are derived from pooled blood of multiple donors, they present high risk of blood-borne infection. These products also cause a variety of biologic side effects [1]. Due to these issues of natural platelet products, there is a significant clinical interest in synthetic platelet substitutes that can render efficient hemostasis while allowing advantages of large-scale preparation, minimum contamination risk via effective sterilization, longer shelf-life, and absence of biologic or pathologic side effects. To this end, we present a unique platelet-inspired hemostat design that combines platelet's key biochemical hemostatic mechanisms of injury site-specific adhesion and aggregation, with its vascular margination promoting physico-mechanical features, on synthetic biomaterials platforms. Our results show enhanced hemostatic efficacy of this platelet-inspired design, both in vitro and in vivo.

Methods: *Combining platelet-inspired adhesion and aggregation mechanisms:* For hemostasis, platelets adhere to the injury site via shear-dependent binding to VWF and shear-independent binding to Collagen [2]. Post adhesion, platelets are activated and the active platelets undergo aggregation via binding of platelet integrin GPIIb-IIIa to Fibrinogen [3]. Inspired by these mechanisms, we have utilized heteromultivalent surface-modification of synthetic particles with VWF-binding, Collagen-binding and active integrin GPIIb-IIIa-binding small peptides. Using lipid-peptide conjugates, we have engineered heteromultivalently decorated liposomal constructs as platelet-inspired model hemostats. These constructs were studied for their hemostatic capability in vitro using a parallel plate flow chamber (PPFC) set-up where fluorescently labeled constructs and low concentration of natural platelets were allowed to flow over VWF/collagen-coated (injury site simulating) versus albumin-coated (negative control) surfaces. Construct-induced platelet recruitment and aggregation was monitored with fluorescence microscopy [4].

Incorporating platelet-inspired physico-mechanical attributes: Platelet's margination to the vascular wall in a hemodynamic flow environment is significantly influenced by its discoid shape and mechanical flexibility [5]. Inspired by these cues, particles mimicking platelet-inspired discoid shape and flexibility were engineered using albumin via a layer-by-layer assembly technique. These particles were heteromultivalently surface-modified with the same peptides as before. The margination of these particles compared to similarly decorated spherical rigid particles was studied in vitro in microfluidic chambers. Following this, 6the particle interaction with platelets in vitro under flow on VWF/collagen surfaces was studied using the PPFC set-up as before. Finally, the hemostatic capability of this integrative synthetic platelet design was evaluated in vivo in a mouse tail transection model.

Results: Combining platelet-inspired biochemical hemostatic mechanisms of injury site-specific adhesion and aggregation, via heteromultivalent decoration of peptides on biomaterial-based particle platforms showed enhanced ability of the constructs to recruit active platelets to injury site-specific protein-coated surfaces under a flow environment [4], thereby increasing site-specific platelet aggregation (primary hemostasis). The integrative platelet-inspired synthetic hemostat design incorporating the biochemical adhesion and aggregation mechanisms on the surface of platelet-mimetic discoid flexible particles, showed high hemostatic efficacy in vivo by reducing tail bleeding time by ~ 70% [5].

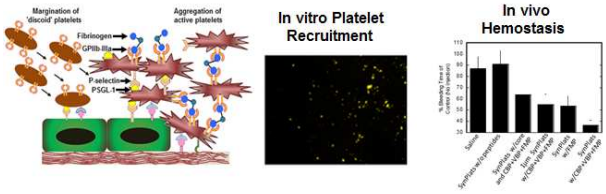

Figure 1. Design inspiration and representative in vitro and in vivo results for platelet-inspired hemostat

Conclusion: Integrating platelet-inspired biochemical and physico-mechanical properties on biomaterial platforms provides a uniquely engineered design of synthetic hemostats for future transfusion applications.

References: [1] Modery-Pawlowski et al, *Biomaterials* 34(2): 526-41 (2013). [2] Ruggeri et al, *Circ Res* 100(12):1673-85 (2007). [3] Jackson SP. *Blood* 109: 5087-95 (2007). [4] Modery-Pawlowski et al, *Biomaterials* 34(12): 3031-41 (2013). [5] Anselmo et al, *ACS Nano* (accepted 2014; DOI: 10.1021/nn503732m)