Evaluation of Safety and Efficacy of Platelet-like Particles in Trauma Models

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Statement of Purpose: Uncontrolled bleeding following trauma represents a significant clinical problem; more than 60,000 Americans die each year from hemorrhaging. Clot formation is crucial to the cessation of bleeding and involves the formation of a platelet plug within a fibrin network. A critical role of platelet function is the ability of their surface receptor GPIIb/IIA to bind fibrin at injury sites, thereby promoting clot formation. However, following traumatic injury, platelet activity is reduced, robust clot formation is not observed, and exsanguination can occur. The current clinical standard of care to treat bleeding after trauma is administration of platelet transfusions; however, donated platelets have a short shelf life and pose immunogenicity risks to the patient. Therefore, there is a critical need to develop hemostatic platelet alternatives. To circumvent these obstacles, our group has developed synthetic platelet-like particles (PLPs) that are capable of enhancing clot formation and stemming blood loss in rodent injury models. PLPs are constructed by functionalizing highly deformable microgel particles with fibrin binding ligands. To improve the translational potential of PLPs, we explored the use of different fibrin binding motifs including a full length antibody specific to fibrin fragment E, fibrin b knob mimicking peptides, and fibrin specific sdFvs clones.

Methods: PLPs were formed from ultralow crosslinked (ULC) poly(N-isopropylacrylamide-co-acrylic acid) microgels coupled to full length anti-fibrin fragment E antibodies (Frag E-PLPs, single domain variable fragment antibodies (df5-C PLPs), or fibrin B knob mimicking peptides(B-knob PLPs). PLP activity was asessed via analysis of clot structure with confocal microscopy, fibrin binding ELISAs, and evaluation of bleeding and safety parameters following intravenous injective of PLPs in murine and porcine liver laceration models.

Results: All three PLP iterations were observed to have high fibrin binding specificity, augment *in vitro* fiber density when incorporated into a fibrin clot, and decrease total blood loss in a liver laceration mouse model of traumatic bleeding. However, we determined that B knob PLPs required a higher therapeutic dose than the other candidates due to lower fibrin affinity. Biodistribution studies conducted in mice with Frag E and df5-C PLPs determined that the majority of the particles were excreted into the urine by 48 hours post injection. df5-c PLPs were selected as the candidate with the highest translational potential due to its low production cost, high fibrin specificity, and optimal *in vivo* activity. Therefore, df5-C PLPs were utilized in a porcine liver laceration injury model to analyze PLP efficacy within a large animal model of traumatic bleeding. Blood loss over two hours, blood chemistry and coagulation parameters, vital signs, and histological analysis indicate improved clotting responses in animals treated with PLPs. Immunohistochemistry of tissue wound sites reveals colocalization of PLPs and fibrin within wound areas. No off target effects were observed in peripheral organ tissue. Analysis of complement C3a activity indicated no significant activation in PLP treated groups compared to saline controls.

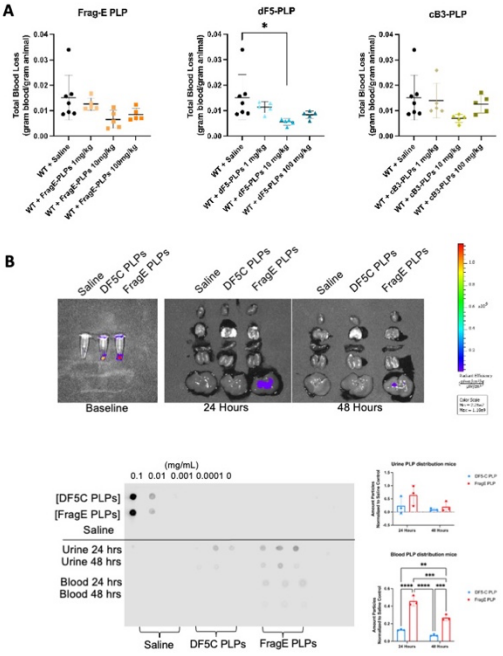

Figure 1. PLPs reduce total blood loss in a murine model of traumatic liver laceration injury (**A**). All three PLP candidates (antibody-based, left; dF5-C-based, middle; peptide-based, right) result in decreased total blood loss in mice treated intravenously with PLPs. Bisodistrubtion studies indicate particle clearance into urine by 48 hours post injection (**B**). *p<0.05; **p<0.01, ***p<0.001, ****p<0.0001.

Conclusions: Overall, these results indicate that all three PLP candidates reduce blood loss *in vivo*. However, df5-C PLPs are superior in augmenting clot formation *in vitro* and improving bleeding outcomes *in vivo* in murine and porcine models.