Intravenously-Administered Polymers for Modulating Clot Properties and Inducing Hemostasis

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Statement of Purpose Trauma is responsible for the largest number of deaths of Americans younger than age 44 in civilian populations and on the battlefield [1]. The majority of these fatalities are attributed to hemorrhage. While well-established technologies such as tourniquets and hemostatic gauzes exist to staunch bleeding in injuries sustained to the extremities, there are currently no effective clinical interventions to stop bleeding in non-compressible injuries (e.g. injuries to the trunk). The goal of this research is to develop a hemostatic agent that can be administered systemically, localize at sites of vascular injury, and induce stable clot formation. In the current work, we have engineered a linear, hydrophilic hemostatic polymer (**HP**) which is able to promote stable clot formation through non-covalent crosslinking of the proteinaceous fibrin fibers that form the supporting matrix in blood clots.

Methods and Materials Hemostatic polymers were synthesized by RAFT polymerization and bioconjugation of fibrin-binding domains. **HP** was subsequently characterized using H^1 NMR, GPC, and UV spectroscopy. To confirm **HP** integration into fibrin networks, fluorescent analogues of **HP** and a scrambled polymer control **SCRP** were added to clotting solutions containing fluorophore-labeled fibrin precursor (fibrinogen) and fibrinogen-cleaving enzyme (thrombin). After fibrin formation, confocal imaging was used to determine **HP** signal in relation to fibrin. SEM imaging was completed to determine the effects of **HP** on fibrin nanostructure. Clotting kinetics, clot strength, and clot lysis were evaluated *in vitro* using thrombelastography (TEG). Finally, **HP** was intravenously injected into a rat model of femoral artery injury. After injection, clamps preventing bleeding from the arterial wound were removed and rats were allowed to bleed and clot within the first 15 min. After 15 min, normal saline was infused to raise and maintain blood pressure above 60 mm Hg thereby testing the stability of control and HP-crosslinked clots when exposed to increasing blood pressures – a relevant challenge that clots face during fluid resuscitation.

Results Fluorescence from **HP** exhibited fiber morphology coinciding with fibrin signal confirming that **HP** is integrated into the fibrin matrix (**Fig. 1**). Furthermore, **HP**-integrated fibrin networks are denser with thinner fibers and smaller pore sizes – consistent with a highly crosslinked network (not shown).

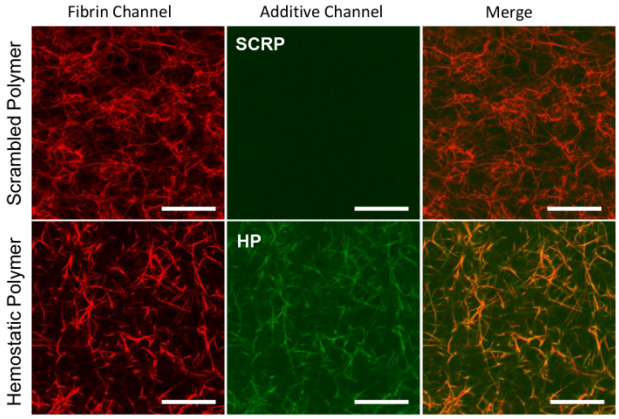

Fig. 1. Confocal imaging of HP interaction with fibrin fibers. Scale bar = 20 µm.

TEG studies showed that the presence of **HP** during fibrin formation significantly accelerated clotting rate by 23-41%, increased clot stiffness by a factor of 2.5, and reduced clot lysis when plasmin was added to clotting solutions to recapitulate hyperfibrinolytic conditions present during trauma-induced coagulopathy (not shown). When injected into rats, **HP** significantly reduced blood loss during saline infusion/fluid resuscitation showing that **HP**-modified clots are able to withstand higher blood pressures with less blood loss due to rebleeding. Additionally, **HP**-treated rats required less saline infusion to maintain blood pressure above 60 mm Hg due to the ability of clots to maintain a closed system. Smaller infusion volumes are beneficial to prevent dilution of circulating clotting factors. Lactate levels in the blood were significantly lower in **HP**-treated rats, indicating better oxygen delivery to tissues.

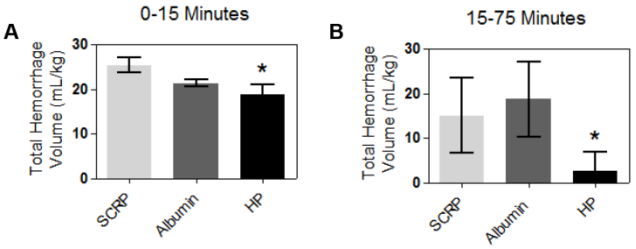

Fig. 2. Cumulative hemorrhage volume during (A) free bleeding and (B) fluid resuscitation in rat femoral artery injury model. * Indicates significance ($p < 0.05$) compared to SCRP and albumin controls in A and B, respectively.

Conclusion Fibrin-crosslinking **HP** increases hemostatic efficacy *in vivo* and can potentially be used to induce clotting in a range of blood clotting disorders.

References [1] E. G. Krug, G. K. Sharma, R. Lozano, The global burden of injuries. *Am J Public Health* **90**, 523-526 (2000).