Engineered Synthetic Platelets and Their Augmentation of Hemostasis

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Introduction: Following severe trauma the body is often unable to establish hemostasis. The most common treatments for aiding this normally intrinsic process include Quik-clot® or recombinant Factor VII (NovoSeven®). While useful, they have limitations. Quik-clot® is limited to external application. NovoSeven® is potentially immunogenic, presents the risk of stroke, and its intended use is limited to bleeding associated with hemophilia. Endogenous platelets play a dynamic role in establishing hemostasis. They not only aggregate at the site of injury, but also facilitate the adhesion of other platelets and hemostatic agents. If one could create a synthetic platelet system that was non-immunogenic, stable at room temperature, and efficient in augmenting hemostasis in a localized manner, one could mitigate the vascular hemorrhage associated with life threatening traumas and disease. To address this need, we created a synthetic platelet consisting of a nanosphere core comprised of a poly(lactic-co-glycolic acid)-poly(ε-carbobenzoxy-**L**-lysine) block copolymer (PLGA-PLL). Polyethylene glycol (PEG) terminated with the cell recognition motif arginine-glycine-aspartic acid (RGD) was conjugated to the core surface to finalize the synthetic platelet structure. We hypothesized that by varying the RGD motif and PEG molecular weight, we could manipulate interactions between synthetic and endogenous platelets.

Methods: The nanosphere core of the synthetic platelet was fabricated using a single emulsion technique. Synthetic platelets and their constituents were characterized using ^{1}H-NMR, UV-vis, amino acid analysis, and dynamic light scattering. Following successful synthesis and fabrication, interactions with rat platelets were determined both *in vitro* and *in vivo*. *In vitro* analysis was used for optimizing material properties for platelet adhesion. Variations included PEG molecular weight and RGD motif (i.e. RGD, RGDS, and GRGDS). Following optimization, a mechanical perturbation to the rat macrovasculature (femoral artery/vein), following an intravenous injection of the synthetic platelets (10, 20, or 40 mg/ml), was utilized for determining hemostatic efficacy *in vivo*. Briefly, a transverse cut in the left femoral vessel, encompassing two-thirds of the vessel circumference, was performed and the cessation of bleeding was recorded.

Results: By varying both PEG molecular weight and the RGD motif we were able optimize our synthetic platelet's ability to induce platelet adhesion *in vitro*, and establish hemostasis *in vivo*. As seen in Figure 1, platelets comprised of PEG molecular weight 4,600 Da and GRGDS mitigated bleed times in the femoral artery the greatest, as compared to saline injection alone, 130±13s 237±21s, respectively. Electron microscopy validated an intimate association between the synthetic platelets and clot formation *in vivo* (Figure 2). We found this

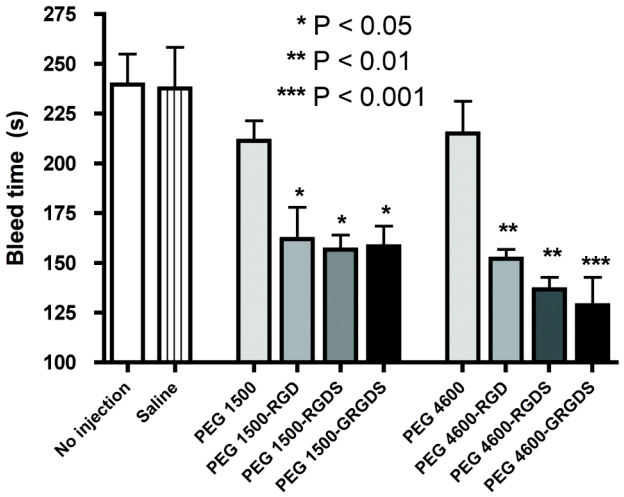

Figure 1. Femoral artery bleed times following 20 mg/ml administration. Mean ± SEM. Statistics compared to saline injection.

hemostatic effect to be dependent on both dose and vessel type. An intravenous injection of 20 mg/ml was optimal, while 40 mg/ml resulted in respiratory irregularities, and 10 mg/ml had no effect. Analysis in the femoral vein found no differences in bleed times between groups and controls.

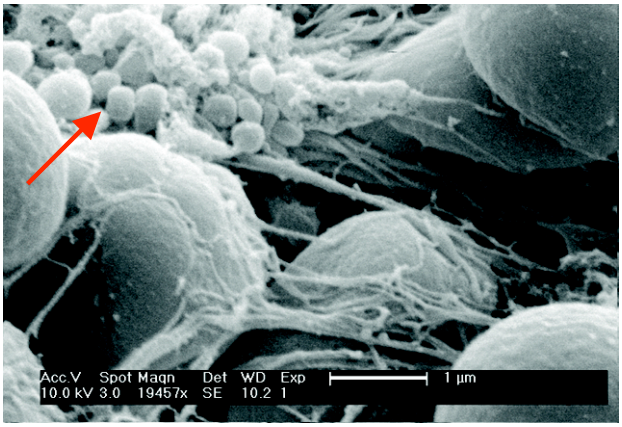

Figure 2. Excised clot from femoral artery injury with PEG4600-RGDS synthetic platelets (red arrow) and RBCs.

Conclusions: By administrating our synthetic platelets, we were able to decrease bleed times in a major vascular injury model by approximately half. The synthetic platelets not only participated in the clot formation but also showed no adverse effects systemically. We found that by varying the molecular weight of our PEG arm, thus varying the proximity of our RGD motif to the nanosphere surface, we were able to mitigate bleed times *in vivo* as well as vary platelet affinity *in vitro*. Our findings suggest that in an injured environment, where platelet aggregation is not the primary means of hemostasis (i.e. vasoconstriction is prominent in an artery/arteriole), synthetic platelets significantly augment clotting. These results imply that a synthetic platelet may be a feasible substitute for current treatments in facilitating hemostasis in an injured or pathological state.