Intravenously Administered Nanoparticles to Control Bleeding

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Statement of Purpose: Seventy percent of all wounds sustained by combatants in action are caused by explosive munitions leading to blast-induced injury. (Coupland) This type of trauma is often associated with severe internal hemorrhaging, specifically in the gas-filled organs and CNS tissues. Following severe trama, the body is often unable to establish hemostasis. Conventional treatments can only be applied externally and are thus ineffective to treat internal bleeding. Intravenous injection of recombinant factor VIIa (NovoSeven®) has been extensively used to treat uncontrollable bleeding in hemophilic patients but its efficacy in non-hemophilic patients is debatable and may be associated with adverse thromboembolic events. (O'Connell)

Endogenous platelets, in concert with clotting factors and thrombin, mediate hemostasis by adhering to the site of injury in the vessel wall and aggregating other activated platelets to impede the flow of blood. Bertram and Lavik previously developed a room-temperature stable polymer nanosphere formulation that mimics the selective aggregatory effects of endogenous platelets. The nanospheres consist of a poly(lactic-co-glycolic acid)-poly(l-lysine) (PLGA-PLL) core with poly(ethylene glycol) (PEG) arms conjugated to the surface and terminated with the arginine-glycine-aspartic acid (RGD) motif. This short peptide selectively targets and binds endogenous platelet glycoprotein IIb/IIIa which is only exposed in activated (prothrombotic) platelets. This specificity for injury site and degradable nanosphere formulation also allows the particles to be used as drug delivery vehicles.

These nanospheres have been previously shown to augment hemostasis in a rat femoral artery injury model (Bertram et al.). To further assess their efficacy in internal bleeding, here we investigate these synthetic platelets in animal models of blunt trauma. This work includes scaling up the synthesis and production of the synthetic platelets and the validation of such efforts. Additionally, biodistribution of the nanopsheres is assessed, building on the previous biodistribution study by Bertram et al.

Methods: PLGA (Resomer 503H) was purchased from Boehringer Ingleheim, Germany. PEG was purchased from Polyciences Inc.. All other reagents were purchased from Sigma Aldrich and used as received. PLGA-PLL-PEG copolymer was made using standard DCC and CDI bioconjugation techniques. The nanosphere core of the synthetic platelet was fabricated using a single emulsion technique. Nanospheres are flash frozen after purification steps, lyophilized and stored until use. Synthetic platelets and their constituents were characterized using ^{1}H-NMR, UV spectroscopy, amino acid analysis (AAA), scanning electron microscopy (SEM) and dynamic light scattering (DLS) to verify intended formulation.

The rat femoral artery injury was performed using the scaled up synthetic platelet formulation to reproduce our baseline results, using the same methodology from Bertram et al.

Additionally, we investigated various models of blunt trauma in the rat. In brief, the left lobe of the liver is cut sharply 1.3 cm away from the superior vena cava. Immediately following induction of injury, the surgical cavity is closed and synthetic platelets are administered. Control groups include no injection, saline injection, scrambled peptide sequence (inactive targeting sequence), and recombinant factor VIIa. An injection of 20 mg/ml synthetic platelets in PBS was used in the experimental group. Bleed times and bleed volumes are assessed using pre-weighted sponges to soak up blood. All major organs are harvested for histology and biodistribution of synthetic platelets.

Results: A dose titration was performed previously for the femoral artery injury model in the rat, and yielded an optimal concentration of nanospheres to be 20 mg/ml in a 0.5 carrier solution (10 mg in 1x PBS).

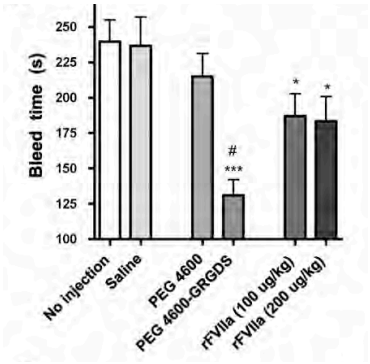

Figure 1. Bleed times in femoral artery injury model shows synthetic platelets reduce bleed times by ~50%, and are significantly more effective than recombinant factor VIIa.

For a study with multiple rodents/models, we deemed scale up of the synthetic platelets to be necessary. We met this challenge, scaling up production from a 3-week protocol that synthesized 60 mg of product to a 1-week protocol that synthesizes 600 mg product.

Particle size was determined to be approximately 154 ± 36 nm diameter by SEM and 345 ± 81 nm hydrodynamic diameter by DLS. ^{1}H-NMR, UV-vis, and AAA confirmed the polymer formulation, and verified that solvents, byproducts and unreacted reagents were removed during purification steps.

Conclusions: The synthetic platelets reduced bleeding times in models of trauma. The room-temperature stable nanospheres have the potential to greatly impact the treatment of uncontrollable hemorrhage and have shown utility as an injury-site targeted drug delivery vehicle.

References: Coupland RM. BMJ. 1999;319:410-412.
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