Heat Stable Polymer for Hemostatic Nanoparticles

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Statement of Purpose: Uncontrolled hemorrhage is a leading cause of death for civilians and soldiers (Champion, H. R. J Trauma. 2003; 54:S13–9. Krug, E. G. Am J Public Health. 2000;90:523–526). Severe bleeding can result in death before the patient ever reaches a hospital and, if the bleeding is internal, there are no treatments available to first responders (Kauvar, D. S Journal Trauma 2006;60:S3–S11). Current treatments for internal bleeding include blood transfusion and use of recombinant coagulation factors, both of which require refrigeration and are therefore undertaken in hospitals. In addition there are serious side effects and high costs associated with these treatments.

As an alternative to these treatments, 250 nm polymer nanoparticles decorated with a glycoprotein IIb/IIIa targeting peptide (GRGDS) have been developed to crosslink platelets and stabilize the platelet plug (Bertram, J. Sci. Transl. Med 2009;1:11-22). However, these particles are currently formulated with a poly(lactic-co-glycolic acid) (PLGA) core, which is an amorphous polymer with a glass transition temperature of 40 ^{o}C. While they remain stable at room temperature, when subjected to higher temperatures (which may occur in battlefield locations such as Afghanistan, where the temperature can reach near 50 ^{o}C) the transition can result in formation of large aggregates.

To mitigate this problem, we will replace the PLGA core with poly(lactic acid) (PLA). PLA is more crystalline (Tm ~160 ^{o}C) and has a glass transition temperature of 55 ^{o}C. This will increase the stability of the core at temperatures around 50 ^{o}C by both increasing the temperature at which the amorphous portion of the core transitions and by maintaining crystallinity while the particles are below the degradation temperature. The crystallinity, and therefore temperature stability, can be further enhanced by including both PLLA and PDLA, which form a stereocomplex. This change in core material will allow particles to be stored up to 50 ^{o}C, making them a more viable option for use by first responders, both in civilian and battlefield internal hemorrhage.

Methods: Materials: Poly (ethylene glycol) (HO-PEG-CM MW 5,000) was purchase from Laysan bio. L-lactide was purchased from Polysciences Inc. D-lactide was from PURAC biomaterials. 1,3-Bis-(2,4,6-trimethylphenyl)imidazole-2-ylidene (IMes) was purchased from Sigma Aldrich. All reagents were ACS grade and purchased from Fisher Scientific.

Methods: PLA and PLA-PEG block copolymer were generated via ring opening polymerization catalyzed by Imes. GRGDS peptide was conjugated to the PEG using EDC/NHS to activate the carboxylic acid on the PEG and conjugate it to the peptide amine. Particles were formed via nanoprecipitation and collected by centrifugation.

Molecular weight of the product was determined by gel permeation chromatography (GPC) (Shimadzu) Glass transition and melting temperatures were determined by differential scanning calorimetry (Q100 TA instruments). Particle size was determined by dynamic light scattering (DLS 90Plus, Brookhaven Instruments Corportation) and scanning electron microscopy (Hitachi S4500).

Particles are stored at 50 ^{o}C before additional testing to confirm that they maintain their size, shape, and effectiveness when exposed to temperature extremes. Effectiveness is assessed by measuring survival and blood loss in a rat liver injury mode.

Results: Our block PLLA-PEG copolymer had a molecular weight of 35 kDa in GPC. This was supported by NMR data, indicating Mn of 40 kDa. The PDLA product had an Mn of 30 kDa. DSC (figure 1) indicates that the thermal properties of the polymer are as predicted. Values are slightly lower in the block copolymer, indicating the effect of the presence of PEG.

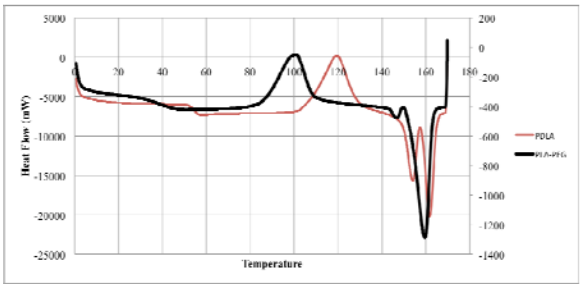

Figure 1. DSC PLLA-PEG and PDLA

Particles formed by blending these polymers can be tuned between 100nm and 500nm by adjusting the ratio of PLLA-PEG and PDLA, confirmed by DLS and SEM results. Storage of the particles at high temperature followed by additional imaging and DLS demonstrates that the particles maintain their size and shape when subjected to temperature extremes.

Finally, in vivo testing in a rat liver injury model demonstrates the effectiveness of the particles in reducing bleeding and increasing acute survival. Previous work with PLGA hemostatic nanoparticles in this model increased survival by 50% at the one hour time point (Shoffstall, AJ, Biomacromolecules 2012;13:3850–3857).

Conclusions: PLA-PEG block copolymer is simple to synthesize and has properties that make it ideal for storage at high temperatures, making it a good replacement for PLGA as the core of our hemostatic nanoparticles. The higher glass transition temperature in addition to increased crystallinity and stereocomplexation will allow the core to maintain more cohesion at higher temperatures, preventing aggregation and loss of function. These particles have properties similar to our previous generation of PLGA particles, which were effective in increasing survival in a rat liver trauma model.