Nanofibrous PLA Mesh is Wound Adherent and Hemostatic in Pig Liver Resection Model

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Statement of Purpose: Liquid fibrin sealants (LFS), which consist of human plasma-derived fibrinogen and thrombin, form an adherent, viscoelastic polymeric clot that is an effective barrier to stop hemorrhage from traumatized blood vessels or blood plasma loss from large surface area, oozing wounds. The current methods used to apply LFS as a tissue glue are inefficient and use about 500 to 1000 mg of biologics per dose. Because of the high protein content, LFS is an expensive hemostatic device for wound treatment. In addition, the fibrinogen is stored as a freeze dried powder which inherently possesses slow dissolution kinetics making the reconstitution and preparation of the LFS cumbersome at these dosages. We hypothesized that minimizing the exogenous application of human plasma-derived LFS could be achieved by shifting the hemostatic barrier burden to a re-absorbable, pliable, Poly(D,L lactide) (PLA) mesh. Thus the role of the LFS is reduced to only a thin layer of adhesive, rather than a polymeric barrier. The PLA mesh was designed to be microporous when made by electrospun formation of approximately 500 nm diameter fibers. The role of the micropores is to enable physico-mechanical intercalation of both endogenous blood clot and LFS as well as the co-polymerization of fibrins within the mesh. We investigated the compatibility of forming fibrin clots within the mesh structure and its wound adhesiveness in a grade 5+ pig liver resection model.

Methods: Ultrafine Poly(D,L lactide) fiber mesh was manufactured from medical grade biopolymer at LNK Chemsolutions LLC by using a proprietary [1], high-speed electrohydrodynamic (EHD) production spinneret housed in a Class 100 enclosure. The PLA mesh for surgical use consists of a 5 cm by 5 cm 4-ply bandage with a thickness of about 800 microns. Macroscopic pores and corrugations were added to facilitate intercalation of blood and exogenous LFS. The intercalation of LFS proteins to the nanofibers were evaluated *in vitro* by impregnating the mesh with fibrinogen, drying the bandage and then exposing it to thrombin. The interaction of the proteins with the nanofibers was analyzed by scanning electron microscopy (SEM). These bandages were also tested *in vivo* on grade 5+ pig liver surgical resection models. A segment of the left lateral liver was resected about 6 cm from its tip with a base of approximately 11.5 cm. LFS, consisting of 9 mg/ml plasma-derived fibrinogen, 2,463 U/ml Factor XIIIa, 105.6 U/ml thrombin and 12 mM calcium, was sprayed onto the PLA mesh and applied to the oozing wound. Nine 5 cm by 5 cm bandages were applied one-by-one to cover the wound surface area. Pressure was applied for approximately 10 minutes after which hemostasis was evaluated. The pigs' mean arterial pressures were maintained at greater than 100 mmHg throughout the procedures.

Results: PLA can be electrospun to make a random felt-like matrix structure with average fiber diameters of 500 nm (Figure 1A). A stable lamina of about 100 to 150 um could be made in 10 inch wide swaths at an extrapolated rate of 90 yards per day. The ability of LFS to intercalate into the hydrophobic PLA mesh was evaluated *in vitro* by SEM. PLA nanofibers could be coated with fibrinogen. When exposed to thrombin, a fibrin clot formed throughout the nanomesh (Figure 1B). PLA nanomesh bandages were tested *in vivo* with and without a coating of LFS to a grade 5+ pig liver resection model. PLA bandage alone resulted in hemostasis and wound adherence; however, the addition of a thin film having a total of about 50 mg (covering the 9 bandage swaths) of sprayed human plasma-derived LFS (Figure 2) increased the hemostatic efficiency. Hemostasis was obtained at >100 mean arterial pressure.

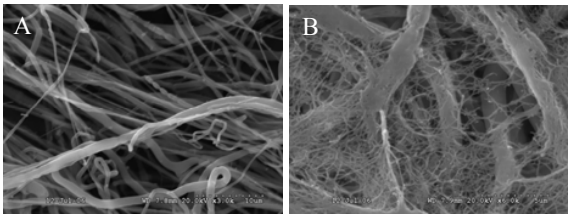

Figure 1. SEM of PLA nanomesh alone (A) and following impregnation with fibrinogen and then exposure to thrombin solution (B).

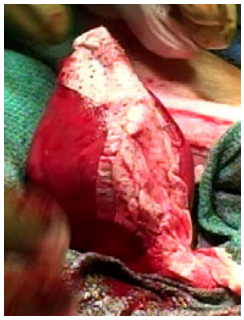

Figure 2. Hemostasis achieved on a lethal grade 5+ pig liver resection model by application of LFS coated PLA nanomesh.

Conclusions: In this work we demonstrate that it is feasible to make an ultrathin resorbable, plyable hemostatic device, which minimizes the use of biopolymer and fibrin sealant. The ultrafine nature of such device allows dispersion of the optimized sealant composition onto a high surface area for an effective formation of an intercalated fibrin-nanomesh structure that exhibits great tissue-adhesive properties. This study shows that LFS sealants and blood borne fibrin can form clot structure that intimately intercalates and coats the nanomesh. The wound adhesiveness with and without exogenous fibrin sealant was sufficient to achieve hemostasis in a grade 5+ pig liver resection model. Wound adhesion depended on only 200 um of mesh lamina thus minimizing both the amount of PLA and exogenous LFS needed to provide enhanced wound adhesiveness needed to be an effective barrier.

References:

[1] Larsen G. PCT Appl. No. IB2007/050107.