Development of a Novel Drug-Loaded Fibrin-Based Nanoparticle Biomaterial for Improved Surgical Wound Healing

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Statement of Purpose: While fibrin biomaterials function well as surgical sealants and glues, their high thrombin and fibrinogen concentrations promote the formation of a high-density matrix that does not promote cellular infiltration and healing. Furthermore, these materials require cold chain storage and have brief work times, allowing for little to no adjustment prior to full polymerization. We have developed pre-polymerized fibrin-based nanoparticles (FBNs), which allows for the use of physiologically relevant fibrin/thrombin concentrations that result in a colloidal biomaterial with optimal porosity for wound healing. Cold chain storage needs are also eliminated by the use of FBNs, as they may be stored in a lyophilized form at room temperature. While bulk fibrin materials can be used for drug delivery, FBNs allow for targeted drug and small molecule administration. Based on FBN's pre-polymerized colloidal structure, we hypothesize that an FBN-based surgical glue will promote enhanced cellular infiltration and wound closure compared to traditional fibrin glues when examined with respect to mechanics, *ex vivo* cellular interactions, and *in vivo* wound healing.

Methods: FBNs were formed through thrombin-mediated fibrin polymerization, followed by sonication, filtration, and lyophilization. As well as varying FBN concentrations, glue included the use of a patient co-factor: either platelet poor plasma or whole blood. $CaCl_2$ and thrombin were used to imitate contact with the native clotting cascade. To analyze clot structure as a function of FBN concentration, FBN biomaterials were prepared, polymerized for 12h, and imaged using cryogenic scanning electron microscopy (cryoSEM). ImageJ was used to quantify material porosity, pore size, density, fiber width, and fiber length. The effects of FBN concentration on the mechanics of wound closure were examined using a 3400 Series Single-column Instron UTS. Wound closure limit was obtained by allowing biomaterial to fully polymerize over a standardized 1 cm wound made in a segment of porcine arterial tissue, then applying tension until biomaterial rupture. n=7 per FBN concentration group for mechanical wound closure testing. To validate drug loading capabilities, 20 mg/ml FBN solutions were exposed to 0.25 ml solutions of varying tazarotene concentration. After 24h at 4°C on shaker, solutions were filtered via centrifugation, and washed twice in diH_2O. Centrifugation was repeated and pellets were resuspended. A standard curve was created and absorbance of loaded FBNs were measured using NanoDrop. To compare the wound closure effects of FBN release of tazarotene to exposure of free tazarotene, a 24h scratch assay was performed using human dermal fibroblasts. ImageJ was used to quantify cellular wound closure over time. Ongoing leporine studies apply this material to internal tissue healing in a vascular injury model.

Results: FBN biomaterial structure varied with FBN concentration, with increasing density and fiber alignment associated with higher FBN concentrations (30-40 mg/mL) and optimal porosity occurring at 10-20 mg/mL. Mechanical wound closure was highest for samples made with 20-30 mg/mL FBNs, with overall data suggestive of a truncated bell curve with left tail. Tazarotene loading studies showed a positive linear relationship between tazarotene in loading solution and measured tazarotene in the loaded FBNs. Cells exposed to FBNs loaded with 5 mg/mL tazarotene loading solution displayed the greatest degree of wound closure.

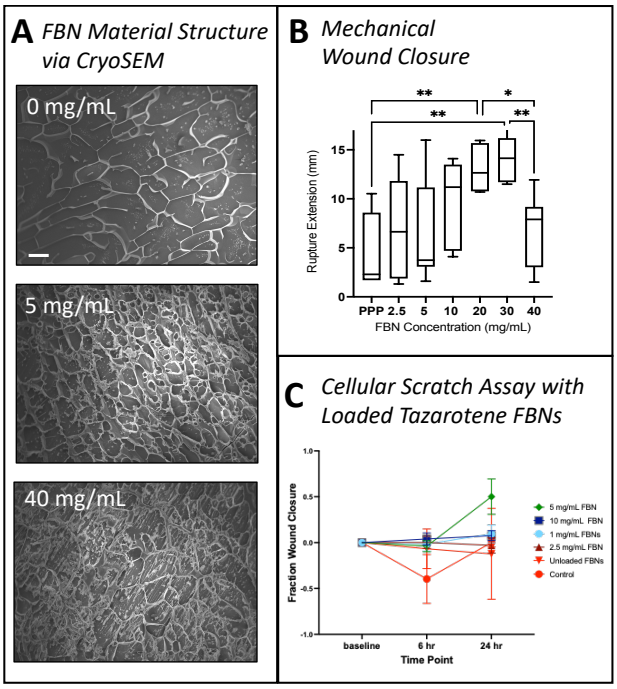

Figure 1: Physical properties of FBN biomaterial vary with FBN concentration and FBNs may be successfully loaded with tazarotene for improved local wound healing. Variation may be noted in (A) gross biomaterial structure and (B) mechanical wound closure. (C) Tazarotene-loaded FBNs increase speed of *ex vivo* wound closure. *p<0.05 **p<0.01. Scale bar = 10 microns.

Conclusion: Together, these data validate that FBNs can be used as a novel surgical sealant with wound healing properties. We have shown that material characteristics may be adjusted through variation in FBN concentration and that FBNs may be variably loaded with small molecules for local delivery, which positively affect *ex vivo* cellular migration. Ongoing studies include further *ex vivo* studies and *in vivo* models of vascular surgery.