Development of a Novel Surgical Glue Using Fibrin-Based Nanoparticles

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Statement of Purpose: Use of fibrin glue has improved surgical times and outcomes in diverse treatment areas including cardiothoracic surgery, trauma, and organ transplant. These glues are often impractical as their high thrombin and fibrinogen concentrations lead to very short working times and form a high-density mixture that does not promote healing. They also require cold chain storage. We have developed colloidal fibrin-based nanoparticles (FBNs) that, unlike traditional fibrin glues, are pre-polymerized, allowing for use of physiologically relevant fibrin/thrombin concentrations with optimal porosity to promote cellular infiltration. Lyophilized FBNs can be stored at room temperature, thereby eliminating cold chain needs. FBN glues can also be used for targeted drug and growth factor 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 and demonstrate tunability in its physical properties including: tensile strength, fiber density, adhesive strength, degradation time, and drug loading.

Methods: FBNs were formed through thrombin-mediated fibrin polymerization, followed by sonication, filtration, and lyophilization. As well as varying FBN concentrations, glue included platelet poor plasma, $CaCl_2$, and thrombin to imitate contact with the native clotting cascade. To analyze clot structure as a function of FBN concentration, FBN glue was prepared with the addition of 2.5% (w/w) Alexa-Fluor-488 fibrinogen, polymerized for 1h, and imaged using confocal microscopy. ImageJ was used to quantify fiber density. The effects of FBN concentration on glue mechanics were examined using a 3400 Series Single-column Instron UTS. Ultimate tensile strength (UTS) was obtained by allowing glue samples to fully polymerize, then applying tension until rupture. To measure shear adhesion, FBN glue was allowed to polymerize between glass coverslips, which were exposed to tensile forces and shear adhesive force was recorded. To analyze degradation, isotonic saline (0.9 mg/mL) was overlaid on FBN glue samples after 2hr polymerization. Samples were drained and weighed at predetermined timepoints. n=7 per FBN concentration group for fiber density, tensile strength, and adhesive testing. To validate drug loading capabilities, 20 mg/ml FBN solutions were exposed to 0.25 ml solutions of varying vancomycin concentration, using 1:9 bodipy fluorescent (488 excitation) to standard vancomycin. After 24 hrs at 4°C on shaker, solutions were centrifuged, supernatant removed, and pellets were washed once in H_2O. Centrifugation was repeated and pellets were resuspended. A standard curve was created and fluorescence of samples and standards were measured using a plate reader to determine the vancomycin concentrations. n=3 per loading solution.

Results: Fiber density of glue structure was highest at 25-30 mg/mL of FBNs, with an overall bell curve dose response. Tensile strength was highest for glue with 5mg/mL FBNs, with overall data suggestive of a truncated bell curve with right tail. A negative dose-dependent relationship was found between FBN concentration and adhesive strength, with increased concentration leading to decreased adhesive capacity. Degradation was slowest in glue with 80 mg/mL FBNs, with overall data suggesting a bell-curve dose response. Loading studies showed a positive linear relationship between vancomycin in loading solution and measured vancomycin in the loaded FBNs.

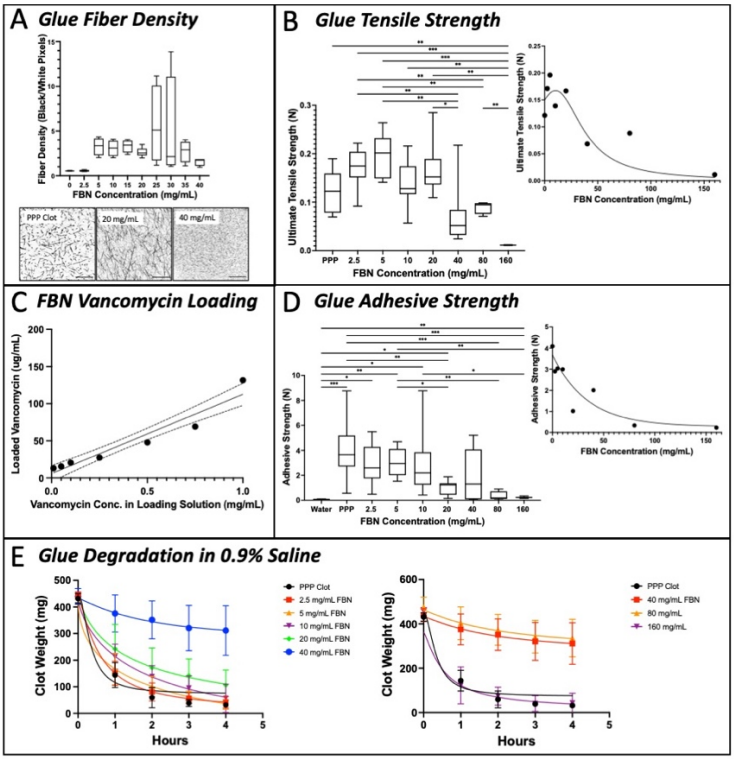

Figure 1: Physical properties of surgical glue vary with FBN concentration and FBNs may be successfully loaded with vancomycin for local drug delivery. Variation may be noted in (A) fiber density, (B) tensile strength, (D) adhesive strength, and (E) degradation curves. (C) FBNs demonstrate variable loading with vancomycin. *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001. Scale bar = 50 microns.

Conclusion: Together, these data validate that FBNs can be used to create a novel surgical glue. We have shown that glue characteristics may be tuned through adjustments to FBN concentration and that FBNs may be variably loaded with vancomycin for local delivery. Ongoing studies include functional drug delivery testing and analysis of wound healing outcomes.