Evaluation of fibrin-based scaffolds implanted in chronic spinal cord injury

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Statement of Purpose: The purpose of this study was to evaluate the effects of implanting fibrin scaffolds following chronic rat spinal cord injury (SCI). Fibrin is a natural wound healing matrix and can be covalently modified easily via the transglutaminase activity of Factor XIIIa during fibrin polymerization. Thus, fibrin presents an ideal material for the fabrication of tissue-engineered scaffolds for the treatment of nerve injury. It has been shown that fibrin provides a permissive environment for the proliferation and differentiation of neural progenitor cells (1). Furthermore, through covalent modification, fibrin scaffolds containing a heparin-binding delivery system (HBDS) can provide controlled release of growth factors. Controlled release of neurotrophin-3 from fibrin scaffolds containing a HBDS have been shown to promote increased neural fiber sprouting following acute rat spinal cord injuries compared to controls (2). In this study, we sought to evaluate the best method of fibrin polymerization for implantation into a dorsal hemisection injury model and to evaluate the biological response to fibrin scaffolds following chronic rat SCI

Methods: Fibrin scaffolds were made by mixing the following components: human fibrinogen (10 mg/mL), fluorescently labeled human fibrinogen (0.4 mg/mL), $CaCl_2$ (2.5 mM), and thrombin (125 NIH /mL) in Tris-buffered saline. The scaffolds were either allowed to polymerize partially into 10 µL beads for 5 min at room temperature prior to implantation or the mixture was injected into the wound site for *in situ* polymerization.

Adult female Long Evans rats (250-280 g) received a 1.2 mm deep dorsal hemisection of the spinal cord at level T9. After 14 days, the injury site was exposed, and scar tissue in the injury site was removed. At this time a fibrin scaffold was implanted or injected at the injury site. The animals were euthanized and perfused transcardially with 4% para-formaldehyde 14 days after scaffold implantation. All studies were performed with IACUC approval and in accordance with NIH guidelines.20 µm sagital sections containing fibrin were stained with anti-β- tubulin III antibody, which stains neurons, or anti-glial fibrillary acidic protein (GFAP) antibody, which recognizes astrocytes, the main cellular component of the scar formed around spinal cord lesions. Sagittal sections lacking fluorescent fibrin were double stained with either anti-beta tubulin III and anti-GFAP and anti-EDI (macrophage/ microglia). Finally, appropriate secondary antibodies were used, and each section was stained with Hoechst nuclear stain.

Two images of the GFAP staining bordering the rostral side of the lesion site and two bordering the caudal side were taken from 6 sections spaced 200 µm apart. The area of GFAP positive pixels was divided by the area of pixels in the entire image giving a percent GFAP density. All statistics were performed with Analysis of Variation (ANOVA, planned comparison post-hoc test).

Results/Discussion: Fibrin scaffolds that were allowed to partially polymerize prior to implantation could be visualized 7 days after implantation (Fig. 1A). Those fibrin scaffolds allowed to polymerize *in situ* were not visible in images taken 7 days after implantation. Analysis of the astroglial scar reveals SCIs receiving fibrin implants showed no significant difference in astroglial density compared to controls in this preliminary study (n = 3) (Fig. 1B).

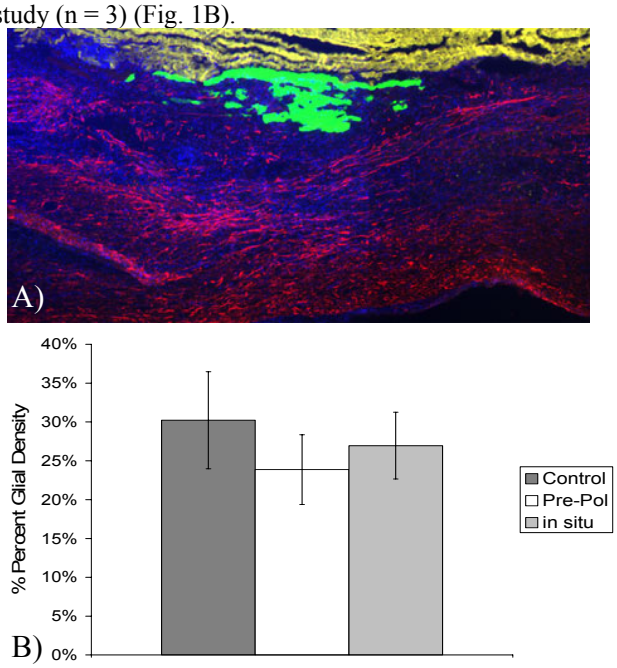

Figure 1. A) 20 µm sagittal sections stained with anti-beta tubulin III (red) showing a chronic SCI with implanted fibrin scaffold. White arrow shows a fluorescent fibrin scaffold (green) still visible after 7 days from the pre-polymerized fibrin group. B) Results from GFAP density analysis showing a control containing no fibrin, pre-polymerized fibrin implant (Pre-Pol), and *in situ* polymerized fibrin implant (*in situ*).

Conclusions: These results suggest that fibrin scaffolds that polymerized *in situ* were degraded at a faster rate than those allowed to polymerize prior to implantation. The increased degradation rate may be the result of components of the scaffold being cleared from the area prior to polymerization resulting in a less stable scaffold. Thus, future studies will utilize pre-polymerized fibrin scaffolds. Based on the density of astrocyte staining observed, we concluded that the fibrin scaffold did not increase astroglial scarring. In a prior time course study of the same injury with no treatment, we also saw a decrease in the immune response (macrophage and microglia) with time surrounding the lesion site. In future studies we will measure the immune and astroglial response of fibrin scaffolds implanted in SCI lesion over time and use the fibrin as a scaffold for stem cell transplantation..

References:

1) Willerth et al. Biomaterials 27 (2006) 5990–6003
2) Taylor and Sakiyama-Elbert Journal of Controlled Release 113 (2006) 226–235