Fibrin-based tissue engineered scaffolds containing neural progenitors cells for subacute spinal cord injury

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Statement of Purpose: Fibrin scaffolds have been shown to enhance neural fiber density and decrease accumulation of reactive astrocytes following spinal cord injury (1). It has also been shown that fibrin provides a permissive environment for the proliferation and differentiation of neural progenitor cells (2). Fibrin scaffolds can be modified to contain a heparin-binding delivery system (HBDS) that can provide controlled release of growth factors. In this study, we evaluate the viability and percent differentiation of transplanted neural progenitor cells embedded within fibrin scaffolds in a subacute spinal cord injury (SCI) model. Functional recovery was also assessed.

Methods: All studies were performed using the CE3 mouse ES cell line that expresses green fluorescent protein (GFP) under the β actin promoter. The undifferentiated ES cells were cultured as described previously (2). The undifferentiated CE3 cells were induced to form neural progenitor containing embryoid bodies (EBs) using the 4-/4+ retinoic acid treatment protocol as previously described (3). 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, and either no implant (control), fibrin scaffold alone (fibrin), 10 EB alone (10 EB no fibrin), 10 EB embedded in a fibrin scaffold (10EB + Fibrin), or 10 EB embedded in fibrin scaffolds with our heparin binding delivery system, 125ng of neurotrophin-3 (NT-3) and 20ng of platelet derived growth factor (PDGF) (10 EB + DS + GF).

The animals were euthanized and perfused transcardially with 4% para-formaldehyde at 2 and 4 weeks after scaffold implantation. All studies were performed with IACUC approval and in accordance with NIH guidelines. Serial 20 μm sagital sections containing fibrin were stained with anti-β- tubulin III antibody (Tuj1; neurons), anti-glial fibrillary acidic protein (GFAP; astrocytes) antibody and O4 (oligodendrocyte). Finally, appropriate secondary antibodies were used, and each section was stained with Hoechst nuclear stain.

To assess differentiation of transplanted cells, images of transplanted neural progenitors (NPs) were taken from every 12th section throughout the cord. The area of GFP positive pixels (representing NPs), and the area of pixels positive for both GFP and one of the three mature neural markers (Tuj1, GFAP, or O4; representing differentiated NPs) was measured in each section. An average across all the sections for each rat was then calculated to determine the average cross sectional area.

To asses the functional recovery, a grid walk behavioral analysis was performed. Rats walked on a grid of evenly spaced bars for 4 min. The total number of missed steps were recorded and divided by the total number of steps that were taken, yielding a fraction of missed steps. The fraction of missed steps correlates inversely to functional recovery.

All statistics were performed with Analysis of Variation (ANOVA, planned comparison post-hoc test).

Results/Discussion: After two weeks of transplantation, neural progenitors cells embedded in fibrin scaffolds with NT-3, PDGF and heparin binding delivery exhibited the largest co-stained cross sectional areas (Fig. 1A). Four weeks following scaffold implantation, all groups treated with neural progenitors exhibited a significant decrease in the fraction of missed steps on the grid walk test and thus a significant increase in functional recovery (Fig. 1B).

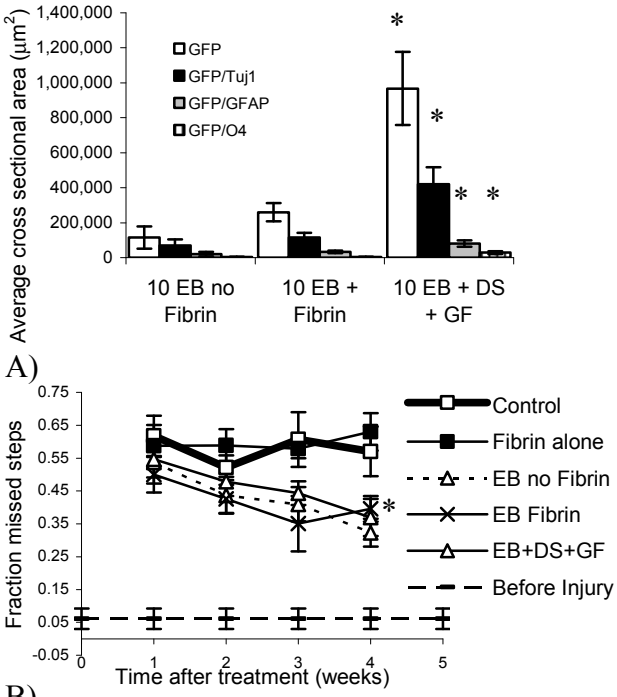

Figure 1. A) Spinal cords were harvested after 2 weeks of implantation. The average cross sectional area of GFP positively stained tissue is greatest in the 10 EB + DS + GF group. Similarly, the average cross sectional area of tissue positively stained for both GFP and TUJ, and GFP and O4 was greatest in the 10 EB + DS + GF group. All other average cross sectional areas were the same. * denotes $p<0.05$ versus 10EB no fibrin and 10 EB+Fibrin groups. B) Results of the gird walk behavioral analysis show that all groups treated with transplanted neural progenitors had a significant decrease in fraction missed steps and thus an increase in functional recovery. * denotes $p<0.05$ versus Control and Fibrin alone groups.

Conclusions: These results suggest that transplanted neural progenitor cell embedded in fibrin scaffolds and exposed to the controlled release of NT-3 and PDGF had an increase in survival and differentiation into all three neural sub types. Also, those groups treated with neural progenitors exhibited an increase in functional recovery 4 weeks after treatment in the grid walk behavioral test.

References:

1) Johnson et al. J Biomedical Materials Research (In Press)
2) Willerth et al. Biomaterials 27 (2006) 5990–6003
3) Bain, G., et al., Dev Biol, 1995. 168(2): p. 342-57.