In vivo assessment of guided neural stem cell differentiation in growth factor immobilized hydrogel scaffolds

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Statement of Purpose: Within the central nervous system, neural stem/progenitor cells (NSPCs) have been isolated that offer potential cellular treatments for spinal cord injury (SCI). Traditionally, biochemical factors and ECM derived proteins have been exploited for specific differentiation of NSPCs. Strategies for delivering stem cells and guiding differentiation are needed to achieve better functional outcomes after SCI. The overall goal of this work is to test a regenerative conduit we have created for treatment of SCI integrating NSPCs and a unique inductive hydrogel scaffold containing specifically tethered engineered signaling proteins we have shown can guide lineage commitment of NSPCs in the soluble form[1,2]. We hypothesize that NSPCs exposed to immobilized signaling molecules and implanted *in vivo* will promote spatial cell differentiation to desired lineages (neurons, astrocytes, oligodendrocytes) with minimal inflammatory response.

Methods

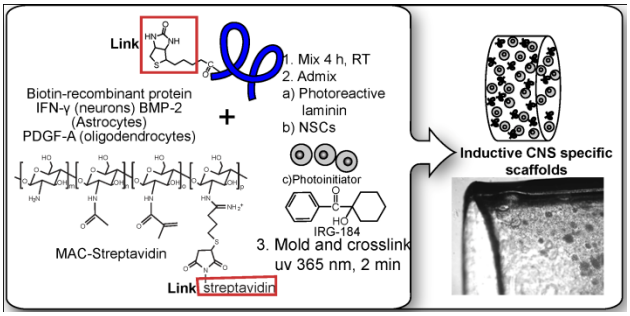

Figure 1. Scheme for creating 3-D recombinant growth factor immobilized stem cell seeded SCI conduits.

Materials synthesis, analyses and in vivo testing: The interior of conduits were filled with a soft hydrogel (E_Y < 1 kPa) composed of methacrylamide-chitosan (MAC), a material that we have previously shown to support NSPC differentiation[1,2]. MAC-SH was formed by reacting Traut's reagent (2-iminothiolane) with MAC, then functionalized with maleimide-streptavidin[1]. We created fusion proteins that were biotinylated on the N-terminus and contain the differentiation factors IFN-γ, PDGF-AA or BMP-2 which we have demonstrated specify neurons, oligodendrocytes and astrocytes respectively from NSPCs[2]. We first tested the degradation and release of each factor *in vitro*. For animal testing, modified MAC was created, factors were tethered, photoreactive laminin, NSPCs and initiator were added, then hydrogels were formed inside chitosan spinal cord guidance tubes (made as previously described[3]). For *in vivo* compatibility testing, we studied conduits containing MAC alone; MAC mixed with NSPCs, MAC with immobilized or soluble factors (IFN-γ, PDGF-AA, and BMP-2) with NSPCs and MAC with immobilized or soluble factors. NSPCs were harvested from donor Fischer 344 rats and expanded for testing. All conduits were placed subcutaneously in Fischer 344 rats and left for 28 d. At day 28, animals were sacrificed and conduit scaffolds were retrieved with surrounding tissue for analyses.

Histology and assessment: After fixation, samples were paraffin embedded and sectioned. Masson's trichrome was performed to quantify host response. Immunostaining was performed using antibodies for βIII-tubulin, NeuN, RIP, anti-oligo, glial fibrillary acidic protein (GFAP), s-100β, nestin, Ki-67 and ED-1 followed by microscopy to assay differentiation and neural tissue formation.

Results and Discussion: We have successfully shown immobilization of our engineered proteins over 28 d and have observed significant 3-D differentiation *in vivo* into desired phenotypes due to the immobilized factors.

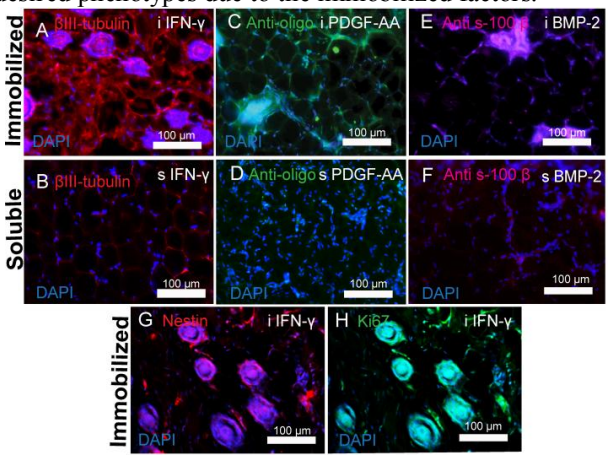

Figure 2. Immunostaining results from subcutaneous studies after 28d in vivo show immobilized signaling factors guide differentiation better then soluble factors.

Results from subcutaneous testing show that immobilized factors are superior to *in vitro* results[1,2] at specifying the desired phenotype within the conduit interior volumes. All immobilized factors showed enhanced proliferation over soluble groups. Interestingly, tethered IFN-γ encourages structures similar in morphology and markers to neural rosettes, which have only been observed previously in development or from pluripotent stem cells (Fig. 2 G, H). Immune responses have shown minimal ED-1 upregulation around the outside tube and collagen capsule thicknesses in all treatments < 200 μm, suggesting limited immune responses.

Conclusions: These results demonstrate that neural stem cells can be guided in defined scaffold volumes to differentiate into neurons, oligodendrocytes and astrocytes with immobilized signaling molecules over long periods *in vivo*. Immobilization of whole signaling proteins provided superior results to soluble administration. Now that safety has been demonstrated we are now applying our treatments as spinal cord bridges in SCI rats, and are especially interested in tethered IFN-γ and its developmental potential for CNS regeneration.

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