Stem cell derived radial glial cells in magnetically aligned scaffold for repair after spinal cord injury

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Abstract—The following was completed as part of the 2011 Route 28 Summit at the International Symposium on Neural Regeneration. The topic of the Route 28 Summit was "Novel Ways to Exploit Stem Cells for Recovery of Human Central Nervous System Function." Traditional cell transplant strategies have not been successful in promoting robust functional recovery following spinal cord injury (SCI). Part of the reason why cell transplant therapies have failed may be related to improper targeting of regenerating axons through the lesion. In this report, we propose a novel therapeutic approach using stem cell-derived radial glial cells in combination with a magnetically aligned fibrin scaffold for targeted axonal regeneration. Animals with a cervical dorsal column injury will receive an injection of embryonic stem cell-derived radial glial cells along with a fibrin-thrombin solution directly into the lesion. The fibrin scaffold will be aligned in situ using magnetic resonance imaging (MRI) prior to polymerization. To further promote axon growth through the lesion, cyclic adenosine monophosphate and chondroitonase ABC will be co-administered rostral and caudal to the lesion. Functional recovery will be evaluated using electrophysiological assessments and the sticker removal and forelimb reaching tasks. Growth of the regenerating or sprouting host axons will be examined at 3 and 6 mo after injury using MRI and diffusion tensor imaging. Histological analyses will be conducted to visualize the underlying circuitry mediating putative functional recovery. It is hypothesized that the fibrin-mediated linear organization of the radial glial cells in the lesion will enhance targeted axon outgrowth and improve functional recovery following SCI.

BACKGROUND

Radial Glial Cells

Radial glial cells play an integral role in guiding cell migration during development of the central nervous system. In birds and fish, radial glial cells play an essential role in regeneration following injury [1]. Furthermore, data suggest that radial glial cells maintain their phenotype and favor the regenerative response in the presence of a patterned substrate (i.e., fibrin) [2]. Embryonic stem cell-derived cells with a specific radial glial phenotype are readily obtainable [3] (**Figure 1**). Therefore, radial glial cell transplant therapy is technically feasible.

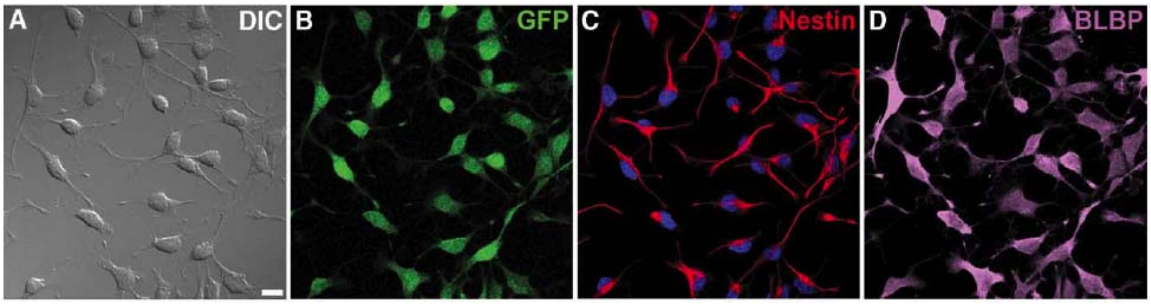

Figure 1. RG3.6 cells showed features of radial glial cells. (**a**) Differential interference contrast (DIC) and (**b**) green fluorescent protein (GFP) showing radial glia morphology. Expression of radial glial markers: (**c**) the intermediate filament protein, nestin, and (**d**) brain lipid binding protein (BLBP). Scale bar = 20 μm. Adapted with permission from Hasegawa et al. [3].

Aligned Substrates Can Enhance Axonal Growth

It is becoming increasingly evident that physical cues such as topography can play a significant role in guiding axons [4]. For instance, following peripheral nerve injury, Schwann cell basal lamina and the associated extracellular matrix provide guidance cues for the regeneration of axons [5–6]. Specifically, laminin and chondroitin sulfate proteoglycans interact with regenerating axons and either promote or inhibit axonal outgrowth. In a recent study, poly-L-lactic acid microfibers in either aligned or in random configuration were examined to determine the effect topography on axon outgrowth [7]. The authors found that neurites of cultured dorsal root ganglia (DRG) neurons, grown on aligned fibers, reached significantly greater distances compared to randomly aligned fibers and film controls (Figure 2). Moreover, DRG neurons cultured on random fibers produced a denser network of neurites than those

cultured on films without any topography (**Figure 2(a–b)**). However, the overall length of neurites did not significantly differ between these two conditions (random versus control) (**Figure 2(b–c)**), suggesting that the path of neurite growth is more circuitous on randomly aligned fibers. Therefore, while the presence of a suitable substrate is essential, alignment of the substrate can maximize targeted neurite extension by limiting or restricting the direction of axonal growth.

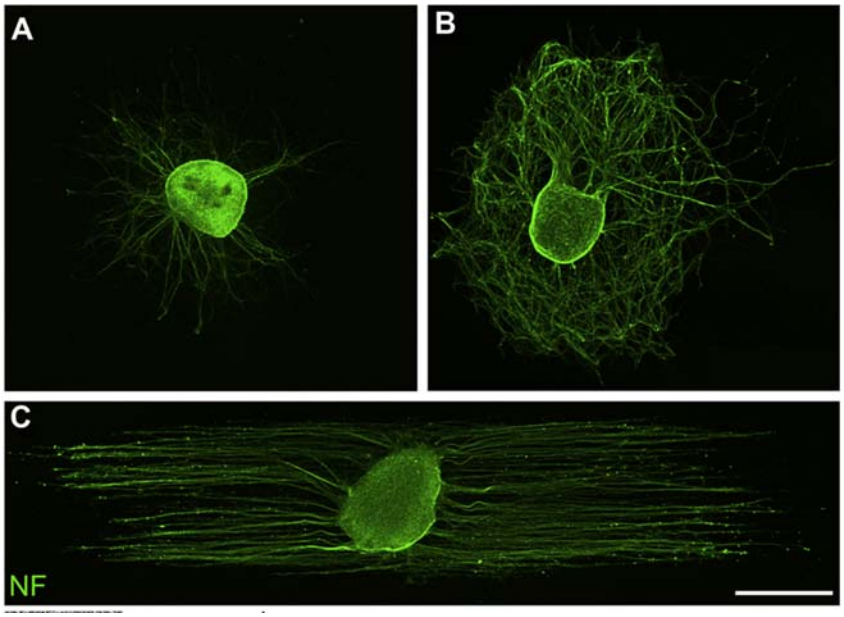

Figure 2. Aligned substrates can enhance axonal outgrowth *in vitro*. Dorsal root ganglia P4 explants were plated onto (**a**) smooth, (**b**) unaligned, and (**c**) aligned fibers. Samples were labelled with neurofilament (NF). Scale bar = 500 µm. Adapted with permission from Hurtado et al. [7].

Study Proposal

We hypothesize that stem cell derived radial glial cells can significantly enhance axonal outgrowth after injury to the spinal cord. We propose a highly innovative approach that uses MRI to produce an aligned fibrin scaffold.

METHODS

Treatments and Animal Groups

Adult Sprague-Dawley rats will be subjected to a complete bilateral removal of dorsal column between C6 and C7. Animals will recover for two weeks while the injury site stabilizes. Two weeks

following injury, the glial scar will be surgically resected and rats will be injected with embryonic stem cell-derived radial glia (GFP-labeled for better tracking in vivo) in a fibrin-thrombin mixture. Rats will be placed in a MRI scanner to align the fibrin scaffold during a crosslinking reaction with thrombin as previously described [8]. chABC will be injected rostrally and caudally to the lesion site, immediately following removal from the MRI. cAMP will be injected into the C7 DRG to further promote axonal outgrowth. Five experimental groups will be used: radial glia + aligned substrate + cAMP + chABC; radial glia + aligned substrate; radial glia + unaligned substrate + cAMP + chABC; aligned substrate only; and radial glia only.

Histology

At 3 and 6 mo post-injury, the anterograde tracer, biotinylated dextran amine, will be injected into the C7 DRG to assess the extent of axon growth.

Functional Analyses

The sticker removal test for proprioception and reach task for fine motor control, as well as electrophysiological assessments as previously described [9], will be used. The following time points will include behavioral assessments: training (pre-injury), post-lesion, pre-graft, post-graft (with biweekly tests up to 6 mo).

In Vivo Imaging

Longitudinal studies using DTI will be used to visualize the growing axons [10–11].

DISCUSSION AND CONCLUSIONS

It is anticipated that this novel application of radial glial cells will enhance axonal outgrowth and improve functional recovery after SCI. However, a few potential limitations to this approach may exist. We proposed to use fetal-derived stem cells; however, previous studies suggest alternative methods for the development of radial glial cells from embryonic stem cells [3]. The use of embryonic stem cells may have more clinical relevance, due to availability and ethical constraints against the use of embryonically derived cells. Secondly, magnetic alignment of the fibrin scaffold has been successful in vitro but has not yet been attempted in vivo. Therefore, additional modifications may be necessary to obtain

polymerization of fibrin scaffolds in vivo. Thirdly, stability of the fibrin scaffold in vivo may be a limiting factor. Though a possible limitation, previous studies suggest that fibrin gel degradation can be significantly prolonged by either the addition of aprotinin [12] or the addition of polyethylene glycol onto the fibrin [13]. Lastly, if the proposed treatment shows efficacy in this specific injury model, additional testing will be needed to determine if this method can be used in a chronic setting and/or in other injury models.

REFERENCES

1. Peterson RS, Lee DW, Fernando G, Schlinger BA. Radial glia express aromatase in the injured zebra finch brain. J Comp Neurol. 2004;475(2):261–69. [PMID:15211466] http://dx.doi.org/10.1002/cne.20157

2. Mattotti M, Alvarez Z, Ortega JA, Planell JA, Engel E, Alcántara S. Inducing functional radial glia-like progenitors from cortical astrocyte cultures using micropatterned PMMA. Biomaterials. 2012;33(6):1759–70. [PMID:22136716] http://dx.doi.org/10.1016/j.biomaterials.2011.10.086

3. Hasegawa K, Chang YW, Li H, Berlin Y, Ikeda O, Kane-Goldsmith N, Grumet M. Embryonic radial glia bridge spinal cord lesions and promote functional recovery following spinal cord injury. Exp Neurol. 2005;193(2):394–410. [PMID:15869942] http://dx.doi.org/10.1016/j.expneurol.2004.12.024

4. Spivey EC, Khaing ZZ, Shear JB, Schmidt CE. The fundamental role of subcellular topography in peripheral nerve repair therapies. Biomaterials. 2012;33(17):4264–76. [PMID:22425024] http://dx.doi.org/10.1016/j.biomaterials.2012.02.043

5. Hudson TW, Zawko S, Deister C, Lundy S, Hu CY, Lee K, Schmidt CE. Optimized acellular nerve graft is immunologically tolerated and supports regeneration. Tissue Eng. 2004;10(11–12):1641–51. [PMID:15684673] http://dx.doi.org/10.1089/ten.2004.10.1641

6. Feneley MR, Fawcett JW, Keynes RJ. The role of Schwann cells in the regeneration of peripheral nerve axons through muscle basal lamina grafts. Exp Neurol. 1991;114(3):275–85. [PMID:1748202] http://dx.doi.org/10.1016/0014-4886(91)90153-4

7. Hurtado A, Cregg JM, Wang HB, Wendell DF, Oudega M, Gilbert RJ, McDonald JW. Robust CNS regeneration after complete spinal cord transection using aligned poly-L-lactic acid microfibers. Biomaterials. 2011;32(26):6068–79. [PMID:21636129]

8. Namani R, Wood MD, Sakiyama-Elbert SE, Bayly PV. Anisotropic mechanical properties of magnetically aligned fibrin gels measured by magnetic resonance elastography. J Biomech. 2009;42(13):2047–53. [PMID:19656516] http://dx.doi.org/10.1016/j.jbiomech.2009.06.007

9. James ND, Bartus K, Grist J, Bennett DL, McMahon SB, Bradbury EJ. Conduction failure following spinal cord injury: functional and anatomical changes from acute to chronic stages. J Neurosci. 2011;31(50):18543–55. [PMID:22171053] http://dx.doi.org/10.1523/JNEUROSCI.4306-11.2011

10. Ramu J, Herrera J, Grill R, Bockhorst T, Narayana P. Brain fiber tract plasticity in experimental spinal cord injury: diffusion tensor imaging. Exp Neurol. 2008;212(1):100–107. [PMID:18482724] http://dx.doi.org/10.1016/j.expneurol.2008.03.018

11. Thuen M, Olsen O, Berry M, Pedersen TB, Kristoffersen A, Haraldseth O, Sandvig A, Brekken C. Combination of Mn(2+)-enhanced and diffusion tensor MR imaging gives complementary information about injury and regeneration in the adult rat optic nerve. J Magn Reson Imaging. 2009;29(1):39–51. [PMID:19097077] http://dx.doi.org/10.1002/jmri.21606

12. Smith JD, Chen A, Ernst LA, Waggoner AS, Campbell PG. Immobilization of aprotinin to fibrinogen as a novel method for controlling degradation of fibrin gels. Bioconjug Chem. 2007;18(3):695–701. [PMID:17432824] http://dx.doi.org/10.1021/bc060265o

13. Zhang G, Wang X, Wang Z, Zhang J, Suggs L. A PEGylated fibrin patch for mesenchymal stem cell delivery. Tissue Eng. 2006;12(1):9–19. [PMID:16499438] http://dx.doi.org/10.1089/ten.2006.12.9