Biodegradable Neuro-Spinal Scaffold Promotes Neuropermissive Remodeled Tissue Following Spinal Contusion Injury in Rats

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BACKGROUND

Contusive spinal cord injury (SCI) produces cellular necrosis and secondary tissue loss culminating in a fluid-filled cystic cavity. Limited endogenous repair results in focal cellular trabeculae composed of Schwann cells, fibroblasts, astrocytes, pericytes, macrophages, collagen, and sprouting axons.[1,2] We hypothesized that implantation of a biodegradable, biomaterial scaffold into the injured spinal cord could serve as a locus for appositional healing and tissue remodeling that would enhance endogenous repair. We evaluated the effect of implantation of Neuro-Spinal scaffolds (NSS) composed of the block copolymer poly(lactic-co-glycolic acid)-poly(L-lysine) (PLGA-PLL) on tissue remodeling in a contusion model of SCI.

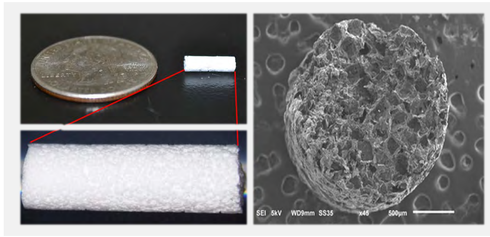

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Figure 1. Porous structure of the Neuro-Spinal Scaffold

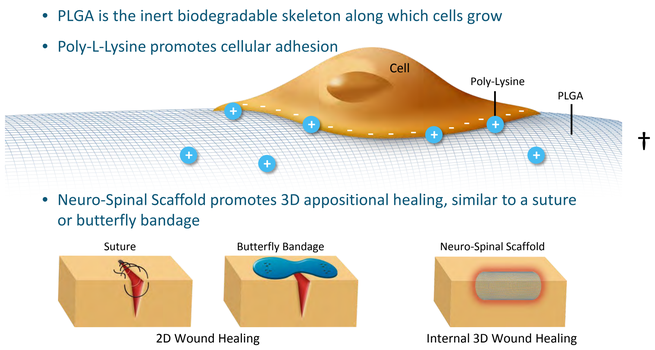

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Figure 2. Neuro-Spinal Scaffold implant promotes appositional healing

HYPOTHESIS

Implantation of a biodegradable, biomaterial scaffold into the injured spinal cord can serve as a physical substrate for appositional healing and tissue remodeling that enhances the endogenous repair process.

MATERIALS AND METHODS

Fabrication of Scaffolds. Cylindrical NSS implants (1.0 mm diameter, 2.0 mm length) were manufactured similar to previously published methods.[3] Glucose porogens of approximately 180 to 430 µm particle size were used, resulting in the formation of a highly interconnected porous structure of sufficient size to permit in-growth of endogenous cells and to facilitate nutrient and waste transport.

Contusion and Implantation. A spinal T10 contusion injury was created in female Sprague-Dawley rats under isoflurane anesthesia with a Precision Systems IH Impactor (220 kDyn). Cylindrical NSSs were surgically implanted via myelotomy at the lesion site between 24 and 72 hours later.

MATERIALS AND METHODS

Histology and Immunohistochemistry. Recovery of coordinated hind limb function using the Basso, Beattie, and Bresnahan (BBB) scale[4] were evaluated for 12 weeks. At 12 weeks, rats were anesthetized and subjected to transcardial perfusion with heparinized saline followed by 4% paraformaldehyde in phosphate buffer. Spinal cord tissue was removed and fixed by immersion in 4% paraformaldehyde in phosphate buffer for at least 24 h, transferred to 10% sucrose phosphate buffer overnight, followed by incubation in 30% sucrose phosphate buffer for 48 hours. Spinal cord tissue was embedded in OCT and frozen sections (20 µm) were prepared with a Leica cryostat and mounted on microscope slides. Standard histopathology (H&E staining or trichrome) and immunohistochemistry methods were used. Sections were incubated overnight with the following primary antibodies: beta-III tubulin (Neuromics, MO15013), laminin (Sigma, L9393), myelin protein 0 (EMD Millipore, ABN363) and neurofilament heavy (Santa Cruz, SC-58553). Alexa 488, 555 and 647 labeled secondary antibodies (ThermoFisher) were incubated for 3 hours, followed by an incubation with 300µM DAPI (ThermoFisher #P369620) in the final washes before being mounting with Prolong Diamond (ThermoFisher, #P36961). H&E and trichrome stained sections were imaged with a NanoZoomer 2.0-RS slide scanner, and fluorescent immunohistochemistry images with a Zeiss Imager A2 epifluorescence microscope with a ThorLabs 4MP cooled CCD camera.

Morphometry. NDP.view software was used to perform morphometric analysis, including cavity areas, areas of remodeled tissue, and widths of residual healthy tissue at the lesion epicenter. Cavity and remodeled tissue area measurements were integrated to obtain volumes. Histomorphometry values (cavity volume, remodeled tissue volume, surviving tissue width) were analyzed by t-test using GraphPad Prism version 5.00 for Windows, GraphPad Software (San Diego California USA, www.graphpad.com).

RESULTS

Thoracic spinal contusion injury resulted in acute hindlimb paralysis, followed by eventual tissue loss and spinal architecture disruption. Rats in the non-treated control group developed large cystic cavities surrounded by a rim of spared tissue. In contrast, in rats treated with scaffold implantation, cavity volume decreased by 86%, and spared white matter width increased by 44%. Although scaffolds were fully resorbed by 12 weeks after implantation, the amount of remodeled tissue at the implantation site in the lesion epicenter increased by 111%.

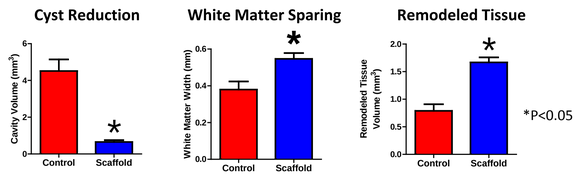

Figure 3. NSS implantation preserves spinal architecture; (A) representative longitudinal sections from control (n=14) and scaffold implanted rats (n=38), (B) histomorphometric analysis (values are means ± S.E.M.).

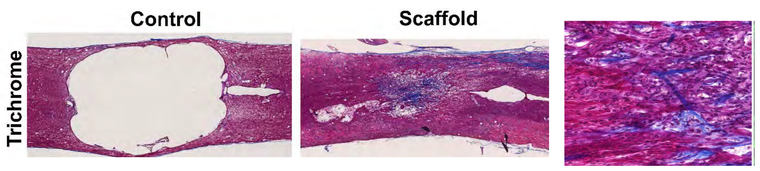

Figure 4. Representative longitudinal sections stained with Masson's trichrome from a control and NSS implanted rat.

RESULTS

Remodeled tissue in scaffold implanted rats contained sparse collagen (Figure 4) and abundant laminin (Figure 5). Sprouting axons (β3-tubulin and neurofilament positive fibers) indicated neural regeneration within the remodeled tissue (Figures 5 and 6). Schwann cells (myelin protein zero positive) were widely present in the remodeled tissue of the lesion epicenter and the perilesional white matter (Figure 6).

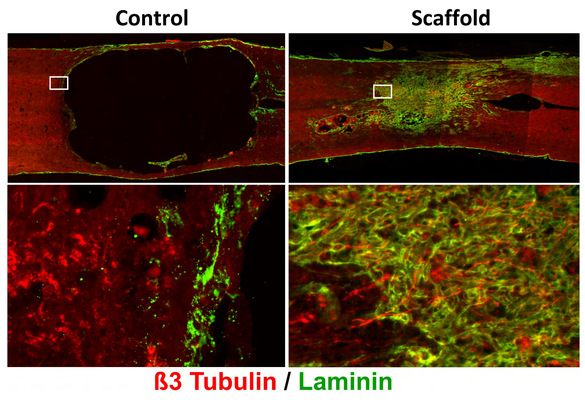

Figure 5. NSS promotes tissue remodeling. Axons (β3 Tubulin, red) extend into laminin (green) rich remodeled tissue in NSS implanted rats.

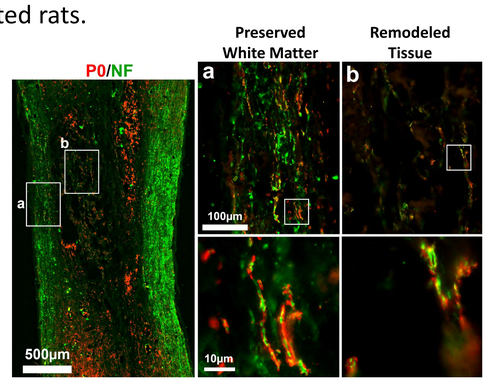

Figure 6. NSS implantation promotes axon (neurofilament, green) myelination by Schwann cells (P0, red) in both spared white matter (a) and remodeled tissue (b).

CONCLUSIONS

- Implantation of the NSS in the acutely injured spinal cord can act as a physical substrate for appositional healing, reducing cavitation and promoting tissue sparing and neuropermissive tissue remodeling.
- NSS-remodeled tissue contains sparse collagen, abundant laminin, extending axons, and myelinating Schwann cells.
- The NSS, currently the subject of an ongoing human clinical trial, may play an important role as a treatment for acute spinal cord injury.

REFERENCES

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