Biodegradable Neuro-Spinal Scaffold Promotes Neuroporative 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.\textsuperscript{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.

MATERIALS AND METHODS

Histology and Immunohistochemistry. Recovery of coordinated hindlimb function using the Basso, Beattie, and Bresnahan (BBB) scale\textsuperscript{6} were evaluated for 12 weeks. At 12 weeks, rats were anesthetized and subjected to transthoracic 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 mounted 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%.

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 neuroporative 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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