Biodegradable Neuro-Spinal Scaffold Preserves Spinal Cord Architecture Following Spinal Contusion Injury in Rats

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BACKGROUND

Severe spinal cord injury (SCI) is accompanied by disruption of spinal cord architecture, including cystic cavitation and tissue loss. We hypothesized that implantation of a biodegradable, biomaterial scaffold (Figure 1) into the injured spinal cord could serve as a physical substrate for appositional healing and tissue remodeling that would preserve spinal cord architecture (Figure 2). We evaluated the effect of implantation of Neuro-Spinal Scaffolds composed of a block copolymer of poly(lactic-co-glycolic acid) and poly(L-lysine) (PLGA-PLL) on preservation of spinal architecture in a rat contusion model of severe SCI.

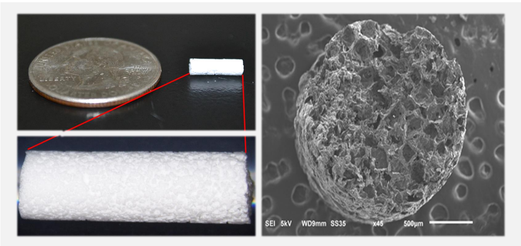


Figure 1. Porous structure of the Neuro-Spinal Scaffold

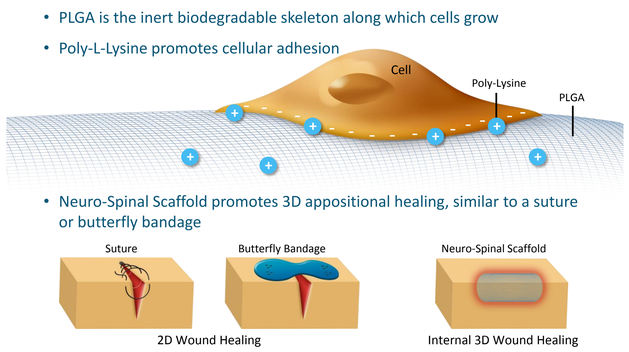


Figure 2. Neuro-Spinal Scaffold implant promotes appositional healing

HYPOTHESIS

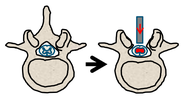
Implantation of a biodegradable, biomaterial scaffold into the injured spinal cord can serve as a physical substrate for appositional healing and tissue remodeling that preserves spinal cord architecture.

MATERIALS AND METHODS

Fabrication of Scaffolds. Cylindrical Neuro-Spinal Scaffold implants (1.0 mm diameter, 2.0 mm length) were manufactured similar to previously published methods[1]. 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.

MATERIALS AND METHODS

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 Neuro-Spinal Scaffolds were surgically implanted via myelotomy at the lesion site between 24 and 72 hours later.



Endpoints. Body weight, development of mechanical allodynia (using an electronic Von Frey device), and recovery of coordinated hind limb function using the Basso, Beattie, and Bresnahan (BBB) scale[2] were evaluated for 12 weeks. At the end of the experiment (week 12) rats were terminally anesthetized with ketamine/xylazine (90 mg/kg i.p./10 mg/kg i.p.) 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 at 4°C. Spinal cord samples were then transferred to 10% sucrose phosphate buffer and incubated 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. H&E staining was performed to visualize the injured area using a commercially available kit. Images were analyzed using a Hamamatsu NanoZoomer 2.0-RS scanner system to scan the slides, creating digital images at a resolution of 40x. NDP.view software was used to evaluate spinal architecture at 12 weeks by 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.

Statistical Analysis. BBB scores, withdrawal thresholds, and body weights were analyzed by two-way repeated measures ANOVA. 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

Most experts agree that the contusion injury model is the most clinically relevant model of human SCI[3]. Spinal contusion injury results in tissue loss and spinal architecture disruption from the initial mechanical trauma as well as a second phase of tissue loss that persists for weeks to months. Damaged and necrotic tissue in the lesion site is cleared over the course of several weeks through the action of resident microglia and circulating leukocytes, leaving a fluid-filled cystic cavity surrounded by a rim of surviving tissue (Figure 3).

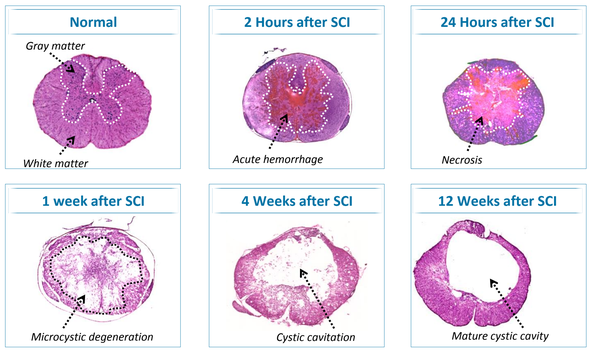


Figure 3. Progression of Spinal Contusion Injury

RESULTS

Scaffold implantation did not result in mechanical allodynia, did not impair body weight gain, and did not interfere with partial recovery from full hind-limb paralysis. Histological analysis revealed that rats in the non-treated control group developed large cavities surrounded by a rim of spared tissue. In contrast, in rats treated with scaffold implantation surgery, cavity volume decreased by 86% and spared tissue width increased by 44%. Although scaffolds were fully resorbed by 12 weeks after implantation, the amount of remodeled tissue at the site of implantation in the lesion epicenter increased by 111%.

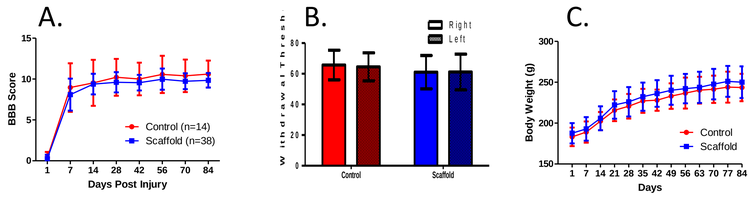


Figure 4. Neuro-Spinal Scaffold implantation does not alter (A) functional recovery, (B) Von Frey withdrawal threshold, or (C) body weight gain (values are means ± standard deviation)

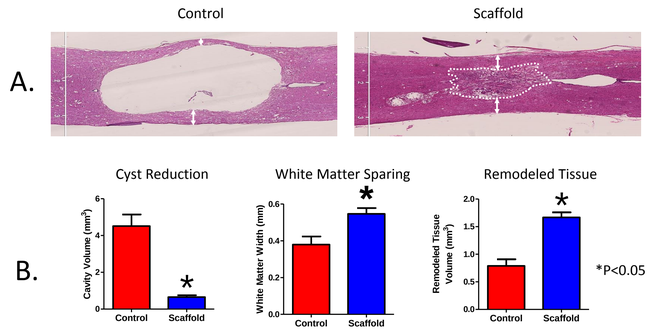


Figure 5. Neuro-Spinal Scaffold preserves spinal architecture; (A) representative longitudinal sections from control (n=14) and scaffold implanted rats (n=38), (B) histomorphometric analysis (values are means ± S.E.M.).

CONCLUSIONS

- These results demonstrate that implantation of a Neuro-Spinal Scaffold in the acutely injured spinal cord can reduce cavitation, promote tissue sparing and remodeling, and act as a locus for appositional healing.
- Implantation of a Neuro-Spinal Scaffold preserves spinal cord architecture and may play an important role as a treatment for acute spinal cord injury.
- The Neuro-Spinal Scaffold is currently the subject of an ongoing human clinical trial.

REFERENCES

1. Teng, Y.D., et al., *Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells.* Proc Natl Acad Sci U S A, 2002. **99**(5): p. 3024-9.
2. Basso, D.M., M.S. Beattie, and J.C. Bresnahan, *A sensitive and reliable locomotor rating scale for open field testing in rats.* J Neurotrauma, 1995. **12**(1): p. 1-21.
3. Kwon, B., et al., *A grading system to evaluate objectively the strength of pre-clinical data of acute neuroprotective therapies for clinical translation in spinal cord injury.* J Neurotrauma, 2011. 28: p. 1525-1543.