Effects of Bridging a Gap in the Rat Spinal Cord with a Collagen Tube and Membrane

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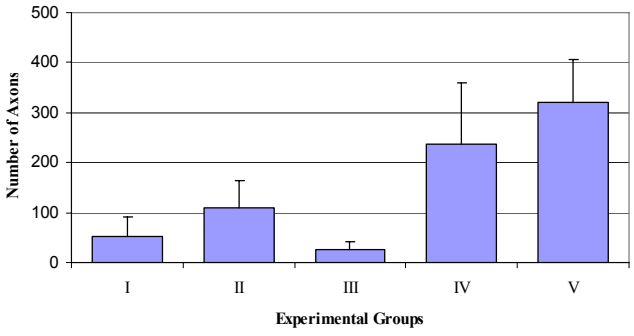
Statement of Purpose: Recent work [1] has shown that an off-the-shelf collagen tube filled with a collagen sponge-like scaffold exceeds the performance of a nerve autograft, the "gold standard," in a rat model The objective of this study was to begin to apply this approach to the spinal cord using an absorbable type I collagen tube and to extend the concept by using a collagen membrane as a more easily applied wrap around the defect to protect the lesion from the collapse of surrounding tissue into the gap and to contain endogenous agents; specific attention was focused on the evaluation of the amount, organization and orientation of the fibrous scar in the defect.

Methods: A 5-mm mid-thoracic gap was created surgically in the rat spinal cord, as has previously been described [2,3]. There were 2 treatment schemes: 1) the ends of the cord were fitted into a collagen tube, and 2) a collagen membrane was wrapped around the cord stumps to contain the defect. A collagen membrane was also used along with both strategies as a dorsal barrier in order to further reduce scar infiltration of the defect. There were 5 experimental groups (n=4):

I. Non- implanted control group
II. Dorsal barrier only
III. Collagen tube
IV. Collagen tube and dorsal barrier
V. Collagen membrane wrap and dorsal barrier

Collagen tubes were fabricated by freeze-drying a type I microfibrillar collagen (from bovine tendon; Integra Life Sciences, Plainsboro, NJ) slurry that was injected into polytetrafluoroethylene (PTFE) molds into which were inserted PTFE coated glass rods, 3 mm in diam. The tubes were dehydrothermally treated at 120°C for 24 hr.
The collagen membrane comprised porcine type I/III collagen (Geistlich Biomaterials, Wolhusen, Switzerland). Histomorphometric evaluation was performed 4 weeks post-implantation. Specimens were fixed in formalin. Sections of osmium-postfixed, Epon-embedded tissue were stained with toluidine blue; sections of paraffin embedded tissues were stained with Masson's trichrome for collagen. Immunohistochemistry was performed using antibodies to anti-glial fibrillary acidic protein (GFAP) to detect astrocyte proliferation and α-smooth muscle actin (SMA) to reveal myofibroblasts.

Results/Discussion: All animals lost hindlimb function distal to the insult but maintained adequate forelimb mobility, grooming and consumption of food and water (provided *ad libidum*). As described previously [2,3], temporary loss of the reflex bladder voiding function required manual expression of the bladders.
The excised spinal cord tissue of every animal group, including Groups I and II, revealed a cord of reparative tissue that bridged the gap between rostral and caudal spinal cord stumps. Grossly, all of the collagen implants showed some degree of resorption. The collagen tubes exhibited fragmentation; it is not clear at what time postoperatively there may have been a loss of entubulation of lesion. Histological evaluation revealed that buckling of the wrap resulted in a degree of collapse into the lumen of the gap.
GFAP and SMA expression in the groups receiving the dorsal barrier was notably less than Groups I or III. Group II expressed the highest levels of GFAP and Group V the least among the groups with a dorsal barrier. SMA expression was minimal and indistinguishable between Groups IV and V, but significantly higher in Group II.
Pronounced fibrous and glial scar formation was found in the groups with no dorsal barrier: control group (I) and the group with the collagen tube, but no dorsal barrier (III); the least scar in the tube and wrap groups with the dorsal barrier (IV and V). The most axons were found in the tube and wrap groups with the dorsal barrier (IV and V; Figure 1), but the numbers were substantially less than normal (>100,000 axons). The majority of the axons were less than 5 μm in diameter.



± std. dev.

Conclusions: The combination of a collagen membrane wrapped around a spinal cord gap and a dorsal barrier may be effective in reducing the formation of fibrous scar in gaps in the spinal cord and thus may contribute to creating a hospitable environment for a regenerative process, which will likely require the implantation of antagonists of nerve growth inhibitors, neurotrophic factors, and stem cells.

Acknowledgments: Department of Defense and Department of Veterans Affairs.

References: 1. Chamberlain LJ, *et al*. J. Neurosci. Res. 2000;60(5):666-677; 2. Spilker MH, *et al*. Tiss. Engr. 1997;3:309-317; 3. Spilker MH, *et al*. Restor Neurol Neurosci 2001;18(1):23-38.