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Matrix inclusion within synthetic hydrogel guidance channels improves specific supraspinal and local axonal regeneration after complete spinal cord transection

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Abstract

We have previously shown that a novel synthetic hydrogel channel composed of poly(2-hydroxyethyl methacrylate-*co*-methyl methacrylate) (pHEMA-MMA) is biocompatible and supports axonal regeneration after spinal cord injury. Our goal was to improve the number and type of regenerated axons within the spinal cord through the addition of different matrices and growth factors incorporated within the lumen of the channel. After complete spinal cord transection at T8, pHEMA-MMA channels, having an elastic modulus of 263 ± 13 kPa were implanted into adult Sprague Dawley rats. The channels were then filled with one of the following matrices: collagen, fibrin, Matrigel™, methylcellulose, or smaller pHEMA-MMA tubes placed within a larger pHEMA-MMA channel (called tubes within channels, TWC). We also supplemented selected matrices (collagen and fibrin) with neurotrophic factors, fibroblast growth factor-1 (FGF-1) and neurotrophin-3 (NT-3). After channel implantation, fibrin glue was applied to the cord-channel interface, and a duraplasty was performed with an expanded polytetrafluoroethylene (ePTFE®) membrane. Controls included animals that had either complete spinal cord transection and implantation of unfilled pHEMA-MMA channels or complete spinal cord transection. Regeneration was assessed by retrograde axonal tracing with Fluoro-Gold, and immunohistochemistry with NF-200 (for total axon counts) and calcitonin gene related peptide (CGRP, for sensory axon counts) after 8 weeks survival. Fibrin, Matrigel™, methylcellulose, collagen with FGF-1, collagen with NT-3, fibrin with FGF-1, and fibrin with NT-3 increased the total axon density within the channel (ANOVA, $p < 0.05$) compared to unfilled channel controls. Only fibrin with FGF-1 decreased the sensory axon density compared to unfilled channel controls (ANOVA, $p < 0.05$). Fibrin promoted the greatest axonal regeneration from reticular neurons, and methylcellulose promoted the greatest regeneration from vestibular and red nucleus neurons. With Matrigel™, there was no axonal regeneration from brainstem motor neurons. The addition of FGF-1 increased the axonal regeneration of vestibular neurons, and the addition of NT-3 decreased the total number of axons regenerating from brainstem neurons. The fibrin and TWC showed a consistent improvement in locomotor function at both 7 and 8 weeks. Thus, the present study shows that the presence and type of matrix contained within synthetic hydrogel guidance channels affects the quantity and origin of axons that regenerate after complete spinal cord transection, and can improve functional recovery. Determining the optimum matrices and growth factors for insertion into these guidance channels will improve regeneration of the injured spinal cord.
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1. Introduction

As our understanding of spinal cord regeneration advances, several regeneration and repair strategies have demonstrated improved outcome after spinal cord injury [1–3], and many of these strategies reflect the importance of a combination of strategies for optimum recovery. To achieve recovery it will be essential to understand the mechanisms underlying the repair so that the appropriate combination of strategies can be selected.

For example, in selecting the appropriate spinal cord injury repair model, it is important to differentiate between repair that promotes axonal collateralization and true axonal regeneration. The quality of a repair will be suboptimal when achieved by axonal branching or collateralization from an intact axon that has been spared from injury. In contrast, the repair would be optimal when achieved through true axonal regeneration from the axonal end of a transected or damaged axon. Ideally, all transected axons would regenerate rather than have spared axons compensate for lost function through collateralization. To differentiate between collateralization from axons spared from injury and true regeneration requires animal models of complete transection of the spinal cord rather than models of partial spinal cord injury. While partial injury models may be more relevant to the majority of human spinal cord injuries, and complete transection injuries to a minority of injuries, partial injury models do not allow precise differentiation between regeneration and collateralization.

While complete spinal cord transection models facilitate the differentiation between factors that stimulate regeneration and collateralization, complete transection models lack a cavity at the injury site making it difficult to apply many types of treatments. For example, administered growth factors, or transplanted cells are not easily contained at the injury site after complete cord transection. As well, the potentially extensive scarring from fibroblasts or astrocytes in complete transection models may inhibit axonal regeneration and prevent evaluation of a repair strategy.

To facilitate the delivery of a combination of factors to enhance repair and to decrease scarring in complete transection models, we and others have utilized the method of entubulation [4–9]. Utilizing a channel to contain regenerative factors allows their efficacy to be compared more uniformly because the channel allows the assessment of the regenerative factors in an environment that contains axons, glia, endothelial cells, inflammatory cells and fibroblasts. We have previously reported that a synthetic hydrogel channel composed of poly(2-hydroxyethyl methacrylate-*co*-methyl methacrylate) (pHEMA-MMA) implanted without a matrix after complete spinal cord transection facilitated motor axon regeneration from the brainstem [4]. In the present study, we examined whether the addition of a matrix and/or growth factor to the channel would improve axonal regeneration and if there was a difference between the matrices tested in terms of the quantity and types of axons that regenerated after complete spinal cord transection. We chose to compare the following matrices—fibrin, collagen, Matrigel^{TM} and methylcellulose—because each of fibrin [1,4,10,11], collagen [12,13], Matrigel^{TM} [5,14–18], and methylcellulose [12,19], have demonstrated regenerative capacity in the nervous system, and may improve regeneration through interactions with ligands present in the matrix. For example, fibronectin in fibrin, laminin in Matrigel, and collagen are all components of the extracellular matrix and are known to provide haptotatic cues to nerve fibers.

We chose to compare fibroblast growth factor-1 (FGF-1) and neurotrophin-3 (NT-3) because both have demonstrated beneficial effects on spinal cord regeneration [1,10,11,20–26]. These neurotrophic factors were added only to collagen and fibrin glue because these matrices have been extensively studied with respect to spinal cord injury repair [1,7,21,27–31]. This is the first study comparing these promising materials/growth factors in pHEMA-MMA channels, which themselves have regenerative capacity [4].

2. Methods

2.1. Channel and small tube fabrication

All chemicals for the fabrication of the channels were purchased from Aldrich (Milwaukee, WI). Water was distilled and deionized at 18 ΩM. Channels were synthesized by the liquid–liquid centrifugal casting technique previously described in detail by our group [32,33]. Briefly, the monomers, methyl methacrylate (MMA) and 2-hydroxyethyl methacrylate (HEMA), were dissolved in excess water in the wt ratios of 2.5:22.5:75, respectively. This mixture was stirred and degassed, then aqueous solutions of ammonium persulfate (APS) and sodium metabisulfite (SMBS) were added to reach a final concentration (with respect to the monomer) of 0.5 and 0.4 wt%, respectively. This solution was injected through rubber septa into a 15 cm length glass mold, with ID of 4.2 mm, which was then placed in the chuck of a horizontally mounted stirrer. Rotation commenced at 4700 rpm, as measured with a tachometer (Model 461893, Extech Instruments, Waltham, MA). As the monomer was polymerized, the polymer became insoluble in the aqueous solution and phase separated with unreacted HEMA monomer. This separated liquid phase is denser than water and pushed to the periphery of the glass mold by centrifugal forces. After approximately 5 h, the glass molds were removed from the horizontally mounted stirrers and the pHEMA-MMA hydrogel channels removed from the molds. The hydrogel channels were cut into 6 mm lengths and Soxhlet-extracted overnight in water.

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