Multifunctional, degradable scaffolds with linear microchannels for spinal cord repair

Dena Shahriari[1], Kayla Felger[1], Yacov Koffler[2], Daniel Lynam[1], Mark Tuszynski[2], Jeffery Sakamoto[1]
[1]Michigan State University- East Lansing, MI
[2]University of California, San Diego- La Jolla, CA

Statement of Purpose: Spinal cord injuries are debilitating and typically cause loss of function. Although some progress has been made towards treatment immediately after trauma and through physical therapy, a viable approach for chronic spinal cord injuries is currently not available. One potential therapy involves the injection of neuronal stem cells (NSCs) to recapitulate the native axonal tracts[1]. However, to improve the efficacy of this approach, precise linear guidance of NSCs is needed over clinically relevant length scales (up to 2cm). Previous work has demonstrated strictly linear axonal guidance using scaffolds with precision microchannels[2-4]. Thus, we believe that NSCs permeated or pre-seeded within multiluminal scaffolds can enhance linear organization to restore function. To achieve this goal, the scaffold should ideally 1. have linear channels to provide NSC linearity 2. degrade after nerve regeneration 3. provide cell attachment to improve NSC survival and 4. continuously release active nerve growth factors to promote neuronal growth. This work explores various forms of alginate-based materials to meet these criteria and tests their efficacies for nerve growth in the spinal cord. These scaffolds will be tested for the survival and growth of NSCs as a potential approach for spinal cord repair.

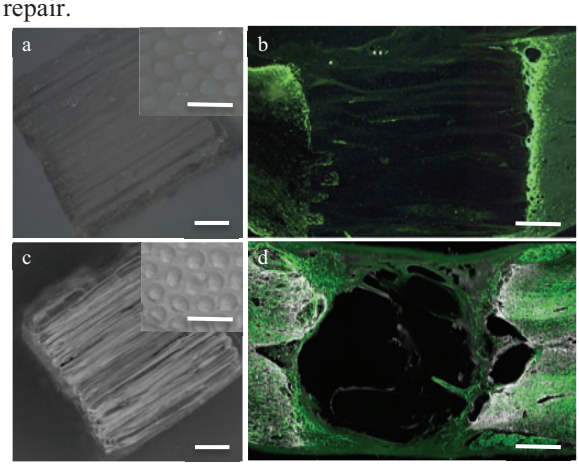

Fig. 1. Longitudinal and cross-section view of (a) agarose and (c) alginate-based hydrogel scaffolds. Neurofilament staining of the spinal cord after implantation of (b) non-degradable agarose scaffolds after 4 weeks[4] and (d) alginate hydrogel scaffold after 2 weeks shows that non-degradable scaffold stays in the lesion while alginate hydrogel degrades within 2 weeks. Both scaffolds show some axonal growth. All scale bars are 0.5mm.

Methods: The *in vitro* degradation of alginate (FMC Novamatrix; Philadelphia, PA) hydrogel was quantified with rheology. Alginate scaffolds were fabricated using a previously described fiber templating process[2]. The scaffolds were sterilized and implanted in Fisher 344 rodent. To prolong the degradation of alginate hydrogel, its chemistry was modified and used to fabricate multiluminal scaffolds. Cell attachment was conducted using fibroblasts and stem cells. The alginate-based materials were further functionalized and tested for drug delivery using lysozyme as an analog of brain derived neurotrophic factor (BDNF) as well as BDNF.

Results: *In vitro* observations ostensibly show alginate hydrogel scaffolds maintain their superficial dimensions; however, mechanical property measurements indicate a dramatic reduction in alginate's shear modulus from 155kPa to 5kPa in 2 days. Thus, although the alginate hydrogel scaffolds kept their initial shape, the drop in shear modulus resulted in noticeable distortion and fragmentation *in vivo* in full T3 transections (Fig. 1). Modifications to the alginate synthesis enabled a significant decrease in the degradation rate as well as enhanced cell attachment (Fig. 2). Finally, to further functionalize alginate-based materials, we explored their potential for drug delivery and progress towards a goal of month-long 50ng/ml BDNF daily release.

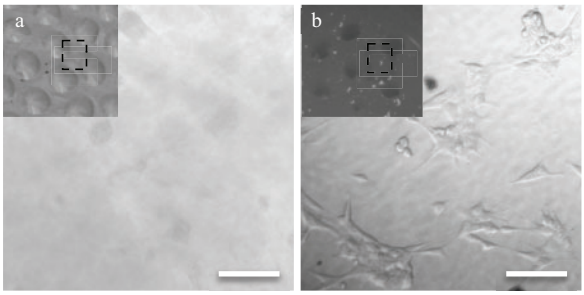

Fig. 2. NIH 3T3 fibroblasts do not attach on (a) alginate hydrogel, but do attach to (b) modified alginate-based materials. Scale bars are 30μm.

Conclusion: Implanted alginate hydrogel scaffolds showed rapid degradation *in vivo*. Modifications were made to the alginate to prolong degradation as well as promote cell attachment. We have engineered this material into scaffolds with linear channels that could be further functionalized for BDNF delivery. The multifunctional scaffolds will be tested for the survival and guidance of NSCs in the T3 full transection rat model.

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