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Targeted tissue engineering: hydrogels with linear capillary channels for axonal regeneration after spinal cord injury

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Spinal cord injury (SCI) frequently results in the permanent loss of function below the level of injury due to the failure of axonal regeneration in the adult mammalian central nervous system (CNS). The limited intrinsic growth capacity of adult neurons, a lack of growth-promoting factors and the multifactorial inhibitory microenvironment around the lesion site contribute to the lack of axonal regeneration. Strategies such as transplantation of cells, delivery of bioactive compounds and gene transfer have been investigated as a means to promote axonal regrowth through the lesion, to form new synaptic connections and to improve functional outcomes. Although growth of some axonal populations can be robustly enhanced by cellular implants alone or in combination with neurotrophic factors, axons usually extend in random orientation and even reverse growth direction in the lesion site (**Figure 1A**) (Gros et al., 2010; Günther et al., 2015). Thus, regenerating axons often fail to approach the distal edge of the lesion site, a pre-requisite for proper contact with spared host neurons. The lack of a 3-dimensional organization in the injury site is

guidance through structured scaffolds and chemical guidance by growth factor gradients, have emerged as potential means to provide directional cues for axonal growth through the lesion.

Scaffolds with linear channels: Advances in tissue engineering and biomaterials have provided promising leads for spinal cord repair. Biomaterials with high bio-compatibility and low toxicity can either be chemically synthesized or derived from natural polymers. After engraftment into sites of SCI, biomaterials can bridge the lesion cavity to restore continuity of the spinal cord. By adjusting the chemical and physical conditions during gelation, hydrogel scaffolds consisting of porous chambers, linear channels or aligned fibers can be fabricated. The internal structure can serve as a conduit to physically guide axon growth across the lesion, reduce contact of regenerating axons to the inhibitory microenvironment and act as a vehicle for cells and bioactive factors, which in turn create a permissive microenvironment for axonal

growth (Figure 1B). Arrangement of cells in parallel channels may also contribute to axon orientation. Cells and blood vessels that are organized in distinct channels attract axons to sprout along the linear pores (Moore et al., 2006). For example, transplantation of freeze-dried agarose scaffolds or alginate hydrogels composed of uniaxial channels stimulate and guide axonal growth in a linear fashion after SCI (Stokols and Tuszynski, 2006; Günther et al., 2015).

Cell filling and growth factors enhance axon growth in hydrogel channels: In most studies, biological effects of biomaterials without additional manipulations are limited following transplantation to the injured spinal cord despite a delicately fabricated microarchitecture. Therefore, biomaterials have frequently been combined with cells to improve morphological and functional outcomes. Biomaterials can provide a matrix for cell adhesion, and enhance cell survival as well as migration. In addition, cells that are co-transplanted within a scaffold can interact with the surrounding host tissue and increase axon growth into and beyond the graft/host border, a major obstacle for long-distance axonal regeneration (Figure 1C). As biomaterials effectively fill portions of the lesion cavity, the number of cells required for transplantation also decreases. This is particularly important for clinical translation and more extended lesions when large cell numbers that might be difficult to obtain are needed. Several cell types used for transplantation in animal models of SCI have been examined in combination with different biomaterials. These include studies using poly(lactic-co-glycolic acid) (PLGA) scaffolds composed of multiple channels together with Schwann cells (SCs), templated agarose scaffolds with bone marrow stromal cells (BMSCs) and alginate hydrogels with BMSC- or SC-filled channels. In PLGA scaffolds, axons were found to grow throughout the full extent of channels containing SCs but not in channels without SCs (Moore et al., 2006). In templated agarose scaffolds, oriented axonal growth in a highly linear topography throughout the channels was demonstrated when channels were pre-loaded with BMSCs and overexpressing brain derived neurotrophic factor (BDNF) robustly increased axon density within the scaffold (Stokols et al., 2006). Similarly, channels in templated agarose scaffolds seeded with neurotrophin-3 expressing BMSCs facilitated axonal growth towards the distal aspect of the graft, in comparison to animals that were grafted with cell suspensions without agarose scaffolds (Gros et al., 2010). As in studies with agarose scaffolds, BMSCs expressing BDNF enhanced axonal regeneration into channels of alginate hydrogels. However, axonal regeneration generally decreases in the central portion of hydrogel implants and axonal extension into the distal host spinal cord tissue was not observed (Günther et al., 2015).

Influences of hydrogel channel diameter: The channel diameter of multi-channel biomaterials used in most studies ranged from 200 μm to 600 μm. In contrast to other techniques, alginate hydrogels with smaller anisotropic channels can be easily fabricated by diffusion of divalent cations through an alginate solution (Prang et al., 2006). Ranging from 10 μm to 100 μm, channel diameters depend on the cations utilized to cross-link alginate polymers (Pawar et al., 2015). *In vitro* cultures of neonatal cortex, spinal cord or dorsal root ganglia on alginate hydrogels have shown that the density of axon growth into the hydrogel positively correlates with the diameter of channels. In contrast, the linear orientation of axons diminishes with increasing channel diameter (Pawar et al., 2015). Other aspects that can be affected by microchannel diameter within the scaffold include the number and type of cells migrating into channels and newly formed blood vessels that occupy part of the channel lumen available for axonal regeneration. The influence of channel diameter in anisotropic alginate hydrogels seeded with BMSCs was recently evaluated in a cervical spinal cord hemisection lesion. Comparing channels with 41 μm (cross-linked by Sr^{2+}) to 64 μm (cross-linked by Zn^{2+}) diameter, axonal growth was supported throughout the lesion and the 50% difference in channel diameter did not influence axon density. While axon growth was overall oriented in a linear pattern irrespective of the diameter of hydrogel channels and similar to that observed in intact white matter of the spinal cord, even this small increase in channel diameter resulted in a measurable decrease in linear axonal orientation (Günther et al., 2015).

Tissue integration of hydrogels and axonal bridging: Similar to transplantation studies with other

biomaterials (Gros et al., 2010), one major obstacle impeding axonal growth beyond an alginate hydrogel is the graft/host interface. Host cells including astrocytes and fibroblasts that generate a myriad of inhibitors respond to the lesion and impede axon extension (Günther et al., 2015; Liu et al., 2017). To overcome the inhibitory environment, neurotrophin gradients extending from the adjacent host spinal cord into the graft can shift the balance from inhibition to growth promotion and induce some longer-distance axonal regeneration (Taylor et al., 2006). Degradation of inhibitors by enzymes such as chondroitinase, or modification of the density of the barrier to allow axon penetration have also shown success in combination with biomaterial implants (reviewed in Günther et al., 2016). In addition, SCs, currently under investigation in an FDA-approved clinical trial in SCI can improve the host-graft continuity. SCs can modify the interface to become more permissive for axonal growth by intermingling with astrocytic processes of the glial scar (Williams et al., 2015). Recent studies using a combination of alginate hydrogels with SCs demonstrate that axons can extend throughout the full length of channels without a decline in the number of axons in the central portion of the scaffold. While axonal growth is enhanced by BDNF delivered *via* viral gene transfer into the caudal tissue, this chemotropic gradient beyond the lesion was not sufficient for axons to bridge the lesion site. Only when SCs are co-injected into the caudal spinal cord, regrowing axons penetrate the caudal interface and extend into the host parenchyma (**Figure 1D**) (Liu et al., 2017). These findings further highlight the importance of the biomaterial/host interface and the critical role of barriers immediately surrounding the scaffold for successful axonal regeneration.

Glial fibrillary acid protein (GFAP)-labeled astrocytes, which are commonly used to define the border of the spinal parenchyma after injury, are generally confined to areas around biomaterials rather than migrating into the channels. These astrocytes are considered to be beneficial by limiting secondary injury and possibly facilitating axonal growth (Anderson et al., 2016). Although implants provide for the structural continuity across the lesion, a “gap” composed of invading cells and small cysts frequently exists between hydrogels and the rostral and caudal astrocytic edges/host parenchyma. *In vitro* studies using alginate hydrogels have shown that growth of fetal CNS axons into alginate channels is always accompanied by astrocytes, suggesting that glia might be required for axon elongation (Pawar et al., 2015). Incorporation of suitable astrocyte subtypes into channels that can mingle and interact with host astrocytes may therefore help to bridge this “gap” and further improve axonal growth into and out of the channels. Studies investigating this hypothesis are ongoing.

Conclusions: Taken together, anisotropic biomaterials composed of channels for axon orientation and guidance provide several advantages compared to isotropic biomaterials or transplantation of cell suspensions. Additional approaches such as a combination with cells and transient neurotrophin delivery can improve anatomical and behavioral effects (**Figure 1**). Further modifications of biomaterials by electrostatic or covalent coating with bioactive molecules and alterations of the stiffness of biomaterials in particular at the host/graft interface might lead to even better tissue integration, may enhance and accelerate vascularization and cell migration, and provide the support necessary for axons to bridge across larger lesions.

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Footnotes

Plagiarism check: *Checked twice by iThenticate.*

Peer review: *Externally peer reviewed.*

Open peer review reports:

Reviewer 1: *Hedong Li, Pennsylvania State University, USA.*

Comments to authors: *This is a nice and concise review on a specific topic in spinal cord regeneration. The review touched on multiple aspects of the problem and provided helpful insights on potential strategies that one may consider to proceed and solve the problem. As complex as it is, spinal cord injury involves multiple steps in its pathogenesis, which would require combinatory strategies to circumvent. The authors nicely dissect out steps of axonal regeneration with the help of combination of biomaterials (hydrogels) and cells, and give readers a overview of the potential problems and their solutions in a step-wise manner.*

Reviewer 2: *Meng-Jen Lee, Chaoyang University of Technology, China.*

Comments to authors: *This paper is an interesting topic-focused review about the alginate hydrogel use for spinal cord injury.*

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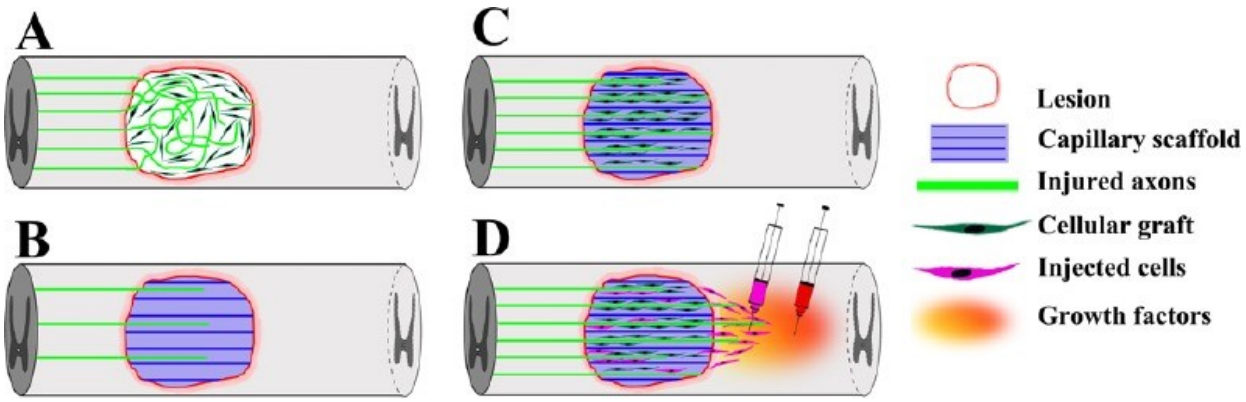
Figures and Tables

Figure 1



Schematic of linear guidance of axonal growth by biomaterials containing a capillary channel structure filled with supporting cells.

(A) Axons regenerate robustly into the lesion implanted with a suitable cellular graft, but growth is randomly oriented rather than extending in rostrocaudal direction toward the distal host parenchyma. (B) Transplantation of an anisotropic biomaterial can effectively fill the lesion cavity and direct a small number of axons to grow in a linear pattern within channels. (C) Linear axonal growth is enhanced when channels contain suitable cells increasing the number of regrowing axons that approach the distal graft/host interface allowing a few axons to re-enter the distal host spinal cord. (D) Additional gradients of neurotrophic factors generated by viral gene transfer and injections of suitable cells such as Schwann cells into the distal spinal cord promotes axonal growth across the distal host/graft interface.
