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·Review·

Biomaterials for spinal cord repair

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Spinal cord injury (SCI) results in permanent loss of function leading to often devastating personal, economic and social problems. A contributing factor to the permanence of SCI is that damaged axons do not regenerate, which prevents the re-establishment of axonal circuits involved in function. Many groups are working to develop treatments that address the lack of axon regeneration after SCI. The emergence of biomaterials for regeneration and increased collaboration between engineers, basic and translational scientists, and clinicians hold promise for the development of effective therapies for SCI. A plethora of biomaterials is available and has been tested in various models of SCI. Considering the clinical relevance of contusion injuries, we primarily focus on polymers that meet the specific criteria for addressing this type of injury. Biomaterials may provide structural support and/or serve as a delivery vehicle for factors to arrest growth inhibition and promote axonal growth. Designing materials to address the specific needs of the damaged central nervous system is crucial and possible with current technology. Here, we review the most prominent materials, their optimal characteristics, and their potential roles in repairing and regenerating damaged axons following SCI.

Keywords: spinal cord injury; axon regeneration; biodegradable materials; extracellular matrix proteins; functional recovery; growth factor; guidance; injury and repair; spinal motor neuron

Introduction

Spinal cord injury (SCI) causes loss of neurons and axons resulting in motor and sensory function impairments. There are thousands of new cases of SCI in the world annually[1, 2] occurring often in young adults. The lack of endogenous repair and the significant costs to the individual, family and society[1] are important motivations behind the continuing efforts to develop effective therapies. Promoting axonal regeneration is considered a potential repair strategy because it could lead to recovery of axonal circuits involved in motor and/or sensory function[3].

The central nervous system (CNS) neurons are intrinsically capable of some regeneration of damaged axons[4] but their attempts after SCI are frustrated by structural and chemical obstructions in the damaged nervous tissue[5]. Spinal cord repair approaches typically lead to small functional gains. One feature that most of these approaches have in common is a poor axonal regeneration response, suggesting that promoting axonal regeneration, including synapse formation, is essential to achieve substantial functional repair after SCI. If so, it is rational to anticipate that effective spinal cord therapies will need to include interventions addressing the axon growth-inhibition that leads to the poor axonal growth responses.

Over the last decades, bioengineering principles have been introduced into the field of spinal cord injury and repair through collaborative efforts between basic, translational, and clinical researchers. This review focuses on the potential application of synthetic and natural biomaterials to modify growth-inhibitory terrain in the injured spinal cord to elicit axonal regeneration and foster functional restoration.

Repair-supporting Biomaterials—Polymers

In general, biomaterials for spinal cord repair are used

for their ability to provide passive structural or active growth support to damaged axons; some offer both through functionalization with biologically-active peptide sequences. Numerous natural and artificial materials have been tested for their efficacy in repairing the injured spinal cord. Application of some of these has resulted in functional improvements, indicating their repair potential. As introduced above, axonal regeneration is considered an important repair mechanism for the injured spinal cord. Therefore, this review focuses on materials that have shown most promise to elicit an axonal regeneration response to foster functional restoration after SCI.

Most biomaterials designed and produced in the laboratory and tested for their efficacy to repair the injured spinal cord are polymers. Polymers have numerous biomedical applications due to their vastly diverse properties. Typically, polymers are large two- or three-dimensional molecules composed of repeating units[6, 7]. Polymers can be obtained from natural sources including plants, animals, and DNA, or be fabricated synthetically in a laboratory. Natural polymers are widely available and tend to undergo highly-controlled synthesis resulting in regular structures; however, they often contain contaminating molecules and are difficult to sterilize[7]. Synthetic polymers are easier to sterilize, but are typically susceptible to the chosen process of synthesis which often causes irregularities in structure and composition[7].

Currently, there is no consensus in the literature on what constitutes the optimal characteristics of biomaterials for spinal cord regeneration and repair. However, there is increasing theoretical and experimental evidence for the use of polymers for the repair of soft tissue such as nervous tissue. An important advantage of some polymers for spinal cord repair is that they can be designed for *in situ* polymerization and/or cross-linking, therefore needing minimally-invasive approaches (injection) to be applied. A variety of techniques have been developed and used to characterize material properties, optimizing biomaterials for use in a tissue-specific repair application. Figure 1 illustrates some examples of these characterization techniques. Using these techniques, biomaterials can be fabricated to degrade within a specific window of opportunity, be non-cytotoxic, and have mechanical properties similar to those of the native tissue to decrease fibrolysis[8]. Hydrogels, a category of polymer that are highly water-sequestering, specifically seem to fulfill many of these requirements that could support the repair of damaged nervous tissue.

Hydrogels

Most SCIs in humans are contusions[1] that result in an irregular-shaped cavity surrounded by spared white matter. The preferable way to introduce a biomaterial for repair would be injection into the cavity, which would minimize additional damage to the nervous tissue. Therefore, a number of research groups have investigated injectable, *in-situ* gelling substrates for spinal cord repair[9-11]. Many of these substrates are hydrogels, water-swollen cross-linked polymer networks capable of imitating the mechanical and, to some degree, the architecture of the soft spinal cord tissue[12]. Some characteristics of hydrogels that can be considered for optimizing their application in spinal cord repair are their porosity, degradation, functionalizability, biocompatibility, *in situ* gelling, and elastic modulus.

Mammalian Extracellular Matrix-based Natural Polymers

A variety of natural biomaterials mostly present within the extracellular matrix (ECM) provide both structural and growth support to axons during development[13, 14] and maturity[15] as well as after injury[16]. Collagen, laminin, fibronectin, vitronectin, and proteoglycans/glycosaminoglycans[14] are all members of this particular group of materials. Most ECM components signal their axonal growth-promotion through integrin receptors[17] that bind, in many, the Arg-Gly-Asp (RGD) peptide sequence[18]. Our continuously increasing understanding of the role of ECM in axonal growth has fueled many studies of these materials to investigate their efficacy for repair (i.e. [19]). For an overview of recent studies that have used mammalian ECM materials, the injury models chosen, and the outcome measures, see Table 1.

Collagen Collagen is found naturally as a triple-helical protein in mammals[20]. It is the most abundant protein in the human body and the main component of the ECM[21]. Collagen self-polymerizes in a two-phase process (nucleation/growth[20, 22]) which is determined by pH, temperature, and polymerization rate[23-26]. The highly-tunable mechanical properties[27, 28] and swelling of fibers[28] allow collagen to form a hydrogel. Collagen signals its

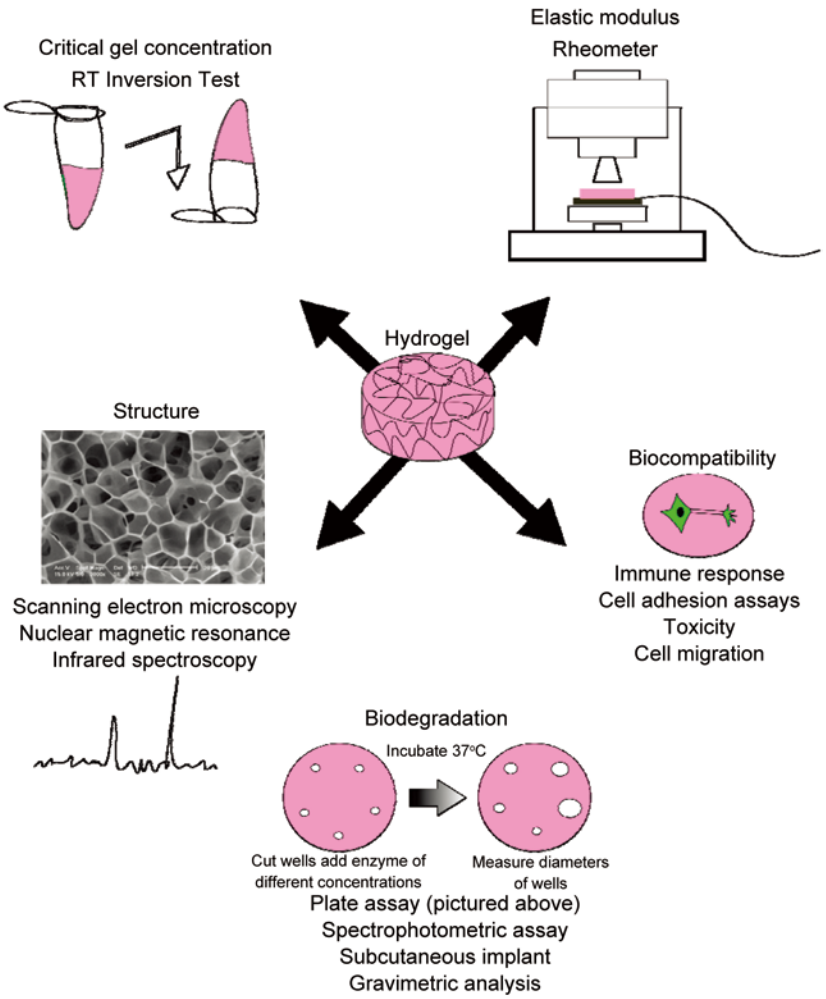

Fig. 1. Overview of general techniques for the characterization of hydrogels for use in spinal cord repair. The scanning electron microscope image is a decellularized extracellular matrix (ECM) hydrogel derived from urinary bladder matrix (ECM concentration 8 mg/mL; courtesy of Matthew Wolf and Stephen Badylak, University of Pittsburgh).

biological actions through α1β1 (monomers) and α2β1 (fibrils) integrins[29] as well as discoidin domain receptors and glycoprotein VI[30]. In nervous tissue regeneration, collagen has been used in scaffolds[31, 32], magnetically-aligned fibrils[33-36], gels[34, 37], and cell-delivery vehicles[38]. Collagen has the innate ability to form magnetically-responsive fibrils which are capable of directing neurite growth and delivering growth factors, both of which can be advantages for spinal cord repair. However, if the mechanical properties are not properly tuned for the environment, collagen may act as a physical barrier for regenerating growth cones[39]. Also, one group found a high mortality rate (40%) with implanted collagen filaments, suggesting that it has potential cytotoxicity[40].

Laminin Laminin is a heterotrimeric glycoprotein consisting of α, β, and γ subunits that self-assemble in a temperature-, pH- and concentration-dependent manner[41, 42]. As a major component of basement membrane ECM, laminin has frequently been shown to influence neurite outgrowth both *in vitro*[42-45] and *in vivo*[44, 46-49]. Laminin forms a sheet-like polymer that is best known for its critical involvement in basal membrane formation. Laminin signals through a variety of integrins[50]. Integrin activation influences neurite outgrowth *via* the formation of a focal adhesion complex and the activation of focal adhesion kinase and the mitogen-activated protein kinase kinase/extracellular

Table 1. Mammalian-derived natural polymers for spinal cord repair

Material	Tests	SCI Model	Main findings
Collagen	Wrathall motor scale[86]	Acute hemi-resection[31, 87] with semi-cylindrical scaffold[31];	Some motor function benefit when combined with cells[31, 38, 87] or ChABC[31]
	IHC[31, 32, 38]	Acute complete transection[32]	Increased neurofilament staining[31]
	Bladder function[31, 32]	Contusion followed by complete transection and collagen filaments[40]	Decreased cyst size[32]
	BBB[87]		Increased synapse formation and neuronal differentiation[87]
	Inclined plane[87]		45% of rats died within one month; survivors had improved walking, coordination, axon regeneration and electrophysiology[40]
	Walking movements[40]		
	Reflexive behaviors[40]		
	Climbing ability[40]		
	Electrophysiology[40]		
Laminin	BBB[49, 88]	Complete transection[49, 88]	Increased axon regeneration[49, 88]
	Combined behavior score[88]	Contusion[49]	Increased motor recovery[49]
	IHC[49, 88]	Dorsal hemisection[49]	
	Robotic recording[88]		
Fibronectin	Von Frey filaments[89]	Dorsal column crush[89]	Blocked development of mechanical allodynia[89]
	IHC[62, 89-91]	Hemisection[91]	Attenuated blood-spinal cord barrier leakage to decrease inflammation[89]
	Horizontal ladder[91]		Improved sensory-motor function[91]
	Beamwalk[91]		Neuroprotective[62, 90, 91]
			Axon regeneration[62, 90]
Hyaluronan	BBB[70, 71, 92]	Compression injury[70, 71, 92]	Improved[70, 71] or no improvement[72, 92] in locomotion
	Inclined plane[70]	Dorsal hemisection[93]	Improved[70] or no improvement[72] in electrophysiology
	Von Frey[70]	Complete transection[72]	Decreased mechanical allodynia[70]
	IHC[70, 92, 93]		Decreased lesion size[70]
	Electrophysiology[70, 72]		No change in lesion size[92]
	Structural MRI[70]		Decreased CSPGs[93] and cytokines[70]
			Increased inflammatory response[72]
Combination ECM	BBB[85, 94, 95]	Lateral hemisection[85, 95]	Increased axon regeneration[85, 94, 95]
	IHC[85, 94, 95]	Complete transection[94]	Improved locomotion alone[85] or when combined with cells[95]
	Electrophysiology[94, 95]		Remyelination with cell transplant[85]
			Increased angiogenesis[94]
			Improved electrophysiology[94, 95]

BBB, Basso, Beattie and Bresnahan score test; ChABC, chondroitinase ABC; CSPG, chondroitin-sulfate proteoglycans; IHC, immunohistochemical staining; MRI, magnetic resonance imaging.

signal-regulated kinase signaling pathways[51].

Laminin does not provide structural support and, therefore, is not often introduced alone as a biomaterial for SCI regeneration. Instead, it is combined with other supportive materials to enhance growth promotion[35, 39, 52, 53] and cell differentiation[54]. Recently, it was shown that the

structure of the laminin polymer may be tied to its signaling capabilities. Certain structural configurations can induce regeneration and functional recovery when injected alone directly into the spinal cord following injury[49].

Fibronectin Fibronectin is a high molecular weight glycoprotein that binds to integrins and to other ECM proteins such as collagen[17, 55-57]. Soluble fibronectin circulates in the blood and is involved in clot formation, while insoluble fibronectin is found in the ECM. Unlike some other ECM proteins, fibronectin does not undergo spontaneous polymerization, but rather does so *via* a highly-regulated, cell-mediated process[58]. The biological activity of fibronectin is mediated by RGD-integrin α5β1 binding, and also has an additional integrin-binding site[59], PHRSN[60, 61], that results in a synergistic effect on cell adhesion and the activation of a variety of pathways leading to changes in >30 genes[59].

Although incapable of forming a hydrogel on its own, as a potential treatment strategy, it has been used in combination with hydrogels for spinal cord repair due to its cell-signaling properties through RGD-integrin binding[62]. After injury, it has been shown to elicit neurite outgrowth[57]. Fibronectin may be most relevant for regenerating damaged blood vessels *via* the α5β1 integrin receptor, which in turn could improve nutrient delivery to cells and reduce the infiltration of circulating macrophages following SCI[63]. Fibronectin has also been used in biosynthetic conduits for regeneration in a complete spinal transection model[64].

Hyaluronan/Hyaluronic Acid Hyaluronan/hyaluronic acid (HA) is a high molecular weight non-sulfated glycosaminoglycan that occurs naturally in the ECM in the adult CNS. It plays a major role as the structural component in brain ECM[65]. Due to its ability to sequester large amounts of water, it readily forms hydrogels both alone[66] and (often) in combination with other natural materials such as fibronectin[67] and methylcellulose[68] or methacrylate[69]. It is biodegradable and functionalizable, and therefore, when combined with materials that are not readily degraded, it permits tunable concentration-dependent degradation rates. HA binds to a variety of membrane-associated proteins including versican, aggrecan and glial HA-binding protein[65]. Modified HA supports cell attachment and migration, and when combined with brain-derived neurotrophic factor, may improve regeneration and motor function as an implanted scaffold after SCI[70, 71]. Due to its natural role as a structural component in the CNS, it has been used both alone as well as in functionalized and combination formats to provide structure and guidance for regenerating axons after injury[71, 72].

Combination ECM-derived Polymers Recently, some groups have focused on creating a more complete ECM environment containing multiple molecules from native ECM[19]. Previously, it was shown that various basement membranes containing a full arsenal of ECM molecules enhance neurite outgrowth *in vitro*[73, 74] and axonal extension in the lesioned adult brain[74]. Basement membrane is a specific type of ECM secreted by support cells and composed of >20 different molecules[75]. They are of specific interest for axon regeneration due to their crucial role in regeneration in the damaged peripheral nerve[76]. Although it is typically difficult to isolate and manipulate these fragile substrates without causing damage, recent advances have made successful isolation of various basement membranes possible[75]. Several combination ECM materials isolated from mammalian sources are commercially available[52, 77], while others are in various stages of development and characterization[19, 78-80].

Matrigel™ is a well-characterized, commercially available ECM-derived hydrogel that is a gelatinous mixture of proteins secreted by tumor cells[52]. The abundance of proteins secreted by this particular cell type makes Matrigel™ an easy-to-obtain source of hydrogel-resembling natural ECM[52]. Previous studies have indicated its ability to promote neurite outgrowth[35, 53] and to serve as a cell-delivery vehicle[81]. It has been used in a variety of studies and in many different combination treatments, with groups reporting mostly positive results; however, in some studies, axonal regeneration occurred with significant tissue loss when Matrigel™ was used to fill a conduit following complete spinal transection[82, 83].

In addition, non-tumor-related sources of ECM have also become available. ECM isolated from de-cellularized pig bladder was shown to have regenerative potential in a variety of organs including esophagus[84] and ligament[78]. This potential is likely due to its ability to provide structural support, growth factors, and a variety of signaling molecules to regenerating tissues[19]. Recently, several groups have started to isolate ECM from CNS to study its potential as a regenerative substrate for SCI[80,85].

Non-mammalian Natural Polymers

Natural polymers for neural tissue engineering and regeneration can also be obtained from non-mammalian sources such as algae, crustaceans, plants, and bacteria. An important aspect of these polymers is their biocompatibility, i.e., how the mammalian body responds to these foreign materials. They typically do not have ligand-associated interactions with mammalian cells and therefore may serve primarily as a supportive matrix. Some of the most investigated non-mammalian natural polymers that have shown some benefits for nervous tissue repair are listed in Table 2.

Synthetic Polymers

Synthetic polymers are championed due to their ability to be modified and designer-made with optimal mechanical properties and functional sequences for cell signaling and controlled degradation. They are also easy to sterilize[7] in contrast to natural polymers. The selection discussed here has been chosen as a representative subset of the most common synthetic polymers that have been used for CNS repair (Table 3).

Poly(2-hydroxyethyl methacylate) (PHEMA) or poly (2-hydroxyethylmethacrylate-co-methylmethacrylate) (PHEMA-MMA) PHEMA and PHEMA-MMA are probably the most studied water-swelling biomaterials. PHEMA is a non-biodegradable, functionalizable hydrogel with many medical applications such as contact lenses. Some studies suggest it may actively increase axon regeneration[53]. PHEMA easily forms designer conduits[114], but these are structurally weak with a risk of collapse[115]. Typically, copolymerization with methyl methacrylate (PHEMA-MMA) offers structurally-stronger scaffolds[114]. The biocompatibility of PHEMA has been recognized as a potential problem in CNS repair studies.

Table 2. Non-mammalian-derived natural polymers for spinal cord repair

Material	Type	Source	Benefits	Limitations
Chitosan	Polysaccharide	Crustaceans	Improves neuronal survival[96] Potentially increases axonal growth[97]	No increase[97] or increase in axonal outgrowth[97, 98]; increases inflammatory response[99, 100]; breakdown controlled to some degree by level of de-acetylation[100-103]
Agarose	Linear polysaccharide	Red algae	Biocompatible; thermal gelling; improves neurite extension[104, 105]; flexible and stable in culture conditions[106]	Biodegradation depends on composition with other materials (i.e. HA)[107]; biologically inert unless modified[106]
Alginate	Linear polysaccharide	Brown algae	Enhances cell resistance to oxidative stress[108]; increases neuronal regeneration[108]; negatively charged[109]; biocompatible; biodegradable	Low cell adhesion without addition of functional groups (i.e. RGD)[110]
Xyloglucan	Structural polysaccharide	Cell wall of plants	Directs stem cells to a neuronal lineage[111]	Increased astrocyte infiltration[112]
Gellam gum	Polysaccharide	Bacteria	Promotes axon regeneration across injury site[113]	Not gel-forming, incompatible with contusion injury
Methylcellulose	Cellulose	Plant cellulose treated with methyl chloride	FDA-approved for use in brain, non-toxic, thermally sensitive, functionalizable	Non-biodegradable unless combined with other materials such as hyaluronic acid

Table 3. Synthetic polymers for spinal cord repair

Material	Tests used	SCI model used	Main findings
PHEMA/PHEMA-MMA	IHC[53, 140, 141] MRI[140] BBB[140]	Complete transection[53, 140] Partial hemisection[141]	Improved axon regeneration from vestibular and red nucleus[53] Decreased cyst volume[140] Increased cell infiltration into scaffold[140] Moderate inflammatory response[141] Angiogenesis[141]
PHPMA	IHC[116, 142] EM[116, 142] Visual examination of movement[116] Treadmill walking[142]	Complete transection[116, 142] Hemisection[116]	Axon regeneration[116, 142] Qualitative motor improvement[116, 142] New synapse formation[116] Angiogenesis[142] Reduction of glial scar[142]
PEG/PEO	Histology[129, 143] Antioxidant assay[129] HRP uptake[129] Cutaneous trunci muscle function[119, 123, 124] Electrophysiology[119, 123, 124]	Compression injury[119, 123, 124, 143] PEG administered directly into cord[119, 123] PEG administered subcutaneously[124] Organotypic SC culture[129]	Decreased membrane permeability[129] Decreased reactive oxygen species[129] Decreased apoptosis[143] Improved muscle function[119, 123, 124] Improved electrophysiology[119, 123, 124]
PVA	IHC[134]	Compression injury[134] Laminectomy only[144]	As a drug-releasing thin film, well-tolerated, does not incorporate with tissue and preserves bioactivity of drug[134] Protects cord after laminectomy[144] Prevents scar formation[144]
PGA/PLA/PLGA/PLCL	IHC[133, 139, 145, 146] BBB[92, 145] Inclined plane[145] Mechanical allodynia[145] Gridwalk[146] Beamwalk[146]	Complete transection[139] Lateral hemisection[145] Dorsal hemisection[146] Clip compression[92] Contusion[133]	As drug-releasing nanoparticles, well-tolerated with optimal release characteristics and preserved bioactivity of drug[92, 133, 146] As scaffold for NSCs, increased sensory, motor and reflex function, increased axon regeneration and non-cytotoxic[145] As conduit, increased axon regeneration, but over time collapse/breakdown of tubes hindered axon survival[139]

BBB, Basso, Beattie and Bresnahan score test; IHC, immunohistochemical staining; MRI, magnetic resonance imaging; EM, electron microscopy.

Poly[N-(2-hydroxypropyl)methacrylamide] (PHPMA) PHPMA is non-biodegradable but has better biocompatibility than PHEMA because of its modified chemical structure[116]. It was developed for drug delivery/gene therapy. In chronic SCI, PHPMA was shown to reduce the lesion cavity volume and induce axon regrowth[116]. One disadvantage compared to PHEMA is that PHPMA shows an increase in regeneration-blocking connective tissue after SCI[117], which suggests it may work more efficiently when coupled with a drug (such as an matrix metalloproteinase)

or therapy to counter the connective tissue deposition.

Poly-ethylene-glycol (PEG)/poly-ethylene oxide (PEO) PEG is a water-soluble hydrogel and can be designed to be hydrolytically unstable, i.e., biodegradable, or inert, i.e., non-degradable[7]. This is an attractive property, especially for drug delivery, because it allows for controlled degradation. Another quality is that PEG gels can be modified to be thermally activated so that they are liquid at room temperature but quickly gel *in situ*[118]. Due to its hydrophilic properties, PEG has been useful in repairing damaged neuronal membranes when applied directly to the injury site immediately following a crush injury[119-122], in a delayed application[123], or as a subcutaneous application[124]. PEG is favorable as it is functionalizable and groups have incorporated cell-binding domains such as RGD[125] and IKVAV[126]. It can be used for cell immobilization[127] and drug release[128]. Another interesting capability of PEG is that it has anti-oxidant actions[129, 130], which may limit secondary injury. PEG hydrogels have been used alone[119-123, 129, 130] and as factors[128] or cellular carriers[131] after SCI and they are generally considered promising candidates for nervous tissue repair.

Poly(vinyl alcohol) (PVA) PVA is a degradable, biocompatible, non-toxic, carbon-carbon backbone polymer[132], which makes it an attractive option for biomedical applications. It is nontoxic, hydrophilic, and has good adhesion properties. PVA is commonly used for drug delivery directly to the injury site after SCI[133, 134]. PVA forms hydrogels with varied mechanical properties based on the water-content of the gel[7]. Unless it is functionalized, PVA does not directly bind to cell surface receptors and causes little immune response[134]. Therefore, unmodified PVA does not elicit axonal growth responses.

Poly(α-hydroxyacids) Poly(α-hydroxyacids)[135] include poly(glycolic acid) (PGA), poly(lactic acid) (PLA), their co-polymer poly(lactic-co-glycolic acid) (PLGA) and poly(lactic-co-caprolactone) (PLCL). Varying the composition of these co-polymers allows for precise control of degradation and mechanical properties[136]; however, this class of polymers does not form hydrogels. Therefore their use is limited to conduits for complete transection. An important advantage over other candidates is that they are FDA-approved for use in the peripheral nervous system. They have been shown to support regeneration after complete spinal transection when combined with either growth factors[137] or cell transplants[138, 139].

Axonal Regeneration: A Balance between Growth-Promoters and Growth-Inhibitors

Many molecules at an injury site directly influence the conduciveness of the environment for axonal regeneration. Balancing these signals in ways that lead to axonal regeneration is an important challenge in the field of spinal cord repair. In essence, there are two ways to influence this balance in favor of axonal growth: (1) remove/degrade growth-inhibitory molecules, such as chondroitin-sulfate proteoglycans (CSPGs), myelin-associated glycoproteins, and others, and (2) introduce growth-promoting molecules, such as neurotrophins (for review see[147]). An important role for biomaterials is that of a delivery vehicle for factors that, when released in a controlled fashion, may affect the growth-inhibition balance so that axonal regeneration is achieved.

Functionalizing Biomaterials to Tip the Balance

Although intrinsically capable of regeneration[4], one reason that CNS neurons fail to do so is the excess of inhibitory signals within and around the injury site in the spinal cord[5]. Many groups address this issue by incorporating molecules into biomaterials to counter these injury-related signals over a controlled period of time. The enzymes chondroitinase (ChABC) (for review see[148]) and sialidase[149, 150], or a combination of the two were shown to decrease growth inhibition[151]. Many groups have shown that administration of ChABC is associated with sensory axonal regeneration[152] and functional recovery[148, 152, 153] when experimentally introduced following SCI. When comparing the two, one group found that sialidase increases axonal regeneration and functional recovery[149-151] to a greater degree than either ChABC alone or a sialidase/ChABC combination[151].

Alternatively, groups have addressed this issue by incorporating growth-promoting molecules into biomaterials. Largely, this approach involves either presenting cell-matrix-binding ligands or growth factors to enhance regeneration and encourage infiltration of growing axons into and through the lesion site[154]. Often, the cell-matrix-binding ligands are covalently attached to the material itself, while growth factors are incorporated during the

polymerization phase and slowly released as the material degrades. Growth factors often used in combination with biomaterials are brain-derived neurotrophic factor[71, 155-159], neurotrophin-3[156, 159], nerve growth factor[160-162], and ciliary neurotrophic factor[155, 159, 163]. These factors appear to act on both specific and shared target neurons and affect the regeneration of different axonal tracts. This argues for the careful choice of growth factor, depending on the deficit and measure of recovery. With damage to multiple tracts, a combination of factors may be necessary.

One technique to deliver multiple growth factors from a biomaterial is to incorporate stem cells and stem cell-like cells that secrete a growth-promoting cocktail of factors to the damaged cells within an injury site (for review see[164, 165]). Using biomaterials as a vehicle for transplants may offer the protection and cues needed to increase survival of the transplants in order to prolong their therapeutic effects and provide meaningful regeneration and functional recovery. In order to best enhance the survival of transplanted cells in spinal cord repair, materials should protect the cells from sources of cytotoxicity within the injury environment (i.e., macrophages and reactive oxygen species), while maintaining biologically-active interactions with the transplant as a pseudo-ECM. Functionalizing biomaterials with specific binding motifs could aid in providing a pseudo-ECM environment and protect cell transplants from anoikis (a form of apoptotic death due to loss of attachment). These cell-binding motifs include laminin-associated peptides[166, 167] and more generally RGDs[165, 167, 168] and have shown some improvements in transplant survival[165-169].

Concluding Remarks

Currently, a plethora of biomaterials is available and many more are in development for use in spinal cord repair. Each material has benefits and pitfalls when incorporated into the injury site. In this review, a subset of materials widely used in the field of spinal cord regeneration is presented. When testing any biomaterial for spinal cord repair, the type of material chosen should depend on the specific needs of the injury. The most clinically-relevant injury in humans is contusion, which occurs in >75% of all SCI cases. Therefore, we argue that injectable, *in situ* gelling materials hold the greatest clinically-relevant treatment potential. Injectable materials can be delivered either directly to the epicenter or adjacent to the lesion site while causing little additional damage. Of the *in situ* gelling materials, it is necessary that the immunogenicity of the material is low and reliable sterilization techniques exist. Currently, there is still some concern over the reliable sterilization of natural polymers while more techniques are available to safely sterilize synthetic polymers. The material should also be biodegradable, preferably in such a way that it slowly degrades as it is replaced by the naturally-regenerated environment. Considering all of this, PEG-based hydrogels meet many of these criteria, and when combined with the innate ability to sequester reactive oxygen species, it is reasonable to expect advances in this particular group of *in situ* gelling materials which could make an important contribution to the development of therapies for spinal cord repair.

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