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Alginate Hydrogels as Scaffolds and Delivery Systems to Repair the Damaged Spinal Cord

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Alginate (ALG) is a lineal hydrophilic polysaccharide present in brown algae cell walls, which turns into a gel state when hydrated. Gelation readily produces a series of three dimensional (3D) architectures like fibers, capillaries, and microspheres, used as biosensors and bio-actuators in a plethora of biomedical applications like drug delivery and wound healing. Hydrogels have made a great impact on regenerative medicine and tissue engineering because they are able to mimic the mechanical properties of natural tissues due to their high water content. Recent advances in neurosciences have led to promising strategies for repairing and/or regenerating the damaged nervous system. Spinal cord injury (SCI) is particularly challenging, owing to its devastating medical, human, and social consequences. Although effective therapies to repair the damaged spinal cord (SC) are still lacking, multiple pharmacological, genetic, and cell-based therapies are currently under study. In this framework, ALG hydrogels constitute a source of potential tools for the development of implants capable of promoting axonal growth and/or delivering cells or drugs at specific damaged sites, which may result in therapeutic strategies for SCI. In this mini-review, the current state of the art of ALG applications in neural tissues for repairing the damaged spinal cord is discussed.

1. Introduction

The use of hydrogels provides a broad range of possibilities in biomedicine, ranging from cell and gene therapy,[1,2] and drug delivery,[3] to regenerative medicine and tissue engineering applications.[4–7] The success of this technology derives from the ability of hydrogels to retain high levels of water, as much as over 99%, which favors the entrapment of biological entities and increases their biocompatibility.[8]

Synthetic (e.g., 2-hydroxyethyl methacrylate [HEMA], polyacrylamide [PAA], polyethylene glycol [PEG]) and natural polymers (e.g., alginate [ALG], carrageenan, chitosan, hyaluronic acid) have been used in appropriate concentrations to trigger gelation processes by applying chemical or physical cross-linking strategies.[9] These two processes permit the self-assembly of such polymers into hydrophilic networks, giving rise to a plethora of 3D materials containing both strong and weak interactions throughout polymer networks.

Although synthetic polymers have played a meaningful role in multiple biomedical applications,[10,11] extensive studies have led to natural polymer-based hydrogels emerging as equally or more suitable materials, due to their low immunogenicity, ready biodegradability, and easy large-scale production.[12,13] To this end, specific polysaccharide-based hydrogels are being engineered. They prove to be efficient depots for living cells, small molecules, growth factors, and liposomes, for use in a wide range of biomedical applications including cell transplantation or drug delivery. Hydrogels,[14] in particular those composed of ALG,[15,16] have recently[17–21] emerged as bridging materials capable of delivering cells and drugs into specific locations, thus promoting tissue regeneration when implanted in injured tissues.[22]

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1.1. Spinal Cord Injury

The brain together with the spinal cord (SC) forms the central nervous system (CNS), which integrates information and coordinates activity across the entire body. Spinal cord injury (SCI) is a dreadful disorder that affects more than 180 000 people worldwide every year, causing permanent disability—including loss of sensory and motor functions in trunk and limbs, together with the loss of autonomous regulation of breathing, bladder emptying, and sexual function.[23]

Traumatic injury to the SC leads to a complex pathophysiology that affects the neural, vascular, and immune systems and largely determines the functional outcome from the SCI. The trauma damages the membranes of spinal cord cells, mainly in the gray matter, causing cell death, axotomy, and blood vessel rupture.[24] The initial damage may consist of rupture of the blood–spinal cord barrier, ionic disbalance, massive excitatory neurotransmitter release, oxidative stress, inflammation, and immune response, among others. The molecular and cellular events triggered by such damage induce cell death among neurons and glial cells for weeks after injury, and extend the damage to regions far from the injury epicenter.[25,26] The cellular responses lead to the formation of a glial scar that reestablishes the blood–brain barrier and isolates the damaged region, where cysts usually form. Functional deficits due to SCI are usually permanent because the regenerative response of the injured neurons is very limited,[27,28] and regenerating axons are exposed to inhibitory molecules,[29] glial scars, and cysts that prevent growth across the injury site.[30]

Despite the resources and efforts applied in research to seek an efficient therapy to restore loss of function, only rehabilitation and epidural electrical stimulation[31] have proven effective in recovering the functional deficits of SCI. Significant efforts have been made to develop therapeutic strategies aiming to protect spared tissue and replace lost cells, promote axonal growth and myelination, and restore or replace lost neural signaling. In this context, novel therapeutic treatments involving regenerative medicine and tissue engineering have emerged as promising strategies focused on promoting axon growth and/or delivering cells or drugs to specific damaged sites for SCI. These approaches rely on using cell transplantation procedures, antioxidative or anti-inflammatory molecules, specific growth or neurotrophic factors, as well as biomaterial scaffolds. The positive outcomes achieved in vitro and in vivo have facilitated launching various pre-clinical and clinical controlled trials to validate such therapeutic effectiveness, giving rise to promising therapeutic strategies for SCI.[32–36]

1.2. Hydrogel-Based Materials in SCI and Neural Regeneration

The rapid development and extensive research sustained by polymer science have facilitated the appropriate design and preparation of a large number of scaffolds for tissue engineering applications. Both synthetic and natural materials have demonstrated their usefulness to reconstruct and replace the great majority of damaged tissues.[6,7,37] To do so, preformed materials should fulfill a series of requirements to be considered as optimal implants for promoting SC repair and neural tissue engineering. These include their lack of toxicity and immunogenicity, thus contributing to high biocompatibility.[38,39] Appropriate degradation rates,[40] shear modulus, substrate stiffness, and superficial geometry are important properties that should also be taken into account.[40]

A good number of 3D materials and drug delivery devices in conjunction with injectable hydrogels have been properly designed for SCI.[16,39,41–44] Some non-degradable materials composed of synthetic polymethacrylates have shown notable success as implants due to their stiffness without causing compression of the SC tissue.[44] This feature makes non-degradable materials favorable to cell encapsulation and to providing the proper 3D networks for bridging neural tissues.[45,46]

Degradable synthetic polymers like PEG and poly-peptide hydrogels,[47–49] among many others,[50–53] have emerged as promising alternatives for reconstructing damaged tissues.[44] This is mainly due to optimizing several parameters like molecular weight, degree of crosslinking, or polymer structures, which tend to favor surface degradation rates and consequently the absence of immune response in the body and nerve compression after the scaffold implantation.[54,55] A selection of other synthetic

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Table 1. Use of alginate (ALG) as a natural hydrogel involved in neural tissue engineering applications such as axon growth, cell-based therapies, drug delivery, and 3D bioprinting.

Entry	Application	Material	Model	Comments	Reference
1	Axon growth	ALG-poly-L-ornithine and laminin	In vitro and in vivo (Fischer 344 rats)	The material was filled with astrocytes and was able to integrate into the damaged site	[77]
2	Axon growth	Capillary ALG hydrogel	Schwann cells encapsulation (primary culture) and in vivo (Fischer 344 rats)	Axon growth was significantly increased when BDNF is expressed	[78]
3	Axon growth	ALG-PHB coated with fibronectin	In vivo (adult female Sprague-Dawley rats)	The presence of PHB and fibronectin aided regeneration and supported neuronal survival	[79]
4	Cell-based therapy	Unmodified soft ALG hydrogel	In vivo (Wistar rats)	Material was implanted, leading to improved locomotor recovery	[80]
5	Cell-based therapy	ALG hydrogel was covalently modified with RGD	In vitro (2D culture and 3D culture)	The RGD modification improved the Schwann cells attachment	[81]
6	Cell-based therapy	Ionically cross-linked unmodified ALG hydrogel	In vitro (primary neurons in 3D culture)	The material was able to promote neuritogenesis, exhibiting good viability and cell proliferation	[82]
7	Cell-based therapy	ALG anisotropic capillary	In vivo (Fischer 344 rats)	Bone marrow stromal cells expressing a neurotrophic factor were seeded	[83]
8	Drug delivery	ALG microfibrous patches (a drug delivery platform)	Local delivery of Rolipram. Characterization of in vitro and in vivo models (athymic rats)	Those animals treated with the prepared patches exhibited greater functional recovery than controls	[84]
9	3D-scaffold	ALG-gelatin blends	Printability tests for bioprinting. Cell in vitro studies (mesenchymal stem cells)	A general study in which 7% ALG and 8% gelatin gave rise to high printability rates and stiffness	[85]
10	3D-scaffold	Neurocompatible 3D-ALG "living" scaffold combined with methylcellulose	Cell lines used: primary human neonatal fibroblasts, hiPSC-derived ventral sNPCs, and miPSC-derived OPCs	A 3D-spinal cord tissue model resulted in successful modeling of CNS tissue	[86]

hydrogels used for SC regeneration[56–59] have been listed in Table S1, Supporting Information.

Extensive efforts have been also made to use natural hydrogels in SC repair.[60–67] Natural materials made up of ALG, agarose, cellulose, chitosan or hyaluronic acid, among others, have been used in vitro and in vivo with the aim of promoting cell adhesion, controlled and localized delivery of neurotrophic factors, cell delivery (including pluripotent or multipotent stem cells), or the formation of filament bridges and scaffolds for neurite regrowth.[3,12,13,16,21,68] Unlike synthetic polymers, natural implanted materials display high biocompatibility in living tissues. In this way, the cytotoxicity and immune response exhibited by these materials are practically zero.[69] Recent uses of natural hydrogels are listed in Table S1, Supporting Information.

Taking advantage of the characteristics of synthetic and natural hydrogels, a combination of both materials has been proposed for attempting SC regeneration. The idea of using synthetic/natural hydrogels is not novel, as they are employed in other biomedical applications.[70,71] In particular, this strategy involves putting the most common features of both hydrogels into practice: biocompatibility and biodegradability in the case of natural hydrogels and the ability to "tune" the physical and mechanical properties of the resultant materials when using synthetic hydrogels. This combination allows the preparation of functionalized materials to provide protection at the injured site as well as deliver neurotrophic factors and neural stem cells.[72–76] Detailed information on the progress and uses of these composites is shown in Table S1, Supporting Information.

In this article, we aim to review the current state-of-the-art strategies employed to prepare natural hydrogels for neural tissue engineering applications. Particularly, this revision is focused on ALG as an artificial scaffold, analogous to the ECM used in regenerative applications to repair the damaged SC. The possibilities of ALG for promoting axon growth, cell-based therapies, drug delivery, or as a 3D-scaffold will be discussed below, and are displayed in **Table 1**.

2. Alginate Biomaterials for the Repair of the Injured Spinal Cord

ALG is a hydrophilic lineal polysaccharide naturally occurring in the cell walls of brown algae. ALG consists of repeated units of (1-4)-β-D-mannuronic acid and α-L-guluronic acid building blocks. These residues are found as flexible coils in aqueous solution. The presence of divalent cations (e.g., Ca^{2+}, Mg^{2+}, Ba^{2+},

and many others) is essential for the chelation process amid the two aforementioned building blocks. As a consequence, cooperative ionic inter-chain forces are spontaneously produced, giving rise to ordered 3D structures that cause gelation of the ALG solution.[87] Physical properties of the resultant hydrogels (e.g., mechanical strength, stability or elasticity) can also vary, depending on the divalent cation used. This tunable property facilitates engineering different ALG-based materials used in numerous minimally invasive biomedical applications, from oral administration to hydrogel injection. Versatility, high biocompatibility and lack of toxicity, ease of gelation, and external structural similarity to the extracellular matrix (ECM) of living tissues are key properties of ALG for biomedical applications. However, despite ALG being a biocompatible and naturally derived hydrogel, some impurities isolated in commercially available samples have proved to be cytotoxic and mitogenic.[88,89] This might lead to unwanted responses after cell transplantation, reducing its potential use unless a prior purification of the commercial sample is carried out.[88]

ALG has proven to be an efficient biomaterial and a model scaffold when working as a bridging material in neural tissue engineering, including SCI and peripheral nerve regeneration, as well as in the delivery of cell and growth factor molecules (EGF and bFGF) for SC repair.[90–95]

2.1. 3D-Scaffolds

3D-scaffolds have resulted in a prominent strategy for CNS regeneration, as they can facilitate cell proliferation and provide cellular adhesion onto the new material.[96] Hydrogels may confer additional advantages as cell and molecule delivery vehicles, due to their high water content and good response to several stimuli like pH or temperature. Natural polymers like chitosan, agarose, fibrin, collagen, gellan-gum, Matrigel, and hyaluronic acid have been used as 3D scaffolds to aid in soft-tissue reconstructions of the injured ECM of the SC, through filling cavities arising from the injury.[97–105] To accomplish this, such bio-scaffolds have been successfully engineered. They exhibit tubular-like structures, besides containing some components of the ECM, including hyaluronic acid[106] and fibronectin.[107]

3D-bioprinting has recently emerged as a potential technique with numerous therapeutic applications, including neural tissue engineering.[108] Alginate has been used in the search of bioinks for 3D-bioprinting applications.[85] On this point, soft ALG hydrogels have proved to be efficient as a bioink for 3D bioprinting experiments to provide an appropriate scaffold to accommodate neuronal cells. These uses are favored by their biocompatible and biodegradable properties in contact with nerve tissues. To achieve this, different viscosities of ALG were tested and mixed with methylcellulose (MC) in a 1:3 volume ratio (ALG:MC) to find the most suitable ink composition for printing.[86] This strategy allowed a microenvironment to be created by preparing a 3D SC-based platform containing neuronal and oligodendrocyte progenitor cells. Interestingly, both progenitor cells were able to proliferate precisely, showing the usefulness of this platform in modeling CNS tissue architectures. Besides MC, other additives like fibrin,[109] chitosan, or genipin[110] have been also tested to produce useful bioinks and 3D-bioscaffolds for neural tissue engineering.[111]

2.2. Axon Growth

In the past century, Aguayo and colleagues demonstrated that SC axons can regenerate if provided with the appropriate substrate.[112,113] Since then, many laboratories have explored the possibilities of biomaterials to build up scaffolds that support and promote axonal growth across the injury region. Biomaterials should have thin walls with appropriate diameters, capable of promoting axon growth and orientation in the SC.[22,42] The generation of cross-linked ALG-based capillaries has facilitated preparing materials with various channel diameters (11, 13, 29, and 89 μm), according to the divalent cation used in vitro (Ba^{2+}, Ca^{2+}, Sr^{2+}, and Zn^{2+}, respectively). In this regard, Pawar and coworkers showed that axon density growth within hydrogel strongly depended on the channel diameter, which ranged from 10 to 100 μm, whereas a decrease in the linear orientation of such axons was obtained with increasing channel diameter.[114]

Not only unmodified anisotropic scaffolds have been used as promising implants to be integrated into the SC without detecting any inflammatory responses. Recently, alternative attempts involving the modification of anisotropic ALG capillary surfaces with cationic polymers (poly-L-ornithine, PLO) and laminin have resulted in enhanced axonal growth and cellular adhesion, after 2 weeks of incubation in vitro.[77] These positive responses were replicated in adult rats after implanting this PLO-laminin-ALG hydrogel scaffold, which promoted cell migration and slight axon growth throughout the capillary channels. Notably, the authors observed that neurite growth was significantly improved in the presence of cationic peptides when additional astrocytes were first seeded within the material before grafting.

A combined therapy involving the transplantation of ALG capillary hydrogels containing Schwann cells followed by the injection of a neurotrophic factor (BDNF) as a supplementary growth stimulus facilitated the regeneration of axons at the lesion site. Injection into the caudal spinal parenchyma led to significant regeneration, compared to transplants that lacked BDNF (**Figure 1**).[78] The positive effect promoted by BDNF after SCI was also observed by Novikov et al., when used a biodegradable synthetic implant made up of fibers containing poly-β-hydroxybutyrate (PHB).[79] These PHB fibers were coated with neonatal Schwann cells as well as a combination of ALG and fibronectin. The resultant 2–3 mm long matrix was evaluated in vivo and surprisingly showed neuronal survival and axonal regeneration after its implantation in the lesion site.

2.3. Cell-Based Therapy

Embryonic, pluripotent, neural, and mesenchymal stem cells as well as Schwann cells, neurons, and other neural cells have been transplanted into specific damaged areas to replace lost neural cells, protect the surviving ones, and facilitate regenerative processes.[31,115] However this strategy, though promising, faces the risk of poor cell survival after transplantation.[116]

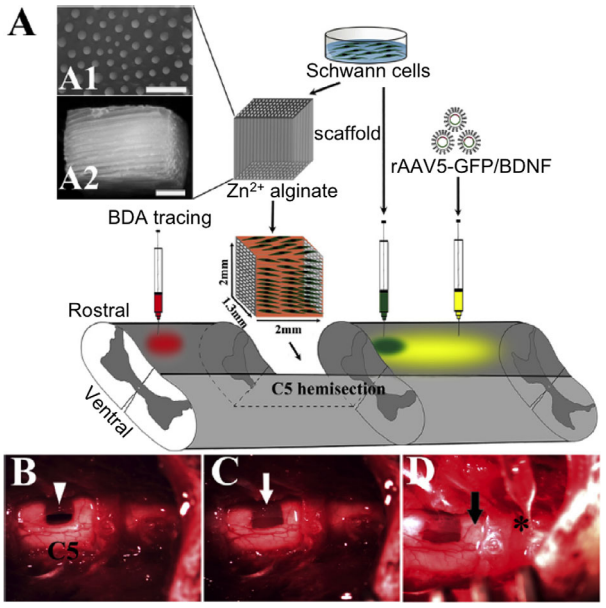

Figure 1. Lesion paradigm and experimental procedures. A) Schematic diagram of the experimental design. A 1.5–2 mm long segment of the SC was removed unilaterally at the C5 level before implanting a Schwann cells (SCs)-seeded alginate scaffold. Subsequently, viral vectors (yellow) for the regulatable expression of green fluorescent protein (GFP) (rAAV5- GFP) or BDNF (rAAV5-BDNF) were injected into the caudal SC, ipsilateral to the lesion. SCs (blue) were also injected into the caudal SC in one group. Biotinylated dextran amine (BDA, red) was injected in the SC rostral to the lesion to trace descending axons 3 or 7 weeks post-lesion. A1) Cross-sectional and A2) longitudinal view of the capillary lumen. B) After a hemisection lesion (arrowhead, ≈2 mm in length) of the rat SC, C) the alginate scaffold loaded with SCs (white arrow) was grafted into the lesion and D) virus (*) or SCs (black arrow) were injected into the SC caudal to the scaffold, using a glass capillary. Scale bar: 300 µm in (A1); 500 µm in (A2). Reproduced with permission[78]. Copyright 2017, Elsevier.

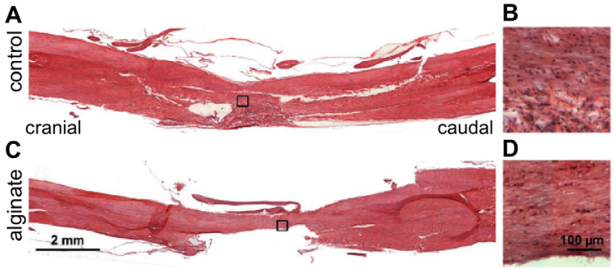

Figure 2. Alginate reduces fibrous scarring. Examples of hematoxylin & eosin (H&E) stained tissue sections 140 days after SCI of 2 mm size. A) Overview of a cross section of control SC. B) Magnification of the area indicated by the black box showing the fibrotic scar and adjacent SC tissue. C) Overview of a cross section of SC that received an alginate implant. D) Magnification of the area indicated in (C) by the black box illustrating the lack of a fibrous scar. Reproduced with permission[80]. Copyright 2018, Springer Nature.

In this regard, ALG has proved to be efficient as a soft hydrogel material capable of adhering to and protecting neurons when acting as an implant. Furthermore, such ALG implants have also favored functional recovery, particularly in the locomotor system after SCI[80] (**Figure 2**), including the outgrowth of axons in the presence of some divalent cations (e.g., Ca^{2+}, Ba^{2+}, or Sr^{2+}).[117,118]

Unfortunately, poor adhesion properties have also been reported when Schwann cells are seeded onto the above-mentioned ALG scaffolds.[81,119] To avoid this limitation and achieve superior adhesiveness, both the preparation of ionically ultrasoft cross-linked ALG hydrogels[82] and modifying the ALG surface with a argininylglycylaspartic acid tripeptide (RGD) peptide[81] led to cell proliferation and facilitated Schwann cell adhesion in 3D cell cultures.

Alginate scaffolds have been also used as an appropriate depot for bone-marrow stromal cells (BMSCs). These cells were embedded into a 2 mm-long capillary hydrogel and finally implanted to evaluate the release of neurotrophic factors (BDNF).[83] Data confirmed ALG was able to integrate into the SC lesion without toxicity, favoring axonal regeneration. Encouragingly, survival of BMSCs and proliferation of Schwann cells and blood vessels were observed when compared to ALG-based biomaterials containing only BMSCs.

Recent studies carried out by Wen et al. showed the feasibility of modifying ALG hydrogels with an integrin ligand ($\alpha 3\beta 1$). This 3D culture system proved to be efficient in favoring the encapsulation and differentiation of neural progenitor cells (NPCs) in vitro, after a 3-month incubation.[120] These authors used surface plasmon resonance (SPR) to measure the specific interactions between the integrin ligands and the NPCs. This 3D platform also enabled differentiation of both mouse and human NPCs into the distinct neural cell types, after being entrapped within ALG hydrogel.

2.4. Drug Delivery

Available evidence has demonstrated that drugs and bioactive molecules, such as nucleotides and proteins, can help repair the damaged SC, promoting neurogenesis, plasticity, or regeneration.[121–126] Hydrogels have been employed to deliver many of these molecules to target tissues and cells under precise conditions. For example, interesting strategies have been proposed to achieve controlled release of rolipram—a blood-brain barrier permeable neuroprotectant with significant effects on functional recovery after SCI[127]—and increase its therapeutic effects at the action site. In this direction, Downing and coworkers prepared series of ALG microfibrous patches for the continuous delivery of rolipram in vivo, achieving good therapeutic results (**Figure 3**).[84] Improvements in the functional recovery of motor function after injury were observed (open-field locomotion, forelimb articular movement, and animal coordination) when small doses of rolipram were released (≈60 µg cm^{-2}) over 12 days through the material. In contrast, when authors used high-doses of rolipram under regular administration conditions, a pronounced decline in animal survival rates (up to 50%) was observed.

3. Conclusions and Future Perspectives

The use of ALG-based hydrogels faces a good number of challenges but also opportunities for tissue repair and regenerative

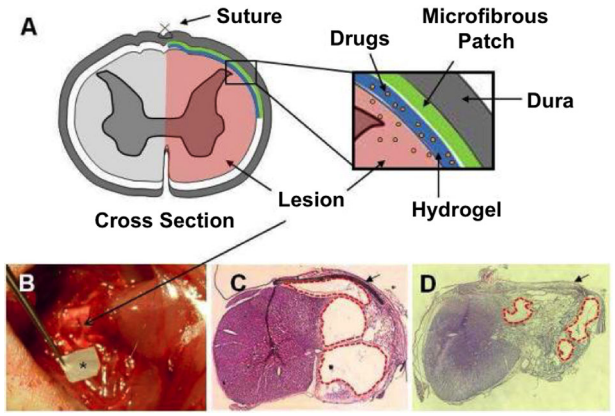

Figure 3. Implantation of drug-eluting microfibrous patches after SCI. A) Schematic of subdural implantation of drug-eluting microfibrous patches into the injured cord. B) Macroscopic view of lesion site and patch during animal surgery. An asterisk was used to mark location of drug-eluting microfibrous patch. C,D) Gross histology of SC cross section 8 weeks post SCI. H&E staining reveals new tissue formation at the lesion site and patch's ability to integrate into the surrounding tissues (D). C) Less regenerated control for comparison. Here, arrows point to the implantation site for drug-eluting microfibrous patches. Red dashes outline areas of less tissue formation for comparison. Reproduced with permission[84]. Copyright 2012, Elsevier.

medicine. The structure, physical properties, and specific functions of hydrogels may vary when tuning these materials. Beneficial properties such as biocompatibility, long-term stability in vivo, or their suitability to be chemically modified with appropriate ligands, make these materials promising scaffolds for embedding drugs, nerve cells, growth factors, etc.

The use of ALG-based hydrogels in regenerative medicine has grown exponentially in the last decade, achieving significant and promising results as shown by a good number of in vivo models. However, the translational step from the bench with new designs of materials for the injured CNS in the field of tissue engineering to implementing an effective treatment in humans is still a daunting challenge. Promotion of axon regeneration and neural cell replacement after SCI by means of ALG and other biomaterials has been addressed through a variety of strategies, for instance embedding and subsequent cell release, the use of neurotrophic factors, and small drug molecules.

In a plethora of in vitro and in vivo models, the development of these combined strategies has shown greater therapeutic effectiveness than those involving single treatments.[128] The design and development of new biodegradable materials capable of entrapping stem cells and/or therapeutic drugs could enhance this combinatorial approach, providing maximal functional recovery and the highest chance of success in the clinic. Regrettably, a full understanding of biomaterial properties as well as choosing the right therapeutic combination (e.g., stem cells, neurotrophic factors, or small drugs) requires great investigation in depth, so as to improve the relevant translational research.

In addition to using cell therapy and biomaterials for treating SCI, tissue engineering offers alternative technologies like 3D-bioprinting and microfluidic devices. These are emerging as potential applications for replacing tissues and creating disease models aimed at developing further new therapies and also understanding more precisely the behavior of the disorder.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

alginate, biomaterial, bioprinting, central nervous system, neural tissue engineering, regenerative medicine, spinal cord injury

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