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Regenerative medicine for the treatment of spinal cord injury: more than just promises?

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Abstract

Spinal cord injury triggers a complex set of events that lead to tissue healing without the restoration of normal function due to the poor regenerative capacity of the spinal cord. Nevertheless, current knowledge about the intrinsic regenerative ability of central nervous system axons, when in a supportive environment, has made the prospect of treating spinal cord injury a reality. Among the range of strategies under investigation, cell-based therapies offer the most promising results, due to the multifactorial roles that these cells can fulfil. However, the best cell source is still a matter of debate, as are clinical issues that include the optimal cell dose as well as the timing and route of administration. In this context, the role of biomaterials is gaining importance. These can not only act as vehicles for the administered cells but also, in the case of chronic lesions, can be used to fill the permanent cyst, thus creating a more favourable and conducive environment for axonal regeneration in addition to serving as local delivery systems of therapeutic agents to improve the regenerative milieu. Some of the candidate molecules for the future are discussed in view of the knowledge derived from studying the mechanisms that facilitate the intrinsic regenerative capacity of central nervous system neurons. The future challenge for the multidisciplinary teams working in the field is to translate the knowledge acquired in basic research into effective combinatorial therapies to be applied in the clinic.

Keywords: spinal cord injury • cell therapies • stem cells • clinical trials • biomaterials • growth factors • gene therapy • conditioning lesion

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Introduction

Spinal cord injury (SCI) results in severe sensory and motor deficits due to the poor regenerative capacity of the adult spinal cord. After SCI, the inhibitory environment of the lesion, the development of a glial scar and the accumulation of extracellular matrix (ECM) proteoglycans lead to the formation of an impermeable barrier that hinders axons from regenerating across the site of injury [1, 2]. The leading causes of SCI are motor vehicle crashes, falls and sport-related injuries that primarily affect young adults. In addition to paralysis, the loss of sensory and motor functions leads to multiple health problems such as urinary, cardiac and respiratory dysfunction. There are no up-to-date incidence or prevalence studies of SCI, but a recent literature survey points to an incidence ranging from 10.4 to 83 cases per million inhabitants per year (average incidence 29.5) and a prevalence of 223–755 per million inhabitants (average prevalence 485) [3]. This estimate, even if conservative, reveals the vast extent of this problem, with serious repercussions for the patients, their families, caregivers, health systems and society in general. Treatment of the injured spinal cord thus represents a major challenge for regenerative medicine. In the past 10 years tremendous attention has been paid to stem cell-based therapies, motivated in part by the increasing number of clinical studies that are underway to evaluate the safety and efficacy of transplanted cells in patients with SCI.

This review addresses recent advances and achievements in cell-based therapies for the treatment of SCI. It will go beyond a discussion of the potential cell types and sources, focusing as well on the recent contributions of biomaterials to the field and how these may constitute key tools for the development of cutting edge cell-based therapies for neural tissue regeneration.

Aspects of stem cell-based therapy for spinal cord injury

Spinal cord injury evokes a cascade of cellular and biochemical events leading to a series of reactive changes such as inflammation, hemorrhagic necrosis, oedema and demyelination, which together result in cell death (loss of neurons and myelinating oligodendrocytes), vascular destruction, scarring and axonopathy with a loss of functional connections (denervation) below the central lesion site [4, 5] (Fig. 1).

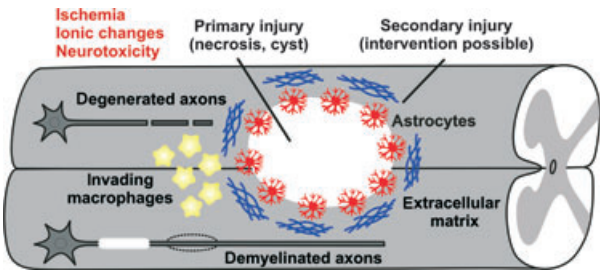

Fig. 1 Schema of acute and chronic events following spinal cord injury.

Therefore, the focus of treatment should be directed towards various goals such as reducing excitotoxicity and the inflammatory response or attenuating the inhibitors of axonal growth, such as myelin-associated proteins or chondroitin sulphate proteoglycans [6–9]. The diversity of aspects of the SCI pathology require combined therapies aimed at targeting various factors that may act synergistically to further enhance the extent of spinal tissue regeneration. In this respect it is important to realize that the potential of cell-based therapy for SCI may also fulfil broader multifactorial roles due to the cells' capacity to do the following:

(i) *Replace missing-lost cellular elements*, not only neurons but also supporting cells, particularly the oligodendrocytes that form myelin sheaths around axons.
(ii) *Deliver trophic factors.* The effect of cell therapy is mediated by the secretion of growth factors or cytokines that reduce neuronal apoptosis and inflammation and that also stimulate endogenous regenerative processes, remyelination, as well as neural plasticity, leading to the collateral sprouting of intact or damaged axons of the descending pathways [10].

A wide range of cell types has been investigated for cell therapy in SCI, including embryonic stem cells (ESCs) induced towards the neural lineage, neural stem cells (NSCs), adult stem cells such as mesenchymal stem cells (MSCs), umbilical cord blood stem cells, macrophages, Schwann cells, olfactory ensheathing cells (OECs) and oligodendrocyte progenitor cells, among others [11]. The selection of appropriate cell types is thus a key factor that determines the therapeutic effectiveness of specific approaches to cell transplantation. According to the recent literature, as well as our own results, it is well documented that transplanted cells of non-neural origin, such as MSCs, umbilical blood cord cells or macrophages, may facilitate functional recovery, most probably by indirect mechanisms by which they provide trophic support, modulate the early inflammatory response, improve vascularization, provide a permissive growth substrate and/or suppress cavity formation at the lesion site after a SCI [12, 13]. On the other hand, the transplantation of NSCs or ESCs neurally induced, which are able to give rise to all types of neural tissue, has resulted in very limited numbers of surviving, fully differentiated neurons, so only a few reports have suggested that they may contribute to the observed functional recovery [14]. Therefore, it is more likely that their beneficial effect may be mediated, similarly as in the case of non-neural grafts, through the release of growth/trophic factors by the transplanted cells, thus sustaining the survival of endogenous cells or supporting axonal sprouting [15].

A significant component of SCI pathology is the loss of oligodendrocytes and the resultant demyelination, leading to the progressive and delayed degeneration of residual axonal tracts. The transplantation of oligodendroglial progenitors (derived from ESCs, neural progenitors or induced pluripotent stem cells) or the recruitment and stimulation of endogenous oligodendroglial progenitors are considered to be promising approaches to rescuing the remaining axons. There is convincing evidence indicating that endogenous sources of neural stem cells can be mobilized from different regions to replace the lost/impaired neurons in neurodegenerative diseases or CNS injuries. In regard to endogenous regenerative mechanisms, it has been shown that pathological as well as physiological stimulation

(e.g., increased physical activity) can promote the proliferation of endogenous ependymal stem cells and thus increase the number of endogenous stem cells that could be used to restore or replace damaged or lost neural cells [16, 17] (Fig. 2).

Criteria for cell-based therapies for spinal cord injury

During the past two decades the methods of stem cell-based therapy have been gradually improved in terms of new approaches that combine stem cells with biocompatible materials or gene therapies capable of introducing/releasing trophic-growth supporting factors that are crucial for the stimulation of nerve tissue regeneration following spinal cord injuries [18–21]. Importantly, these pre-clinical trials and experiments (see reference [22] for a recent review) have demonstrated that the effectiveness of cell-based therapy for SCI depends on several factors:

(i) Selection of the best source of stem cells (adult, embryonic, foetal, induced pluripotent stem cells);
(ii) characterization and expansion of stem cells in an attempt to achieve the desired amount of well defined stem cells for further transplantation;
(iii) development of minimally invasive but highly effective delivery strategies (systemic, local transplantation/intraspinal, intrathecal);
(iv) optimal dosing of stem cells (single dose, continuous administration of stem cells during several days);
(v) proper timing of cell transplantation (during the acute or chronic phase of disease);
(vi) reduction of the risks of stem cell treatment, thus fulfilling safety and regulatory considerations;
(vii) survival of transplanted cells in the host;
(viii) efficacy of the transplants, ability to restore function of specific types of damaged or lost cells.

An important factor in stem cell selection for transplantation is their compatibility with the host tissue. Therefore, in human clinical trials the most preferred stem cells are considered to be those that may be derived from the patients' own tissues. The autologous transplantation of stem cells obtained from a patient's bone marrow is not only commonly used, for example, in the treatment of haematopoietic disorders or to repair bone and cartilage defects, but also for the treatment of myocardial tissue or traumatic SCI [23]. Furthermore, some studies have utilized other autologous sources of stem cells such as Schwann cells derived from peripheral nerves or OECs [24, 25] from the olfactory bulb or mucosa, and grafted them into rodents

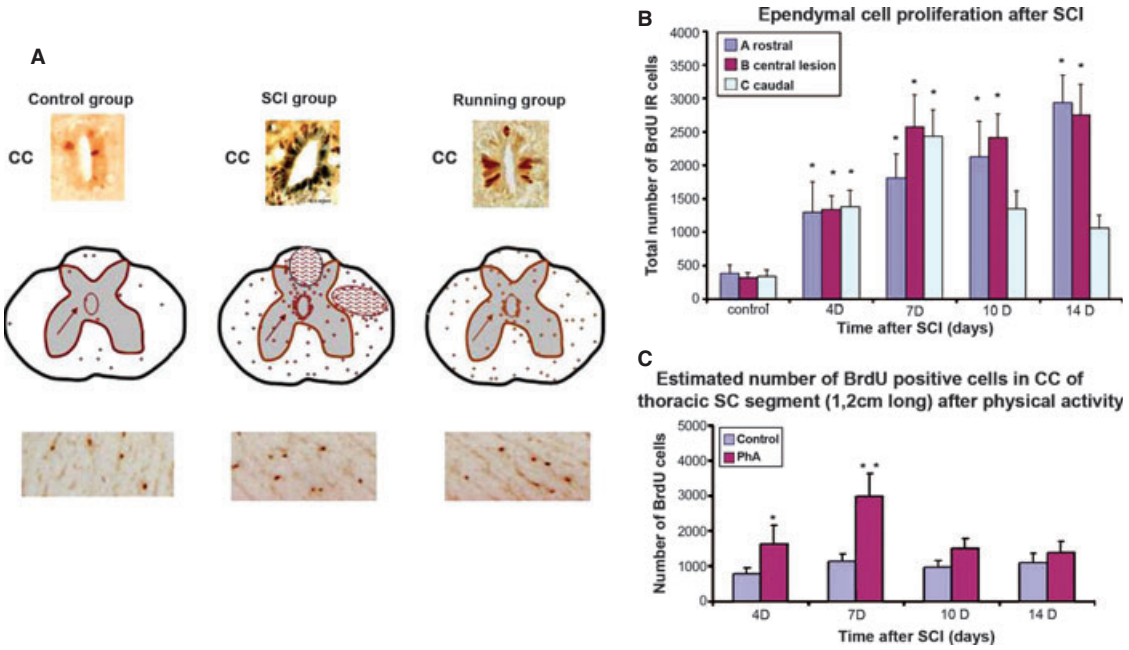

Fig. 2 Increased proliferation of endogenous stem cells in the spinal cord after SCI or after increased physical activity in comparison to control. (A) Schematic illustration of BrdU staining in thoracic spinal cord sections (Th8) of control, SCI and physical activity (running) groups. Note, the highest BrdU expression in the central canal (CC), parenchyma and around the lesion site in the SCI and running groups. Below each schematic drawing is a panel showing BrdU staining in the corresponding ventral white matter. (B) Quantitative analysis of the BrdU-positive nuclei in the ependyma of the thoracic spinal cord segments Th7-9 after SCI and (C) physical activity, revealing that the highest concentration of BrdU-labeled cells was restricted to the ependymal zone of the CC at 7–14 days after SCI and at 4–7 days after physical activity, ($*P < 0.1$ and $**P < 0.05$, Student's *t*-test). Modified from [16], Copyright (2009), with permission from Elsevier.

after SCI. However, in most human clinical trials it is still more common to transplant allogeneic stem cells that have to meet the established criteria of compatibility (ABO, HLA), but even then these patients have to undergo immunosuppressive therapy, which brings side effects and disadvantages.

In an attempt to find new, additional sources of immunologically compatible adult stem cells with minimal risks of rejection following cell grafting, a very powerful tool has been developed, namely induced pluripotent stem cells (iPSC) [26, 27]. This new therapeutic approach can re-programme fully differentiated somatic cells, for example fibroblasts, back to an embryonic stem-like state by forcing the cells to express genes and factors important for maintaining the properties of pluripotent stem cells. However, a recent study indicates that iPSC isolated from mouse fibroblasts can induce a T cell-dependent immune response in syngeneic recipients [28]. This means that the immunogenicity of iPSCs should be carefully evaluated before any clinical trials are performed.

Sources of stem cells for spinal cord injury repair

In the next few paragraphs the main sources of stem cells being investigated both in pre-clinical and clinical studies will be presented and the main outcomes of these studies discussed (see Table 1 for an overview).

Embryonic stem cells

ESCs, which are derived from the inner cell mass of blastocyst-stage embryos and which are characterized by pluripotency and unlimited propagation, represent one of the most promising candidates for a wide range of cell replacement therapies [29]. With proper culture protocols, ESCs maintain a normal karyotype, not undergoing mitochondrial or epigenetic changes [30]. Nevertheless, chromosomal instability of human ECSs (hESCs) in culture due to centrosomal amplification has been recently demonstrated [31].

In vitro as well as *in vivo* studies involving mouse ESCs have served as the basic stem cell culture model that could be adopted for future strategies using hESCs. Pioneering studies using ESC transplantation into various animal SCI models, confirmed in most cases the ability of these cells to promote recovery through remyelination mechanisms or by providing trophic support [32, 33]. Similarly to rodent-murine ESCs that can be driven towards a neuronal [34] or glial fate [32], hESCs may also generate multipotent neural precursors [35] or desired cell types such as motor neurons [36] or oligodendroglial progenitors [37]. Moreover, it has been shown that the transplantation of purified hESC-derived neural precursors prevents graft-derived tumour formation [38].

Cumulatively, results from different studies have confirmed that one of the main challenges for cell-based SCI treatment is promoting the differentiation of hESCs into a highly purified population of oligodendroglial progenitors that could remyelinate damaged axons. This strategy has been tested in various pre-clinical experiments in which oligodendroglial progenitors were transplanted into spinal cord injured or myelin-deficient rodents, where they integrated into the spinal cord, differentiated into oligodendrocytes, restored myelination and improved locomotor function [39–41]. Currently, a Phase I clinical trial conducted by the US company Geron Corporation using hESC-derived oligodendroglial progenitor cells (GRNOPC1) is underway in patients with spinal cord injuries. The initial analyses showed a very good safety profile, with no serious adverse events, no evidence of cavitation at the injury site and no immune responses to the transplanted cells even after the complete withdrawal of immune-suppression. Recently, Geron Corporation has announced that due to changes in their strategic development plans it will "discontinue further development of its stem cell programs" (Geron News Release, CA, US, November 14, 2011). With the closing of the GRNOPC1 trial for SCI to further enrolment, despite the fact that Geron will continue to follow all enrolled patients, hESC therapies for SCI will probably need some more time to be reintroduced back into clinical testing.

Neural stem cells

NSCs are defined as multipotent, self-renewing stem cells found in both embryonic and adult tissue. They are mostly derived from rat or mice embryonic tissue, maintaining some capacity for self-renewal, and generating differentiated neurogenic and gliogenic progeny that can functionally integrate and repair damaged nerve tissue, if grafted into the neurogenic areas of the CNS [42–44]. However, data from initial studies have showed that NSCs transplanted into SCI differentiate mainly into astrocytes, with limited potential to generate neurons and oligodendroglia *in vivo* [45]. Therefore, new *in vitro* cell culture techniques and protocols had to be developed, that would allow one to direct NSCs into neuronal or oligodenroglial progenitors.

Unlike ESCs, NSCs are already committed to a neural phenotype, which gives the advantage of making them easier to differentiate into the desired pro-oligodendroglial cells that could be used for further transplantation applications. One of the ways to achieve this is to engineer NSCs to express noggin, a bone morphogenetic protein (BMP) agonist that stimulates their differentiation towards a population lacking astrocytes. Thus, after transplantation into a SCI, they give rise to an increased number of mature neurons and oligodendrocytes while promoting an increase in locomotor recovery [46].

Another useful strategy to achieve a highly purified oligodendroglial population from a heterogeneous population of spinal NSCs is based on magnetic cell sorting (MACs) technology, involving specific antibodies attached to nanoparticles together with a cocktail of cultivation media [47] (Fig. 3).

Although it seems that NSCs are a powerful source of neural progenitors that constitutively secrete a variety of growth stimulating factors such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF), they are often genetically modified to further enhance their potential to secrete additional factors such as neurotrophin 3 (NT-3) [48], or are combined with antibodies that neutralize ciliary neurothrophic factor (CNTF) in an attempt to attenuate astrocytic differentiation [49].

Table 1 Overview of the main stem cell types currently being investigated in both pre-clinical and clinical studies for the treatment of spinal cord injuries

Type of trial	Cell type	SCI treatment approach	Main results	References
Pre-Clinical	ESCs	Human ESC-derived oligodendrocyte progenitor cells (OPCs), mice ESC in rodent model of acute, subacute, chronic SCI and SCI-myelin-deficient shiverer (shi/shi) mutant mice, SCI rat-chemical demyelination model.	Implanted cells differentiated into oligodendrocytes; improved remyelination of host axons; functional improvement.	[32, 37, 39–41]
	NSCs	Mice, foetal neural precursor cells engineered to express BMP inhibitor, rat neural precursor cells neutralizating ciliary neurotrophic factor (CNTF), human foetal NSCs (neurospheres) in rodent model of acute, subacute, chronic SCI and SCI - myelin-deficient shiverer (shi/shi) mutant mice, spinal cord-injured NOD-scid mice, SCI-ischaemia.	Implanted cells differentiated into neurons (cholinergic, GABA-ergic), oligodendrocytes, low astrocytes; increased number of regenerated CST fibres both at the lesion and at caudal sites; improved remyelination, trunk stability, suppression of spasticity and rigidity; functional improvement.	[14, 46, 49–51, 54]
	SCs	Autologous, syngeneic, allogeneic or xenogeneic transplantation of adult, new-born, skin-derived, bone-marrow derived or SC precursors in rodent model of acute, subacute and chronic SCI. Combinatorial therapies.	Improved axonal regeneration and remyelination; functional improvement; limited integration of SC into the host tissue.	[22, 59–62]
	OECs	Allogeneic OEC implantation, and chondroitinase addition, olfactory-ensheathing glia grafts and SC bridges with chondroitinase in rodent models of acute, subacute and chronic SCI. Combinatorial therapies.	Improved axonal regeneration and remyelination; increased serotonergic axons in the bridge and beyond; significant correlation with functional improvement.	[62, 69]
	MSCs	Autologous, syngeneic, allogeneic or xenogeneic MSC transplantation in rodent, large animals and primate models of acute, subacute and chronic SCI. Combinatorial therapies.	Reduced lesion volume, anti-apoptotic effect, enhanced axonal regeneration and remyelination; functional improvement.	[13, 18, 22, 72, 73]
Clinical	ESCs	Human ESC-derived oligodendrocyte progenitor cells (OPCs) GRNOPC1 in complete T3-T9 subacute SCI.	Safety of the therapy. No adverse or beneficial effect.	Geron News Release, CA, US, November 14, 2011
	NSCs	Human foetal derived neural progenitor (HuCNS-SC) in SCI Injury. Phase I/II.	Safety of the therapy. No adverse or beneficial effect.	[55]
	SCs	Autologous intramedullar SC transplantation into chronic SCI.	Safety of the therapy. No adverse or beneficial effect.	[168]
	OECs	Autologous olfactory ensheathing cell transplantation in human SCI: a pilot clinical study; a Phase I and a 3-year clinical trial (Phase I/IIa).	Feasibility and safety.	[66–68]
	MSCs	Autologous bone marrow mononuclear cells or *ex vivo* expanded autologous MSCs transplantation. Combination with granulocyte–macrophage colony-stimulating factor. Phase I/II.	Safety of the therapy. Modest functional improvement in acute and sub-acute patients, mild improvement in chronic patients.	[12, 78–84]

ESCs – embryonic stem cells; NSCs – neural stem cells; SCs – Schwann cells; OECs – olfactory ensheathing cells and MSCs – mesenchymal stem cells.

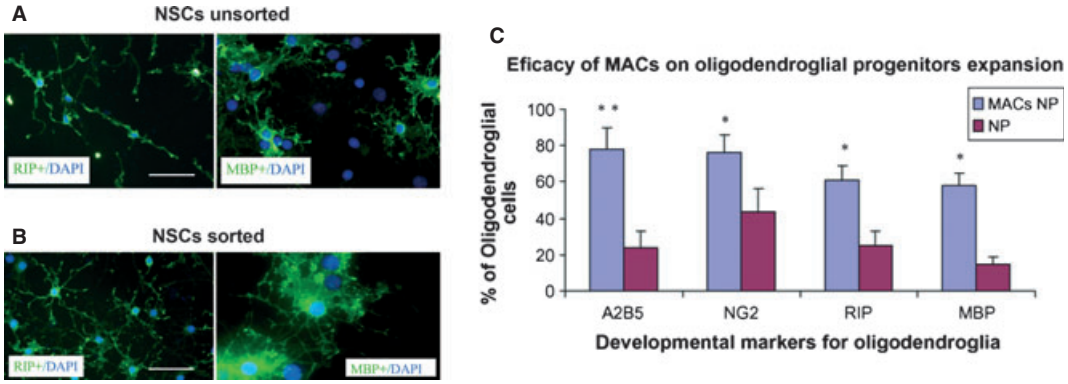

Fig. 3 MAC enrichment of rat oligodendroglial progeny from spinal cord-derived neural stem cells (NSCs). *In vitro* differentiation to oligodendrocytes (RIP staining) at day 14 and staining for myelin basic protein (MBP) of mature oligogodendrocytes at day 21 in unsorted NSCs (A) and MAC-sorted NSCs (B). Scale bars: left hand side images = 100 μm; right hand side images = 50 μm. (C) Comparison of oligodendroglial lineage development in MAC-sorted (blue bars) and unsorted spinal NSCs (purple bars). Modified from [47], Copyright (2009), with permission from Elsevier.

Several studies have investigated the potential of NSCs harvested from human tissue to promote functional recovery in various pre-clinical animal models of SCI [33, 50]. For example, NSCs derived from human foetal brain improved recovery after a contusion SCI in both severe combined immunodeficiency (SCID) and myelin–deficient shiverer mice [51]. Another study showed that neuronal precursors (hNT) isolated from the human teratocarcinoma cell-line or rat spinal neuronal precursors (SNPs) grafted into ischaemic spinal segments depleted of inhibitory neurons, restore local inhibitory tone and ameliorate spasticity [52]. In addition, when human-derived NSCs were treated with a cocktail of growth factors and subsequently transplanted into the injured spinal cord, they differentiated preferentially into cholinergic neurons [53]. Recent data using human foetal NSCs show that after transplantation into a SCI, these cells survive in the lesion, differentiate into motoneurons and improve motor as well as sensory function [54].

An additional study has shown that NSCs derived from human foetal spinal cord and grafted into a rat model of ischaemic spastic paraplegia resulted in the progressive recovery of motor function with a concurrent improvement in motor evoked potentials [14]. The functional recovery was associated with the long-term survival of the grafted neurons, neuronal differentiation and the development of a GABAergic phenotype in a sub-population of grafted cells that most probably contributed to the suppression of spasticity (Fig. 4).

In 2011, the Stem Cells Inc. has initiated a first Phase I/II clinical trial (12 patients) using human foetal derived neural progenitor (HuCNS-SC) cells for the treatment of SCI (Stem Cells Inc. News Release, CA, US, March 14, 2011). According to their pre-clinical studies, these HuCNS-SC cells could be directly transplanted in the CNS showing long–term survival and no sign of tumour formation or adverse effects [55].

On the other hand, it has been reported that transplantation of ESCs or naive NSCs derived from adult rat spinal cords may cause aberrant host fibre sprouting associated with development of allodynia-like hypersensitivity or neuropathic pain [56]. Therefore, in an attempt to reduce these pain behaviours following injury, the transplantation of pre-differentiated ESC cells, serotonergic or GABAergic neural precursor cells has been successfully used in various pre-clinical animal studies [57].

Schwann cells

Schwann cells are the myelinating cells of the peripheral nervous system (PNS) that sustain peripheral axon regeneration, but which may also support CNS axon regeneration [58]. These cells can be easily isolated from the peripheral nerve and expanded *in vitro*, representing a valuable source of autografts for spinal cord repair. The remyelinating capacity of Schwann cells has been demonstrated in a number of different animal models of SCI that presented conduction impulses by regenerated axons [59–62]. For example, after transection of the spinal cord and grafting of Schwann cells, damaged axons can extend into the grafts and even become myelinated, but they are unable to leave the grafts distally and re-innervate the caudally located target tissue. Similarly, after a contusion injury, transplanted Schwann cells reduced the extent of cavitation, and the spinal axons that were growing into the graft were mostly remyelinated [58]. The mechanisms by which Schwann cells promote axonal regeneration may involve the secretion of various trophic factors, such as NGF, fibroblast growth factor-2 (FGF-2), BDNF or NT-3 [63]. Although many studies have demonstrated the beneficial effects of Schwann cells on spinal cord repair, they show very limited migration from the grafted site and an inability to intermingle with host astrocytes. This negative Schwann cell–astrocyte interaction is probably mediated by ephrin-related mechanisms *via* VAV signalling affecting integrin function [64].

Olfactory ensheathing cells

OECs are specialized glial cells surrounding olfactory sensory neurons. Numerous pre-clinical studies have demonstrated that when transplanted into the spinal cord, OECs, similarly as Schwann cells,

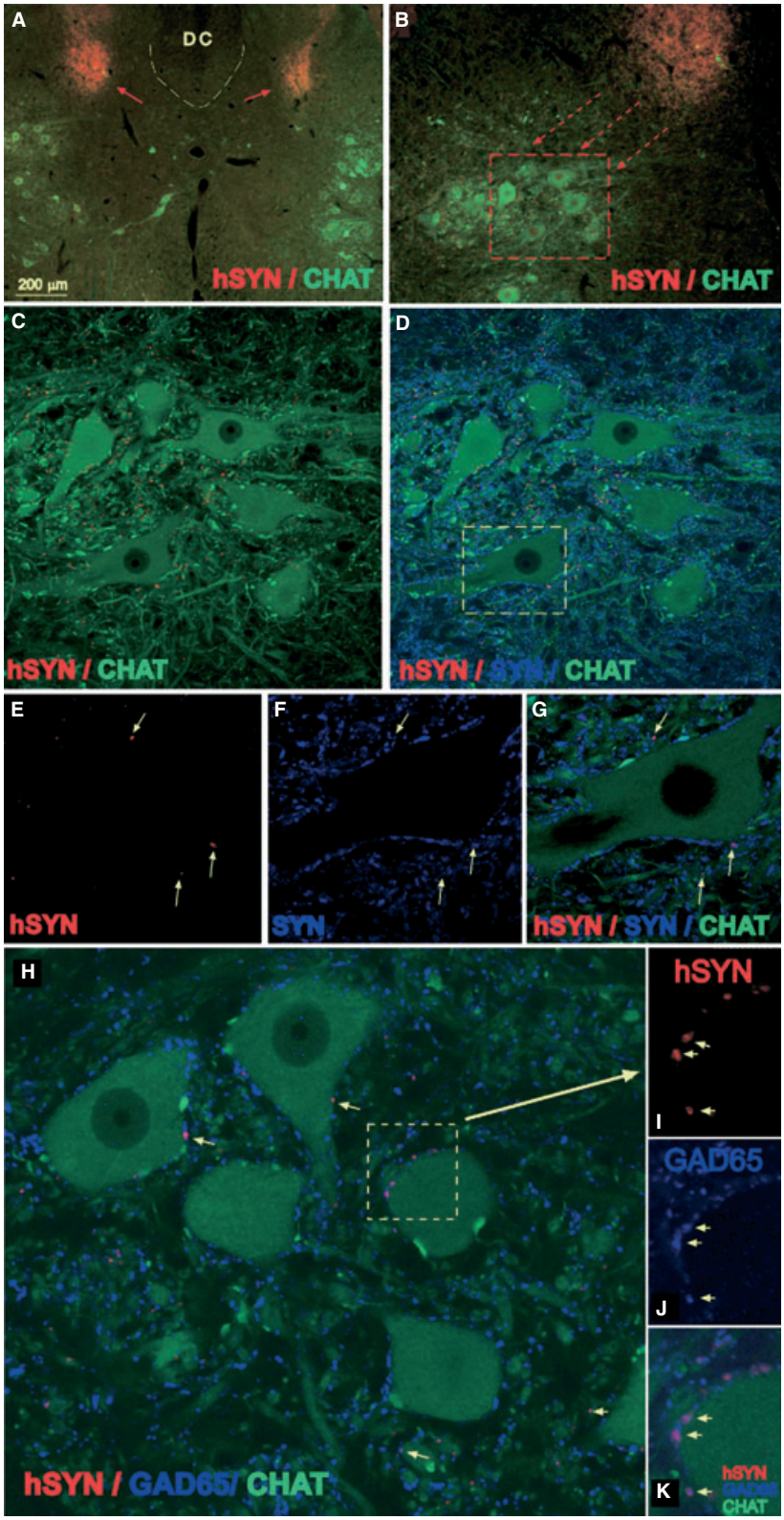

Fig. 4 Survival of grafted human stem cells identified by hSYN immunoreactivity with a dense population of inhibitory terminals (hSYN/GAD65 immunoreactive) in the vicinity of persisting α-motoneurons. (A–G) Fluorescent microscopy images (A, B) and projected confocal images (C, D) of transverse spinal cord sections taken at 3 months after grafting and stained with human-specific SYN antibody (red), CHAT antibody (green) and SYN antibody that cross-reacts with both human and rat SYN (blue). Intense hSYN staining was found within the two bilateral grafts (A; red arrows). Numerous hSYN-stained terminals were localized in the base of the dorsal horn and extending into ventral-motoneuron pools (B, C; red). (E–G) Single optical images showing the colocalization of hSYN and SYN IR in the vicinity of CHATα-motoneurons (yellow arrows). (H-K) The majority of hSYN terminals (red) co-localized with GAD65 (blue), purple dots. Modified from [14], Copyright (2007), with permission from Elsevier.

myelinate the axons in the region of injury, secrete growth factors and enhance functional recovery [65]. As an autologous cell source, OECs can be isolated *via* biopsy from the olfactory mucosa, and have already moved into human clinical testing being currently in Phase I/IIa trials to test the feasibility and safety of their transplantation [66–68]. In addition, combined therapies using Schwann cells with the simultaneous delivery of neurotrophins, or OECs and chondroitinase, enhanced the regeneration of axons capable of exiting the grafted site [69]. On the other hand, the co-transplantation of OECs and MSCs into SCI resulted in functional improvement, but did not show synergistic effects [70].

Mesenchymal stem cell transplantation and a clinical study

MSCs currently represent the most promising cell source for clinical transplantation [71]. They are present in adult tissue, primarily in the bone marrow, but they can also be found in fat, skin, liver, peripheral blood and umbilical cord, among other tissues. These cells are easy to isolate and expand for autologous application. As multipotent cells, MSCs can differentiate into cells of mesenchymal origin, and controversy still exists as to their capacity to be pluripotent i.e., to differentiate into non-mesenchymal cell types.

Many studies in animal models of SCI have documented that transplanted MSCs promote remyelination, reduce lesion volume and induce functional improvement [13, 18, 72–74]. However, despite various reports suggesting the trans-differentiation of MSCs into cells of neuronal lineages *in vitro* [75], it is still questionable if MSCs can give rise to functional neurons *in vivo* [76]. The therapeutic effect of MSC transplantation in the neural tissue is generally thought to be due to their ability to produce a variety of anti-apoptotic and neurotrophic factors [77]. In addition, not only the transplantation of expanded MSCs but also the transplantation of bone marrow mononuclear cells or bone marrow cells mobilized by granulocyte colony-stimulating factor results in an increase in the extent of spared white matter, smaller cavities and behavioural improvement in a rat model of SCI [74].

In clinical applications, the administration of autologous bone marrow mononuclear cells (BMC) into SCI patients has been most commonly used [12, 78–82]. In a Phase I/II clinical study, the autologous transplantation of BMC was combined with granulocyte–macrophage colony-stimulating factor. This therapy was found to be safe and led to modest functional improvement in acute and sub-acute but not in chronic patients [80]. Pilot clinical studies have been also reported using the transplantation of *ex vivo* expanded autologous MSCs [83, 84].

Autologous BMC implantation was therefore used in an ongoing Phase I/II clinical trial in patients with an acute or chronic SCI at the cervical or thoracic level [12]. Bone marrow was harvested from the iliac bones of 41 patients with a transversal spinal cord lesion under local anaesthesia. Mononuclear cells were separated *via* sedimentation, and about 1.5×10^8 cells were administered *via* intra-arterial catheterization or intravenously. For functional evaluation, the American Spinal Cord Association (ASIA) score was used, together with the measurement of motor and somatosensory evoked potentials and magnetic resonance imaging (MRI). The evaluation of the lesion size using MRI is usually difficult, as patients have been stabilized using a metal implant. Intra-arterial administration *via* catheterization of a vertebralis led to a significant improvement in 90% of the acute patients with cervical injuries (up to 4 weeks after injury); however, mild improvement in the ASIA score was found in chronic patients even 467 days post-injury. Until now, no serious complications associated with the cell therapy have been observed (the first patients were transplanted 7 years ago). Nevertheless, to evaluate the efficacy of this therapy, a larger and more homogeneous group of patients is needed. A future clinical study will be based on the combination of expanded MSCs with the bridging of the spinal cord lesion with an appropriate biomaterial, either a hydrogel or a nanofibre scaffold.

Stem cells and gene therapy

Stem cells also serve as the main tool for gene therapy because their genetic modification can drive them to produce several trophic factors that are essential for the regeneration of injured nerve tissue [63]. Because the exogenous application of several neurotrophic factors has been shown to have serious functional limitations due to their fast degradation, this may be overcome with the development of non-toxic, non-immunogenic viral vectors with long-term transgene expression. This "new age generation" of viral vectors could be either applied into stem cells before transplantation is performed (*ex vivo*) or injected directly *in situ* into the damaged-impaired CNS tissue [21]. In this respect, the adeno-associated viral vectors (AAVs), particularly serotype AAV-2 [85] or AAV-1 and AAV-5 [86], represent one of the most attractive gene delivery systems for targeted gene therapy to the nervous tissue. They are able to efficiently transduce neurons while inducing minimal immune responses in the host brain [85, 87, 88]. Nowadays, it is well established that advances in gene transfer strategies represent a realistic prospect of delivering therapeutic genes to the nervous tissue for neuroprotection, the restoration of function and/or the replacement of deficient proteins in various neurodegenerative disorders [86].

Nevertheless, the use of the AAVs in pre-clinical animal models has also shown some drawbacks of these systems, such as viral spread from the injection site to adjacent tissue, or an increased level and prolonged duration of transgene expression, which are often difficult to control and may lead to unwanted side effects [21]. Thus, in view of a clinical application there is a need to emphasize the relevance of inducible and regulatable transgene expression of AAVs. Furthermore, previous or ongoing clinical trials have also identified additional problems with the presence of neutralizing antibodies to the AAV vector, as well as the loss or low expression of the transgene over time that were not observed in animal models. Therefore, discrepancies found between pre-clinical and clinical trials indicate that more research is needed to determine which AAV serotype should be selected to achieve a safe, widespread and controlled transduction of the CNS [89].

Biomaterial-based matrices for spinal cord injury repair

In acute SCI, transplanted cells may either replace dead cells or provide various bioactive factors that promote endogenous regeneration and prevent apoptosis and cavity formation. However, in the case of a chronic spinal cord lesion, when the cystic cavity is already developed, cell transplantation alone is not sufficient to promote tissue regeneration. In these cases tissue repair requires "bridging" the lesion with a matrix that provides a permissive environment, fills the tissue gap and, concomitantly, provides structural support for axonal re-growth and functional reconnection.

The design of a biomaterial must carefully consider parameters such as biocompatibility, mechanical properties that match those of the neural tissue, porosity and permeability and, in addition, the ability to support cell attachment, growth and differentiation.

Biomaterial nature

A number of materials have been proposed for use in spinal cord tissue engineering [20, 90, 91]. These include biodegradable, either natural or synthetic, as well as non-biodegradable polymers.

Biodegradable polymers hold the potential for the ultimate restoration of function and full regeneration of the tissue. To achieve this goal, both the material degradation and the tissue regeneration and maturation rates should match. However, some concern about the potential inflammatory response triggered by the degradation process and by-products still exists within the medical community [92]. Among synthetic biodegradable polymers, aliphatic polyesters, such as poly (lactide), poly (glycolide) and their copolymers, and poly (ε-caprolactone) are the most explored, probably encouraged by their approval by the FDA for medical applications [93–95].

Non-biodegradable synthetic materials, including cross-linked synthetic polymers based on methacrylate hydrogels, such as poly (2-hydroxyethyl methacrylate) (PHEMA) [91, 96] and poly [N-(2-hydroxypropyl) methacrylamide] (PHPMA) [91, 97], are also being studied. Their application in a clinical scenario requires the establishment of their safety in terms of foreign body reaction upon implantation. Interestingly, efforts to use synthetic conducting polymers such as poly (3, 4-ethylenedioxythiophene) (PEDOT) [98] for the preparation of substrates for nerve regeneration opened new possibilities for exploring the enhancement of neural growth through electrical stimulation by the use of materials that combine the properties of polymers with those of electrically conductive materials.

Natural polymers are generally biocompatible, can support cell adhesion and minimize the occurrence of cytotoxic effects. These properties make natural polymers advantageous materials for nerve tissue engineering [99], despite the fact that naturally harvested materials have higher batch-to-batch variability and can, in some cases, induce immunogenic reactions. Naturally derived polymers comprising collagen [100], fibrin [101, 102], hyaluronic acid [103, 104], agarose [105], alginate [106], chitosan [102, 107], fibroin [108] or poly (β-hydroxybutyrate) [109], have been reported in numerous studies to be promising scaffolding materials for the treatment of spinal cord lesions. The use of natural and synthetic composites can combine the biocompatible properties of natural materials and the mechanical strength and tunable degradation rates of synthetic materials [110].

Nevertheless, as most natural and synthetic polymers do not have cell-adhesion properties, an additional surface modification is needed to promote cell-surface interactions. Factors affecting cell adhesion to a polymer surface include the chemical composition, the net charge of the surface, and the balance between hydrophilic and hydrophobic micro-domains. To improve the biocompatibility of PHEMA scaffolds, the introduction of groups with positive charges [111] or modification with cholesterol [112, 113] has been studied. Modifying the surface of PHEMA-based hydrogels with different surface charges showed that, after implantation into the hemisected spinal cord, hydrogels with positively charged functional groups promoted connective tissue infiltration and extended axonal ingrowth into the hydrogel bridge, compared to negatively or uncharged hydrogels [111]. Considerable efforts have also been made to modify biomaterial surfaces by the pre-coating or immobilization of full-length ECM proteins or their functional protein sequences for integrin receptor binding sites, such as those from fibronectin, laminin and collagens. Well-characterized cell adhesion ligands include fibronectin-derived RGD (Arg-Gly-Asp) and laminin-derived peptides such as YIGSR (Tyr-Ile-Gly-Ser-Arg), IKVAV (Ile-Lys-Val-Ala-Val), *etc.* [114]. The advantage of using these short epitopes rather than the whole protein is their easy production, high stability and the possibility to incorporate the epitopes available for receptor binding at high surface density relative to the natural extracellular matrix. PHEMA hydrogels modified with the IKVAV peptide resulted in improved cell attachment and spread, as well as the improved differentiation of neural foetal precursor cells [115].

More recently, Stupp and co-workers have designed and synthesized a broad range of peptide amphiphiles to create new self-assembling biomaterials [116]. The structure of these molecules is composed of a short hydrophobic block bound to a short peptide sequence with overall hydrophilicity relative to the other block. These synthetic compounds combine the structural features of amphiphilic surfactants with the functions of bioactive peptides. One of these systems incorporates the laminin-derived sequence IKVAV and has been used to prepare *in situ* forming hydrogels to promote SCI regeneration [117].

Implantable scaffolds *versus in situ* forming hydrogels

The use of implantable biomaterials to promote spinal cord regeneration in a chronic lesion scenario aims not only at filling the gap formed in the spinal cord upon scar tissue removal but also preventing the formation of a secondary scar, moving the lesion site towards a pro-regenerative environment, while providing physical cues to axonal extension. Based on these requirements, the microstructure of the biomaterial is essential for directing cell attachment, migration and axonal re-growth across the lesion [118]. In this regard, a number of designs have been proposed, involving

porous conduits with or without longitudinal aligned channels. There is no study in the published literature that systematically assessed the effect of pore size on spinal cord regeneration, but a consensus exists in the field that the scaffolds should be highly porous with interconnected pores to allow fluid and nutrient exchange as well as neovascularization of the implant, thus creating a permissive environment for axonal growth [20]. More studies have focused on scaffold design in terms of architectural features [119]. Although the presence of longitudinally aligned channels was found to promote regeneration [94, 120–122], their number, dimensions and architecture are a matter of debate.

More recently, electrospinning has been proposed as a promising technique to prepare nanosized fibres, from both synthetic and natural polymers that can closely mimic the morphology of the extracellular matrix. When aligned, these fibres can promote axonal extension in the direction of the long axis of the fibre [123–125] and were found to mediate regeneration *in vivo* in a complete spinal cord transection model [126].

However, the regenerative capacity of guidance channel materials has mostly been evaluated in acute models of SCI, such as a transection, whereas in the case of a more complex SCI, when irregularly or multiple-shaped cavities develop, the shape of the scaffolds may not conform to the cavity and thus can pose structural and integration difficulties; in addition, the implantation of the scaffold may be surgically problematic. Therefore, polymers that can be easily shaped or even injected to fill the entire lesion cavity are highly attractive for CNS regeneration as they offer a minimally invasive delivery.

Hydrogels, either physically or chemically cross-linked materials that exhibit the ability to swell in water and to retain a significant fraction (>20%) of water within their structure, have the advantage over other matrices of better mimicking the aqueous environment of the extracellular matrix. Various injectable hydrogels are under study, and parameters such as mesh-size, setting time and softness can be optimized according to need. Moreover, self-assembling nanofibres that spontaneously aggregate from an aqueous solution into a stable nanofibre gel due to multiple non-covalent interactions in the presence of a physiological salt solution or by changing the pH have recently been shown to be an effective tool for implantation into soft neuronal tissue [117, 127].

In Table 2 a summary of the discussed materials, as well as their main features in terms of degradability and processability is presented.

Biomaterials in combination therapies

The use of matrices to promote axonal regeneration following a spinal cord lesion can be further improved by their combination with cells or therapeutic agents, such as neurotrophins. Cells can be either adhered to the pores of the scaffolds or incorporated as a cell suspension in a hydrogel that can also be combined with a more rigid scaffold. Such matrices may enhance cell survival after transplantation and promote differentiation into desired phenotypes based on the scaffold's properties. Sakiyama-Elbert and co-workers have recently published a study that evaluated the viability and differentiation of embryonic stem cell-derived neural progenitor cells transplanted within fibrin scaffolds containing growth factors to enhance cell survival and direct differentiation into neurons [128]. They report that the combination of growth factors and fibrin scaffold enhanced the total number of embryonic stem cell-derived neural progenitors present in the treated spinal cords and increased the number of NeuN-positive neurons 8 weeks after transplantation.

As cell-seeded scaffolds implanted into a spinal cord transection, Schwann cells within a poly (β-hydroxybutyrate) scaffold [109] or Schwann cells and NSCs seeded in poly(lactic-co-glycolic acid) aligned channels [129] have been investigated. In another study, a poly (D, L-lactic acid) guidance scaffold was seeded with Schwann cells genetically modified to produce bi-functional neurotrophin with brain derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3) activity [130]. Woerly *et al.* developed macroporous PHPMA hydrogels modified with the RGD peptidic sequence (Neurogel^{TM}) that have been shown to promote tissue regeneration, axonal ingrowth and angiogenesis when implanted into SCI [131]. Further enhancement of PHPMA-RGD hydrogels with fibroblasts producing BDNF or CNTF factor significantly increased axonal ingrowth into the hydrogel [132]. In our studies, PHPMA-RGD hydrogels were seeded with MSCs and implanted into rat chronic spinal cord lesions (5 weeks after injury) [133]. The hydrogels successfully bridged the spinal cord cavity and provided a scaffold for tissue regeneration (Fig. 5). Behavioural analysis showed a statistically significant improvement of motor and sensory scores 4–6 months after implantation. This effect was only achieved in rats with combined treatment, hydrogel and MSCs, compared with the control group and a group implanted with a hydrogel only. This therapy also prevented tissue atrophy.

By incorporating growth factors or other drugs in the polymeric structure, one can achieve the controlled delivery of the therapeutic agents [134], overcoming the limitations of the systemic administration of these molecules – short half-life, off-target effects and cytotoxicity. As an injectable drug-delivery system, a highly concentrated collagen solution containing growth factors has been administered intrathecally into a spinal cord compression injury [135]. An *in situ* gelling agarose scaffold embedded with microtubules releasing BDNF has been shown to promote neurite extension in spinal cord hemisection [136]. A photo-polymerizing PEG-derived hydrogel containing NT-3 was injected into the injured spinal cord cavity and exposed to light for *in situ* gelation; hydrogel-NT3-treated animals showed improved behavioural recovery and axonal growth [137].

The local expression of neurothrophic factors, or other relevant therapeutic proteins, achieved by gene transfer may also be used to achieve the desired outcome. A number of viruses have been tested as vectors of therapeutic genes to the nervous system [138–140]. Although these proved to efficiently mediate gene delivery, their use in clinical applications raises obvious safety concerns. Also here, biomaterials can be used in the development of non-viral gene delivery strategies [140–142]. With the rise of RNA interference (RNAi) technology, one also has the possibility to downregulate the expression of inhibitory molecules that prevent spinal cord nerve regeneration. RNAi involves double stranded small interfering RNA (siRNA) molecules about 20–30 nucleotides in length that mediate the sequence-specific enzymatic cleavage of target mRNA through complementary

Table 2 Materials with potential application in spinal cord lesion and their main features in terms of biodegradability and processing

Material	Degradability *in vivo*	Form (processing)	Examples of application in tissue engineering of the nervous system
Natural			
Collagen	Degradable	Hydrogel; porous scaffold; (electrospun) fibres	[100, 169–172]
Hyaluronic acid	Degradable	Hydrogel; (electrospun) fibres	[103, 104, 173]
Fibrin	Degradable	Hydrogel; porous scaffold; (electrospun) fibres	[101, 102, 128]
Agarose	Degradable	Hydrogel; porous scaffold	[105, 136, 174]
Alginate	Poorly degradable	Hydrogel; porous scaffold	[106, 175]
Chitosan	Degradable	Hydrogel; porous scaffold; (electrospun) fibres	[102, 107, 176, 177]
Fibroin	Degradable	Porous scaffold; (electrospun) fibres	[108]
Poly (β-hydroxybutyrate)	Degradable	Porous scaffold; (electrospun) fibres	[109, 178, 179]
Synthetic			
Poly (lactide) (PLA) and its copolymers with glycolide (poly (glycolide-co-lactide), PGLA)	Degradable	Porous scaffold; (electrospun) fibres	[93, 126, 129, 180]
Poly (ε-caprolactone) (PCL)	Degradable	Porous scaffold; (electrospun) fibres	[94]
Poly (trimethylene carbonate-co-ε-caprolactone) (P(TMC-CL))	Degradable	Porous scaffold; (electrospun) fibres	[95, 181]
Peptide amphiphiles	Degradable	Hydrogel	[182]
Poly (2-hydroxyethyl methacrylate) (PHEMA)	Non-degradable	Hydrogel	[91, 96, 111–115]
Poly [N-(2-hydroxypropyl) methacrylamide] (PHPMA)	Non-degradable	Hydrogel	[91, 97, 131–133]
Poly (3, 4-ethylenedioxythiophene) (PEDOT)	Non-degradable	Coating; particle form (to be used in composite materials)	[98]

base pairing. This allows the design of siRNA specific to a particular protein, which has resulted in several clinical trials for cancer, viral infections and senescence-associated diseases. Nevertheless, delivery into the cell remains a challenge for the clinical application of siRNA. This is due to the susceptibility of naked siRNA for degradation in the bloodstream, renal clearance and inadequate entry into cells [143]. A recent non-viral delivery approach is the use of chitosan-based nanoparticles formed by the electrostatic interaction between the anionic phosphates of the RNA and the cationic amino-bearing chitosan. In a previous study [144] we have combined chitosan/siRNA nanoparticles with microstructured implants as a method for the local delivery of RhoA-specific siRNA for guided neuroregeneration (Fig. 6) with promising results.

Future perspectives: increasing the intrinsic axonal regenerative capacity

Upon the embryonic to adult transition, the intrinsic axonal growth capacity of vertebrate CNS neurons is repressed to allow for correct synaptogenesis. As such, under physiological conditions, adult CNS

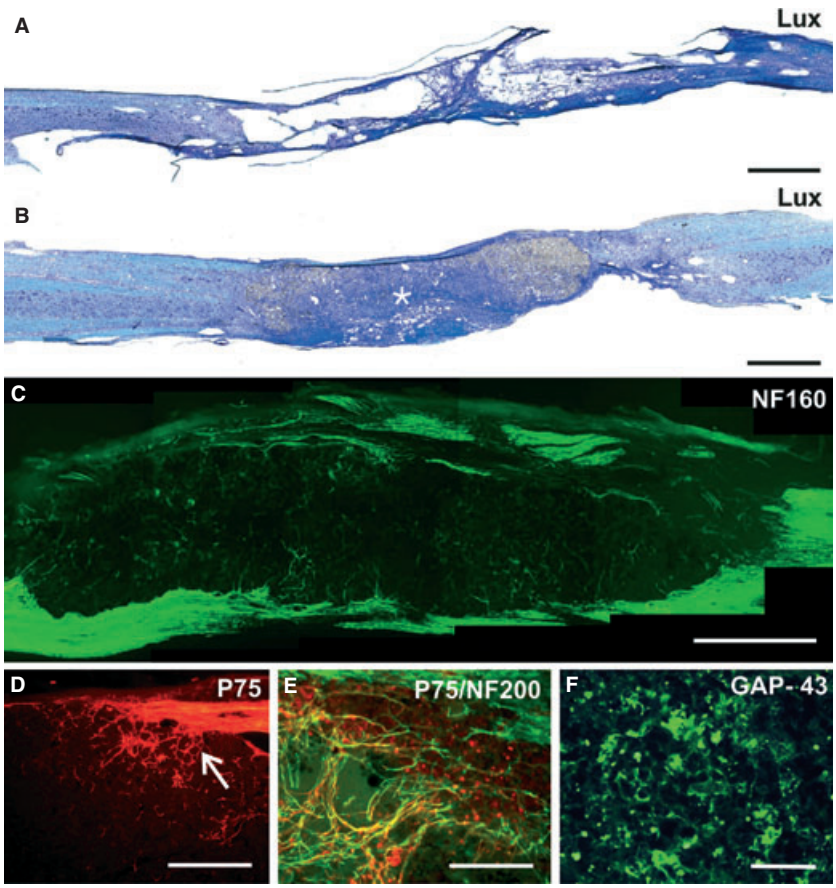

Fig. 5 Histological evaluation of the lesion in rats with chronic SCI treated with a PHPMA-RGD hydrogel seeded with MSCs. (A, B) Longitudinal section (Luxol blue staining for myelin, Lux) of a spinal cord lesion 6 months after SCI. (A) The untreated lesion was dominated by tissue atrophy due to progressive cavitation. (B) A hydrogel seeded with MSCs (white asterisk) and implanted into a chronic spinal cord lesion (5 weeks after injury) formed a bridge across the epicenter of the chronic lesion. (C) The hydrogel was completely filled with infiltrating axons (staining for neurofilament NF160) throughout its whole volume. (D) Schwann cells (p75 staining), originating from the spinal root entry zone, crossed the spinal cord-hydrogel border and infiltrated the hydrogel (white arrow). (E) Double staining showing axons (NF 160 staining, green) growing inside the implant in close proximity with Schwann cells (p75 staining, red). (F) Regenerating axons present inside the hydrogel scaffold showed GAP-43 positivity. Scale bar: (A, B) 2 mm, (C) 500 μm, (D) 100 μm, (E) 50 μm, (F) 25 μm. Modified from [133].

neurons are in a non-regenerative state. However, little is known about the nature of the signals responsible for the repression and the possible re-activation of the autonomous capacity of CNS axons to grow. In the following paragraphs, the possible mechanisms enabling the activation of intrinsic axonal regeneration competence in adult vertebrate CNS neurons will be discussed.

It is widely acknowledged that upon injury, CNS axons mostly fail to spontaneously regenerate whereas PNS axons promptly re-grow following a lesion. This disparity between the axonal regeneration capacity of the CNS and PNS is unrelated to any intrinsic inability of CNS axons to sprout after injury, as these axons are able to regenerate in the presence of a permissive growth environment [145, 146]. As a consequence of these findings, the differential regenerative capacities of the CNS and PNS have been attributed to environmental differences. In this respect, the PNS permissiveness to regeneration has been related to the following: (*i*) the absence of axonal regeneration inhibitors such as Nogo-A in PNS myelin [147] and (*ii*) a faster immune response, as PNS macrophages and Schwann cells rapidly clear myelin after injury [148], precluding the accumulation of myelin inhibitors and the formation of a glial scar. Although progress has been made in characterizing the extrinsic cues that inhibit axon growth, the cell-intrinsic mechanisms that govern axon growth and regeneration remain poorly understood.

Despite the general inability of CNS axons to regenerate when a permissive growth environment is absent, CNS regeneration within a highly inhibitory milieu, such as the one formed upon SCI, is possible. Dorsal root ganglia (DRG) neurons have a peripheral branch that regenerates after a lesion and a central branch that enters the spinal cord and does not regenerate upon injury. However, when the peripheral branch is injured approximately 1 week prior to a lesion to its

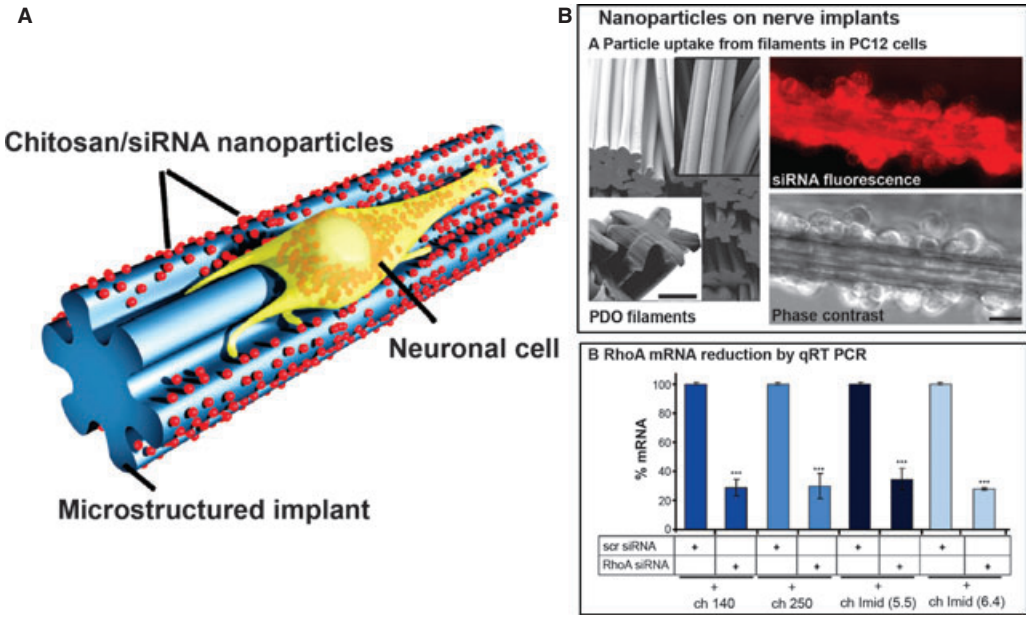

Fig. 6 Nanobiofunctionalized implants for spinal cord regeneration. (I) Proposed strategy: nerve implants are biofunctionalized by chitosan/siRNA nanoparticles that are taken up by cells, and enable neurite outgrowth. (II) Nanoparticles on nerve implants. (A) Particle uptake from nerve implants (filaments) into PC12 cells. Chitosan/siRNA nanoparticles (NP) were immobilized on polydioxanon (PDO) filaments (scale bar left image: 10 μm) by lyophilization. PC12 cells were seeded onto the filaments carrying NP and after 48 hrs the uptake was analyzed by microscopy. PC12 cells grew well on coated filaments (phase contrast image) and showed the efficient uptake of fluorescently labeled siRNA (red fluorescence). Scale bar right image: 20 μm. (B) Chitosan/siRNA NP functionality and RhoA mRNA reduction were determined by real time quantitative reverse transcription polymerase chain reaction (qRT PCR). Cells were lysed and mRNA was isolated and processed using the TaqMan Gene Expression Cell-to-CT Kit. RhoA mRNA levels were normalized to GAPDH mRNA levels. RhoA siRNA initiated the degradation of target mRNA compared to scr siRNA. Mean value of three independent experiments. ***$P < 0.001$ *versus* scr siRNA NP treatment. The transfection of PC12 cells with RhoA nanoparticles resulted in a 65–75% RhoA mRNA reduction compared to cells transfected with scr nanoparticles. Modified with permission from [144]. Copyright 2010 American Chemical Society.

central branch (conditioning lesion), the central axon does not undergo retraction and dieback, but instead overcomes the inhibitory environment of the injured spinal cord and regenerates, with some axons even being able to grow beyond the injury site [149–151]. Although this CNS axonal regeneration is only on the millimetre scale, it has fuelled efforts to understand the mechanism through which a conditioning lesion leads to the gain of regenerative capacity. Interestingly, this effect is not restricted to DRG neurons as it has also been described in motor neurons [152] and retinal ganglion cells of certain species [153]. Moreover, this effect is also observed *in vitro* as conditioned DRG neurons have increased neurite outgrowth in culture and are able to overcome myelin inhibition [154].

Although the sequence of events responsible for the conditioning lesion effect is far from being understood, the effect is probably the consequence of the activation of the regenerative machinery prior to the CNS lesion. The increase in the intrinsic growth state of the primed neurons most likely encompasses changes in both gene expression and axonal transport induced by the initial PNS lesion. In fact, it has been shown that sensory neurons have an increased regenerative ability as soon as 1 day after a conditioning lesion [151] and that this ability to express known regeneration-associated genes lasts for as long as 2 months following the priming injury [150]. Several molecules have been put forward as required for the conditioning effect, namely the transcription factors smad1 [155] and ATF3 [156], cytokines of the gp130 family (leukaemia inhibitory factor, LIF and interleukin-6, IL-6) [157–159] and tissue plasminogen activator [160], among others. However, none of them has been clearly proven to be sufficient and necessary to mimic a conditioning lesion. Among the several putative candidates responsible for the gain of regenerative capacity following conditioning, increased cAMP in DRG neurons has assumed a central role [151, 154]. Despite the fact that cAMP alone is insufficient to reproduce the magnitude of the effect of conditioning lesions [154], agents that increase cAMP levels promote axonal regeneration in SCI models [151, 154, 161, 162]. The regenerative effects of cAMP were shown to be transcription-dependent and mediated by arginaseI [163], a gene target of cAMP that catalyzes a rate limiting step in the synthesis of polyamines. In fact, an established approach to increase cAMP and promote regeneration following SCI is the administration of phosphodiesterase inhibitors (such as rolipram) that suppress cAMP degradation [161, 162]. However, the clinical relevance of these inhibitors is limited as they induce disabling nausea. To overcome this problem, a large-scale screen was

performed to identify other regulators of the arginasel promoter [164]. Daidzein (a soy isoflavone) was identified in this screen as a clinically approved small molecule that can promote axonal regeneration through a cAMP-independent pathway. Despite the fact that daidzein is more potent than cAMP analogues in inducing arginasel expression, its potency in promoting regeneration is probably not sufficient to be effective in humans [164]. As such, the quest for clinically relevant molecules that mimic a conditioning lesion, as well as the dissection of cAMP-independent pathways capable of increasing the intrinsic regenerative capacity of CNS neurons, should be pursued and the possibility of translation into clinical practice explored.

Conclusions

This review highlights some of the most promising clinical approaches to promoting spinal cord regeneration that are under investigation at present, including ones already in clinical trials. It is, however, important to note that despite the growing list of studies providing evidence for axonal regeneration after SCI, even when the environmental inhibitory cues are cleared and a more permissive environment is created to support axonal regeneration, the distance of axon re-growth is very modest [165, 166] specially in the context of human anatomy. Moreover, in rodent models, the proportion of injured axons that are able to regenerate at least two spinal segments is generally less than 10% and in the majority of the cases, the small proportion of axons that regrow after injury usually regenerate misdirected [167] and fail to reform functional connections. Importantly, the contribution of axonal re-growth to functional recovery is mostly unknown. As such, future studies should distinguish re-growth of injured axons from sprouting of spared fibres i.e., to establish the contribution of plasticity to the observed recovery.

Clearly, cell therapies will have a key role in future strategies to treat SCI, regardless of the discussion that surrounds the field concerning the selection of the best cell source. Based on our current knowledge of all the possible candidates, the use of MSCs presents obvious advantages over other cell sources, including their safety and availability. The use of biomaterials is also receiving increased attention, either as vehicles for the cells or as vectors of therapeutic agents (neurothropic factors, genes, siRNA, among others) for the spinal cord regenerative process. In addition, the advantages of using a biomaterial-based scaffold to promote axonal regeneration are becoming more apparent in the treatment of chronic spinal cord lesions, when the cystic cavity is already developed and cell transplantation alone is not sufficient to promote tissue regeneration. Finally, the possibility of stimulating the action of molecules and mechanisms that can increase the intrinsic regenerative capacity of adult vertebrate CNS neurons was also addressed, exploring the knowledge gained from the conditioning lesion model.

Undoubtedly, SCI treatment is heading towards the use of combinatorial strategies due to the broad series of events that occurs after this type of lesion that lead to the failure of regeneration in this tissue. One of the challenges currently facing the multidisciplinary teams working in this field is the development of safe and effective therapies to be applied in the clinic based on the advances achieved in basic research.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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