

● INVITED REVIEW

Advances in regenerative therapies for spinal cord injury: a biomaterials approach

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Abstract

Spinal cord injury results in the permanent loss of function, causing enormous personal, social and economic problems. Even though neural regeneration has been proven to be a natural mechanism, central nervous system repair mechanisms are ineffective due to the imbalance of the inhibitory and excitatory factors implicated in neuroregeneration. Therefore, there is growing research interest on discovering a novel therapeutic strategy for effective spinal cord injury repair. To this direction, cell-based delivery strategies, biomolecule delivery strategies as well as scaffold-based therapeutic strategies have been developed with a tendency to seek for the answer to a combinatorial approach of all the above. Here we review the recent advances on regenerative/neural engineering therapies for spinal cord injury, aiming at providing an insight to the most promising repair strategies, in order to facilitate future research conduction.

Key Words: tissue engineering; neuroregeneration; repair; central nervous system; biomaterial; regenerative medicine; nanotechnology; spinal cord injury

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Introduction

Spinal cord injury (SCI) is a highly debilitating disorder with no effective therapeutic plan until now. Regardless of the extensive research conducted nowadays, it still remains one of the most daunting challenges in all neuroscience research. Due to the development of novel cell-based and scaffold-based treatment strategies with the advances of neural tissue engineering, there are now some promising results that raise hope for the treatment of SCI in the future. Given the rapid pace of the advancement in the field of neural engineering and neuroregeneration, there is a need for constant vigilance. The aim of the current review is to summarize all the current therapeutic options for SCI and brief the scientists for novel emerging therapies of great potential that could soon be applied to the clinic.

Epidemiology

SCI is a devastating disorder worldwide. Excluding the number of people who die at the scene of the accident, it is estimated that the annual incidence of SCI is approximately 40 cases per million population in the United States, or, in other words, about 12,000 new cases of SCI patients each year, while at the same time it primarily affects young adults

(Sebastià-Alcácer et al., 2013; National Spinal Cord Injury Statistical Center, March 2013). The main cause of SCI refers to motor vehicle crashes, followed by falls and violent acts (Akdemir et al., 2013; Chen et al., 2013). There is tremendous impact of the disorder to their social, personal and professional life and a great financial and psychological burden for the patient himself and the whole family (Selvarajah et al., 2013). Nevertheless, despite the fact that there are certain benefits on the overall outcome of the SCI patients due to the medical and surgical management, there is currently no effective treatment plan for the major SCI-related neurological deficits (Garcia-Altes et al., 2012; National Spinal Cord Injury Statistical Center, March 2013). The different types of the injuries, as well as the complex pathophysiology of SCI, may account for the difficulty in establishing an efficient treatment plan (Blesch and Tuszynski, 2009; Lo et al., 2013).

Pathophysiology

In fact, traumatic injury to the spinal cord can be caused by compression, laceration or contusion, which in turn leads to motor, sensory and/or autonomic deficits at the injured site and below. The range of signs and symptoms (sensory and motor impairment, neuropathic pain, bowel and bladder

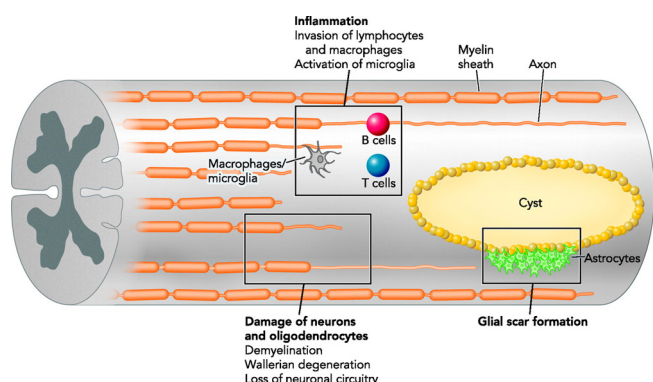


Figure 1 Schematic illustration of glial scarring in spinal cord injury (SCI).

The figure schematically demonstrates the process of cavitation during the subacute and chronic phases of SCI and the inflammatory response triggered. Around the cyst there are hypertrophic astrocytes initiating the cavitation process. The inflammatory response is shown with inflammatory cells invading the central nervous system from the periphery. Many neuronal axons are interrupted and undergo Wallerian degeneration. From: Obermair et al. (2008). Copyright © and courtesy of the American Physiological Society (2008).

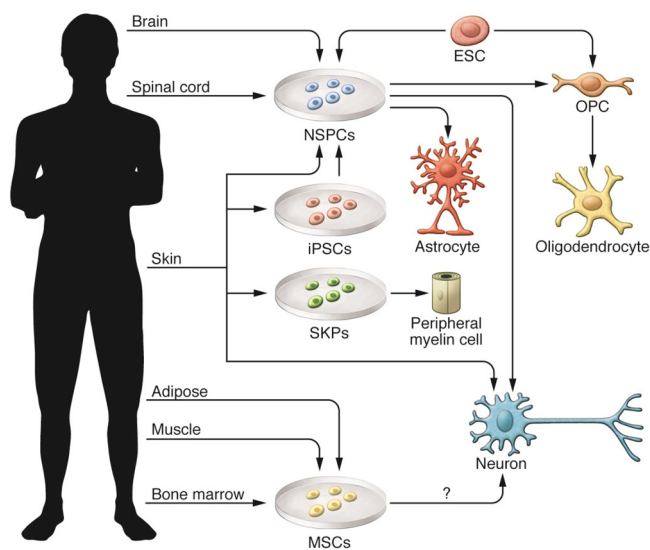


Figure 2 The figure demonstrates transplantation of different sources of stem cells into the injured spinal cord.

The stem cells depicted include neural stem/progenitor cells (NSPCs), induced pluripotent stem cells (iPSCs), skin-derived precursors (SKPs) and mesenchymal stem cells (MSCs) and direct conversion methods are used for yielding nerve cells for transplantation. The differentiation of NSPCs can either lead to oligodendrocyte precursor cells (OPCs), mature oligodendrocytes, astrocytes or neurons depending on the culture conditions and the growth factors exposure. There are certain conditions that can promote OPC generation derived by embryonic stem cells (ESCs) even though by default they differentiate to neural cells. MSCs can be harvested by a variety of different tissues such as the bone marrow, umbilical cord, adipose tissue, muscle and dental pulp from deciduous baby teeth and, *in vitro* in culture, they show neural cell properties. Many studies have used a variety of methods to reprogram fibroblasts from the skin into iPSCs or even to directly convert them to neurons and NSPCs without the need to pass into the pluripotent stage. This opens a window of great potential for the neural cell transplantation techniques. From: Advances in stem cell therapy for spinal cord injury. (Mothe and Tator, 2012). Copyright © and courtesy of the American Society for Clinical Investigation (2012).

Why do spinal cord axons not regenerate?

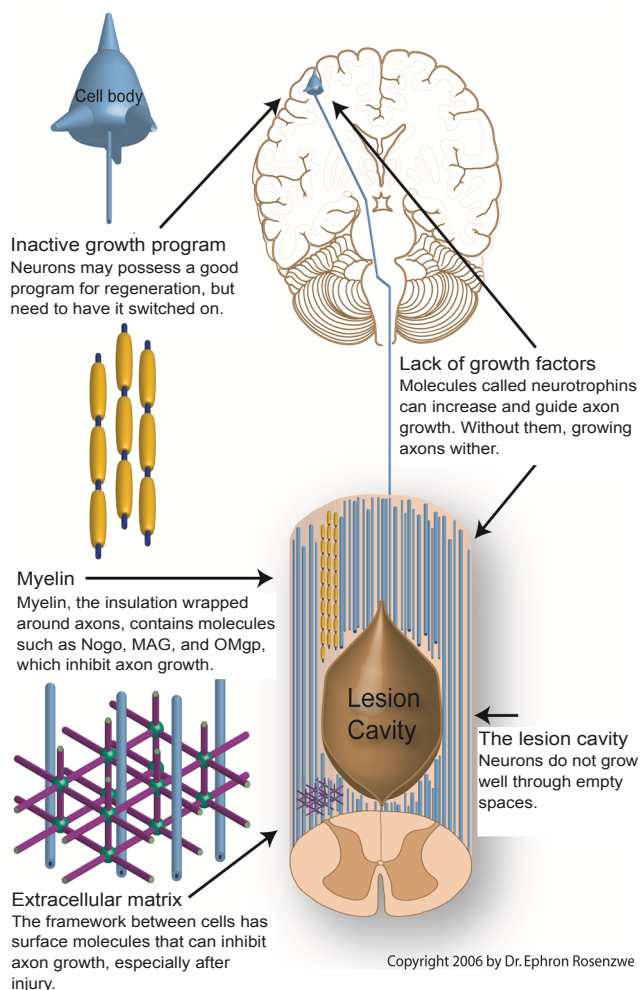


Figure 3 The figure illustrates the various inhibitory factors which are blocking the axon regeneration in the central nervous system (CNS).

This schematically demonstrates the imbalance within the CNS between the factors inhibiting nerve regeneration and the factors enabling it. The CNS is a “hostile” environment for nerve regeneration after an injury and this is why the nerves can not regrow properly even though there is such a potential. This inhibition can either be central due to the “switched off” growth program of the CNS neurons, or it can be caused by environmental factors around the lesion site. The latter one can be either due to the increase of inhibitory molecules or due to the reduction of promoting factors. Around the lesion site inhibitory molecules of nerve regeneration such as Nogo, MAG, and OMgp, which are being included in the myelin sheath, are up-regulated; the same applies for the inhibitory surface molecules within the extracellular matrix. Contrary to that, neurotrophins, which are growth factors facilitating nerve growth are down-regulated, further inhibiting the regenerative potential within the CNS. Mechanically speaking, it is proven that neurons can not grow well through empty spaces and this is the concept of neural tissue engineering which uses scaffolds to mechanically support neurons to enable their regrowth. From: <http://tuszynskilab.ucsd.edu/rosenz.php> Copyright © and courtesy of Dr. Ephron Rosenzweig (2006). With the kind permission of Dr. Ephron Rosenzweig, Center for Neural Repair, University of California, San Diego, USA.

Table 1 Common materials for hydrogel fabrication

Material	Source	Inherently cell adhesive	Injectable	Comments	References
Matrigel	Mouse tumor cells	Yes	Yes	Coating for cell adhesion, mainly cell carrier, unlikely approval for human use	Hawryluk et al., 2012b; Kubinova and Sykova, 2012; Silva et al., 2013
Collagen	Animals	Yes	Sometimes*	Great biocompatibility, mimicking natural ECM	Lanfer et al., 2010; Suri and Schmidt, 2010; Macaya et al., 2013
Hyaluronic acid	Animals, bacteria	No	Yes	Injectable gels after chemical derivatization, mostly for brain injuries	Austin et al., 2012; Caicco et al., 2013; Collins and Birkinshaw, 2013; Mothe et al., 2013
Fibrin	Blood	No	Yes	Cell and biomolecule delivery suitability after extensive <i>in vivo</i> tests	King et al., 2010; Lu et al., 2012; Liu et al., 2013a
Alginate	Algae	No	Sometimes*	Preformed scaffold for cell transplantation and regeneration studies	Mekhail et al., 2012; Collins and Birkinshaw, 2013
Agarose	Seaweed	No	No	Preformed scaffold, regeneration promoting potential	Wylie and Shoichet, 2008; Gros et al., 2010
Chitosan	Insects and crustaceans	Sometimes	Sometimes*	Abundance in nature, crosslinked and injectable gel formation after derivatization	Crompton et al., 2006; Collins and Birkinshaw, 2013; Donoghue et al., 2013
Dextran	Bacteria	No	Sometimes*	Recent macroporous scaffold fabrication	Hiemstra et al., 2007; Collins and Birkinshaw, 2013
Peptide amphiphiles	Synthetic	No	Yes	Easy production, modification with peptide domains, gel formation	Tysseling-Mattiace et al., 2008; Tan et al., 2012
PEG	Synthetic	No	Sometimes*	Controlled studies of scaffold degradation and cell adhesion use	Park et al., 2011; Chen et al., 2012; Mekhail et al., 2012; Collins and Birkinshaw, 2013
PHEMA	Synthetic	No	No	Biomolecule delivery support, tunability of mechanical properties	Hejcl et al., 2008; Kubinova and Sykova, 2012; Kubinova et al., 2013
PHPMA	Synthetic	No	No	Functionalization with peptide domains, similar mechanical properties to CNS	Cholas et al., 2012; Kubinova and Sykova, 2012; Mekhail et al., 2012

ECM: Extracellular matrix; PEG: polyethylene glycol; PHEMA: poly(2-hydroxyethyl methacrylate); PHPMA: poly[N-(2-hydroxypropyl) methacrylamide]; CNS: central nervous system. *The material is used either in an injectable hydrogel form or not interchangeable within the literature.

dysfunction, autonomic dysreflexia, etc.) is dependent on the level and severity of SCI (Akdemir et al., 2013; Ovechkin et al., 2013). The pathophysiology relies on two separate mechanisms: primary injury mechanisms and secondary injury mechanisms (Lis et al., 2013; Silva et al., 2013). In the acute phase of SCI (seconds to minutes after the injury), the initial mechanical impact leads to direct damage of the tissue, meaning hemorrhage, local edema, necrosis, and laceration of the tissue (Kakulas, 2004; Silva et al., 2013). During this phase, various systemic and local events emerge (Hulsebosch, 2002), such as systemic hypotension, spinal shock, vasospasm, plasma membrane compromise, ischemia, neurotransmitter/ionic disturbances (Pineau and Lacroix, 2007; Rowland et al., 2008; Silva et al., 2013). Some of the acute phase events pass into the subacute phase (minutes to weeks after the injury), just like some subacute phase events continue into the chronic phase of SCI (months to years after injury). In the subacute phase, a cascade of secondary events take place, including further edema, vasospasm, excitotoxicity, inflammation, free radical production, lipid peroxidation, ischemia, apoptosis, demyelination and neurotransmitter/electrolyte disturbances (Donnelly and Popovich, 2008; Silva et al., 2013). In the subacute and chronic phases, the central part of the spinal cord contains a lentiform-shaped

cyst filled with fluid, while hypertrophic astrocytes are found around that cyst, initiating a process called “cavitation” process (Rowland et al., 2008; Bauchet et al., 2009; Silva et al., 2013) (Figure 1). Those astrocytes along with other cells secrete extracellular matrix and inhibitory molecules, thereby inhibitory factors such as chondroitin sulfate proteoglycans (CSPGs) get up-regulated locally. In turn, that leads to the glial scar formation, which sets both a physical and a chemical barrier to the process of neuroregeneration (Yiu and He, 2006; Liu et al., 2013b). Interestingly though, it has been observed that the subpial region contains a certain amount of preserved tissue (Hulsebosch, 2002), thereafter, trying to remyelinate the axons of the region is for sure one of the future treatment targets (Mekhail et al., 2012).

Current Management

The current therapeutic approach to the SCI patient mainly aims at eliminating further damage to the spinal cord. The spinal cord gets operatively decompressed, any unstable lesions are stabilized and fused, the secondary complications are addressed and the patient enters a rehabilitation program to improve functional outcome (Wilson et al., 2013). Even though they do improve the clinical outcome of SCI patients, no therapeutic approach targets the neurologic

Table 2 Common materials for nerve guidance channel construction

Material	<i>In vivo</i> studies in the CNS?	Source	Cell adhesive	Electrically active?	Comments	Common outcomes	References
PAN/PVC	Yes	Synthetic	No	No	Mainly SC or OEC delivery	Large numbers of peripherally myelinated axons in SC bridges created	Moon et al., 2006
PHEMA	Yes	Synthetic	No	No	Similar mechanical properties to the spinal cord	The elastic modulus of the spinal cord can be approximated with such nerve guides but their biocompatibility is still being assessed	Dalton et al., 2002
*Poly-(α -hydroxy acids)	Yes	Synthetic	No	No	Family of synthetic polymers, spectrum of mechanical properties	Successful long-term drug/biomolecules release (<i>i.e.</i> , NT-3 with NSCs and SCs), promising carriers to facilitate neuroregeneration, multi-channel scaffolds and tubes facilitating axonal regeneration	Xiong et al., 2012
*Chitosan	No	Insects and crustaceans	Sometimes	No	Neural adhesion improvement possibly due to its cationic nature	Promising carriers of drugs/biomolecules (<i>i.e.</i> , NT-3 with NSCs), for channels with NSPCs increasing cell survival and differentiation percentage but no functional improvement but for channels with peripheral nerve grafts great increase in remyelinated axons, amine content can be tuned to optimize	Freier et al., 2005; Nomura et al., 2008a, b; Li et al., 2009
*Collagen	Yes	Animals	Yes	No	Cell growth and differentiation enhancement	Scaffolds facilitate neural differentiation, axon regeneration and functional recovery, delivery of sustained cells and genes is achieved	Li et al., 2013; Yao et al., 2013
*PHB	No	Bacteria and algae	Yes	No	Neurons and glia-guided longitudinally oriented fibers	Surface functionalization can achieve better cell attachment and proliferation, scaffolds promote SCs' attachment, proliferation and survival, facilitating neuroregeneration, similar modulus to human spinal cord, good biocompatibility	Novikova et al., 2008; Ribeiro-Samy et al., 2013
PVDF	No	Synthetic	No	Yes	Electrical stimulation without external source	Inhibitory effect on NSCs differentiation, influenced/ altered by environmental factors	Hung et al., 2006
PP	No	Synthetic	No	Yes	Controlled external electrical stimulation	Electrically conductive meshes support neural growth and differentiation with aligned nanofibers improving axon growth, the content of the material and the porosity can be tuned for optimization	Wan et al., 2005; Lee et al., 2009

CNS: Central nervous system; PAN/PVC: polyacrylonitrile/polyvinylchloride; SCs: Schwann cells; OECs: olfactory ensheathing cells; PHEMA: poly(2-hydroxyethyl methacrylate); NT-3: neurotrophin-3; NSCs: neural stem cells; NSPCs: neural stem/progenitor cells. PHB: poly(3-hydroxybutyrate); PVDF: polyvinylidene fluoride; PP: polypyrrole. *Indicates biodegradable.

Table 3 Currently running stem cell clinical trials for spinal cord injury

Stem cell type	Country; sponsor	Phase; ASIA scale	No. of patients	Timing of transplantation after injury	Route of cell delivery	Estimated completion date
Human fetal brain NSPCs (Hu-CNS-SC)	Switzerland; StemCells Inc.	I/II; ASIA A–C	12	3–12 months (chronic stage)	Intraspinal, single dose	March 2016
Autologous BMSCs	USA; Memorial Hermann Healthcare System	I/II; ASIA A–D children	10 estimated enrollment (eligible ages 1–15 years)	6 months–4 years (chronic stage)	Intravenous	October 2014
Autologous BMSCs	China; Guangzhou General Hospital of Guangzhou Military Command	I/II; ASIA A–B	20 estimated enrollment (eligible ages 16–60 years)	2 weeks–1 year	Combined intravenous and intrathecal <i>via</i> LP	June 2014
Autologous BMSCs	India; TotipotentRX Cell Therapy Pvt. Ltd.	I/II; ASIA A–C	15 estimated enrollment (eligible ages 18–60 years)	6 months–8 years (chronic)	Not indicated	October 2013
UCB MNCs	China; Spinal Cord Injury Network	I/II; ASIA A	20 (aged 18–60 years)	> 1 year (chronic stage)	Intraspinal	August 2013
UCB MNCs	China; Spinal Cord Injury Network	I/II; ASIA A	60 (aged 18–65 years)	< 4 weeks (acute/subacute stage)	Intraspinal, single dose	May 2013
Autologous BMSCs	Brazil; Hospital Sao Rafael	I; ASIA A	20 (aged 18–50 years)	Not indicated	Intraspinal	January 2013
Autologous BMSCs	USA; TCA Cellular Therapy, LLC	I; ASIA A	10 (aged 18–65 years)	> 2 weeks	Intrathecal <i>via</i> LP, single dose	June 2012
MSCs (umbilical cord-derived)	China; General Hospital of Chinese Armed Police Forces	I/II; ASIA scale not indicated	60 estimated enrollment (eligible ages 20–50 years)	Interval time not indicated; acute and chronic transplants	Not indicated	May 2012

ASIA: American Spinal Injury Association; NSPCs: neural stem/progenitor cells; BMSCs: bone marrow stromal cells; UCB: umbilical cord blood; MNCs: mononuclear cells; LP: lumbar puncture; LLC: limited liability company; MSCs: mesenchymal stem cells.

deficits after SCI. The ultimate goal for the management of SCI patients is: 1) to reduce cell death and minimize the extent of the injury, while 2) to facilitate the process of neuroregeneration to repair the damaged tissue (Wilcox et al., 2012). To this direction, there are a few ongoing clinical trials which are currently testing the use of neuroprotective agents for SCI patients (Kwon et al., 2011; Tator et al., 2012). This might serve the first part of the aforementioned goal but it is not thought to promote regeneration and tissue repair. Thus, in terms of neuroregeneration, stem cell therapy is thought to provide several attractive potentials for neural repair (Mothe and Tator, 2012; Wilson et al., 2013). Strategies improving the survival and the function of the grafted stem cells are needed, leading to further research to optimize the therapeutic strategies used (Guest et al., 2011; Silva et al., 2013), *i.e.*, stem cell seeding on various biomaterials and scaffolds, growth factor administration, *etc.*

It is beyond the scope of this review to analyze all the therapeutic approaches for SCI patients. There is a tremendous amount of ongoing research projects, *in vivo*, *in vitro* models and clinical trials for SCI, making it hard to follow the advances on the field as well as the advantages and disadvantages of each method tested. There are also many

questions, which need to be addressed in order to maximize the efficiency of future research experiments. The aim of this article is to gather all the pieces of this puzzle in order to provide insight into the recent advances on the regenerative therapies for SCI. The main focus is the use of biomaterials, trying to find the missing parts, which will give rise to the future perspectives and facilitate the research of the scientific community worldwide.

Treatment strategies

Cell-based treatment strategies

There was an accepted dogma for several years up to the 1960s, supporting the opinion that the central nervous system (CNS) has no regenerative ability. Shortly after that period though, specific regions of the adult human brain were found to maintain the capacity for neuroregeneration for a lifetime (Mothe and Tator, 2012; English et al., 2013). In particular, the subependymal zone of the lateral ventricles and the subgranular zone of the hippocampus are two major regions of neuroregeneration in the adult human brain (Aimone et al., 2010; Mothe and Tator, 2012; English et al., 2013). This microenvironment, which facilitates neurogenesis in the subependymal zone and subgranular zone is called neural

Table 4 Previously completed stem cell clinical trials for spinal cord injury

Stem cell type	Country; sponsor	Phase; ASIA scale	Number of patient receiving transplantation	Timing of transplantation after injury	Route of cell delivery	Comments	References
ESC-derived OPCs (GRNOPC1)	USA; Geron	I; ASIA A	5 transplanted, (18–65 years old)	1–2 weeks (subacute stage)	Intraspinal, single dose	No safety issue reported but complete results not published	–
Autologous BM-MNCs	Brazil	I; ASIA A–C	10 (median age 34 years)	mean 3 years (chronic stage)	Intrathecal <i>via</i> LP, single dose	No adverse effects, but patients only followed for 12 weeks; efficacy not reported	Callera and do Nascimento, 2006
Autologous BM-MNCs	Czech Republic	I/II; ASIA A	20 (aged 19–41 years)	10–30 days (subacute stage), 2–17 months (chronic stage)	Intra-arterial or intravenous	No complications reported, 5/6 patients who received cells intra-arterially reported improvement, 5/7 acute but only 1/13 chronic patients reported improvement	Syková et al., 2006
Autologous BM-MNCs	Korea	I/II; ASIA A	35 (aged 15–57 years)	> 2 weeks (acute stage), 2–8 weeks (subacute stage), > 8 weeks (chronic stage)	Intraspinal; single dose	Some improvement reported in acute and subacute stages but not in chronic stage	Yoon et al., 2007
Autologous BM-MNCs	Ecuador	I/II	8 (aged 27–44 years)	5 days–6 months (acute stage), 5–21 years (chronic stage)	Intraspinal, spinal canal, or intravenous, single dose	No adverse effects reported; improvement in bladder function	Geffner et al., 2008
Autologous BMSCs	Korea	I/II; ASIA A–B	10 (aged 34–61 years)	> 1 month (chronic stage)	Intraspinal followed by intrathecal <i>via</i> LP (2 doses)	3 patients showed improvement in upper limb recovery with MRI and electrophysiological changes	Park et al., 2012
Autologous BMSCs	Egypt; Cairo University	I/II; ASIA scale not indicated	80 (aged 10–36 years)	10 months–3 years (chronic stage), >2 months	Not indicated	No published reports	–
Autologous MSCs (adipose-derived)	Korea; RNL Bio Company Ltd.	I; ASIA A–C	8 (aged 19–60 years)	> 2 months	Intravenous; single dose	No serious adverse events during 3 month follow-up were reported	Ra et al., 2011
Autologous BMSCs	India; International Stemcell Services Ltd.	I/II; ASIA A	12 (aged 20–55 years)	> 2 weeks (acute stage), 2–8 weeks (subacute stage), > 6 months (chronic stage)	Intrathecal for acute and subacute; intraspinal for chronic	No published reports	–

ASIA: American Spinal Injury Association; ESCs: embryonic stem cells; OPCs: oligodendrocyte precursor cells; BM: bone marrow; MNCs: mononuclear cells; LP: lumbar puncture; BMSCs: bone marrow stromal cells; MRI: magnetic resonance imaging; MSCs: mesenchymal stem cells. “–” means the data are taken from the website clinicaltrials.gov.

Table 5 Selected *in vivo* neural engineering studies for spinal cord injury repair

Author, year	Scaffold composition and architecture	Cell encapsulation	Neurotrophic factor	Spinal cord injury model	Results
Kubinova, 2013	(SILVAV)-modified highly superporous PHEMA scaffolds	MSC	Not studied	Laminectomy and T ₈ hemisection	The surface functionalization and oriented porosity of the hydrogel optimized the promotion of neural tissue bridging and aligned axonal ingrowth
Li, 2013	Functionalized collagen-cetuximab scaffolds	NSPC expressing GFP	Not studied	Lateral hemisection at T ₁₃ -L ₂ level	The functionalization with an epidermal growth factor receptor (EGFR) neutralizing antibody improved functional recovery and facilitated neural regeneration
Liu, 2013	Injectable fibrin scaffolds	EMSCs expressing GFP	Not studied	Laminectomy and T ₁₀ transection	EMSCs significantly improved axonal regeneration and remyelination induced by fibrin alone
Mothe, 2013	Hydrogel blend of HAMC; covalently modified with rPDGF-A	NSPCs	Not studied	Laminectomy and clip compression injury at T ₂ level	Significant cavitation reduction, graft survival improvement and oligodendrocytic differentiation were noted in comparison to NSPC implantation alone
Yao, 2013	Multichannel collagen neural conduits	Fibroblast cells and adipose-derived stem cells	NT-3	T ₈₋₁₀ complete transection	Significant increase of NT-3 levels and aligned axon regeneration after effective non-viral PEGylated transfection vectors
Lu, 2012	Fibrin matrices	NSCs expressing GFP	Growth factor cocktails (BDNF, NT-3, PDGF-AA, IGF-1, EGF, bFGF, aFGF, GDNF, HGF, MDL28170 calpain inhibitor)	T ₃ complete transection	The supportive fibrin matrix with the growth factors cocktail achieved long-distance axonal regeneration with no need for further manipulation of the inhibitory environment of the adult CNS, high density of axons is achieved with rapid rates of elongation (1–2 mm/day) even through inhibitory white matter
Liu, 2012	Collagen-coated glass cover slips and randomly oriented or aligned collagen fibers	Astrocytes and DRGs	Not studied	C ₃ complete unilateral hemisection	The aligned fibers provided the anticipated contact guidance, inducing aligned axonal outgrowth, thicker fibers seemed to have greater effect on axon outgrowth, synergistic topographical and biochemical signaling enhancing neural regeneration
Cholas, 2011	DHT-cross-linked collagen scaffold alone or containing laminin, or incorporating pGDNF or carbodiimide-cross-linked collagen scaffold containing laminin	Adult NSCs	pGDNF	Dorsal laminectomy T ₇₋₁₀ level	Collagen tubes, as previously reported, could induce longitudinal axonal alignment and reduce cyst formation
Fan, 2010	Linear collagen scaffold	Not studied	CBD-NT-3	T ₈₋₁₀ transection	Enhancement of the corticospinal tract axon growth across the transplant, the linear collagen scaffold has strong structure for nerve guidance, increasing the affinity of NT-3 and collagen was essential due to the cerebrospinal fluid flow
Gros, 2010	Templated agarose scaffold	BMSCs	NT-3 added within the scaffold as well as distal to the site of injury	C ₄ transection	The linearized microstructure design of the scaffold linearly guides long-tract axons through the lesion site causing a 4-fold increase in the number of axons reaching the distal aspect of the lesion cavity; 83% of axons penetrate the scaffold channel over the full length of the lesion cavity; the reactive cell matrices formed is a problem under investigation
Han, 2009	Linear ordered collagen scaffold	Not studied	CBD-BDNF	T ₈₋₁₀ hemisection	Higher concentration and stronger bioactivity of BDNF was achieved; sustained release growth factor delivery system, linear ordered collagen induced linear ordered nerve growth
Hatami, 2009	3D type 1 collagen scaffold	Human ESC-derived neuronal progenitor cells	Not studied	T ₁₀ hemisection	Human ESC-neuronal progenitor cells in collagen scaffolds improve the motor and sensory function; transplanted cell migration and differentiation were noted

Table 5 Continued

Author, year	Scaffold composition and architecture	Cell encapsulation	Neurotrophic factor	Spinal cord injury model	Results
Itosaka, 2009	Thin fibrin fibers forming uniform mesh with pore size 2–5 mm	BMSCs	Not studied	T ₈ hemisection	The survival and migration of transplanted cells was markedly increased and functional recovery was significantly more pronounced; use of injectable and biodegradable matrix was a promising and minimally invasive method
Johnson, 2009	Fibrin-based scaffold created in situ from 10 µl fibrin spheres	Not studied	NT-3	T ₃ hemisection	The local concentration of NT-3 was effectively increased and enhanced the therapeutic effect; enhanced migration of astrocytes into fibrin scaffolds led to a more permissive environment to axonal sprouting; controlled release of NT-3 increased neural fiber density within the lesion, enhancing neuroregeneration even in a subacute stage, representing a dose-response curve for axonal regeneration
Olson, 2009	85:15 PLGA	SCs, NSCs	Not studied	T ₈₋₉ transection	Regeneration was facilitated and axonal regeneration was quantifiable due to the multichannel scaffolds, only 1% of axons regenerated so no functional recovery was noticed, NSC-treated group showed evidence of allodynia
Nomura, 2008a	Chitosan channels; Fibrin sealant used	Intercostal nerve grafts	Not studied	T ₈ clip compression	Peripheral nerve graft-filled channels were biocompatible, resulted in thicker bridge with more myelinated axons; no significant difference in functional recovery was noted though, biostability and biocompatibility of the channels facilitate long-term survival of SCs
Nomura, 2008b	Laminin-coated chitosan channels	NSC-derived progenitor cells	Not studied	T ₈ transection	The combination of NSPCs with chitosan channels led to long-term survival of the cells, the tissue bridge, even though containing many surviving transplanted cells and host axons led to no functional improvement
Pan, 2008	Fibrin glue	NSCs	G-CSF (50 µg/kg)	T ₈₋₉ transection	The combinatorial approach was found to be the most beneficial with combined therapy of NSCs and G-CSF exerting a better proliferative effect on cells and up-regulating the neuronal cell markers levels, G-CSF helped in the reduction of inflammation
Zhang, 2007	Gelatin foam	SCs, NSCs	NT-3	T ₉ transection	The transplants appeared to promote remyelination, the co-transplantation of SCs; NSCs fabricated a micro-environment favoring axonal regeneration (reduction of inhibitory molecules and increase of axonal regeneration promoting factors)
Guo, 2007	Type 1 collagen scaffold	SCs, NSCs	NT-3	T ₈₋₁₂ transection	The co-transplant of NSCs and SCs appeared to have a better curative value; NSCs and NT-3-SCs manifested better results due to the neurotrophic factors released
Stokols, 2006	Templated agarose scaffold	BMSCs	BDNF	C ₄ aspiration lesions	Individual uniaxial channels, high aperture/wall ratio and physical texture resembling to spinal cord, uniform wall thickness which are not limited by the fabrication process
Yarygin, 2006	Spherogel surrounded with collagen film	Embryonic nerve cells	Not studied	T ₁₀ transection	SCI model with extremely severe conditions for potential regeneration, partial functional improvement
Hurtado, 2006	Freeze-dried Poly D, L lactate tubular scaffold with longitudinally oriented pores	Modified SCs	NT-3, BDNF	T ₉₋₁₀ transection	Only few of the seeded SCs survived after the implantation so the neurotrophic support was less than anticipated, limited functional improvement in hindlimb performance but good BBB scores indicative of the good biocompatibility of PLA with spinal cord
Iwata, 2006	80% collagen-based hydrogel cylinders with uniaxial orientation of DRG constructs	Elongated DRG constructs	NGF	T ₉₋₁₁ hemisection	Promise of the development of nervous tissue constructs consisting of stretch-grown neural axons that could bridge even extensive lesions

Table 5 Continued

Author, year	Scaffold composition and architecture	Cell encapsulation	Neurotrophic factor	Spinal cord injury model	Results
Piantino, 2006	PLA-PEG-PLA hydrogel; in situ photopolymerization of the hydrogel	Not studied	NT-3	T ₈ hemisection	Far greater axon ingrowth for hydrogels/NT-3 scaffolds, long-distance regeneration
Rochkind, 2006	Dextran-gelatin tube with nanofibers, filled with NVR-N gel	Nasal olfactory mucosal cells and human embryonic spinal cord cells	NVR-N gel containing NGF, BDNF	T ₇₋₈ transection	Tissue-engineered tubular scaffold, containing bundles of parallel nanofibers provided guidance for neuroregeneration, functional recovery in comparison to controls after 4 mm spinal cord gap
Joosten, 2004	Collagen gel	Neonatal astroglial cells	No	Thoracic hemisection	Suppression of astroglial scar formation in the collagen implant did not allow the regrowth of injured CNS axons, no invasion of astroglial and microglial cells in the collagen matrix, no significant functional recovery
Patist, 2004	Freeze-dried PLA tubular scaffold with longitudinally oriented pores	Not studied	BDNF	T ₉₋₁₀ transection	BDNF exerted neuroprotective effects only in the rostral spinal cord stumps, which might be linked to vasculature damage, the porous polymer structure supported limited axonal ingrowth, fibrin only implant had better regenerative response, FGF-1 in fibrin glue promoted regeneration, SCs in foam improved number and myelination of axons
King, 2003	Fibronectin unidirectional mats	Not studied	NT-3, BDNF, NGF	Thoracic hemisection	The mats provide a substrate permissive for robust oriented axonal growth, SCs stimulate axonal growth only soon after the mat's implantation but may limit the degree of growth at longer time intervals
Novikov, 2002	PHB fibers, coated with alginate hydrogel and fibronectin	SCs	Intrathecal BDNF-NT-3 used in comparison group	C ₃ hemisection	Implantation of the PHB graft caused a 50% reduce in cell loss (neuroprotective), no effect on neural survival observed in controls (alginate hydrogel and fibronectin without PHB), seeding of SCs in the graft promotes axons sprouting
Teng, 2002	PLGA (50-50) with polylysine bilayered scaffold	NSCs	Not studied	T ₉₋₁₀ hemisection	Combination of NSCs and the scaffold lead to improved functional recovery, the scaffold itself reduces epidural and glial scar formation
Oudega, 2001	PLA50 and PLA100/10 tubes	SCs	Not studied	T ₈₋₉ transection	SC grafts contained in a resorbable tubular scaffold enhance fiber growth and myelination up to 2 months after the implantation and then causing the opposite results, changes in geometry of the scaffolds had an impact in neural regeneration, SC-filled PLA100/10 tubes demonstrated much better regenerative response, poor outcomes for both tubes (limited walls permeability)

SILVAV: Ser-Ile-Lys-Val-Ala-Val; PHEMA: poly(2-hydroxyethyl methacrylate); MSCs: mesenchymal stem cells; NSPCs: neural stem/progenitor cells; GFP: green fluorescent protein; EMSCs: ectomesenchymal stem cells; HAMC: hyaluronan and methyl cellulose; rPDGF-A: recombinant rat platelet-derived growth factor-A; NT-3: neurotrophin-3; NSCs: neural stem cells; BDNF: brain-derived neurotrophic factor; PDGF: platelet-derived growth factor; IGF-1: insulin-like growth factor-1; EGF: epidermal growth factor; bFGF: basic fibroblast growth factor; aFGF: acidic fibroblast growth factor; GDNF: glial cell-derived neurotrophic factor; HGF: hepatocyte growth factor; DRG: dorsal root ganglion; DHT: dehydrothermal; pGDNF: plasmid glial cell line-derived neurotrophic factor; CBD-NT-3: collagen-binding NT-3; CBD-BDNF: collagen-targeting BDNF; ESCs: embryonic stem cells; BMSCs: bone marrow stromal cells; PLGA: poly(lactic-co-glycolic) acid; SCs: Schwann cells; G-CSF: granulocyte colony-stimulating factor; NGF: nerve growth factor; PLA: polylactic acid; PEG: polyethylene glycol; NVR-N-Gel: co-polymer neurotube containing viscous gel; PHB: poly(3-hydroxybutyrate).

stem cell niche (Mothe and Tator, 2012; English et al., 2013).

Ever since then, neural stem cells (NSCs) have been isolated from several areas in the CNS (Ourednik et al., 2001; English et al., 2013), opening the pathway for stem cell based therapies to facilitate regenerative processes in the adult brain. Cell-based therapies aim at facilitating neuroregeneration, either directly *via* having the cells to replace and/or repair the damaged cells themselves or indirectly *via* secreting factors, which alter the environment, thereafter making it more conducive for regeneration (Miller and Gauthier-Fisher, 2009; Bliss et al., 2010; Mothe and Tator, 2012). Mesenchymal stem cells (MSCs) (Kode et al., 2009; English et al., 2013; Silva et al., 2013), neural stem/progenitor cells (Kokaia et al., 2012; English et al., 2013; Silva et al., 2013), embryonic stem cells (English et al., 2013; Silva et al., 2013), induced pluripotent stem cells (Willerth SM., 2011; English et al., 2013), and their differentiated progeny have been used as treatment strategies into the injured CNS (Fehlings and Vawda, 2011; Tetzlaff et al., 2011; Thomas KE., 2011; Mothe and Tator, 2012). Recently, ectomesenchymal stem cells have shown promise for spinal cord repair as well (Ibarretxe et al., 2012; Liu et al., 2013a). At the moment, adipose-derived MSCs have been thought to be the most promising cells for tissue engineering since they can easily be obtained in larger quantities than bone marrow (BM). They proliferate more rapidly and undergo more efficient neural differentiation in comparison to BM-MSCs (Zhang et al., 2012). Each of those types of cells can be obtained from various sources (Figure 2) and has its own advantages and disadvantages for treating SCI patients (Fehlings and Vawda, 2011; English et al., 2013), even though this goes beyond the scope of this review.

The main issue in regard to this therapeutic approach for CNS disorders, is the limited clinical efficacy of stem cell transplantation techniques primarily due to the inhospitable environment at and around the injury site (inhibitory molecule up-regulation, glial scar formation, inflammation, absence of astrocytes to guide axon regrowth), which lead to the poor cell survival, uncontrolled differentiation and ineffective integration into the host tissue (Parr et al., 2008; Mothe et al., 2013). There are several reports supporting the aforementioned problems. For example, in the adult rat lesioned brain, rosettes (Schulz et al., 2004), teratomas (Brederlau et al., 2006; Sonntag et al., 2007) or cellular masses inducing a gliotic host response have been reported after the free injection of hESC-derived neural cells. After non-human ESC injection, the formation of a mass, showing signs of overgrowth in the core and deformations, has been found (Erdo et al., 2003; Dihne et al., 2006; Hayashi et al., 2006). Since the injection of stem cells can lead to tumor formation (Hansmann et al., 2012), and given that most of the effects obtained with free stem cells injection have been mainly attributed so far to the growth factors rather than the ability of the cells to differentiate and form new tissue (Joyce et al., 2010; English et al., 2013), there was a hypothesis that the delivery of specific growth factors may cause a more favorable outcome in terms of regeneration. It is actually known today that lack of growth factors, as well as inhibiting

biomolecules and the lesion cavity itself are some of the factors which inhibit the effective regeneration in CNS (Liu et al., 2013a; Silva et al., 2013) (Figure 3).

Taking this to the next level, the development of advanced novel biomaterials, which will mimic the natural stem cell niche's microenvironment in order to support the cell growth effectively, while providing structural support at the same time, could hold the key of success in neuroregeneration for SCI.

Biomolecules delivery treatment strategies

Bioactive molecules, such as growth factors, are implicated in neuroregeneration mechanisms since, as mentioned above, the trophic microenvironment plays a crucial role in the whole process. The supporting cells in the neurogenic niches normally release extrinsic factors such as epidermal growth factor, vascular endothelial growth factor, brain-derived neurotrophic factor, Noggin, Sonic hedgehog, bone morphogenetic protein, etc. (Panchision and McKay, 2002; Jiao and Chen, 2008). In addition, the extracellular matrix proteins (laminin, fibronectin, collagen) of the niches (Kazanis et al., 2010) are highly important for activating stem cell integrins to bind growth factors (Campos et al., 2004); this way they facilitate the formation of a protein scaffold is supportive of the survival, proliferation, migration and differentiation of the cells.

Trying to mimic this system, the delivery of such molecules in the CNS has been pursued in order to promote neural regeneration. The grafted stem cells themselves are capable of producing neurotrophic factors such as nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3, ciliary neurotrophic factor, glial cell-derived neurotrophic factor, leukemia inhibitory factor (Sahni and Kessler, 2010; Hawryluk et al., 2012a; Hawryluk et al., 2012b). Although the proteins such as laminins, fibronectin and collagen I/III and IV (White and Jakeman, 2008; Fortun et al., 2009), promote CNS neural repair, but there are several concerns linked to free stem cell injection for CNS repair (Kozłowska et al., 2007; Sahni and Kessler, 2010). Another option is the *in situ* growth factor injection, which facilitated neurogenesis and improved functional recovery. Nevertheless, the low permeability of the blood-brain barrier and blood-spinal cord barrier limits diffusion of the molecules (Pardridge, 2012); therefore, conventional delivery strategies require high systemic doses of the growth factors in order to achieve a therapeutic concentration at the injury site, resulting in systemic cytotoxicity. Once again, tumor formation, as well as fibrosis and other undesired effects are possible due to the off-target therapeutic distribution of the molecules (Sahni and Kessler, 2010). To avoid these, local delivery strategies have been pursued. In terms of SCI, the injections into the intrathecal space surrounding the spinal cord can in fact yield higher local concentration of the therapeutic agent, but the rapid distribution and the elimination of the therapeutics by the cerebrospinal fluid affect the efficiency of this method too (Pardridge, 2012). Thus, the encapsulation of growth factors in liposomes, nanoparticles or different kinds

of scaffolds is considered a better approach (Mekhail et al., 2012; Collins and Birkinshaw, 2013; Silva et al., 2013). The delivery of the growth factors can be controlled and guided, showing promising research results so far.

Apart from the use of growth factors, drug delivery therapeutic strategies have been applied, affecting different pathways. Some of those include inhibition of inflammatory response (Silva et al., 2013), inhibition of inflammatory angiogenesis, administration of classical immunosuppressives (Hawryluk et al., 2012a; Silva et al., 2013), stimulation of inflammatory response, vaccination with myelin self-antigens (Hauben et al., 2001; Tetzlaff et al., 2011), suppression of myelin-associated inhibitor molecules (e.g., NOGO-A, myelin-associated glycoprotein (MAG), OMgp) and its pathways (Borisoff et al., 2003; Wright et al., 2011), and CSPGs digestion (e.g., administration of chondroitinase ABC (ChABC) or hyaluronidase) (Tetzlaff et al., 2011; Wilcox et al., 2012; Silva et al., 2013). Even though some of the above seems controversial, in fact it has been indicated that the inflammatory response has a dual role; therefore, even though acute inflammation leads to increased neuronal loss and reduced neurogenesis (Liu et al., 2012b; Mekhail et al., 2012), there is also growing evidence that inflammation may support neurogenesis and recovery, facilitating the migration of progenitors to the lesion site and the expression of neurotrophic factors (Liu et al., 2012b; Mekhail et al., 2012). In support to that, the inhibition of the Rho/ROCK signaling pathway, which is the pathway activated by the axonal growth-inhibitory molecules (i.e., Nogo, MAP, CSPGs) (Raad et al., 2012; Forgione and Fehlings, 2013; Silva et al., 2013; Wu et al., 2013), has demonstrated beneficial effects *via* the modulation of the inflammatory response after the injury (Silva et al., 2013; Wilson et al., 2013; Wu et al., 2013). There have been several studies though which are associated with negative effects, such as increased spinal cord tissue atrophy (Silva et al., 2013), decreased axonal sprouting/regeneration, impaired functional recovery (Sung et al., 2003; Chan et al., 2005) and increased astroglial activation and CSPGs deposition (Chan et al., 2005). A recent promising technique regards the administration of ChABC, which has been shown to degrade CSPGs, allowing significant axon regeneration both *in vitro* and *in vivo* (Karimi-Abdolrezaee et al., 2012; Liu et al., 2012a; Silva et al., 2013; Zhao et al., 2013; Zhao and Fawcett, 2013). Yet, the short half-life of the enzyme remains an obstacle, which needs to be addressed (Tester et al., 2007; Liu et al., 2012a).

Thus, the complexity of the CNS and its response to the injury imposes the advancement of those strategies or the combination of them with other more effective techniques, which will overcome the obstacles of neuroregeneration in CNS.

Scaffold-based or combination strategies

Scaffold-based strategies have established a very attractive alternative for neuroregeneration after SCI. Scaffolds are, by definition, temporary supporting structures for growing cells and tissues (Murugan and Ramakrishna, 2007; Zhong and Bellamkonda, 2008). Different types of scaffolds have been used for CNS repair, taking into account aspects such

as the biodegradability, mechanical strength, channels/fibers, porosity, capability of cell adhesion, and electrical activity of the scaffold (O'Brien, 2011). Up to date, electrospun guidance channels and hydrogels, seem to be very promising for neural engineering in SCI (Liu et al., 2012b, 2013b; Collins and Birkinshaw, 2013; Silva et al., 2013).

Hydrogels are found to be biocompatible implants for SCI repair. They not only can mechanically support the injured spinal cord, forming a local bridge for nerve regeneration, but also can prevent scarring, thereafter creating a permissive environment for tissue regeneration. The three-dimensional porous structure of the hydrogels provides a matrix for the ingrowth of supportive tissue, while it can be combined with other regenerative strategies (i.e., growth factors, stem cells), further contributing to neural regeneration after SCI. They can also be synthesized in large quantities, while they have similar elastic modulus to the spinal cord, something that has been proven to contribute to axonal regrowth. Especially, fabrication of injectable hydrogels is highly beneficial for treatment of SCI since it is a minimally invasive technique and is easily applied by neurosurgeons (Macaya and Spector, 2012).

Electrospun nanofiber guidance channels have also been highly promising, either alone or after being implemented in a hydrogel. Various techniques have been reported to develop nanofibers namely, template synthesis, phase separation, self-assembly, drawing and electrospinning. Among these techniques, electrospinning offers more advantages due to its ease of fabrication (Subramanian et al., 2009). The nanofibers provide a three-dimensional network, which is proven to be better for cell attachment, migration, proliferation and differentiation in comparison to traditional scaffolds (Fan et al., 2013). The fibers morphology and diameter highly resemble to the native extracellular matrix, providing an excellent supportive environment for neuroregeneration. The extent of the axonal ingrowth is dependent on the fibers density and the spatial orientation of the nanofiber layers. There is strong evidence that electrospun nanofibers, especially aligned nanofibers, are suitable for neural tissue engineering due to their extraordinary mechanical strength and high surface area/volume ratio (Liu et al., 2012b).

Scaffolds can often be based on particular extracellular matrix molecules (e.g., fibrin, collagen, fibronectin) (Collins and Birkinshaw, 2013; Li et al., 2013; Liu et al., 2013a; Macaya et al., 2013; Yao et al., 2013), other natural polymers (alginate, agarose, chitosan) (Mekhail et al., 2012; Tan et al., 2012; Collins and Birkinshaw, 2013; Silva et al., 2013) or synthetic polymers (e.g., poly(α -hydroxy acids), poly(2-hydroxyethyl methacrylate), polyethylene glycol) (Xiong et al., 2012; Donoghue et al., 2013; Hejcl et al., 2013; Kubinova et al., 2013). Their aim is to provide structural and active growth support to the damaged axons (Brock et al., 2010; Park et al., 2010; Lu et al., 2012; He and Lu, 2013); some biomaterials can provide both through the biofunctionalization with biologically active peptide sequences (Park et al., 2011; Hejcl et al., 2013; Kubinova et al., 2013). The implantation of a scaffold not only aims at the mechanical and trophic support of the spinal cord, or at the seeding of stem cells to

facilitate nerve regeneration, but it also discourages the scar formation through the bridging of the lesion site. To this direction, a study by Liu et al. (2012b) using nanofibrous collagen nerve conduits not only demonstrated that this type of scaffold is capable of promoting neural ingrowth after SCI, but it is also capable of inhibiting glial scar hyperplasia. Therefore, scaffolds can not only be used as space filling agents, but they can also act as bioactive molecule delivery systems (Lu et al., 2012; Macaya et al., 2013; Zhao et al., 2013; Zhao and Fawcett, 2013) and as cell delivery systems (Chen et al., 2012; Xiong et al., 2012; Caicco et al., 2013; Hejcl et al., 2013). In the latter cases, their aim is to enhance cell survival and integration after cell transplantation and to achieve local delivery of therapeutic factors locally, avoiding any systemic side effects.

Materials Used for Scaffold Fabrication

The biomaterials used for scaffold fabrication can be natural or synthetic polymers (degradable or non-degradable). Each of those has its own advantages and disadvantages (Kubinova and Sykova, 2012; Liu et al., 2012b). Natural polymers are easily obtained from natural sources and they have predictable physical, mechanical and biologic properties since they undergo highly controlled synthesis, resulting in regular structures. They are biodegradable and contain signals for cell adhesion, but they are also hard to be sterilized, thereby containing contaminating molecules often. Another thing that we need to consider is the low reproducibility of the research results, since the exact parameters, which affected the experiment, are unknown like their impact on the results (Kubinova and Sykova, 2012; Saracino et al., 2013). The fast biodegradation rate of natural materials (*i.e.*, collagen) and the low mechanical strength come as great disadvantages, which need to be addressed *via* cross-linking techniques in order to achieve the optimal results (Mitra et al., 2013). On the other hand, synthetic biomaterials are easy to sterilize. Key parameters of the synthetic biomaterials are easily controlled and modified according to our needs (*e.g.*, porosity, architecture, stiffness, degradation rate). Even though they lack recognition signals and they usually have poor biocompatibility, their biofunctionalization can easily overcome such issues (Kubinova and Sykova, 2012; Saracino et al., 2013). Below, we summarize some of the most commonly used biomaterials in the construction of hydrogel scaffolds or conduits (Tables 1, 2).

Studies and Clinical Trials on Regenerative Therapies for SCI

There is a growing number of research studies on regenerative therapies for SCI, since the investigation remains ongoing due to the complexity of the condition and the lack of effective treatments. Some studies have investigated the role of stem cells alone for the treatment of SCI, others have studied the role of growth factor delivery systems in neuroregeneration, while some others have looked into the possibility of developing a novel scaffold to facilitate nerve regeneration or even better combine the aforementioned

approaches.

The aim of this section is not to comprehensively analyze the various studies conducted on the field of regenerative medicine in regard to SCI management. However, since there is an enormous amount of information on nerve regeneration for SCI patients, the main concept is to summarize most of the significant advances on neuroregeneration/neural engineering SCI-related research.

Stem cell therapy clinical trials

Ever since 1998, stem cell *in vivo* studies have started manifesting positive results in terms of CNS repair, leading to a gradually increased number of stem cell clinical trials that are currently running today. The preclinical data of stem cell transplants in SCI models *in vivo* were that the US Food and Drug Administration (FDA) first approved the conduction of a human ESC trial in 2009. With the stem cells-related clinical trials reaching almost 5,000, there is a growing number of ongoing (Table 3) and completed clinical trials (Table 4) on CNS repair and SCI in particular.

The first attempts from 2005 up to date also assessed the safety of stem cells use, indicating that there are no adverse effects from their use in spinal cord injured people. Several studies have demonstrated that there are functional improvements in the acute and subacute stages of SCI but no significant improvement was manifested in the chronic stage of SCI. The transplantation of autologous Bone Marrow Stromal Cells (BMSCs) also showed improvement in hand-limb function according to one study conducted in Korea. The results of those clinical trials, as well as the results of the ongoing clinical trials of stem cell therapy for SCI are anticipated by 2016 according to the researchers' estimates, in order to provide insight into the effectiveness of stem cells on neuroregeneration. It is definitely too early to jump into conclusions since this research field is still in its infancy.

Tissue engineering therapy studies

On the other hand, tissue engineers have started their own combinatorial approaches in order to help to regenerate neurons in the CNS (Table 5). The approaches are using scaffolds encapsulated with cells and or embedded with molecules to achieve neural regeneration.

Fibrin/fibronectin (Itosaka et al., 2009; Johnson et al., 2009; Liu et al., 2013a) and collagen (Guo et al., 2007; Han et al., 2009; Hatami et al., 2009; Fan et al., 2010; Cholas et al., 2012) are the mostly used natural biomaterials for scaffolds to be used for neural tissue engineering application in the CNS repair and they have manifested very promising results so far. On the other hand, poly(α -hydroxy acid) are the most commonly used biomaterials for scaffolds in SCI repair from the synthetic biomaterials point of view (Hurtado et al., 2006; Piantino et al., 2006; Olson et al., 2009; Xiong et al., 2012). The stem cells are the most commonly studied supporting cells (Nomura et al., 2008b; Hatami et al., 2009; Itosaka et al., 2009; Gros et al., 2010; Mothe et al., 2013; Ribeiro-Samy et al., 2013), followed by Schwann cells

(SCs) (Hurtado et al., 2006; Guo et al., 2007; Zhang et al., 2007; Olson et al., 2009; Suri and Schmidt, 2010; Xiong et al., 2012). Other cell types have also been studied though, such as modified SCs releasing various neurotrophic factors (Hurtado et al., 2006), neonatal astroglial cells (Joosten et al., 2004), nasal olfactory mucosal cells (Rochkind et al., 2006; Ribeiro-Samy et al., 2013), human embryonic spinal cord cells (Rochkind et al., 2006; Lu et al., 2012), embryonic nerve cells (Yarygin et al., 2006), neural stem cells-derived progenitor cells (Nomura et al., 2008b; Li et al., 2013; Mothe et al., 2013), human embryonic stem cells-derived neuronal progenitor cells (Hatami et al., 2009) and recently ectomesenchymal stem cells (Liu et al., 2013a). The transplanted cell survival has been shown to be prolonged in most of the studies, even though there are studies which withhold the respective information (Rochkind et al., 2006; Pan et al., 2008; Gros et al., 2010). Even though in most studies stem cells seem to remain undifferentiated, there are studies commenting on the increased differentiation of stem cells into neuronal cell lines in the presence of 3D collagen (Guo et al., 2007; Hatami et al., 2009; Li et al., 2013), and fibrin scaffolds (Pan et al., 2008; Liu et al., 2013a).

In terms of the neurotrophic factors, most studies have used the NT-3 (Johnson et al., 2009; Fan et al., 2010; Gros et al., 2010; Liu et al., 2012a; Xiong et al., 2012; Yao et al., 2013), or BDNF (Hurtado et al., 2006; Stokols et al., 2006; Han et al., 2009; Horne et al., 2010), NGF (King et al., 2003; Iwata et al., 2006) and granulocyte colony-stimulating factor (G-CSF) (Pan et al., 2008). Scaffold binding domains (Han et al., 2009; Fan et al., 2010; Kubinova et al., 2013) as well as heparin-based delivery systems (Johnson et al., 2009; Liu et al., 2012a) are some of the delivery methods commonly employed. Interestingly, a dose-response curve for axonal regeneration has been shown by Johnson et al. (2009), suggesting that a target delivery of 500 ng/mL of NT-3 incurs more growth in comparison with a delivery of 1,000 ng/mL.

Still, all those attempts are limited to *in vivo* animal models and mainly to transection (Nomura et al., 2008b; Pan et al., 2008; Olson et al., 2009; Fan et al., 2010; Gros et al., 2010; Lu et al., 2012; Liu et al., 2013a; Zhao and Fawcett, 2013) or hemisection models (Han et al., 2009; Hatami et al., 2009; Itosaka et al., 2009; Johnson et al., 2009; Hejcl et al., 2013; Kubinova et al., 2013; Li et al., 2013; Ribeiro-Samy et al., 2013), with promising results so far. The majority of publications reported axonal regeneration with only a couple of exceptions (Hatami et al., 2009; Itosaka et al., 2009). The most significant study so far seems to be the study of Lu et al. (2012) which has manifested a rapid, enormous growth of axons in high density, with elongation rates of 1–2 mm per day, despite the inhibitory white matter, after having grafted fibrin matrices, which were embedded with green fluorescent protein (GFP)-expressing NSCs, and also contained growth factors cocktails, to sites of severe SCI. It is evident that the fibrin matrices along with the growth factors cocktail could trigger long-distance axonal growth, leading to the functional improvement of severe SCI models, with no need of manipulating the inhibitory environment of

the adult CNS. This study might soon lead to the initiation of clinical trials on SCI repair in humans, but once again further studies need to be conducted in order to secure those promising results and optimize the techniques to achieve a better outcome.

Conclusion and Future Perspectives

While stem cells alone have been investigated before as a potential answer for nerve regeneration, there is now a growing number of researchers who are turning to tissue engineering or even better to combinatorial approaches which are more likely to give the answer. It is the complexity of the condition of SCI itself, which urges researchers to seek for a combinatorial approach. This approach could, thereafter, provide not only structural support, but also a trophic microenvironment *via* biomolecule and cell delivery strategies, in order to mimic nature, trying to achieve effective neuroregeneration and functional improvement in SCI patients.

Some of these approaches have shown promising results *in vitro* and have also met some success in small-animal models, promoting nerve regeneration. Stem cells therapeutic strategies have even reached the stage of clinical trials, but it is too early to determine the effectiveness on neuroregeneration. Most of the studies were conducted to test the safety of stem cell use during the acute or subacute stages of SCI, so more studies are needed, especially during the chronic stage of SCI in order to investigate the real regenerative capacity of stem cells transplanted to SCI patients.

On the other hand, tissue engineering techniques have developed a variety of scaffolds taking into account the biodegradability, mechanical strength, channels/fibers, porosity, cell adhesion-capability or electrical activity of the scaffolds. There are many factors which seem to influence the effectiveness of a scaffold. It is suggested that the biomaterials used in a scaffold should have similar mechanical properties to the spinal cord.

Given that the elastic modulus of the spinal cord (including pia/dura) is approximately 230 kPa (Dalton et al., 2002), while that of the gray/white matter is 2–5 kPa (Ozawa et al., 2001), there is a tendency to consider that an elastic modulus between 2–230 kPa could be the range for an ideal scaffold construction. Therefore, hydrogels have preferable properties for SCI repair, even though gel patterning in a relatively new approach in this area of research. Another parameter which is widely discussed is whether nano- or micro-scale fibers are better for SCI repair with an increasing number of researchers supporting the necessity to go for the nanoscale in the CNS to achieve better outcomes (Xie et al., 2009; Silva et al., 2013). The different fabrication and micro-patterning techniques of conduits or hydrogels have provided such a wide variety of options that the studies are hard to follow. For now, electrospinning, self-assembly and phase separation techniques seem to be very promising for designing a good scaffold for SCI repair (Kubinova and Sykova, 2012; Tan et al., 2012). In addition, the 3D architecture of CNS imposes to seek better patterning techniques, since most of them are currently producing only 2D structures. While the biomaterial choice

seems crucial for scaffold fabrication, researchers have not yet determined the optimal choice of biomaterial for CNS repair. Fibrin/fibronectin and collagen-based scaffolds seem to manifest currently the more promising results though. As for the nanofibers, not only the alignment of the fibers in a scaffold is significant, but also there is also evidence suggesting that the density of the fibers can also affect the outcome (Lanfer et al., 2010).

Taken all together, it is well understood that there is room for major research advances on neural tissue engineering in order to investigate all the different parameters which could help to optimize the results in terms of neuroregeneration in SCI. The combination of the scaffolds with stem cells and/or growth factors and biomolecules such as the enzyme ChABC seems beneficial in order to regulate the balance between the inhibitory and excitatory factors implicated in neuroregeneration. Thus, it is more and more prominent that a combinatorial therapeutic approach will be the one that will probably provide the conclusive solution to the complex problem of SCI repair.

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