Review

Cell and biomolecule delivery for tissue repair and regeneration in the central nervous system

Irja Elliott Donaghue, Roger Tam, Michael V. Sefton *, Molly S. Shoichet *

Department of Chemical Engineering and Applied Chemistry, Institute of Biomaterials and Biomedical Engineering, University of Toronto, Donnelly Centre, 160 College Street, Toronto, Ontario M5S 3E1, Canada

ARTICLE INFO

ABSTRACT

Tissue engineering frequently involves cells and scaffolds to replace damaged or diseased tissue. It originated, in part, as a means of effecting the delivery of biomolecules such as insulin or neurotrophic factors, given that cells are constitutive producers of such therapeutic agents. Thus cell delivery is intrinsic to tissue engineering. Controlled release of biomolecules is also an important tool for enabling cell delivery since the biomolecules can enable cell engraftment, modulate inflammatory response or otherwise benefit the behavior of the delivered cells. We describe advances in cell and biomolecule delivery for tissue regeneration, with emphasis on the central nervous system (CNS). In the first section, the focus is on encapsulated cell therapy. In the second section, the focus is on biomolecule delivery in polymeric nano/microspheres and hydrogels for the nerve regeneration and endogenous cell stimulation. In the third section, the focus is on combination strategies of neural stem/progenitor cell or mesenchymal stem cell and biomolecule delivery for tissue regeneration and repair. In each section, the challenges and potential solutions associated with delivery to the CNS are highlighted.

© 2014 Elsevier B.V. All rights reserved.

Contents

1. Introduction

1.1. Development of cellular encapsulation in tissue engineering

Over 30 years ago, Lim and Sun [1] demonstrated that pancreatic islets could be microencapsulated in alginate–polylysine, borrowing a technology that already had a significant impact in drug delivery [2]. Together with yet earlier hollow fiber membrane-based methods for cell transplantation [3], the encapsulation of cells delivering therapeutic agents (such as insulin or neurotrophic factors) became a cornerstone of tissue engineering and a new strategy for controlled release.

Simple controlled release devices for insulin and other molecules generated constant or slowly decreasing rates of release [4]. However, the artificial endocrine pancreas [5] showed that closed loop insulin delivery produced better glucose control, by using an algorithm to relate insulin demand to actual glucose levels. This better control had significant

* Corresponding authors.
  E-mail addresses: Michael.sefton@utoronto.ca (M.V. Sefton),
Molly.shoichet@utoronto.ca (M.S. Shoichet).

impact on the complications of diabetes [6] and good diabetes management now uses multiple subcutaneous insulin injections, sometimes with basal insulin release to achieve good control. The artificial pancreas work also led to the development of wearable or implantable insulin pumps, operating in an open-loop, patient operated mode to provide better control than could be obtained with even multiple injections. From a controlled release perspective this created an interest in variable rate drug delivery systems — that combined materials and the drug but with some ability to vary release rate, for example using glucose oxidase to both sense glucose and then alter membrane permeability [7].

Using the pancreatic islet as the source of insulin emerged from the realization that the islet was a superb glucose sensor, obviating the limitation of open-loop control, and was a source of the protein itself. Early insulin delivery systems suffered from insulin aggregation and instability [8] and this was clearly not an issue if the islet could be harnessed as a drug delivery system. Lim and Sun used alginate–polylysine and one of us (MVS) used polyacrylate to microencapsulate islets and other cells [9,10] while many others have used different materials or have developed the alginate–polylysine system into a robust platform for islet encapsulation. A recent review summarizes some of this work [11].

In the context of neural diseases (the focus of this review), one of us (MVS) microencapsulated dopamine producing PC12 cells in a hyroxyethyl methacrylate–methyl methacrylate copolymer. *In vivo* studies showed good biocompatibility in the striatum in a rat model of Parkinson's Disease (PD) [12], but limited impact on the disease symptoms, presumably because of the challenges of introducing capsules into the brain by stereotactic placement and delivering enough cells to produce sufficient dopamine to have a therapeutic effect [13]. On the other hand, *in vitro* studies highlighted what was then a new phenomenon — the challenge of central necrosis when too many cells were placed in too large a capsule, as a consequence of their self assembly in a 3-D spheroidal form [14].

A related effort was to use macroencapsulation methods instead of microencapsulation, particularly for the delivery of neurotrophic factors to the brain. Hollow fiber membranes sealed at one end and then filled with pancreatic islets was one approach to the insulin delivery problem [15]. Aebischer and his team at Brown University and then in Switzerland, and at Cytotherapeutics, used the same hollow fiber approach to treat PD and chronic pain using neural tissue and genetically modified cells. The latter was an early attempt at *ex vivo* gene therapy [16]. One of us (MSS) worked at Cytotherapeutics and then continued at the University of Toronto to combine porous nerve guidance channels with drug delivery to promote tissue repair and recovery after spinal cord injury [17] (Fig. 1).
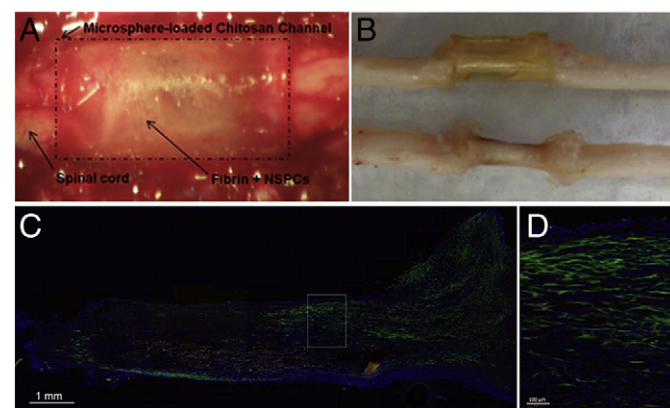
The focus on tissue engineering began through these early efforts in using live cells as drug delivery vehicles. Langer and colleagues used cells and porous scaffolds to produce liver and cartilage substitutes [18–21]. The focus was in a sense primarily structural (rather than therapeutic agent delivery), although the functional attributes of both the liver and cartilage were key outcome measures. Recapitulating structure and function has been a dominant theme throughout the continued development of tissue engineering which has expanded to consider the repair or replacement of nearly every tissue in the body [22], including as is relevant to this article, the spinal cord and brain.

Regardless of the specific tissue, addressing problems of cell number, cell phenotype and cell survival is a convenient means of summarizing the challenges of developing replacement tissues. One needs, certainly in large organs, many cells and typically at high density (say 10^8–10^9 cells/mL) and so how to provide the requisite vasculature to provide the nutritional support and oxygen for large numbers of cells is a critical issue [23]. Transplanted cells need to behave like native cells and this often involves controlling the 3-D extracellular cell matrix and the array of soluble cues [24]. Moreover, the cells must display the desired function for long periods of time — this leads to problems with biocompatibility and immune response [25]. These are all tough, high priority problems [26] and explain, in part, the gap between the promise of tissue engineering and the more limited clinical impact.

Tissue engineering began with a focus on an alternative to therapeutic agent delivery, yet therapeutic delivery itself is non-trivial. Here we illustrate some of the challenges associated with first drug and then cell delivery to the central nervous system (brain, spinal cord, and retina). We could also have discussed the controlled release of growth factors to promote vascularization or cytokines to limit inflammation, but these topics and the many other uses of controlled release in tissue engineering and regenerative medicine are outside the scope of this paper.

Diseases and injuries of the central nervous system (CNS), such as PD, multiple sclerosis (MS), stroke, traumatic brain injury (TBI) and spinal cord injury (SCI), have varied etiologies and symptoms, but all result in cell death, tissue degeneration and permanent disability. Current clinical options for treatment, including small molecule drugs and rehabilitation therapy, are limited [27–29] and do not restore lost tissue or full recovery of function. Biomolecular delivery and cellular delivery are therefore necessary and promising areas of research.

2. Biomolecule delivery for CNS regeneration

In the field of CNS regeneration, biomolecule delivery has been extensively explored to stimulate endogenous repair mechanisms, promote regeneration, and target inhibitory factors. Many biomolecules, such as growth factors, require sustained tissue concentrations for efficacy (from days to months), are rapidly cleared from tissue, and have poor penetration across the blood brain barrier (BBB) and blood–spinal cord barrier (BSCB), often rendering oral and intravenous delivery methods ineffective. [30]. Consequently, the controlled, local release of biomolecules is crucial for effective regeneration of the CNS. Six types of drug delivery strategies will be reviewed here: active biomaterials; direct and systemic delivery; polymer micro/nanoparticles; implanted polymer scaffolds; injectable hydrogels; and particle/scaffold composites. Key literature will be described to examine the progress of biomolecule delivery for CNS regeneration and future directions.

2.1. Active biomaterials

Active biomaterials – *i.e.*, materials that promote cell survival and regeneration without the delivery of additional factors – have been investigated in CNS repair and have shown some promise; however, they may have limited or transient effects that are insufficient to improve functional outcomes. The combination of active biomaterials with the controlled release of biomolecules may be a more promising approach.

Fig. 1. Chitosan channel implantation after spinal cord transection facilitates tissue bridging, NSPC survival, and behavioral improvement over time. (A) Photograph of the surgical implantation of fibrin-filled chitosan channels. (B) Tissue bridges obtained from animals 2 weeks after implantation. (C and D) Longitudinal section of tissue bridge demonstrating NSPC survival after 6 weeks in an animal receiving dbcAMP pre-treatment (dbcAMP, 4div). Boxed area in (C) is magnified in (D).
Reproduced with permission from Kim et al. [17] PLoS One. Copyright 2011 Kim et al.

Some natural polymers have shown limited beneficial tissue effects in the injured spinal cord without the delivery of exogenous factors. Collagen showed mixed results by promoting the growth of both astroglial cells and CST axons when injected as a fluid into the transected spinal cord [31]. A chitosan tube filled with type 1 collagen showed greater axonal growth in a partially transected SCI model than an unfilled chitosan tube [32]. Fibrin that was injected into a hemisection SCI also showed increased neurite density and reduced astroglial response relative to injury alone; however, there was no functional recovery [33].

Modified hyaluronan (HA) formulations have also shown efficacy in the CNS. In a model of chronic subarachnoid scarring after SCI, a physical blend of hyaluronan and methylcellulose (HAMC) was injected intrathecally 1 d after injury and was shown to reduce the extent of inflammation, decrease cavity volume, improve axonal conduction, and increase locomotor recovery [34]. The tissue benefits were attributed to the attenuated inflammatory response associated with HA [35]. A cross-linked HA covalently modified with laminin [36] or the laminin peptide sequence isoleucine–lysine–valine–alanine–valine (IKVAV) [37] implanted into the lesioned rat cortex was able to support the integration of cells, blood vessels, and axons.

Injectable scaffolds formed of self-assembling peptide nanofibers improved outcomes following CNS injury. An arginine–alanine–aspartate–alanine (RADA) scaffold induced tissue and axon growth as well as some functional repair following injection into the severed optic nerve tract [38]. An IKVAV-based self-assembling peptide scaffold was able to inhibit glial scar formation and induce axonal growth and functional recovery after both compression and severe contusion injury [39]. Importantly, it was shown that not only were rostral motor and sensory axons able to respond to the injected IKVAV scaffold [39], but serotonergic fiber density caudal to the lesion also increased [40]. The broad regenerative effects of self-assembling peptide nanofibers may be promising for treatment of many CNS disorders.

2.2. Direct and systemic delivery

Bolus injection into or near the site of injury or disease provides a localized, transient dose and has been used to gain insight into the mechanism of action of many biomolecules, such as the neurotrophins [41]. For example, neurotrophin-3 (NT-3) was first shown to induce sprouting of the corticospinal tract (CST) axons after injection of 300–500 μg into the transected rat spinal cord [42]. The enzyme chrondroitinase ABC (chABC) was also first demonstrated to degrade the inhibitory matrix and promote CST growth and functional recovery after repeated intrathecal bolus injection [43]. Bolus delivery into tissue provides important insights, but the biomolecules are rapidly taken up and/or degraded, resulting in a transient effect.

Intrathecal or intraventricular infusion *via* an osmotic minipump has been widely used to provide sustained delivery of biomolecules and thus enhanced efficacy, but it is highly invasive and can cause scarring, compression of the spinal cord [44], and infections in clinical use [45]. In a series of studies examining rodent models of SCI, brain-derived neurotrophic factor (BDNF), NT-3, and factors targeting the Nogo receptor (NgR) resulted in axonal growth and improved locomotor function after intrathecal infusion for 14–28 d [46–50]. These, and other similar studies, rarely investigated the effects of dosage or determined release kinetics and the total delivered dose of biomolecule ranges from 100 to 5000 μg per animal. The potential of minipump infusion for combinatorial drug delivery was recently demonstrated in a SCI model, with the combined release of chABC and NT-3 [51]. The intracerebral infusion of glial-derived neurotrophic factor (GDNF, 126 μg over 7 d) increased cell proliferation and the formation of new neurons in a middle cerebral artery occlusion (MCAO) model of stroke [52]. Similar results, as well as functional recovery, were observed in a rat forebrain stroke model after the sequential intraventricular infusion of epidermal growth factor (EGF) and erythropoietin (EPO) at concentrations of 10 μg/mL and 1365 IU/mL, respectively [53]. In this study, endogenous neural stem

cells were stimulated to promote brain tissue repair; however, the highly invasive nature of this strategy limits its ultimate translation to the clinic. Thus, notwithstanding the benefit of sequential delivery for tissue regeneration, less invasive strategies are required, yet still must circumvent or overcome the BBB.

Systemic delivery is advantageous for the clinical translation of strategies for CNS regeneration, yet may result in off-target effects and require specific strategies to overcome the BBB. Focused ultrasound opens up the BBB in specific brain areas and has demonstrated beneficial effects, yet is non-specific to what crosses into the brain [54–56]. An alternative strategy is to couple a molecular chaperone to cross the BBB. For example, the Pardridge lab has examined intravenous delivery of proteins coupled to antibodies to enable transport across the BBB for the treatment of stroke. The delivery of BDNF, fibroblast growth factor 2 (FGF2), GDNF, EPO and tumor necrosis factor α (TNF-α) receptor was able to successfully reduce the stroke infarct volume and improve functional recovery after 24 h [57–62] and 7 d [63]. A similar strategy showed functional improvements at 14 d after BDNF-fusion protein delivery in a mouse model of Alzheimer's disease (AD) [64]. However, the long-term effects of this delivery strategy on tissue regeneration and behavior have not been examined. The clinical potential of systemic delivery is illustrated by the delivery of the glucagon-like peptide 1 antagonist exendrin-4. Intraperitoneal (i.p.) injections of exendrin-4 in a mouse model of PD resulted in neurogenesis and behavioral improvements [65] and subcutaneous delivery in a randomized controlled trial in humans also demonstrated modest functional effects [66]. Unfortunately, high doses of biomolecules are often required for systemic delivery because they are dispersed throughout the body where they can have off-target effects, (*e.g.*, uncontrolled cell growth, neuropathic pain) and/or be degraded prior to entering the brain. Furthermore, many biomolecules are recycled out of the brain rapidly, if they do not have a specific target therein.

2.3. Micro/nanoparticles

Polymeric micro/nanoparticle formulations have been extensively developed for the controlled release of both hydrophobic and hydrophilic biomolecular drugs. Challenges in the development of micro/nanoparticle formulations for CNS regeneration include the preservation of biomolecule activity and biomolecule diffusion from the delivery site to the target site. Most micro/nanoparticles used in the CNS have been composed of poly(lactic-*co*-glycolic acid) (PLGA), a biodegradable polymer approved for clinical use. After CNS delivery *via* tissue injection, PLGA microparticles induce a mild microglial/astroglial response and the microparticles can remain at the injection site for up to 4 months [67,68].

PLGA microparticles encapsulating nerve growth factor (NGF) or GDNF have been extensively studied by Benoit and Mantero-Manei for the treatment of AD, PD, and retinal degeneration [69–73]. The neurotrophins were released over 6 weeks *in vitro* with a high burst release characteristic of PLGA microparticles and resulted in both tissue regeneration and functional improvements. High concentrations of surfactants were required to disperse the microparticles for injection, which is undesirable as the surfactants can be cytotoxic [69–73]. PLGA microparticles have also been applied in the treatment of glaucoma, with the delivery of GDNF alone [74,75] and in combination with vitamin E [76].

PLGA nano/microparticles have also been studied for CNS regeneration. For example, sonic hedgehog (SHH) was encapsulated in PLGA microparticles and shown to promote both axon growth and functional improvement after SCI [77]. In another study, GDNF encapsulated in PLGA nanoparticles induced the growth of neuronal fibers and promoted functional improvement [78]. Interestingly, 20 nm nanoparticles were found along the spinal cord both rostral and caudal to the injection site, while 100 nm nanoparticles remained at the injection site, suggesting that the 20 nm nanoparticles were transported by axons. An

unusual nanoparticle formulation, consisting of a highly branched poly(amidoamine) dendrimer grafted to carboxymethyl chitosan, was used to deliver methylprednisolone to the injured spinal cord [79]. A biphasic release with an initial burst was observed *in vitro* and locomotor recovery in a hemisection SCI model was observed *in vivo*. The diffusion distance of nanoparticles injected into the brain was found to increase after coating with poly(ethylene glycol) (PEG) [80], which is known to limit protein adsorption and macrophage engulfment. Coating nanoparticles with peptide sequences that enhance their delivery across the BBB has also been pursued; however, this has not yet been exploited clinically for CNS regeneration [81].

2.4. Implanted scaffolds

Surgically implanted polymer scaffolds are of interest for CNS regeneration due to their ability to physically support the infiltration of host cells as well as deliver biomolecules locally to the injury site. Release kinetics can be zero-order [82,83] or higher [84–86] with release periods from 7 d up to 42 d. However, solid scaffolds require more invasive surgeries than injectable systems and are thus inherently limited to sites that allow implantable materials, such as the transected or hemisected spinal cord.

A scaffold formed of PLGA microparticles was able to deliver a DNA plasmid or the growth factors NGF, vascular endothelial growth factor (VEGF), or FGF2, promoting beneficial endothelial cell infiltration but not significant neurite outgrowth in a hemisection model of SCI [82,84]. In a similar injury model, extensive angiogenesis and axon extension were observed after the implantation of freeze-dried agarose scaffolds containing BDNF [87]. A surgically implanted poly(hydroxyethylmethacrylate) (PHEMA) scaffold was also able to deliver BDNF and induce angiogenesis and axonal sprouting, but axon outgrowth diminished between 2 and 4 weeks, indicating that the BDNF was quickly released from the scaffold and thus had a transient effect [88]. The release of BDNF from a collagen scaffold was extended to 14 d *in vitro* by modifying BDNF with a collagen-binding peptide, and resulted in axonal sprouting and functional recovery *in vivo* [89].

Implanted polymer scaffolds have also been studied by the Shea lab for the delivery of DNA and viral vectors to induce regeneration in the spinal cord. A multichannel PLGA scaffold was able to deliver a lipid–DNA complex (lipoplex) and induce transfection of primarily Schwann cells, macrophages and fibroblasts for up to 3 weeks after implantation in a hemisection SCI model [85,90]. Lentivirus encoding NT-3 or BDNF was also delivered from a PLGA multichannel bridge and was able to stimulate axon growth and myelination inside the scaffold [91]. A polymer–DNA complex (polyplex) encapsulated in a multichannel collagen scaffold resulted in NT-3 expression and increased axon growth into the scaffold, but did not improve behavioral outcomes in a complete transection SCI [86].

A cross-linked hyaluronan (HA) scaffold for delivery of a Nogo receptor antigen was implanted in both an MCAO model of stroke and a hemisection SCI [83,92,93]. The implanted scaffold was able to deliver the antibody for up to 4 weeks in the brain and 8 weeks in the spinal cord. In the stroke injury model, the scaffold promoted tissue repair and functional improvements [92] while in the SCI model, extensive cell and axon growth into the scaffold was observed [93]. Notwithstanding these exciting results, the highly invasive nature of these strategies limits their clinical translation.

2.5. Injectable hydrogels

Injectable hydrogels are perhaps the most widely investigated materials for local drug delivery to the CNS for regeneration. Hydrogels are advantageous because they have similar mechanical properties to native CNS tissue and provide a minimally invasive strategy for drug delivery. Hyaluronan, which comprises the extracellular matrix in the CNS, fibrin, and agarose is often used for local delivery [94]. The challenges of

effective hydrogel delivery strategies include maintaining growth factor activity and achieving a dose high enough to induce significant repair and functional recovery.

Fibrin is a natural hydrogel, commonly studied for the delivery of growth factors after SCI. Fibrin has been developed for the controlled release of several growth factors by Sakiyama-Elbert and colleagues [95–99]. A heparin binding domain was conjugated to the growth factors to transiently immobilize them within the gel and achieve near-linear release for up to 14 d *in vitro* [96]. NT-3 released from the drug delivery system (DDS) was able to induce axon sprouting following SC transection, while the fibrin gel reduced the astroglial response to injury [96–98]. Similar results were observed in a subacute hemisection model of injury [99]. However, no significant functional recovery was observed after NT-3 delivery after 12 weeks [98], suggesting that the dose of NT-3 delivered (approximately 4 ng per animal) was insufficient for regeneration. A fibrin gel without the heparin binding domain was used to deliver chABC [100]. The chABC was able to degrade the glycosoaminoglycans in the glial scar; however, no evaluation of functional recovery was performed. Fibrin was also used to deliver Cethrin, a Rho inhibitor, to the epidural space, where it promoted tissue sparing and functional recovery without allodynia in pre-clinical rodent models of SCI [101]. In a promising SCI phase I/II clinical trial [102], functional improvements were observed mainly in cervical SCI patients following epidural delivery of Cethrin in fibrin during spinal surgery [103,104]. However, the dura and subarachnoid space are formidable barriers, limiting diffusion into spinal cord tissue.

Agarose has also been applied for local delivery to the spinal cord. For example, an agarose gel incorporating lipid microtubules containing BDNF resulted in an increase in axonal density after hemisection SCI [105]. Although agarose was able to form a gel upon application, gelation was cumbersome, requiring cooling, and agarose induced an inflammatory response. The same drug delivery system (DDS) was able to release a thermostabilized formulation of chABC and NT-3 to promote the growth of sensory axons and serotonergic fibers and improve locomotor function [106]. The sustained release of proteins and small molecules at μg/mL concentrations from agarose *in vitro*, but not yet *in vivo*, was achieved using a multilayer film of PEG and poly(acrylic acid) [107,108].

A combination of natural and synthetic polymers has been studied by the Shoichet, Tator and Morshead labs for the minimally invasive delivery of proteins to the injured spinal cord and brain [109–113]. An injectable collagen gel incorporating EGF and FGF2 was delivered intrathecally to recruit spinal cord ependymal cells after compression injury [109]. The DDS promoted tissue sparing, indicated by reduced cavitation and greater white matter density, as well as ependymal cell proliferation. When FGF2 was delivered from a hyaluronan/methyl cellulose (HAMC) hydrogel following SCI, improved blood flow through the injured cord was seen at 7 d by dynamic flow computed tomography (CT) in addition to greater blood vessel density and reduced vessel permeability [110]. HAMC delivered epi-cortically to the stroke-injured brain provided the sustained release and penetration of PEGylated EGF (EGF–PEG) through the cortex, inducing stem cell proliferation in the subventricular zone (SVZ) [111]. EPO was also delivered in HAMC to both the spinal cord and brain, resulting in neuroprotection and reduced inflammatory response [112,113]. The challenge for this exciting strategy is to overcome the limited diffusion distance possible in brain tissue (usually less than 2–3 mm). While convection-enhanced delivery (constant, positive pressure flow at 0.5–3 μL/min) is useful for greater tissue penetration in the brain, the technique is highly invasive and can cause tissue damage [114,115].

Synthetic, cross-linked PEG has been studied to deliver neurotrophins to the injured CNS [116,117]. Ciliary neurotrophic factor (CNTF), released from an acrylated poly(lactide)-poly(ethylene glycol) (PLA-PEG-PLA) tri-block copolymer, stimulated neurite outgrowth in the explanted retina [116]. NT-3 was released over 2 weeks *in vitro* from the acrylated PLA-PEG-PLA hydrogel, and the DDS was injected into a hemisection model

of SCI and photopolymerized *in situ* [117]. NT-3 release from the DDS was detectable at 14 d and increased axon density in the CST and the rubrospinal tract (RST) at 42 d. Furthermore, NT-3-treated animals showed improved locomotor function in comparison with those treated with hydrogel alone [117].

A poly(*N*-isopropylacrylamide)-*g*-polyethylene glycol (PNiPAAm-PEG) hydrogel was tested for its ability to delivery BDNF to the injured spinal cord, exploiting the lower critical solution temperature (LCST) of PNiPAAm-PEG for *in situ* gelation and entrapment of BDNF [118] (Fig. 2). Following a cervical dorsal funiculus lesion that destroyed skilled reaching ability, the delivery of BDNF promoted RST axon growth into and near the lesion site at 8 weeks. Improvements in behavior were not consistent across all outcome measures and the study period, but BDNF delivery promoted modest recovery of both fine and spontaneous motor functions [118].

Recently, several novel hydrogels have been investigated for drug delivery in the CNS. A diblock copolypeptide was used to deliver bioactive NGF for 4 weeks to the uninjured mouse brain [119]. A tunable, sustained release strategy was recently developed, in which the protein of interest is expressed as a fusion with a Src homology domain 3 (SH3) and the hydrogel is modified with a corresponding SH3 binding peptide [120,121]. By controlling both the amount and affinity of the immobilized SH3 binding peptide coupled to the hydrogel, the release profile of the SH3 fusion protein can be moderated. Controlled release of bioactive FGF2 [120] and chABC [121] was demonstrated with this strategy *in vitro*, which obviates the use of PLGA particles and the protein degradation often associated with their formulation.

2.6. Particle/scaffold composites

Composite DD devices, which incorporate micro/nanoparticles into a polymer scaffold or hydrogel, allow for the release of multiple factors with differing release profiles [116]. The dispersion of micro/nanoparticles within a scaffold can also provide better localization and slower clearance *in vivo*. However, challenges in the development of a composite DDS for CNS regeneration include the maintenance of biomolecule activity and the delivery of an effective dose.
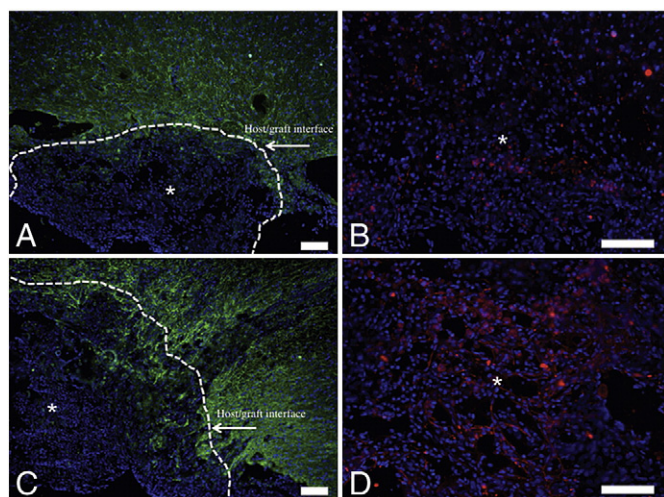

Fig. 2. PNiPAAm-PEG hydrogel-only controls and hydrogel + BDNF animals show a similar presence of glial scar formation; hydrogel + BDNF is permissive to host axonal growth. GFAP (green) stain astrocytes around the graft site, and RT-97 label host axons. DAPI labeling (blue) was used to identify cell nuclei. (A and C) GFAP staining for hydrogel-only controls and hydrogel + BDNF, respectively. (B and D) Images obtained at a higher magnification, showing RT-97 labeling for hydrogel-only controls and hydrogel + BDNF, respectively. Asterisks indicate the specific area of the lesion that was taken at a higher magnification in B and D. Scale bar = 100 mm.
Reproduced with permission from Grous et al. [118], J Neurosurgery: Spine. Copyright 2013 AANS.

A composite DDS consisting of PLGA microparticles dispersed in a photopolymerized PLA-PEG-PLA hydrogel was tested *in vitro* for the simultaneous delivery of CNTF and NT-3, where the CNTF rapidly released from the hydrogel and the NT-3 released from the microparticles over 60 d [116]. A similar system was used in a proof-of-concept study for the delivery of BDNF and GDNF to the brain [122]. Intended for the treatment of PD, the PLA-PEG-PLA hydrogel was formulated with GDNF microparticles at one end of the implant, to promote the survival of transplanted neural stem cells, and BDNF microparticles at the other, to stimulate neurite outgrowth. In healthy rats, BDNF was detected for up to 56 d whereas GDNF was completely released within 28 d [122]. This microparticle/hydrogel system is promising for the delivery of neurotrophic factors, but has not yet shown efficacy in a model of CNS injury or disease.

Composite DDS combining natural polymers with PLGA micro/nanoparticles have also been investigated for SCI [123,124]. In a hemisection SCI model, methylprednisolone (MP) was encapsulated in PLGA nanoparticles and delivered in an agarose gel. The delivery of MP resulted in reduced apoptosis, lesion volume and astroglial response, while improving locomotor function. However, the side effects commonly associated with systemic MP delivery were not investigated [123]. An alginate hydrogel/PLGA microparticle DDS was also investigated in a hemisection SCI model for the delivery of GDNF [124]. The composite DDS resulted in greater neurite density at 3 months in comparison with delivery from alginate alone, which was most effective at 6 weeks. Functional recovery was only observed for the alginate hydrogel with GDNF, suggesting that early release of GDNF is critical to promote repair [124].

The delivery of both hydrophobic and hydrophilic drugs to the CNS *via* a composite HAMC/PLGA micro/nanoparticle DDS has been investigated by the Shoichet lab [125–129]. The delivery of FGF2 to the spinal cord was found to improve blood vessel density without the development of proliferative lesions observed with intrathecal infusion [125]. Cyclosporin A was delivered epi-cortically in a stroke model and detected in the brain for up to 24 d, providing an alternative to high dose systemic delivery or intraventricular infusion [126]. Sequential controlled release of EGF–PEG and EPO from a composite DDS to the stroke-injured mouse brain resulted in tissue repair without the tissue damage associated with catheter/minipump infusion [127] (Fig. 3). Specifically, the sequential delivery of EGF–PEG and EPO resulted in increased numbers of neural precursor cells in the subventricular zone (SVZ), a reduced inflammatory response, a smaller cavity volume, and greater survival of neurons in the cortex. Sequential delivery was achieved by encapsulating EGF–PEG in PLGA nanoparticles for release from days 1–14 and EPO first in PLGA nanoparticles that were then coated in surface-eroding poly(sebacic acid), yielding biphasic composite microparticles for release from days 7–21 [127]. *In vitro* release of bioactive NT-3 [128] and anti-NogoA [129] was also demonstrated with a composite comprised of PLGA nano/microparticles in HAMC.

3. Combination strategies: cell and biomolecule co-delivery

Combination strategies that include the controlled and sustained delivery of both biomolecular therapeutics and cells capable of regenerating damaged tissue are promising with regard to the enhancement of tissue regeneration in the injured CNS. In the context of injury to the brain and spinal cord, functional capacity is lost due to the death of neurons (*e.g.*, Parkinson's and Alzheimer's Diseases, SCI) and oligodendrocytes (*e.g.*, multiple sclerosis, demyelination in SCI). Neural stem/progenitor cells (NSPCs) have the capacity to differentiate into neurons, astrocytes and oligodendrocytes upon exposure to physical [130] and chemical cues [131]. Thus, the delivery of NSPCs offers a potential strategy to replace these lost cells. However, significant hurdles that face the field of stem cell delivery are low cell viability, host tissue integration and uncontrolled differentiation upon transplantation. The latter issue is of significance because it is essential that stem cells differentiate into the desired cell type, and do not become tumorigenic.
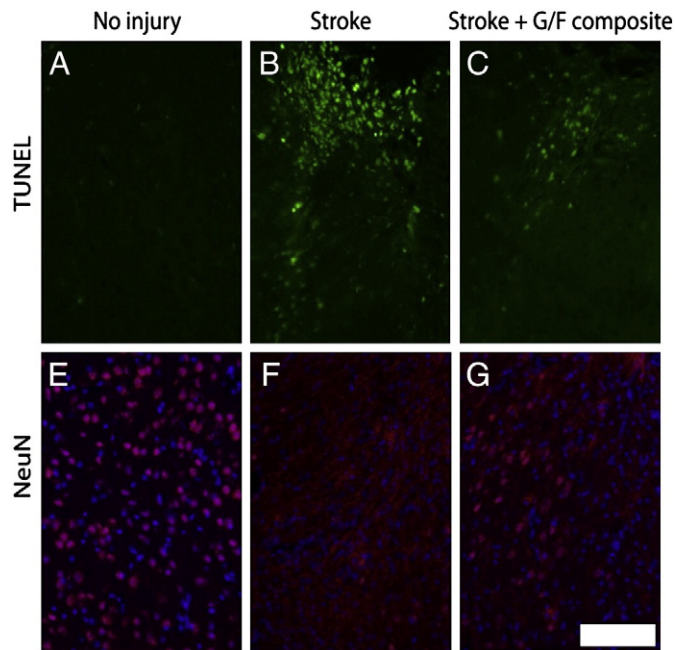
Fig. 3. Sequential delivery of EGF–PEG and EPO from a nanoparticle/hydrogel composite attenuates the injury response and cell death, and increases NeuN + mature neurons in the penumbra 18 d after stroke. At 18 days post stroke, (a–c) the level of TUNEL + cells decreases significantly in the injured cortex following growth factor treatment compared to stroke + vehicle controls. (e–g) Stroke + G/F composite animals have significantly more NeuN + cells in the peri-infarct region compared to vehicle controls, yet still significantly less than uninjured tissue controls. Scale bar = 100 μm.
Reproduced with permission from Wang et al. [127], Biomaterials. Copyright 2013 Elsevier.

Fig. 4. Effect of delivering neural stem cells (NSCs) in a biomolecule-containing biopolymer (RADA$_{16}$-IKVAV) into the injured brain. Gross morphological examinations of the brain wound defect (G–I), histology (H/E staining) of brain neural tissue in coronary sections (g–i), and immunohistochemistry of coronal sections (g′–i′), 6 weeks after surgery. The white dashed lines outline the wound margin to distinguish the area of original host tissue and neoregenerated neural tissue. Neurons were labeled with Nissl stain (red) and the nuclei were counterstained with DAPI (blue). The arrows indicate the presence of the hydrogel vehicle remaining in the injured cavities. Wound healing is enhanced in the group treated with NSCs in RADA$_{16}$-IKVAV (G, g, g′) compared to the groups treated with RADA$_{16}$-IKVAV alone (H,h,h′), or saline (I, i, i′). Scale bar = 200 μm.
Reproduced with permission from Cheng et al. [133], Biomaterials. Copyright 2013 Elsevier.

Efforts to overcome these limitations in cell delivery have included co-delivery with therapeutic molecules such as growth factors. The therapeutic molecule can be beneficial to the cells that are being delivered (*e.g.* to improve cell viability or direct cell differentiation), as well as to the target tissues themselves (*e.g.* enhance host tissue regeneration, glial scar degradation). Moreover, exogenous cells that are transplanted into the injury site can integrate into the host tissue to provide cellular functions, or they may secrete paracrine factors that promote the regeneration of the host tissue. The ability to control and sustain the delivery of both cells and therapeutic molecules is vital to ensure that they reside in the target tissue for a sufficient period of time to interact with each other and also with the injured tissue. As described in the previous section, biomaterials can be used to effectively control the release of therapeutic molecules; importantly, they can also provide both physical support and chemical cues for transplanted cells. The following section describes efforts to use biomaterials to control the delivery of both therapeutic molecules and cells to promote tissue regeneration in the injured CNS.

Strategies for using biomaterials to co-deliver cells and growth factors into the CNS include directly immobilizing growth factors to the biomaterials, or encapsulating them within particles. Direct immobilization of GDNF to fibrous poly(ε-caprolactone) (PCL) has been reported to increase the survival and neuronal differentiation of NSPCs upon transplantation into the rat brain compared to cells delivered in the absence of the PCL vehicle. Interestingly, the presence of immobilized GDNF to the PCL vehicle increased neurite outgrowth compared to PCL alone [132]. Increased neuronal differentiation of NSPCs delivered into the brain has also been reported for co-delivery with self-assembling peptide (SAP) polymers immobilized with the bioactive laminin-derived peptide sequence IKVAV [133] (Fig. 4). Differentiation of NSPCs into oligodendrocytes has also been achieved by culturing NSPCs in a hyaluronan/methyl cellulose (HAMC) biopolymer composite that was immobilized with recombinant platelet derived growth factor-
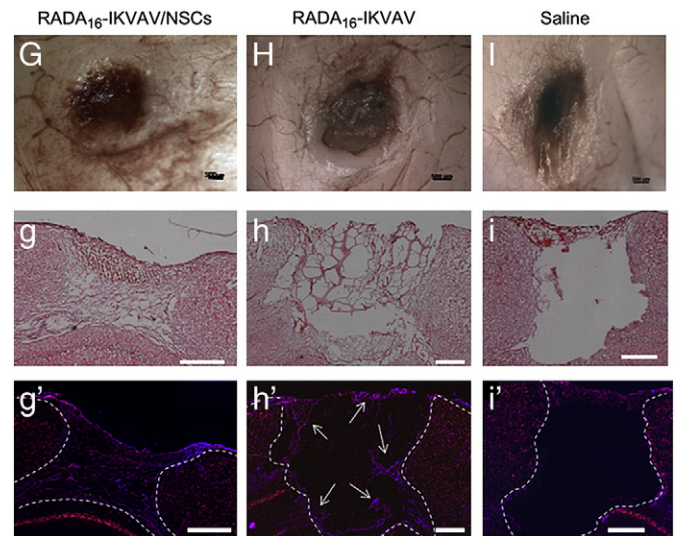
A (rPDGF-A); delivery of NSPCs in rPDGF-A-modified HAMC into a rat spinal cord (clip compression) injury model demonstrated improved behavioral function and improved tissue repair relative cells delivered in media alone [134].

Another strategy for the sustained release of growth factors is to take advantage of the binding affinity between the polymer and growth factor to be delivered. Johnson et al. used fibrin-based hydrogels covalently modified with heparin-binding peptides to bind heparin and subsequently the heparin-binding growth factors PDGF-AA and NT-3. Upon delivery with embryonic stem cell-derived neural progenitor cells, functional recovery and tissue repair were observed; however, tumor formation was also observed, underlying the importance of controlling cell phenotype *in vivo*. [135].

Encapsulation of growth factors into stable microparticles offers another method for sustained release. Moreover, microparticles can be coated with extracellular matrix proteins such as fibronectin or laminin to promote cell adsorption onto the particles. By coating VEGF-encapsulated PLGA particles with fibronectin, Bible et al. demonstrated improved adhesion of human NSPCs to these particles, which were then transplanted into the stroke-injured brain. This strategy enabled dual functionality in that the VEGF was able to promote vascularization into the graft site by recruiting host endothelial cells that were able to interact with the transplanted NSPCs to form 'primary neurovascular networks' [136]. Similarly, NT-3-encapsulated PLGA microspheres coated with laminin and poly-D-lysine were used to deliver mesenchymal stromal cells (MSCs) into hemi-Parkinsonian rats; after 8 weeks post-transplantation, MSCs delivered in PLGA particles in the absence or in the presence of NT-3 increased MSC survival two- or three-fold, respectively, relative to the cells delivered in the absence of PLGA particles [137]. Importantly, rats treated with NT-3-PLGA particles and MSCs showed significant behavioral improvements compared to treatment groups with MSCs + PLGA alone and MSCs alone. Matsuse et al. have also demonstrated that using collagen sponges containing bFGF-

releasing gelatin microspheres to deliver bone marrow-derived MSCs into the stroke-injured rat brain decreased infarct volume and improved behavioral activity relative to various vehicle controls [138].

The combination of delivering cells and therapeutic molecules into the injured CNS offers a multi-functional approach to repairing the injured CNS tissue. However, the complex interactions between the drugs, transplanted cells and host tissue must be clearly defined and understood to enable the clinical translation of this strategy.

4. Conclusion

Cell and/or biomolecule delivery offers promise for the treatment of CNS injury and disease, yet key challenges in delivery remain. In the CNS, the blood–brain barrier serves as a formidable barrier, limiting diffusion of many molecules into the brain and thus requiring local release strategies. These in turn are often highly invasive, resulting in tissue damage to the very tissue that one is trying to repair. This is particularly true for implanted polymer scaffolds in, for example, hemi-sected models of spinal cord injury. While an excellent model to study repair, patients do not present with a hemi-sected cord, making these structures less viable for translation to the clinic. Less invasive strategies, such as those that use injectable biomaterials, provide great promise, yet care must be taken that, after injection, the biomaterial does not swell, which itself could compress neural tissue and cause further tissue and functional loss. Even with local delivery, however, the challenge of penetrating deep within the brain is significant. While studies in small animal models have demonstrated success in, for example, stimulating the endogenous stem cells in the brain, scaling this to the human brain is non-trivial, requiring additional strategies for enhanced tissue penetration — some of which have yet to be invented. Taking advantage of the leaky vasculature, for example in applications of brain cancer, may be a useful strategy to achieve deep brain penetration as the brain is highly vascularized. Thus, in some cases the vascular system may be used to achieve local delivery; however, systemic toxicity and/or off-target effects would remain as undesired side effects.

Cell transplantation offers great promise in the CNS especially in light of the advent of human induced pluripotent stem cells, where the patients own cells can be re-programmed prior to transplantation. This overcomes many of the hurdles of immune rejection, yet cell survival and integration remain as two key challenges. Innovative delivery strategies could be the key to achieving greater success in cell delivery. One advantage of the CNS is that defined cell populations are missing/degenerating in defined regions of the CNS in some of the diseases, such as Parkinson's Disease (*i.e.* the dopaminergic cells), retinitis pigmentosa (*i.e.*, photoreceptors) or age-related macular degeneration (*i.e.*, retinal pigmented epithelium, RPE, cells). With a defined tissue in which to transplant, one can envision transplanting cells exactly where they are missing, yet key to survival is their integration with the neural circuitry of the CNS. Biomaterials that promote greater cell survival serve as excellent carriers for use in cell transplantation. The concomitant strategy to overcome barriers to cell integration further enhances the likelihood for greater cell survival. The co-delivery of these factors that provide a suitable microenvironment for cell survival and integration after transplantation is an area of active research.

Overall, in this review, we have highlighted several methods of delivery that have demonstrated efficacy in pre-clinical models, providing controlled biomolecule release, tissue repair, and functional benefit. Nevertheless, each method has associated challenges that must be overcome for clinical translation. The highly invasive nature of direct delivery and implanted polymer scaffolds has raised concerns in clinical trials (for direct delivery) and limits their use to some types of CNS injuries (*i.e.*, for implanted polymer scaffolds). The biomolecule dose currently achievable for polymer micro/nanoparticles, implanted scaffolds, injectable hydrogels, and particle/scaffold composites may prove to be too low for clinical application. Implanted scaffolds and injectable, hydrogels also often have short release periods that may not be suitable

for many biomolecules. Active biomaterials may be able to be quickly translated to the clinic, but have shown limited efficacy thus far. The co-delivery of cells and biomolecules has demonstrated improved cell survival, cell function, and tissue regeneration in comparison with cell delivery alone. Yet, key mechanisms underlying the success of co-delivery remain to be elucidated, and the challenges of large-scale cell production and the long-term control of immunogenicity and cell fate must be addressed for clinical use.

Acknowledgments

References

[1] F. Lim, A.M. Sun, Microencapsulated islets in diabetic rats, Science 213 (1981) 1146.
[2] J.R. Nixon, Microencapsulation, M Dekker, New York, 1976.
[3] W.L. Chick, A.A. Like, V. Lauris, et al., Hybrid artificial pancreas, Trans. Am. Soc. Artif. Intern. Organs 21 (1975) 8–15.
[4] H.M. Creque, R. Langer, J. Folkman, One month of sustained-release of insulin from a polymer implant, Diabetes 29 (1980) 37–40.
[5] A.M. Albisser, B.S. Leibel, T.G. Ewart, et al., An artificial endocrine pancreas, Diabetes 23 (1974) 389–396.
[6] H. Shamoon, H. Duffy, N. Fleischer, et al., The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes-mellitus, N. Engl. J. Med. 329 (1993) 977–986.
[7] J. Kost, T.A. Horbett, B.D. Ratner, et al., Glucose-sensitive membranes containing glucose-oxidase — activity, swelling, and permeability studies, J. Biomed. Mater. Res. 19 (1985) 1117–1133.
[8] H. Thurow, K. Geisen, Stabilization of dissolved proteins against denaturation at hydrophobic interfaces, Diabetologia 27 (1984) 212–218.
[9] M.E. Sugamori, M.V. Sefton, Microencapsulation of pancreatic islets in a water insoluble polyacrylate, ASAIO Trans. 35 (1989) 791–799.
[10] H. Uludag, J.P. Horvath, J.P. Black, et al., Viability and protein secretion from human hepatoma (Hepg2) cells encapsulated in 400 μm polyacrylate microcapsules by submerged nozzle liquid jet extrusion, Biotech. Bioeng. 44 (1994) 1199–1204.
[11] D.W. Scharp, P. Marchetti, Encapsulated islets for diabetes therapy: history, current progress, and critical issues requiring solution, Adv. Drug Deliv. Rev. 67-68 (2013) 35–73.
[12] E.G. Campioni, J.N. Nobrega, M.V. Sefton, HEMA/MMMA microcapsule implants in hemiparkinsonian rat brain: biocompatibility assessment using [3H]PK11195 as a marker for gliosis, Biomaterials 19 (1998) 829–837.
[13] J.J. Vallbacka, J.N. Nobrega, M.V. Sefton, Tissue engineering as a platform for controlled release of therapeutic agents: implantation of microencapsulated dopamine producing cells in the brains of rats, J. Control. Release 72 (2001) 93–100.
[14] T. Roberts, U. De Boni, M.V. Sefton, Dopamine secretion by PC12 cells microencapsulated in a hydroxyethyl methacrylate–methyl methacrylate copolymer, Biomaterials 17 (1996) 267–275.
[15] P. Aebischer, G. Panol, P.M. Galletti, An intraperitoneal receptacle for macroencapsulated endocrine tissue, ASAIO Trans. 32 (1986) 130–133.
[16] P. Aebischer, N.A.M. Pochon, B. Heyd, et al., Gene therapy for amyotrophic lateral sclerosis (ALS) using a polymer encapsulated xenogenic cell line engineered to secrete hCNTF, Hum. Gene Ther. 7 (1996) 851–860.
[17] H. Kim, T. Zahir, C.H. Tator, et al., Effects of dibutyryl cyclic-AMP on survival and neuronal differentiation of neural stem/progenitor cells transplanted into spinal cord injured rats, PLoS One 6 (2011).
[18] J.P. Vacanti, M.A. Morse, W.M. Saltzman, et al., Selective cell transplantation using bioabsorbable artificial polymers as matrices, J. Pediatr. Surg. 23 (1988) 3–9.
[19] C.A. Vacanti, R. Langer, B. Schloo, et al., Synthetic-polymers seeded with chondrocytes provide a template for new cartilage formation, Plast. Reconstr. Surg. 88 (1991) 753–759.
[20] L.G. Cima, J.P. Vacanti, C. Vacanti, et al., Tissue engineering by cell transplantation using degradable polymer substrates, J. Biomech. Eng. Trans. ASME 113 (1991) 143–151.
[21] J.C.Y. Dunn, R.G. Tompkins, M.L. Yarmush, Long-term *in vitro* function of adult hepatocytes in a collagen sandwich configuration, Biotechnol. Prog. 7 (1991) 237–245.
[22] A. Khademhosseini, J.P. Vacanti, R. Langer, Progress in tissue engineering, Sci. Am. 300 (2009) (64–+).
[23] J. Rouwkema, N.C. Rivron, C.A. van Blitterswijk, Vascularization in tissue engineering, Trends Biotechnol. 26 (2008) 434–441.
[24] M.W. Tibbitt, K.S. Anseth, Dynamic microenvironments: the fourth dimension, Sci. Transl. Med. 4 (2012).
[25] M.V. Sefton, J.E. Babensee, K.A. Woodhouse, Innate and adaptive immune responses in tissue engineering, Semin. Immunol. 20 (2008) 83–85.
[26] P.C. Johnson, A.G. Mikos, J.P. Fisher, et al., Strategic directions in tissue engineering, Tissue Eng. 13 (2007) (2827–+).
[27] J.C. DiNunzio, R.O. Williams, CNS disorders—current treatment options and the prospects for advanced therapies, Drug Dev. Ind. Pharm. 34 (2008) 1141–1167.

[28] M. Cully, Drug development: chemical brace, Nature 503 (2013) S10–S12.
[29] A. Rogalewski, A. Schneider, E.B. Ringelstein, et al., Toward a multimodal neuroprotective treatment of stroke, Stroke 37 (2006) 1129–1136.
[30] F.-M. Chen, M. Zhang, Z.-F. Wu, Toward delivery of multiple growth factors in tissue engineering, Biomaterials 31 (2010) 6279–6308.
[31] E.A.J. Joosten, P.R. Bar, W.H. Gispen, Collagen implants and corticospinal axonal growth after mid-thoracic spinal-cord lesion in the adult-rat, J. Neurosci. Res. 41 (1995) 481–490.
[32] X.G. Li, Z.Y. Yang, A.F. Zhang, et al., Repair of thoracic spinal cord injury by chitosan tube implantation in adult rats, Biomaterials 30 (2009) 1121–1132.
[33] P.J. Johnson, S.R. Parker, S.E. Sakiyama-Elbert, Fibrin-based tissue engineering scaffolds enhance neural fiber sprouting and delay the accumulation of reactive astrocytes at the lesion in a subacute model of spinal cord injury, J. Biomed. Mater. Res. A 92A (2010) 152–163.
[34] J.W. Austin, C.E. Kang, M.D. Baumann, et al., The effects of intrathecal injection of a hyaluronan-based hydrogel on inflammation, scarring and neurobehavioural outcomes in a rat model of severe spinal cord injury associated with arachnoiditis, Biomaterials 33 (2012) 4555–4564.
[35] D. Gupta, C.H. Tator, M.S. Shoichet, Fast-gelling injectable blend of hyaluronan and methylcellulose for intrathecal, localized delivery to the injured spinal cord, Biomaterials 27 (2006) 2370–2379.
[36] S.P. Hou, Q.Y. Xu, W.M. Tian, et al., The repair of brain lesion by implantation of hyaluronic acid hydrogels modified with laminin, J. Neurosci. Methods 148 (2005) 60–70.
[37] Y.T. Wei, W.M. Tian, X. Yu, et al., Hyaluronic acid hydrogels with IKVAV peptides for tissue repair and axonal regeneration in an injured rat brain, Biomed. Mater. 2 (2007) S142–S146.
[38] R.G. Ellis-Behnke, Y.X. Liang, S.W. You, et al., Nano neuro knitting: peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 5054–5059.
[39] V.M. Tysseling-Mattiace, V. Sahni, K.L. Niece, et al., Self-assembling nanofibers inhibit glial scar formation and promote axon elongation after spinal cord injury, J. Neurosci. 28 (2008) 3814–3823.
[40] V.M. Tysseling, V. Sahni, E.T. Pashuck, et al., Self-assembling peptide amphiphile promotes plasticity of serotonergic fibers following spinal cord injury, J. Neurosci. Res. 88 (2010) 3161–3170.
[41] P. Lu, M.H. Tuszynski, Growth factors and combinatorial therapies for CNS regeneration, Exp. Neurol. 209 (2008) 313–320.
[42] L. Schnell, R. Schneider, R. Kolbeck, et al., Neurotrophin-3 enhances sprouting of corticospinal tract during development and after adult spinal-cord lesion, Nature 367 (1994) 170–173.
[43] E.J. Bradbury, L.D.F. Moon, R.J. Popat, et al., Chondroitinase ABC promotes functional recovery after spinal cord injury, Nature 416 (2002) 636–640.
[44] L.L. Jones, M.H. Tuszynski, Chronic intrathecal infusions after spinal cord injury cause scarring and compression, Microsc. Res. Tech. 54 (2001) 317–324.
[45] K.A. Follett, R.L. Boortz-Marx, J.M. Drake, et al., Prevention and management of intrathecal drug delivery and spinal cord stimulation system infections, Anesthesiology 100 (2004) 1582–1594.
[46] L.B. Jakeman, P. Wei, Z. Guan, et al., Brain-derived neurotrophic factor stimulates hindlimb stepping and sprouting of cholinergic fibers after spinal cord injury, Exp. Neurol. 154 (1998) 170–184.
[47] E.J. Bradbury, S. Khemani, V.R. King, et al., NT-3 promotes growth of lesioned adult rat sensory axons ascending in the dorsal columns of the spinal cord, Eur. J. Neurosci. 11 (1999) 3873–3883.
[48] T. GrandPre, S.X. Li, S.M. Strittmatter, Nogo-66 receptor antagonist peptide promotes axonal regeneration, Nature 417 (2002) 547–551.
[49] S.X. Li, S.M. Strittmatter, Delayed systemic Nogo-66 receptor antagonist promotes recovery from spinal cord injury, J. Neurosci. 23 (2003) 4219–4227.
[50] B.X. Ji, M.W. Li, S. Budel, et al., Effect of combined treatment with methylprednisolone and soluble Nogo-66 receptor after rat spinal cord injury, Eur. J. Neurosci. 22 (2005) 587–594.
[51] R.-R. Zhao, M.R. Andrews, D. Wang, et al., Combination treatment with anti-Nogo-A and chondroitinase ABC is more effective than single treatments at enhancing functional recovery after spinal cord injury, Eur. J. Neurosci. 38 (2013) 2946–2961.
[52] T. Kobayashi, H. Ahlenius, P. Thored, et al., Intracerebral infusion of glial cell line-derived neurotrophic factor promotes striatal neurogenesis after stroke in adult rats, Stroke 37 (2006) 2361–2367.
[53] B. Kolb, C. Morshead, C. Gonzalez, et al., Growth factor-stimulated generation of new cortical tissue and functional recovery after stroke damage to the motor cortex of rats, J. Cereb. Blood Flow Metab. 27 (2007) 983–997.
[54] M. Kinoshita, N. McDannold, F.A. Jolesz, et al., Targeted delivery of antibodies through the blood–brain barrier by MRI-guided focused ultrasound, Biochem. Biophys. Res. Commun. 340 (2006) 1085–1090.
[55] J.F. Jordao, E. Thevenot, K. Markham-Coultes, et al., Amyloid-beta plaque reduction, endogenous antibody delivery and glial activation by brain-targeted, transcranial focused ultrasound, Exp. Neurol. 248 (2013) 16–29.
[56] A. Burgess, K. Hynynen, Noninvasive and targeted drug delivery to the brain using focused ultrasound, ACS Chem. Neurosci. 4 (2013) 519–526.
[57] Y. Zhang, W.M. Pardridge, Conjugation of brain-derived neurotrophic factor to a blood–brain barrier drug targeting system enables neuroprotection in regional brain ischemia following intravenous injection of tbe neurotrophin, Brain Res. 889 (2001) 49–56.
[58] Y. Zhang, W.M. Pardridge, Neuroprotection in transient focal brain ischemia after delayed intravenous administration of brain-derived neurotrophic factor conjugated to a blood–brain barrier drug targeting system, Stroke 32 (2001) 1378–1383.
[59] B.W. Song, H.V. Vinters, D.F. Wu, et al., Enhanced neuroprotective effects of basic fibroblast growth factor in regional brain ischemia after conjugation to a blood–brain barrier delivery vector, J. Pharmacol. Exp. Ther. 301 (2002) 605–610.
[60] Y. Zhang, W.M. Pardridge, Blood–brain barrier targeting of BDNF improves motor function in rats with middle cerebral artery occlusion, Brain Res. 1111 (2006) 227–229.
[61] R.J. Boado, Y. Zhang, Y. Zhang, et al., GDNF fusion protein for targeted-drug delivery across the human blood–brain barrier, Biotech. Bioeng. 100 (2008) 387–396.
[62] A.L. Fu, E.K.W. Hui, J.Z. Lu, et al., Neuroprotection in experimental stroke in the rat with an IgG-erythropoietin fusion protein, Brain Res. 1360 (2010) 193–197.
[63] R.K. Sumbria, R.J. Boado, W.M. Pardridge, Combination stroke therapy in the mouse with blood–brain barrier penetrating IgG-GDNF and IgG-TNF decoy receptor fusion proteins, Brain Res. 1507 (2013) 91–96.
[64] J.P. Zhou, Z.G. Feng, B.L. Yuan, et al., Transduced PTD–BDNF fusion protein protects against beta amyloid peptide-induced learning and memory deficits in mice, Brain Res. 1191 (2008) 12–19.
[65] G. Bertilsson, C. Patrone, O. Zachrisson, et al., Peptide hormone exendin-4 stimulates subventricular zone neurogenesis in the adult rodent brain and induces recovery in an animal model of Parkinson's disease, J. Neurosci. Res. 86 (2008) 326–338.
[66] I. Aviles-Olmos, J. Dickson, Z. Kefalopoulou, et al., Exenatide and the treatment of patients with Parkinson's disease, J. Clin. Invest. 123 (2013) 2730–2736.
[67] P. Menei, V. Daniel, C. Monteromenei, et al., Biodegradation and brain-tissue reaction to poly(D, L-lactide-co-glycolide) microspheres, Biomaterials 14 (1993) 470–478.
[68] A.P. Nicholas, C. McInnis, K.B. Gupta, et al., The fate of biodegradable microspheres injected into rat brain, Neurosci. Lett. 323 (2002) 85–88.
[69] J.M. Pean, P. Menei, O. Morel, et al., Intraseptal implantation of NGF-releasing microspheres promote the survival of axotomized cholinergic neurons, Biomaterials 21 (2000) 2097–2101.
[70] C. Jollivet, A. Aubert-Pouessel, A. Clavreul, et al., Striatal implantation of GDNF releasing biodegradable microspheres promotes recovery of motor function in a partial model of Parkinson's disease, Biomaterials 25 (2004) 933–942.
[71] C. Jollivet, A. Aubert-Pouessel, A. Clavreul, et al., Long-term effect of intra-striatal glial cell line-derived neurotrophic factor-releasing microspheres in a partial rat model of Parkinson's disease, Neurosci. Lett. 356 (2004) 207–210.
[72] C. Andrieu-Soler, A. Aubert-Pouessel, M. Doat, et al., Intravitreous injection of PLGA microspheres encapsulating GDNF promotes the survival of photoreceptors in the rd1/rd1 mouse, Mol. Vis. 11 (2005) 1002–1011.
[73] E. Garbayo, C.N. Montero-Menei, E. Ansorena, et al., Effective GDNF brain delivery using microspheres—a promising strategy for Parkinson's disease, J. Control. Release 135 (2009) 119–126.
[74] M.S. Ward, A. Khoobehi, E.B. Lavik, et al., Neuroprotection of retinal ganglion cells in DBA/2J mice with GDNF-loaded biodegradable microspheres, J. Pharm. Sci. 96 (2007) 558–568.
[75] C. Jiang, M.J. Moore, X. Zhang, et al., Intravitreal injections of GDNF-loaded biodegradable microspheres are neuroprotective in a rat model of glaucoma, Mol. Vis. 13 (2007) 1783–1792.
[76] P. Checa-Casalengua, C. Jiang, I. Bravo-Osuna, et al., Retinal ganglion cells survival in a glaucoma model by GDNF/Vit E PLGA microspheres prepared according to a novel microencapsulation procedure, J. Control. Release 156 (2011) 92–100.
[77] N. Lowry, S.K. Goderie, P. Lederman, et al., The effect of long-term release of Shh from implanted biodegradable microspheres on recovery from spinal cord injury in mice, Biomaterials 33 (2012) 2892–2901.
[78] Y.-C. Wang, Y.-T. Wu, H.-Y. Huang, et al., Sustained intraspinal delivery of neurotrophic factor encapsulated in biodegradable nanoparticles following contusive spinal cord injury, Biomaterials 29 (2008) 4546–4553.
[79] S.R. Cerqueira, J.M. Oliveira, N.A. Silva, et al., Microglia response and in vivo therapeutic potential of methylprednisolone-loaded dendrimer nanoparticles in spinal cord injury, Small 9 (2012) 738–749.
[80] E.A. Nance, G.F. Woodworth, K.A. Sailor, et al., A dense poly(ethylene glycol) coating improves penetration of large polymeric nanoparticles within brain tissue, Sci. Transl. Med. 4 (2012).
[81] H. Gao, Z. Pang, X. Jiang, Targeted delivery of nano-therapeutics for major disorders of the central nervous system, Pharm. Res. 30 (2013) 2485–2498.
[82] M. Nof, L.D. Shea, Drug-releasing scaffolds fabricated from drug-loaded microspheres, J. Biomed. Mater. Res. 59 (2002) 349–356.
[83] W.M. Tian, C.L. Zhang, S.P. Hou, et al., Hyaluronic acid hydrogel as Nogo-66 receptor antibody delivery system for the repairing of injured rat brain: in vitro, J. Control. Release 102 (2005) 13–22.
[84] Y. Yang, L. De Laporte, C.B. Rives, et al., Neurotrophin releasing single and multiple lumen nerve conduits, J. Control. Release 104 (2005) 433–446.
[85] L. De Laporte, A.L. Yan, L.D. Shea, Local gene delivery from ECM-coated poly(lactide-co-glycolide) multiple channel bridges after spinal cord injury, Biomaterials 30 (2009) 2361–2368.
[86] L. Yao, W. Daly, B. Newland, et al., Improved axonal regeneration of transected spinal cord mediated by multichannel collagen conduits functionalized with neurotrophin-3 gene, Gene Ther. 20 (2013) 1149–1157.
[87] S. Stokols, M.H. Tuszynski, Freeze-dried agarose scaffolds with uniaxial channels stimulate and guide linear axonal growth following spinal cord injury, Biomaterials 27 (2006) 443–451.
[88] A. Bakshi, O. Fisher, T. Dagci, et al., Mechanically engineered hydrogel scaffolds for axonal growth and angiogenesis after transplantation in spinal cord injury, J. Neurosurg. Spine 1 (2004) 322–329.
[89] Q. Han, W. Sun, H. Lin, et al., Linear ordered collagen scaffolds loaded with collagen-binding brain-derived neurotrophic factor improve the recovery of spinal cord injury in rats, Tissue Eng. A 15 (2009) 2927–2935.

[90] L. De Laporte, Y. Yang, M.L. Zelivyanskaya, et al., Plasmid releasing multiple channel bridges for transgene expression after spinal cord injury, Mol. Ther. 17 (2009) 318–326.

[91] H.M. Tuinstra, M.O. Aviles, S. Shin, et al., Multifunctional, multichannel bridges that deliver neurotrophin encoding lentivirus for regeneration following spinal cord injury, Biomaterials 33 (2012) 1618–1626.

[92] J. Ma, W.-M. Tian, S.-P. Hou, et al., An experimental test of stroke recovery by implanting a hyaluronic acid hydrogel carrying a Nogo receptor antibody in a rat model, Biomed. Mater. 2 (2007) 233–240.

[93] Y.-T. Wei, Y. He, C.-L. Xu, et al., Hyaluronic acid hydrogel modified with nogo-66 receptor antibody and poly-(L)-lysine to promote axon regrowth after spinal cord injury, J. Biomed. Mater. Res. B Appl. Biomater. 95B (2010) 110–117.

[94] M.M. Pakulska, B.G. Ballios, M.S. Shoichet, Injectable hydrogels for central nervous system therapy, Biomed. Mater. 7 (2012).

[95] S.E. Sakiyama-Elbert, J.A. Hubbell, Development of fibrin derivatives for controlled release of heparin-binding growth factors, J. Control. Release 65 (2000) 389–402.

[96] S.J. Taylor, J.W. McDonald, S.E. Sakiyama-Elbert, Controlled release of neurotrophin-3 from fibrin gels for spinal cord injury, J. Control. Release 98 (2004) 281–294.

[97] S.J. Taylor, E.S. Rosenzweig, J.W. McDonald III, et al., Delivery of neurotrophin-3 from fibrin enhances neuronal fiber sprouting after spinal cord injury, J. Control. Release 113 (2006) 226–235.

[98] S.J. Taylor, S.E. Sakiyama-Elbert, Effect of controlled delivery of neurotrophin-3 from fibrin on spinal cord injury in a long term model, J. Control. Release 116 (2006) 204–210.

[99] P.J. Johnson, S.R. Parker, S.E. Sakiyama-Elbert, Controlled release of neurotrophin-3 from fibrin-based tissue engineering scaffolds enhances neural fiber sprouting following subacute spinal cord injury, Biotech. Bioeng. 104 (2009) 1207–1214.

[100] A.J.T. Hyatt, D. Wang, J.C. Kwok, et al., Controlled release of chondroitinase ABC from fibrin gel reduces the level of inhibitory glycosaminoglycan chains in lesioned spinal cord, J. Control. Release 147 (2010) 24–29.

[101] S. Lord-Fontaine, F. Yang, Q. Diep, et al., Local inhibition of Rho signaling by cell-permeable recombinant protein BA-210 prevents secondary damage and promotes functional recovery following acute spinal cord injury, J. Neurotrauma 25 (2008) 1309–1322.

[102] J.R. Wilson, N. Forgione, M.G. Fehlings, Emerging therapies for acute traumatic spinal cord injury, Can. Med. Assoc. J. 185 (2013) 485–492.

[103] M.G. Fehlings, N. Theodore, J. Harrop, et al., Phase I/IIa clinical trial of a recombinant Rho protein antagonist in acute spinal cord injury, J. Neurotrauma 28 (2011) 787–796.

[104] L. McKerracher, K.D. Anderson, Analysis of recruitment and outcomes in the phase I/IIa cethrin clinical trial for acute spinal cord injury, J. Neurotrauma 30 (2013) 1795–1804.

[105] A. Jain, Y.T. Kim, R.J. McKeon, et al., In situ gelling hydrogels for conformal repair of spinal cord defects, and local delivery of BDNF after spinal cord injury, Biomaterials 27 (2006) 497–504.

[106] H. Lee, R.J. McKeon, R.V. Bellamkonda, Sustained delivery of thermostabilized chABC enhances axonal sprouting and functional recovery after spinal cord injury, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 3340–3345.

[107] S. Mehrotra, D. Lynam, R. Maloney, et al., Time controlled protein release from layer-by-layer assembled multilayer functionalized agarose hydrogels, Adv. Funct. Mater. 20 (2010) 247–258.

[108] S. Mehrotra, D. Lynam, C. Liu, et al., Time controlled release of arabinofuranosylcytosine (Ara-C) from agarose hydrogels using layer-by-layer assembly: an in vitro study, J. Biomater. Sci. Polym. Ed. 23 (2012) 439–463.

[109] M.C.J. Hamann, C.H. Tator, M.S. Shoichet, Injectable intrathecal delivery system for localized administration of EGF and FGF-2 to the injured rat spinal cord, Exp. Neurol. 194 (2005) 106–119.

[110] C.E. Kang, R. Clarkson, C.H. Tator, et al., Spinal cord blood flow and blood vessel permeability measured by dynamic computed tomography imaging in rats after localized delivery of fibroblast growth factor, J. Neurotrauma 27 (2010) 2041–2053.

[111] M.J. Cooke, Y. Wang, C.M. Morshead, et al., Controlled epi-cortical delivery of epidermal growth factor for the stimulation of endogenous neural stem cell proliferation in stroke-injured brain, Biomaterials 32 (2011) 5688–5697.

[112] C.E. Kang, P.C. Poon, C.H. Tator, et al., A new paradigm for local and sustained release of therapeutic molecules to the injured spinal cord for neuroprotection and tissue repair, Tissue Eng. A 15 (2009) 595–604.

[113] Y. Wang, M.J. Cooke, C.M. Morshead, et al., Hydrogel delivery of erythropoietin to the brain for endogenous stem cell stimulation after stroke injury, Biomaterials 33 (2012) 2681–2692.

[114] R.H. Bobo, D.W. Laske, A. Akbasak, et al., Convection-enhanced delivery of macromolecules in the brain, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 2076–2080.

[115] A.J. Sawyer, J.M. Piepmeier, W.M. Saltzman, New methods for direct delivery of chemotherapy for treating brain tumors, Yale J. Biol. Med. 79 (2006) 141–152.

[116] J.A. Burdick, M. Ward, E. Liang, et al., Stimulation of neurite outgrowth by neurotrophins delivered from degradable hydrogels, Biomaterials 27 (2006) 452–459.

[117] J. Piantino, J.A. Burdick, D. Goldberg, et al., An injectable, biodegradable hydrogel for trophic factor delivery enhances axonal rewiring and improves performance after spinal cord injury, Exp. Neurol. 201 (2006) 359–367.

[118] L.C. Grous, J. Vernengo, Y. Jin, et al., Implications of poly(N-isopropylacrylamide)-g-poly(ethylene glycol) with codissolved brain-derived neurotrophic factor inject-able scaffold on motor function recovery rate following cervical dorsolateral funiculotomy in the rat, J. Neurosurg. Spine 18 (2013) 641–652.

[119] B. Song, J. Song, S. Zhang, et al., Sustained local delivery of bioactive nerve growth factor in the central nervous system via tunable diblock copolypeptide hydrogel depots, Biomaterials 33 (2012) 9105–9116.

[120] K. Vulic, M.S. Shoichet, Tunable growth factor delivery from injectable hydrogels for tissue engineering, J. Am. Chem. Soc. 134 (2012) 882–885.

[121] M.M. Pakulska, K. Vulic, M.S. Shoichet, Affinity-based release of chondroitinase ABC from a modified methylcellulose hydrogel, J. Control. Release 171 (2013) 11–16.

[122] K.J. Lampe, D.S. Kern, M.J. Mahoney, et al., The administration of BDNF and GDNF to the brain via PLGA microparticles patterned within a degradable PEG-based hydro-gel: protein distribution and the glial response, J. Biomed. Mater. Res. A 96A (2011) 595–607.

[123] Y.T. Kim, J.M. Caldwell, R.V. Bellamkonda, Nanoparticle-mediated local delivery of methylprednisolone after spinal cord injury, Biomaterials 30 (2009) 2582–2590.

[124] E. Ansorena, P. De Berdt, B. Ucakar, et al., Injectable alginate hydrogel loaded with GDNF promotes functional recovery in a hemisection model of spinal cord injury, Int. J. Pharm. 455 (2013) 148–158.

[125] C.E. Kang, M.D. Baumann, C.H. Tator, et al., Localized and sustained delivery of fi-broblast growth factor-2 from a nanoparticle-hydrogel composite for treatment of spinal cord injury, Cells Tissues Organs 197 (2012) 55–63.

[126] M.J. Caicco, M.J. Cooke, Y.F. Wang, et al., A hydrogel composite system for sustained epi-cortical delivery of cyclosporin A to the brain for treatment of stroke, J. Control. Release 166 (2012) 197–202.

[127] Y. Wang, M.J. Cooke, N. Sachewsky, et al., Bioengineered sequential growth factor delivery stimulates brain tissue regeneration after stroke, J. Control. Release 172 (2013) 1.

[128] J.C. Stanwick, M.D. Baumann, M.S. Shoichet, Enhanced neurotrophin-3 bioactivity and release from a nanoparticle-loaded composite hydrogel, J. Control. Release 160 (2012) 666–675.

[129] J.C. Stanwick, M.D. Baumann, M.S. Shoichet, In vitro sustained release of bioactive anti-NogoA, a molecule in clinical development for treatment of spinal cord injury, Int. J. Pharm. 426 (2012) 284–290.

[130] N.D. Leipzig, M.S. Shoichet, The effect of substrate stiffness on adult neural stem cell behavior, Biomaterials 30 (2009) 6867–6878.

[131] Y. Aizawa, N. Leipzig, T. Zahir, et al., The effect of immobilized platelet derived growth factor AA on neural stem/progenitor cell differentiation on cell-adhesive hydrogels, Biomaterials 29 (2008) 4676–4683.

[132] T.Y. Wang, J.S. Forsythe, D.R. Nisbet, et al., Promoting engraftment of transplanted neural stem cells/progenitors using biofunctionalised electrospun scaffolds, Biomaterials 33 (2012) 9188–9197.

[133] T.Y. Cheng, M.H. Chen, W.H. Chang, et al., Neural stem cells encapsulated in a functionalized self-assembling peptide hydrogel for brain tissue engineering, Biomaterials 34 (2013) 2005–2016.

[134] A.J. Mothe, R.Y. Tam, T. Zahir, et al., Repair of the injured spinal cord by transplan-tation of neural stem cells in a hyaluronan-based hydrogel, Biomaterials 34 (2013) 3775–3783.

[135] P.J. Johnson, A. Tatara, A. Shiu, et al., Controlled release of neurotrophin-3 and platelet-derived growth factor from fibrin scaffolds containing neural progenitor cells enhances survival and differentiation into neurons in a subacute model of SCI, Cell Transplant. 19 (2010) 89–101.

[136] E. Bible, O. Qutachi, D.Y.S. Chau, et al., Neo-vascularization of the stroke cavity by implantation of human neural stem cells on VEGF-releasing PLGA microparticles, Biomaterials 33 (2012) (7435-+).

[137] G.J.R. Delcroix, E. Garbayo, L. Sindji, et al., The therapeutic potential of human multipotent mesenchymal stromal cells combined with pharmacologically active microcarriers transplanted in hemi-parkinsonian rats, Biomaterials 32 (2011) 1560–1573.

[138] D. Matsuse, M. Kitada, F. Ogura, et al., Combined transplantation of bone marrow stromal cell-derived neural progenitor cells with a collagen sponge and basic fibroblast growth factor releasing microspheres enhances recovery after cerebral ischemia in rats, Tissue Eng. A 17 (2011) 1993–2004.