Review

Creating permissive microenvironments for stem cell transplantation into the central nervous system

Howard Kim[1,2], Michael J. Cooke[2,3] and Molly S. Shoichet[1,2,3,4]

[1] Institute of Medical Science, University of Toronto, 1 King's College Circle, Toronto, ON, Canada, M5S 1A8
[2] Institute of Biomaterials and Biomedical Engineering, 164 College Street, Room 407, Toronto, ON, Canada, M5S 3G9
[3] Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, ON, Canada, M5S 3E5
[4] Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, ON, Canada, M5S 3H6

Traumatic injury to the central nervous system (CNS) is highly debilitating, with the clinical need for regenerative therapies apparent. Neural stem/progenitor cells (NSPCs) are promising because they can repopulate lost or damaged cells and tissues. However, the adult CNS does not provide an optimal milieu for exogenous NSPCs to survive, engraft, differentiate, and integrate with host tissues. This review provides an overview of tissue engineering strategies to improve stem cell therapies by providing a defined microenvironment during transplantation. The use of biomaterials for physical support, growth factor delivery, and cellular co-transplantation are discussed. Providing the proper environment for stem cell survival and host tissue integration is crucial in realizing the full potential of these cells in CNS repair strategies.

Introduction

Traumatic injury to the central nervous system (CNS) includes spinal cord injury (SCI), traumatic brain injury (TBI) and stroke. These conditions are characterized by cell death and/or axonal damage, with subsequent loss of neuronal circuitry resulting in functional deficits. Typically these deficits are permanent because the human body has limited capacity for self-repair after CNS injury. Neuroprotective strategies have been developed to minimize the extent of injury, however to be effective, these must be administered rapidly following the insult. Groups have also investigated how remodeling of the injury environment can promote endogenous repair, for example chondroitinase ABC can promote regeneration by degrading the glial scar formed after SCI. An alternative strategy is to replace the dead and damaged tissue to re-establish functional connections and promote recovery. There are currently two types of regenerative strategies for the replacement of lost cells in the CNS: pharmacological stimulation of endogenous stem cells [1,2] and exogenous stem cell transplantation, which is the focus of this review.

Exogenous stem cell transplantation aims to replace cells at the injury site (e.g. neuronal replacement), repair the damaged cells (e.g. remyelination) or to alter the local environment to be more conducive for regeneration (e.g. trophic support). Although many cell types have been transplanted into injured spinal cord and brain tissue, including Schwann cells, olfactory ensheathing glia, activated macrophages, and mesenchymal stem cells (reviewed in [3,4]), the focus of this review is on neural stem cells.

Neural stem cells are multipotent cells capable of both self-renewal, allowing for expansion in culture, and differentiation into the three main cell types of the CNS: neurons, astrocytes, and oligodendrocytes. There are some distinctions between 'true' neural stem cells and neural progenitor cells, which have limited capacity for self-renewal. For the purpose of this review, these cells are collectively referred to as neural stem/progenitor cells (NSPCs). Adult NSPCs can be isolated from the subventricular zone (SVZ) of the lateral ventricles or hippocampal dentate gyrus of the brain [5,6], or from the ependymal lining of spinal cord central canal [7]. Alternatively, NSPCs can be derived from embryonic stem (ES) cells [8] or induced pluripotent stem cells (iPSCs) [9]. An extensive review of preclinical transplantation studies in the injured spinal cord was recently published [4]. Of the 20 published articles (up to summer 2008) that performed behavioral assessment, 17 reported some measure of improved functional outcome with NSPC transplantation compared to non-transplanted controls [4]. Of the three studies that did not show improved behavior, two reported poor survival of NSPCs [10,11] and the other reported significant neuropathic pain, possibly associated with a high level of astrocytic differentiation [12]. Although these studies varied widely in design, they clearly demonstrate the potential of NSPCs as a cellular treatment. However, there is much room for improvement. In particular, the concept of directing stem cell fate and function *in vivo* is becoming increasingly attractive.

The task of repairing the CNS is daunting; however, it has been shown that by creating a permissive microenvironment, CNS regeneration is possible. Peripheral nerve

Corresponding author: Shoichet, M.S. (molly.shoichet@utoronto.ca)

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grafts (PNGs) can be grafted into the CNS to provide a permissive supportive substratum that allows CNS axons to regenerate over long distances. PNGs have shown regeneration after SCI [13], with this regeneration being further enhanced when combined with endogenous tissue remodeling [14]. This demonstrates that regeneration of the CNS is possible with the correct permissive environment. The approaches investigated for creating a permissive environment can be divided in three categories: (i) providing physical extracellular support; (ii) providing cytokine support; and (iii) presenting a combination of physical and cytokine support. Here, we discuss the bioengineering strategies to create a permissive transplantation environment. This review aims to highlight the recent advances towards enhancing transplant efficacy through the use of tissue engineering.

Biomaterials

The term biomaterial can be applied to a diverse set of natural and synthetic materials with a wide range of physical and chemical properties [15]. Natural biomaterials are derived and purified from biological sources and can include polysaccharides such as chitosan, alginate, and methylcellulose, hyaluronan or proteins such as collagen, fibronectin, and fibrin. The advantages of natural polymers derive from their inherent roles in biological systems, with many containing natural binding sites for mammalian cells [16,17]. Synthetic biomaterials are produced chemically, which usually allows for greater product consistency and tunable properties compared to natural biomaterials. Of note are the polyesters poly(glycolide), poly(lactide), and poly(lactic-co-glycolic acid) (PLGA), which have been used clinically as absorbable sutures, orthopedic fixation devices, and drug delivery vehicles [18,19].

The design criteria for a biomaterial scaffold depend greatly on the application. Material properties, such as hydrophilicity, cell-adhesion, degradability, and scaffold properties, such as shape, porosity, and mechanical strength, must all be considered. In neural tissue applications, hydrogel networks are attractive for their open porous networks, which allow for cell migration and free exchange of nutrients [20,21]. In many cases, hydrogels can also be designed to be form-filling [22]. Fiber networks and channel designs have also been investigated in neural applications to provide physical guidance cues for directed regeneration [23].

Creating a biomimetic microenvironment *in vitro*

In vitro, biomaterial systems aim to provide a suitable substrate for cell encapsulation or attachment. For optimal cell behavior, biomaterials designs are evolving to include stimuli from the niche microenvironment. For example, several factors can be considered in the biomaterial design including: mechanical stiffness, cell-adhesion signals, soluble or immobilized factors, and the availability of other cell types (Figure 1). The following section elucidates the contribution of these factors.

Cell adhesion

Many biopolymers are inherently non-cell adhesive (e.g. agarose and polyethylene glycol), requiring modification with extracellular matrix (ECM)-derived peptides or proteins [24–26]. Coating ECM proteins onto a surface is relatively simple because they adsorb spontaneously when applied as a solution. However, adsorption is limited by the propensity for desorption and protein denaturation following adsorption, leading to batch inconsistency and potentially irreproducible results [27]. This has the advantage of simplicity but the protein might either diffuse away or be buried within the biomaterial and be unavailable for cellular interaction. Covalent modification of a protein to the biomaterial has the advantage of ensuring inclusion of all protein active sites in the biomaterial; however, protein modification also has the risk of substantial loss of protein activity due to chemical modification of active sites, denaturation and/or random orientation resulting in inaccessibility to cellular receptors. Given that the active sites of the protein are identified and known to promote cell adhesion (usually through integrin receptors), peptide modification is advantageous because the active sites of the protein can be specifically designed to allow covalent modification while maintaining bioactivity. ECM adhesion proteins play significant roles in proliferation, motility, and differentiation [25,28,29]. Moreover, cell adhesion might play a large role in cell survival upon transplantation [30]. Addition of laminin, fibronectin, or peptide sequences derived from these two basement membrane adhesion proteins (e.g. YIGSR and RGD) is typically used to enhance cell adhesion to biomaterial substrates. *In vitro*, adhesive substrates can be used to dictate the distribution and movement of cells. In neural tissue engineering, several physical and chemical strategies have been investigated for cell guidance (reviewed in [23,31]), including photochemical patterning methods that result in distinct 3D cell-adhesive architectures [32]. Interestingly, cell adhesion molecules can affect stem cell differentiation where, for example, peptide sequences derived from fibronectin and collagen, in combination with the correct medium conditions, enhance neuronal differentiation from NSPCs [33].

Elastic modulus

Cell differentiation can be influenced, in part, by the mechanical properties of the surrounding matrix [34,35]. For example, matching substrate stiffness to brain, muscle, and bone tissue could induce mesenchymal stem cell (MSC) differentiation into neurons, myocytes, or osteoblasts, respectively [36]. Even small changes in elastic modulus can affect differentiation of neuron, astrocyte, and oligodendrocyte populations [37]. Mechanical properties can also affect cell morphology, because stiff substrates have been shown to increase astrocyte cell body area and number of processes [38]. Indeed, the mechanical properties of the surrounding matrix influence the cytoskeletal architecture, which in turn affects cell growth, motility, and behavior [39–41]. Substrate stiffness of 3D biomaterial scaffolds is typically controlled by varying polymer concentration or crosslinking density.

Cellular co-culture

The interactions between different cell types are important in regulation of cell behavior *in vivo*. For example, oligodendrocytes are known to play a trophic role in controlling

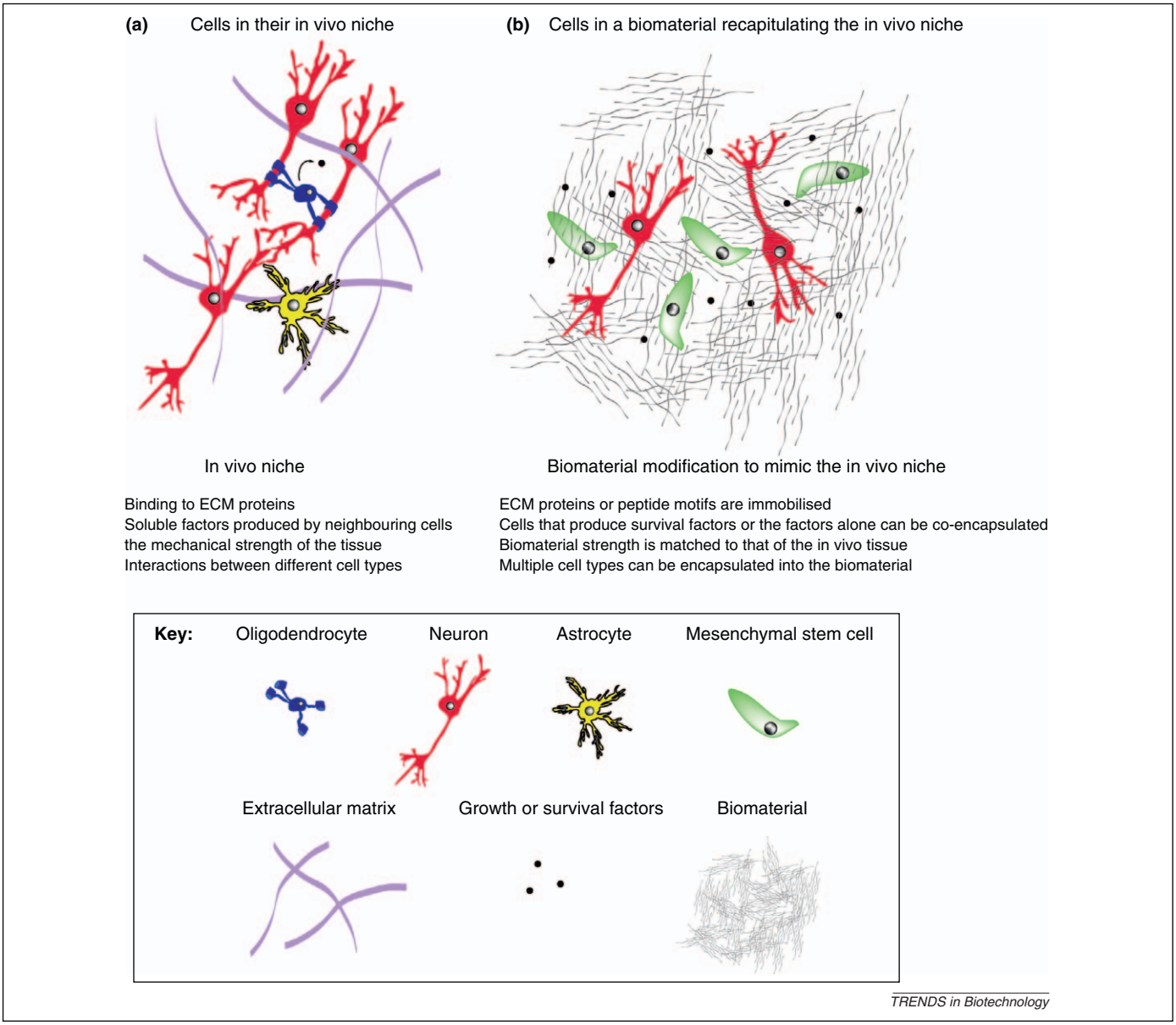

Figure 1. The *in vivo* niche and strategies to include a permissive microenvironment in a biomaterial. **(a)** *In vivo,* cells within their niche have the correct ECM composition, soluble factors, mechanical strength and interactions with other cells. **(b)** Niche factors are included in a biomimetic biomaterial scaffold for cell transplantation.

neurons by the expression of brain-derived neurotrophic factor and neurotrophin (NT)-3 [42], and close association with the vasculature is important in adult neural stem cell regulation *in vivo* [43]. Co-culture with endothelial cells has been shown to increase NSPC proliferation through paracrine signaling of vascular endothelial growth factor (VEGF) [44], an important factor of the neurovascular niche [45]. MSCs can also provide trophic support, and have been shown to upregulate trophic factor release *in vitro* in response to chemical cues present after brain injury [46].

Bioactive factors

The availability of survival, growth and/or differentiation factors can greatly influence stem cell behavior. In culture, these molecules are simply dissolved into the culture medium at the optimum concentrations, which is difficult to translate *in vivo*. Drug delivery is used to overcome this limitation by either releasing factors through a controlled delivery platform or immobilizing the factors to the cell delivery matrix. Co-delivery of cells and factors in a given biomaterial facilitates local effects and obviates the need for the factor to cross the blood–brain barrier. The simplest method of soluble drug delivery is to mix the factor directly into the matrix; however, this generally results in only short-term release that is dictated by diffusion. If cells are transiently exposed to a growth factor, it can be expected that a transient biological response will be observed. *In vivo* growth factors are regulated; this can be mimicked using a biomaterial approach where, for example, the cells are actively involved in the availability of the growth factor [47]. Chemical immobilization or continuous release is advantageous because it prevents the loss of growth factors and maintains the desired concentration; however, over-stimulation can be detrimental in some cases as well. Scaffolds can be modified with affinity-based binding

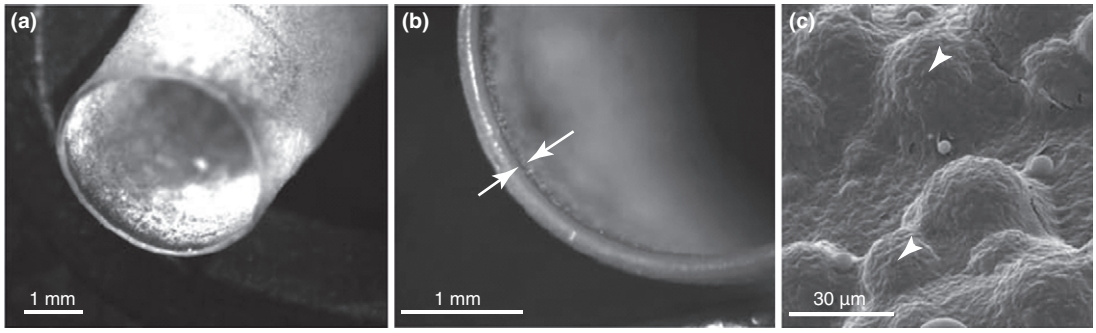

Figure 2. Microsphere-loaded chitosan channels can be fabricated to release survival and differentiation factors locally to control cell behavior. **(a and b)** Low magnification images showing chitosan channels. **(c)** High magnification images showing microspheres attached to the wall of chitosan cannels. Reproduced with permission from [51].

partners to slow diffusional release. For example, hyaluronan hydrogels modified with heparin, a binding partner for basic fibroblast growth factor (bFGF) and VEGF, showed prolonged release from gels [48]. Sustained release can also be achieved from polymeric nano/microspheres, where encapsulated drugs are released according to percolation theory. These systems can be used to achieve drug release in the scale of days to months, and can be incorporated into injectable hydrogels [49], cell-seeded scaffolds [50], and/or nerve guidance channels [51]. Microspheres can be incorporated into the guidance channel to release locally survival or differentiation factors (Figure 2). Finally, factors can be chemically immobilized onto the biomaterial to provide constant signaling for cell stimulation. For example, agarose-immobilized platelet-derived growth factor stimulates differentiation of NSPCs to oligodendrocytes [24], whereas chitosan-immobilized interferon-γ promotes differentiation of NSPCs to neurons [52].

Enhancing transplantation outcomes in the CNS

Regeneration following SCI requires a multifaceted approach to address the numerous challenges the injury poses, including extensive cell death, demyelination, glial scarring, and the growth-inhibitory environment. Stem cell transplantation is one of many strategies aimed towards replacing or repairing lost and damaged cells after injury. For successful treatment, the cell delivery method is important. While intravenous or lumbar puncture administration of cells is minimally invasive, the majority of the cells do not home to the injury site [53,54]. Direct transplantation of cells into the target tissue offers an alternative method. For example, intravenous administration was directly compared to transplantation into the brain using a rat cortical impact model [55]. MSCs transplanted on collagen I scaffolds, relative to tail vein cell delivery, had improved behavioral recovery, reduced cavity volume, increased localization of cells to the injury site, and increased vascularization (Figure 3). This shows that localization of cells to a niche within the target tissue is advantageous compared to systemic cell administration.

When NSPCs are injected into an acute spinal cord or brain lesion, poor survival is generally observed [56]. Moreover, the adult mammalian CNS is not inductive towards neurogenesis, so very few NSPCs differentiate into neurons after transplantation. Biomaterial and tissue engineering strategies aim to increase the efficiency of stem cell therapy by providing greater control of the local microenvironment during transplantation. These strategies are designed to enhance the ability of stem cells to survive, differentiate, and integrate with host tissue.

Improving stem cell survival

One of the key aims for the use of biomaterials in stem cell transplantation to the CNS is the increase in cell survival. Cell transplantation studies commonly use saline or medium solution as the vehicle. To improve the efficiency of transplantation, researchers are beginning to deliver cells in biomaterials to localize cells to the injection site and provide a niche that enhances the viability of transplanted cells. In a recent review, we highlighted how biomaterials

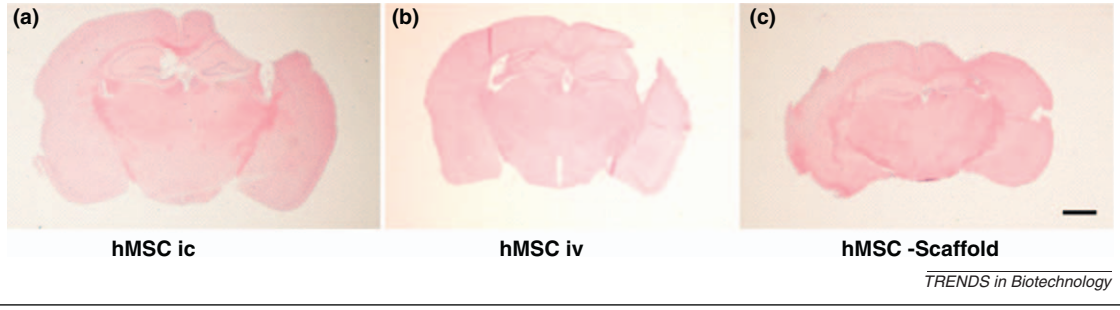

Figure 3. Improvement of stem cell transplantation using a collagen I scaffold. MSCs were injected **(a)** intracerebrally (ic) directly into the lesion core, **(b)** intravenously (iv) into the tail vein after TBI, or with **(c)** a scaffold into the lesion core. Cells seeded with the collagen I scaffold have a beneficial effect and show a reduction in infarct size. Reproduced with permission from [55].

can be used to increase cell survival [56]. Maintaining cell survival is a high priority because without survival, cells can neither differentiate nor integrate with the host tissue.

An important aspect of the *in vivo* niche is its complexity and the presence of multiple different ECM proteins. Biomimetic strategies incorporate some of these ECM proteins into their design to enhance cell survival. Fetal NSPCs have been transplanted in a collagen I gel with either adsorbed laminin or fibronectin [57]. Eight weeks post-transplantation, there were significantly greater number of cells present in the graft site when transplanted in the collagen I/laminin compared to collagen I/fibronectin or medium alone. The observations were attributed to cell survival and not increased cell proliferation.

Few studies have directly compared stem cell survival in a biomaterial versus medium or saline vehicle. In a study by Zhong *et al.*, a hyaluronan–heparin–collagen hydrogel was tested with murine ES-derived neural progenitor cells for cell survival. *In vitro*, cells cultured in the hydrogel showed significantly less cell death than cells cultured without the hydrogel matrix. Importantly, *in vivo* studies reflected the *in vitro* results: 2 weeks after transplantation into mice with cortical strokes, cells transplanted in the hydrogel showed a significant twofold increase in cell number compared to saline delivery [58]. Biomaterial systems incorporating drug delivery can also improve transplant survival through the delivery of growth factors [59] or mediators of inflammation [60]. Adult rat NSPCs show high survival, up to 100%, when seeded onto chitosan guidance channels and transplanted across rat spinal cord transaction injury. As early as 5 weeks post-transplantation, a robust tissue bridge forms between two previously separated stumps. The NSPCs contributed greatly to this newly formed tissue compared to animals receiving no transplant (Figure 4) [81].

Clearly, enhancing survival is an important prerequisite to realizing the potential of stem cell therapy. Unfortunately, stereological quantification of stem cell survival is tedious, labor-intensive and often omitted as an outcome measure. Moreover, cells injected into blood or fluid-filled cavities are dispersed and not easily accounted. Often, the engrafted area is used as a proxy for survival quantification, and in many cases, survival is only assessed by qualitative observation. It is also important to note that delivery of the vehicle is only one of many factors that might affect survival. Variables such as stem cell type (e.g. brain vs. spinal cord, fetal vs. adult), stage of maturation (e.g. progenitor vs. differentiated), aggregation state (e.g. neurospheres vs. dissociated cells), injury model and severity, transplantation location, and transplant time-after-injury are all important factors. Taken together, these data demonstrate that bioengineering is a niche that includes components of the ECM, and growth factors can produce a favorable niche to improve cell survival.

Influencing stem cell differentiation

The phenotypic fate of transplanted NSPCs into the injured CNS is largely dictated by the local tissue environment. In some cases, this can be beneficial, for instance, transplantation studies of NSPCs into the dysmyelinating Shiverer mouse model have resulted in differentiation of

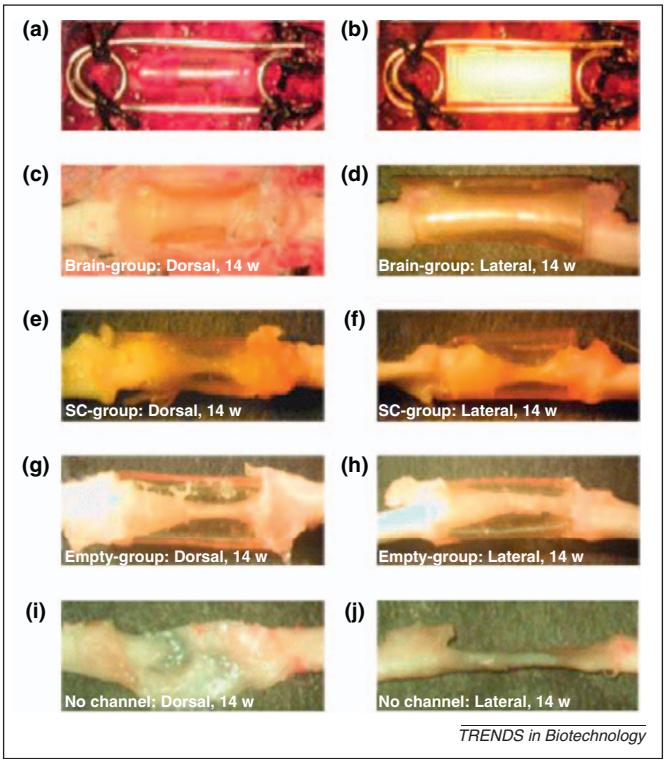

Figure 4. Chitosan channels can be used to support stem cell transplantation and bridge the gap of a transected spinal cord. **(a)** Dorsal view of the transected spinal cord stumps placed within the transparent chitosan channel. **(b)** Spinal fusion with wire for spinal stabilization and the expanded polytetrafluoroethylene membrane placed on the dorsal aspect of the channel for prevention of scar invasion. After 14 weeks, gross appearance of the implanted channel **(c–h)** or spinal cord transection alone: **(i and j)**. A tissue bridge can be seen within the brain, (c and d), spinal cord (e and f), and empty-channel groups (g and h). Reproduced with permission from [81].

NSPCs into myelinating oligodendrocytes [61]. However, it might be advantageous in many cases to provide researchers some measure of control over cell fate decisions. In particular, the generation of neurons is relatively rare when transplanting naïve NSPCs into the injured spinal cord environment [4]. Although it is unknown which cell type (or combination of cell types) is optimal for repairing injured CNS tissue, the idea of directing cell fate decisions is appealing to researchers.

Influence over stem cell differentiation can be performed in culture before transplantation via selection [62], tailored medium conditions [63,64], or genetic manipulation [65]. A recent study has compared transplantation of ES cells at different stages of differentiation into a Parkinson's rat model [66]. Naïve ES cells, ES-derived neural precursors, and ES-derived neurons showed no significant differences in survival, but measurable differences in behavioral improvement that was attributed to dopaminergic competency. Directed differentiation into specific neuronal subtypes might also be possible [67]. Genetic manipulation can also enhance stem cell maturation. Transfected NSCs that overexpress Olig2 have been compared to naïve NSCs after transplantation into the injured spinal cord [68]. Olig2-modified cells resulted in greater white matter migration, better myelination measures, and improved hindlimb function.

An alternative approach to transplanting pre-differentiated cells is to influence cell fate decisions *in vivo*. Most

commonly, this is achieved through factor immobilization or drug delivery systems of molecules that promote phenotype specification. For example, transplanted ESC-derived embryoid bodies within fibrin scaffolds, with or without a growth factor sequestering system for PDGF and NT3 enhanced neuronal differentiation with growth factor delivery [69]. Interestingly, this was also associated with greater cell numbers, although it is probably attributed to effects on proliferation and not necessarily survival.

We directly compared pre-differentiation versus *in situ* differentiation on NSPCs seeded in fibrin scaffolds transplanted into injured spinal cord injured rats [70]. The neuron-promoting factor dibutyryl cyclic-AMP was used to treat NSPC 4 days before transplant, or was encapsulated within PLGA microspheres for *in vivo* release. Although both methodologies resulted in enhanced neuronal differentiation compared to untreated NSPCs, the pre-differentiated group resulted in significantly higher survival. Unfortunately, because of this great difference in survival, it was difficult to assess whether timing of differentiation affected other outcomes such as cell migration and synaptic connectivity with host tissue.

Enhancing functional integration and repair

Although increasing cell survival and being able to promote the cell type of interest are important, the full realization of NSPCs as a cell replacement therapy relies on their proper integration with the host tissue and functional recovery. Host integration is a loosely defined term that describes the ability of the transplanted cells to interact with the host tissue in a beneficial way, and is cell-dependent. For example, in the case of oligodendrocytes, it might be a measure of the quantity and quality of remyelination. Other integration measures might include growth-factor- or cell-contact-mediated promotion of axonal growth and plasticity, or direct formation of new neuronal circuitry via synaptic connections between transplant and host. Ultimately, the goal of promoting interactions between transplanted NSPCs and the host is functional recovery.

Tissue-engineered strategies have been developed to promote host interaction. For example, retinal stem cells (RSCs) can be delivered in an injectable gel to promote better distribution into the subretinal space. Specifically, RSCs are delivered via an injectable hyaluronan/methyl cellulose matrix and report decreased cellular aggregation in the subretinal space compared to cells transplanted in saline alone [71]. Following transplantation, the majority of cells integrate in the retinal pigment epithelial layer (RPE), adopting a cuboidal morphology [71]. Retinal cell transplants have also shown promise when transplanted on laminin-coated poly(glycerol-co-sebacic acid) membranes into porcine eyes, demonstrating organized integration at 3 months [72]. In the spinal cord, MSCs seeded on a fibrin scaffold survive and migrate significantly further compared to MSCs delivered via direct injection [73]. NSPC-seeded PLGA scaffolds performed better than either scaffold alone or NSPCs alone in promoting functional recovery after rat spinal cord hemisection, which was attributed to the presence of corticospinal tract fibers that were only found in the injury epicenter of scaffold-treated animals [74]. NSPC-seeded scaffolds have also shown promising results in larger animal models. For example, human NSPCs were transplanted into PLGA scaffolds in a canine model of SCI and viable stem cells migrating into the host tissue were observed [75]. Recently, human NSPCs were transplanted into primates, utilizing PLGA scaffolds [76]. The goal of this work was to use a primate model of SCI to demonstrate that an approach using cells in combination with a biomaterial is a viable solution to SCI. Notwithstanding these promising results, the surgery is highly invasive and the sample size is very small.

Drug delivery systems have also been used in conjunction with biomaterials-based therapy for cell transplantation. For example, *in vitro*, the addition of heparin to a fibrin gel retards the release of bFGF fibrin. By controlling the concentrations of fibrinogen and thrombin, which comprise fibrin, the release of bFGF can be further tuned. When human MSCs were transplanted in fibrin gels into rats with TBI, the addition of bFGF significantly decreased infarct volume and apoptosis of transplanted cells, increased the number of neurons in the TBI area, and significantly increased behavioral recovery relative to injury alone, with no treatment [77] (Figure 5).

Although there are many mechanisms by which stem cells can contribute to functional recovery, it is often difficult prove these mechanisms experimentally. Tools such as immunostaining, electrophysiology, electron microscopy, and axonal tracing are certainly informative, but in many cases are insufficient to explain the reason for functional recovery in animal studies following cell therapy. Few studies, if any, have been rigorous in this respect however, it is proposed that transplanted cells can both stimulate endogenous repair processes [78] or directly establish new synaptic connections with host cells [79].

Translational considerations

Despite the potential of stem cell therapy, there are several issues that must be considered as the field moves towards clinical applications. The first issue concerns stem cell source. Notwithstanding the ethical issues, ES cells are pluripotent, offering numerous advantages yet, because there is a risk of tumor formation from transplantation of undifferentiated ES cells [80], there is a need for careful purification strategies to isolate the differentiated progeny. Significantly, human ES-derived oligodendrocyte progenitor cells are currently in clinical trials in North America for treatment of SCI, and positive safety data from these trials could accelerate future stem cell therapies. Indeed, RPE cells derived from ES cells have been recently approved for phase I/II clinical testing for age-related macular degeneration in the eye, stemming from preclinical data showing effectiveness in rodents. This could potentially provide an alternative source to fetus-derived or autologous RPE transplants.

Adult neural stem cells derived from organ donors or biopsy patients have the advantages of being restricted to CNS cell lineages, can be expanded long-term, and are amenable to cryopreservation. However, it is still not established whether relevant cell numbers can be generated from adult brain or spinal cord for clinical practice. Fetal neural stem cells might have greater expansion potential, and indeed clinical trials using human fetal-brain-derived

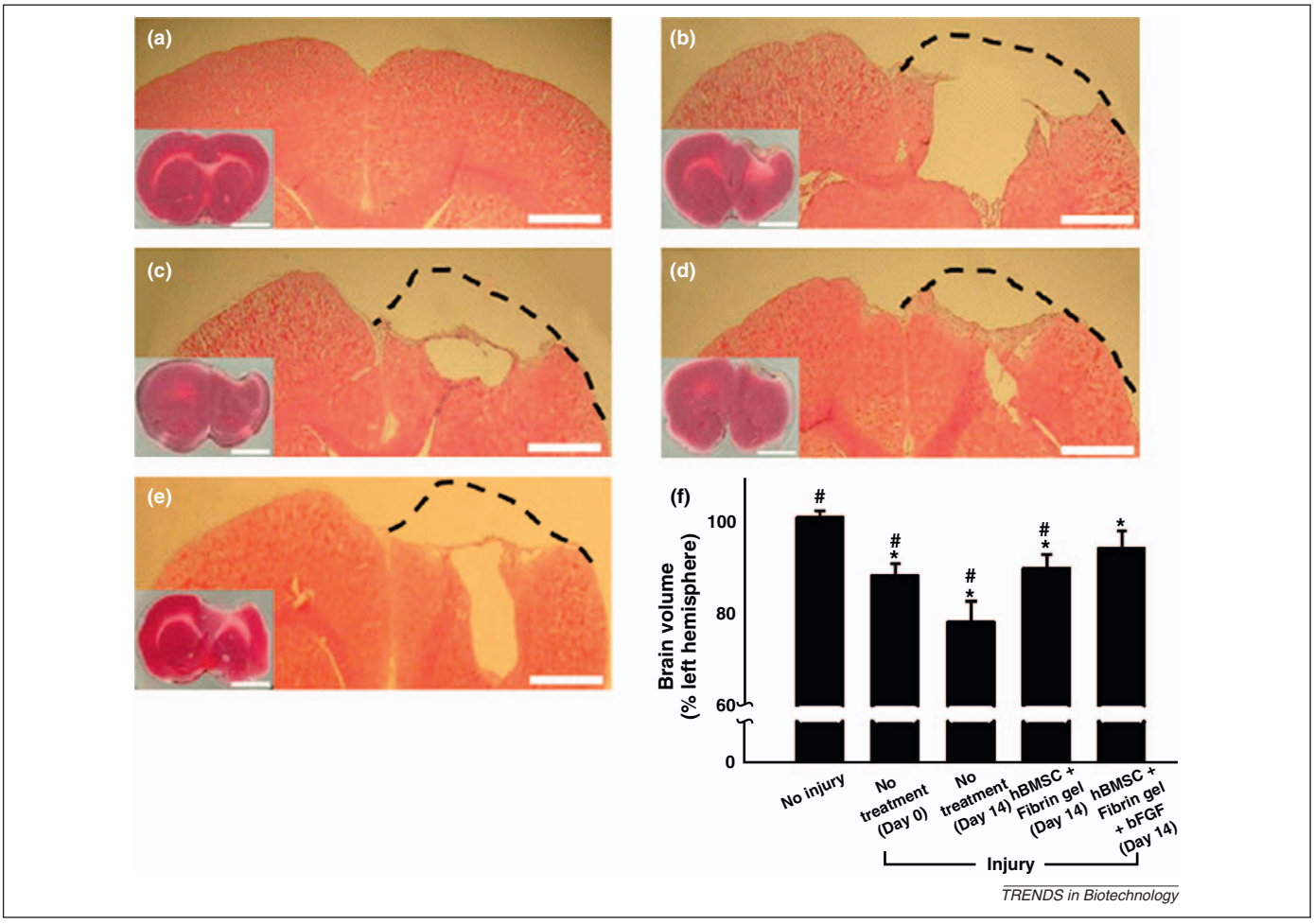

Figure 5. Addition of bFGF to the transplantation biomaterial helps decrease infarct size. Haematoxylin and eosin stained sections of TBI brains 14 days after treatment. **(a)** No injury. **(b)** Injury without treatment. **(c)** Injury with human bone marrow stromal cell (hBMSC) transplantation. **(d)** Injury with hBMSC transplantation in a bFGF-containing scaffold. **(e)** Brain 6 days after TBI before stem cell treatment. The dotted lines show brain tissue size before injury. **(f)** Volume of TBI brains (*$P<0.05$: compared with no injury group, #$P<0.05$: compared with hBMSC transplantation and bFGF treatment group). The scale bars indicate 0.5 cm, 1 cm in inserts. Reproduced with permission from [77].

NSPCs are in progress for the treatment of two separate fatal brain disorders in children, Pelizaeus–Merzbacher Disease (see: http://clinicaltrials.gov/ct2/show/NCT01005004) and neuronal ceroid lipofuscinosis (i.e. Batten disease) (see: http://clinicaltrials.gov/ct2/show/NCT01238315). These cells are also been approved for phase I/II trials for chronic SCI in Europe. In the UK, trials of immortalized fetal-brain-derived NSPCs are underway for treatment of stroke, where preclinical data have shown effectiveness in improving motor deficits in rats.

Autologous sources such as adult brain or spinal cord stem cells are impractical to biopsy, whereas others such as human skin-derived precursors, umbilical cord blood stem cells, or bone-marrow-derived MSCs might not have the differentiation potential to repopulate CNS tissue. iPSCs are a promising source of patient-specific stem cells, particularly in traumatic injuries such as SCI, TBI and stroke, which are not genetic diseases. Patient-specific cell sources are ideal because it is hypothesized this lessens the likelihood of transplant rejection and obviates the need for immunosuppression. However, research into iPSCs is still relatively early and many of the concerns over ES cells exist with iPSCs.

Another issue that must be considered going forward is the use of relevant injury models. In SCI, the most clinically relevant model is the contusion/compression model, which typically results in cavity formation in the center of the cord, surrounded by a ring of spared tissue. However, many scaffold implantation studies rely on hemisection or transection models, largely due to ease of implantation surgery. One can view these as proof-of-concept studies that help elucidate mechanisms or modes of regeneration that can then be used to inform future study design. Transection models are useful because they provide unambiguous information about regeneration, as opposed to the more clinically relevant contusion/compression models where it is difficult to distinguish between neuroprotection and regeneration. Ultimately, accessibility to the injury site is a major concern, and minimally invasive strategies such as injectable or *in situ* gelling hydrogels will be desirable from a clinical perspective.

Future perspectives

CNS injuries are complex and require a multifaceted approach to provide a conducive environment for transplantation and regeneration. For successful transplantation, cells

must survive and integrate into the host tissue – cells need to survive in order to integrate, yet without integration, cells will not survive. Biomimetic strategies aim to enhance cell survival sufficiently for cell integration by providing signaling molecules such as cell adhesion molecules and cell differentiation, proliferation and/or survival factors. After CNS injury, however, merely enhancing cell survival is often insufficient for cell integration because of the presence of the glial scar and inhibitory environment. Here, strategies such as delivering factors to neutralize the inhibitory signals and degrade the glial scar are also required to achieve success. As in all transplantation strategies, sufficient numbers of cells are required. In addition, the method of *in vitro* preparation influences their differentiation profile and *in vivo* fate.

Importantly, an emerging question in stem cell transplantation is the timing of transplantation, for example, how the stage of maturity along the differentiation pathway affects the ability of cells to integrate with the host tissue. It is not clear where on the differentiation profile (stem/progenitor to fully differentiated cells) provides optimal results. The biomimetic niche plays a significant role in stem cell differentiation and survival *in vitro* and *in vivo*; however, other factors such as source and type of cell to be transplanted are equally important.

Biomimetic niches might provide the necessary environmental cues for optimizing stem cell transplant efficacy. With defined 3D culture systems, where the mechanical, chemical and biological milieu is controlled, our understanding of stem cell biology will be enhanced and this knowledge will lead to greater success *in vivo*. Stem cell therapy holds great promise for CNS injury, however, the full potential of cell replacement strategies will only be realized once we have a better understanding of how to manipulate their survival, migration, differentiation, and integration with host tissue.

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