

Regenerative Therapies for Central Nervous System Diseases: a Biomaterials Approach

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The central nervous system (CNS) has a limited capacity to spontaneously regenerate following traumatic injury or disease, requiring innovative strategies to promote tissue and functional repair. Tissue regeneration strategies, such as cell and/or drug delivery, have demonstrated promising results in experimental animal models, but have been difficult to translate clinically. The efficacy of cell therapy, which involves stem cell transplantation into the CNS to replace damaged tissue, has been limited due to low cell survival and integration upon transplantation, while delivery of therapeutic molecules to the CNS using conventional methods, such as oral and intravenous administration, have been limited by diffusion across the blood–brain/spinal cord-barrier. The use of biomaterials to promote graft survival and integration as well as localized and sustained delivery of biologics to CNS injury sites is actively being pursued. This review will highlight recent advances using biomaterials as cell- and drug-delivery vehicles for CNS repair.

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INTRODUCTION

The central nervous system (CNS), composed of the brain, spinal cord, and retina, has a limited capacity to spontaneously regenerate and this, coupled with few regenerative strategies available, provides few treatment options for patients with CNS injuries or diseases. With only pharmacological treatments available that delay the progression of CNS diseases, there is a critical need for regenerative medicine strategies that overcome disease progression and promote tissue regeneration. CNS diseases are complex, resulting in loss of sensory, motor, and cognitive functions, as is the case in Parkinson's disease (Davie, 2008), Alzheimer's disease (Citron, 2010), multiple sclerosis (Lassmann *et al*, 2012), traumatic injuries (Bruns and Hauser, 2003; Lo *et al*, 2003; Sekhon and Fehlings, 2001), and impaired vision for retinal diseases such as retinitis pigmentosa (RP) (Shintani *et al*, 2009) and age-related macular degeneration (AMD) (de Jong, 2006). A multitude

of mechanisms may contribute to CNS injury, including apoptotic and necrotic death of neurons (including photoreceptors), astrocytes and oligodendrocytes, axonal injury, demyelination, excitotoxicity, ischemia, oxidative damage, and inflammation (Fitch and Silver, 2008). The lack of tissue regeneration is attributed to an overall absence of axon-growth promoting factors (eg, the local presentation of growth promoting factors and extracellular matrix (ECM) proteins) and presence of axonal growth inhibitory/repulsive molecules (eg, myelin-associated proteins, and the glial scar which constitutes a chemical and physical barrier) at and around the lesion site (Fitch and Silver, 2008), see Box 1.

Successful therapeutic strategies have been difficult to achieve due to the complexity of the CNS and an inhospitable environment in and around the lesion site for cell transplantation. Limited diffusion of drugs/biologics across the blood–brain barrier (BBB) further restricts the utility of common delivery methods (ie, oral and intravenous). This review will focus primarily on regenerative medicine strategies—that is cell transplantation and endogenous cell stimulation—with particular focus on the role of biomaterials to promote recovery following traumatic brain and spinal cord injuries, and degenerative diseases, such as AMD and RP, which cause degeneration of the photoreceptors and the retinal pigment epithelium (RPE).

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Overview of Cell-Based Treatment Strategies

Several strategies to promote tissue regeneration after injury are currently being pursued including cell-based therapies and delivery of bioactive molecules such as small molecules, growth factors, and antibodies (Pakulska *et al*, 2012; Shoichet *et al*, 2008). Cell-based therapies aim to replace and/or promote the survival of damaged cells or alter the local environment to be more conducive for regeneration by, for example, providing trophic support. Treatment strategies include transplantation of mesenchymal stromal cells (MSCs) (Bang *et al*, 2005; Kode *et al*, 2009), neural stem/progenitor cells (NSPCs) (Kokaia *et al*, 2012), embryonic stem cells (ESCs) (Lerou and Daley, 2005), induced pluripotent stem cells (iPSCs) (Willerth, 2011) and their differentiated progeny into the injured/diseased CNS (Box 2). Such cells can act to regenerate damaged host tissue either by directly integrating into the tissue (ie, cell replacement) or indirectly by secreting factors, which promote neuroprotection or neurogenesis (Bliss *et al*, 2010). For the latter approach, cells are used that are known to produce a variety of factors that have been shown to be beneficial after injury including neurotrophic factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), ciliary neurotrophic factor (CNTF), glial cell-derived neurotrophic factor (GDNF), and leukemia inhibitory factor (LIF), and ECM proteins, such as laminin, fibronectin, collagen I/III and IV (Fortun *et al*, 2009; White and Jakeman, 2008; Wright *et al*, 2003). Early clinical trials showed that cell transplantation of MSCs (Bang *et al*, 2005) or an immortalized cell line of immature neurons derived from human teratocarcinomas (Kondziolka *et al*, 2005), into the stroke-injured brain of human patients had no adverse effects (Wechsler, 2009). Unfortunately, the clinical efficacy of cell transplantation techniques has been limited by poor cell survival, uncontrolled differentiation, and ineffective integration into the host tissue, primarily due to an inhospitable environment at and around the injury site. Biomaterial hydrogels, described in detail herein, are being

investigated to enhance cell survival, host-tissue integration, and even attenuate the inflammatory response.

Overview of Drug/Biologics-Based Treatment Strategies

Bioactive molecules delivered to the CNS have been pursued to promote tissue regeneration—ie, neurogenesis, plasticity, axonal regeneration, and neuroprotection. For example, intraventricular sequential delivery of epidermal growth factor (EGF) and erythropoietin (EPO) into the stroke-injured rat brain showed enhanced migration of endogenous NSPCs (see Box 2) to the injury site, resulting in neurogenesis and improved functional recovery (Kolb *et al*, 2007). Likewise, growth factors such as interferon- γ (Victorio *et al*, 2010) and GDNF (Zhang *et al*, 2009) have been shown to be neuroprotective and promote axonal outgrowth, respectively, following SCI. However, the low permeability of the BBB and blood-spinal cord barrier limit diffusion of therapeutics using conventional delivery strategies (Pardridge, 2012), requiring either high systemic doses to reach therapeutic concentrations at the injury site, which often leads to systemic cytotoxicity, or local delivery strategies. Systemic administration leads to off-target distribution of therapeutic molecules, and can result in undesired side effects such as tumor formation and fibrosis (Lee *et al*, 2000). Several strategies are being pursued to enhance drug permeability across the BBB, including drug delivery via liposomes or nanoparticles (NPs) (Patel *et al*, 2009). Alternative strategies that circumvent the BBB, resulting in direct tissue delivery, include direct injection into the injury site or intraventricular injection, but these too are associated with possible risks such as cerebral edema and convulsions. Injections into the intrathecal space that surrounds the spinal cord can yield higher concentrations of the therapeutic molecule in the target tissue immediately following injection compared to systemic injections, but this approach is limited by the rapid distribution and elimination of the therapeutics by the cerebrospinal fluid (Groothuis, 2000).

BOX 1 Pathophysiology of CNS Diseases

BRAIN

Stroke injuries are widely acknowledged to be highly heterogeneous disorders and the characteristics of the lesion site are known to depend on a number of parameters including type of injury, severity, and time after injury (Werner and Engelhard, 2007). The consequences vary greatly and include neurocognitive deficits, such as speech or movement problems, and mental handicap. Causes include ischemic (lack of blood flow) or a hemorrhagic stroke. The primary injury usually results in cell death and inflammation is a major contributor to the secondary injury. Low cell replacement, glial scarring, and inhibitory/repulsive molecules at and around the lesion site limit regeneration and plasticity.

SPINAL CORD INJURY

The devastating consequences of traumatic spinal cord injury (SCI) depend on the level and severity of the injury but include permanent loss of locomotor and sensory function, neuropathic pain, spasticity, urinary and respiratory dysfunction, and metabolic problems. The loss of function is due to a primary mechanical insult and followed by a secondary injury, which leads to cell death, axonal degeneration, demyelination, inflammation, a cystic cavity surrounded by axon-growth inhibitory/repulsive molecules (eg, NOGO-A, chondroitin sulfate proteoglycans), and glial scarring (Schwab and Bartholdi, 1996).

DISEASES OF THE RETINA

Common causes of blindness due to retinal degenerative diseases include age-related macular degeneration (AMD) and retinitis pigmentosa (RP). AMD is associated with cellular debris between the retinal pigment epithelium (RPE) and the choroid, and blood vessel ingrowth from the choroid into the retina (Nowak, 2006). It can lead to detachment of the retina as well as atrophy of the RPE and loss of photoreceptors. RP is an inherited neurodegenerative disease, associated with different mutations in at least 50 genes (Shintani *et al*, 2009). It usually affects the photoreceptors directly, leading to blindness.

Combination Treatment Strategies

Combination strategies involving cells, bioactive molecules and biomaterials have been pursued over the past several years as a means to both enhance cell survival and integration after cell transplantation and achieve local delivery to the brain, thereby circumventing the BBB and systemic side effects (Orive *et al*, 2009; Pakulska *et al*, 2012). Similar to drug delivery, cell transplantation also poses potential systemic side effects when the cells are injected intravenously and not directly into or adjacent to the injury (Quertainmont *et al*, 2012). Biomaterials can serve as delivery vehicles for therapeutic molecules such as growth factors, proteins, and small molecules to provide a sustained and tunable drug release profile, without the need for multiple, high-dosage treatments (Hoare and Kohane, 2008). They can also be used as cell delivery vehicles, where they can provide physical support for cells to ensure their retention and distribution at the site of transplantation. Hydrogels are water swollen materials, which are particularly compelling for transplantation into soft tissue, such as the CNS, because they can match the mechanical properties of the tissue, are non-cytotoxic and allow both facile migration of cells and diffusion of biomolecules out of the scaffold while maintaining a physical structure (Drury and Mooney, 2003; Tibbitt and Anseth, 2009; Zhu and Marchant, 2011).

We will focus on recent advances using biomaterial hydrogels to study cell–substrate interactions and their use in cell transplantation and bioactive molecule delivery to the injured CNS, with specific focus on the brain and spinal cord after traumatic injury, and the retina due to disease.

CELL–SUBSTRATE INTERACTIONS: DESIGNING HYDROGELS TO MIMIC THE ECM

Cells and their extracellular matrix (ECM) define the cellular microenvironment in terms of chemical, physical and mechanical properties. The CNS ECM comprises proteoglycans such as chondroitin sulfate proteoglycans (CSPGs), glycosaminoglycans such as hyaluronan (HA), and proteins such as laminin, collagen, and fibronectin (Zimmermann and Dours-Zimmermann, 2008). Hydrogels can mimic the chemical, physical, and mechanical properties of the ECM to promote cell adhesion, proliferation, and differentiation (see Box 3). A commonly used hydrogel is Matrigel; however, as it is derived from a mouse sarcoma, its composition is complex and variable (Kleinman and Martin, 2005), making well-defined studies difficult to achieve and reproduce. In order to mimic the native ECM, a hydrogel that provides a blank palette on which biomolecules can be painted is often used to promote specific cell–substrate interactions. Recent advances on neural cell–biomaterial interactions are highlighted below, including chemical signals (the role of cell-adhesive molecules, growth factors and other cells); mechanical cues;

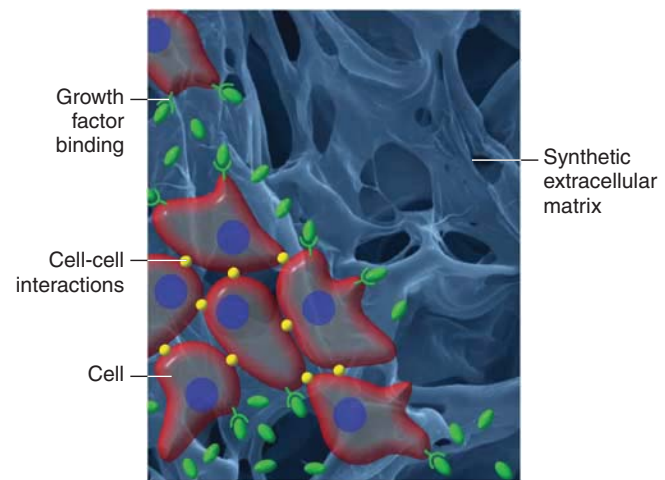


Figure 1. Schematic representation of the multiple interactions of the cellular microenvironment, including: cell–cell and cell–substrate interactions where the ECM is defined by its chemical, physical, and mechanical properties: stiffness and elasticity, matrix degradability, permeability and density of ECM components. Figure adapted with permission Owen and Shoichet (2010); copyright 2010 Wiley Periodicals Inc.

and physical cues (ie, architecture of the biomaterial) on neural cellular response (Figure 1).

Chemical Signals

ECM proteins represent key components in the cell niche that dictates cell fate. These proteins are recognized by cell surface receptors and are involved in cellular processes such as proliferation, differentiation, and migration. For example, fibronectin is a major component of the ECM that binds with cell surface receptors, known as integrins, to promote cell adhesion and viability (Prowse *et al*, 2011). Although the uninjured adult CNS contains limited fibronectin (DeQuach *et al*, 2011; Volpato *et al*, 2013), it has an important role in the developing CNS and has been shown to promote axonal regeneration of adult neurons (Tonge *et al*, 2012). Pierschbacher and Ruoslahti (1984) discovered that a short synthetic peptide, arginine–glycine–aspartate (RGD), derived from fibronectin also binds to integrins and promotes cell adhesion and viability (Hersel *et al*, 2003). Since this initial discovery, numerous other ECM-derived synthetic peptides have been identified to bind to integrin receptors. Given the importance of laminin in the neural ECM, laminin-derived peptides have been investigated: tyrosine–isoleucine–glycine–serine–arginine (YIGSR) (Graf *et al*, 1987) and isoleucine–lysine–valine–alanine–valine (IKVAV) (Tashiro *et al*, 1989) promote neural cell adhesion and neurite outgrowth, respectively, and similarly, the neural cell adhesion molecule (NCAM)-derived amino-acid sequence, EVYVVAENQQGKSKA, induces neurite outgrowth and increases neuronal survival (Neeiendam *et al*, 2004).

To take advantage of key cell–ECM interactions, these cell-adhesive peptides have been incorporated into biomaterial strategies. The conformation of the peptide is critical

to its binding with the corresponding integrin receptor. For this reason, longer peptide chains (Beer *et al*, 1992; Craig *et al*, 1995), cyclic peptides (Haubner *et al*, 1996; Kato and Mrksich, 2004), and/or peptide pairs (Grant *et al*, 1997; Mardilovich *et al*, 2006) are often immobilized to biomaterial surfaces to optimize cellular interaction. Presentation of the immobilized peptide is also critical to its cellular interaction (Maheshwari *et al*, 2000; Massia and Hubbell, 1991) and thus careful attention has been devoted to the conjugation chemistry. Peptide adsorption and/or non-specific conjugation disrupts peptide conformation, resulting in poor receptor binding and irreproducible results. Bio-orthogonal, water-based reactions provide superb peptide immobilization strategies, as they rely on specific coupling chemistry yielding controlled biomolecule orientation, which are key in subsequent bioactivity studies (Azagarsamy and Anseth, 2013). For example, Silva *et al* (2012) used orthogonal Diels–Alder click chemistry to immobilize the GRGDS peptide to a chemically-modified gellan gum hydrogel and showed greater cell adhesion and viability of NSPCs (Silva *et al*, 2012). Similarly, increased dorsal root ganglia neurite outgrowth was observed on elastin-mimetic polypeptide hydrogels functionalized with RGD (Lampe *et al*, 2013) and acrylate-modified dextran hydrogels functionalized with thiolated YIGSR and IKVAV peptides (Levesque and Shoichet, 2006).

Growth factors represent another key component in the cell niche that dictates cell fate. These proteins have cellular receptors and are involved in intracellular signaling processes resulting in proliferation, differentiation, and migration. Site-specific conjugation of growth factors to hydrogels is also key to cellular recognition. Taking advantage of the bio-orthogonal, high-affinity binding between biotin and streptavidin, biotin-platelet-derived growth factor (PDGF-AA) was conjugated to streptavidin-containing hydrogels, such as agarose (Aizawa *et al*, 2008) or hyaluronan/methylcellulose (HAMC) (Tam *et al*, 2012), and shown to promote the differentiation of rat NSPCs into oligodendrocytes. Similarly, when biotin–interferon- γ was conjugated to streptavidin-modified chitosan hydrogels, NSPCs differentiated preferentially into neurons (Leipzig *et al*, 2010). In these examples, the immobilized growth factors were repeatedly shown to promote similar NSPC differentiation as soluble growth factor controls, thereby demonstrating the potential of the immobilized growth factor, not only for *in vitro* differentiation but also for use in cell transplantation studies.

To understand cell–cell and cell–substrate interactions in the cell niche, defined biomimetic three-dimensional (3D) microenvironments can be created. The cytoarchitecture of the CNS is intricate and important for correct function. For example, the retina consists of seven cell types arranged in six layers, which are required for vision. Immobilization of specific growth factors and/or adhesion peptides within spatially defined volumes of a 3D hydrogel may promote preferential differentiation of retinal stem cells (RSCs) to a given phenotype in such spatially defined volumes, thereby

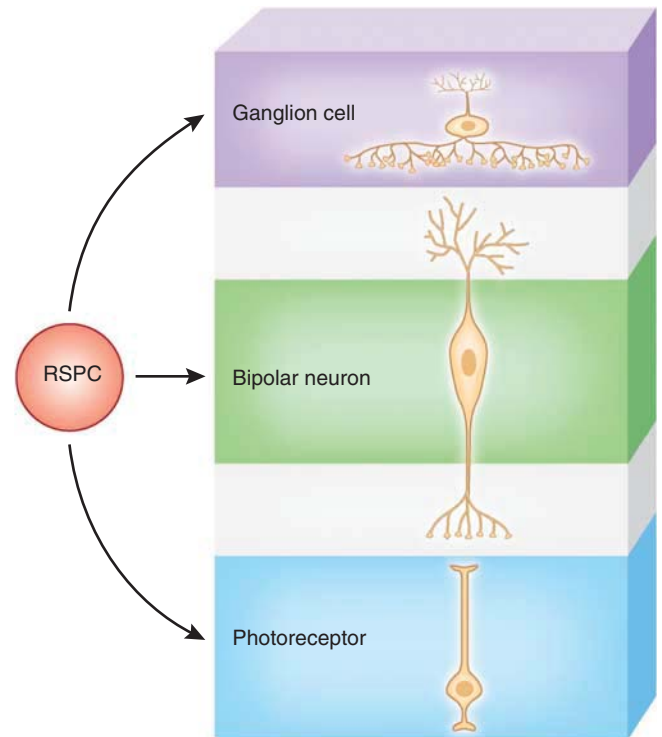


Figure 2. Concept schematic for the spatial immobilization of growth factors that will preferentially and spatially differentiate retinal stem/progenitor cells to progeny of the retina in a layered structure.

providing a platform to study cellular interactions (and disease progression) *in vitro* (Figure 2). Bio-orthogonal chemistry is particularly compelling for selective and spatially controlled immobilization of biomolecules in 3D. For example, protein concentration gradients and protein patterns were created in 3D hydrogels using multi-photon irradiation to precisely bind a given protein (Wosnick and Shoichet, 2008). In this technique, a photo-labile molecule is conjugated to a reactive functional group (eg, thiol) of the hydrogel. Exposure of these photo-labile protecting groups to multi-photon irradiation can be achieved in a spatially defined manner in 3D with micrometer resolution, thereby cleaving the photo-labile groups in a specific region of the hydrogel; subsequent conjugation reactions with bioactive molecules modified with the complimentary bio-orthogonal functional group (eg, maleimide or acrylate) results in spatially controlled immobilization of bioactive molecules. Numerous growth factors, such as CNTF, sonic hedgehog (SHH) (Wylie *et al*, 2011), vascular endothelial growth factor-165 (VEGF₁₆₅) (Aizawa *et al*, 2010), and EGF (Owen *et al*, 2013), as well as cell-adhesive RGD peptides (DeForest and Anseth, 2011) have been photopatterned into various hydrogels such as agarose, hyaluronan, and poly(ethylene glycol) (PEG). When a photopatterned concentration gradient of VEGF₁₆₅ was immobilized in a GRGDS-modified agarose hydrogel, Aizawa *et al* (2010) demonstrated primary brain endothelial cell migration, resulting in tubule-like structures in 3D. Moreover, a symbiotic interaction between endothelial cells and retinal progenitor cells

(RPCs) was observed in these gels: RPCs only grew into gels when cultured with endothelial cells, while endothelial tubules were stabilized by co-culture with RPCs (Aizawa and Shoichet, 2012). This may provide some insight into the RSC niche. Interestingly, Wylie *et al* (2011) reported the concomitant immobilization of multiple growth factors (CNTF and SHH) within distinct volumes of an agarose hydrogel in a spatially controlled fashion. NSPCs cultured on a GRGDS-modified agarose hydrogel migrated into the gel along a SHH concentration gradient (Wylie *et al*, 2011). These studies demonstrate that photopatterning can be used to control the spatial orientation of growth factors and peptides to create biomimetic 3D environments. The defined matrices can be used to study cell–cell and cell–substrate interactions, which may provide insight into disease progression, developmental biology, or drug screening.

The synergism between immobilized growth factors and cell adhesion molecules has been shown to influence cell receptor activation, resulting in improved signaling and bioactivity (Comoglio *et al*, 2003). Martino *et al* (2011) reported that by including growth factors (ie, PDGF, VEGF, and bone morphogenetic protein-2) on the same fibrin polymeric backbone as the integrin-binding domain, greater bioactivity was observed compared with binding them on separate polymer backbones. It was hypothesized that synergistic activation of integrins and receptor kinases occurs when the corresponding ligands of these receptors are in close spatial proximity to each other. This was further supported by Tam *et al* (2012) who demonstrated that co-conjugation of PDGF-A and the cell adhesive RGD peptide to the same polymer backbone promoted greater differentiation of NSPCs into oligodendrocytes compared with controls of each bioactive molecule alone or even both on separate polymeric backbones. Together, the studies demonstrate that cell–substrate interactions are influenced not only by the presence of specific ligands but also by their spatial orientation relative to each other. These are important considerations when designing biomaterials to promote specific cellular responses such as adhesion and/or differentiation whether for *in vitro* culture or *in vivo* transplantation.

Mechanical Cues

An effective biomimetic hydrogel will also emulate the physical and mechanical properties of the 3D environment. For example, Leipzig and Shoichet (2009) showed that gel stiffness affected the differentiation profile of rat neural stem cells. NSPCs cultured on softer (<1 kPa) methacrylamide chitosan hydrogels differentiated predominantly into astrocytes and neurons, whereas those cultured on hydrogels stiffer than 7 kPa differentiated largely into oligodendrocytes. Furthermore, NSPCs cultured on gels with an intermediate stiffness (3.5 kPa) exhibited the greatest amount of proliferation. Thus, even subtle differences in mechanical properties (while maintaining similar chemical

properties) can influence cell fate. Recently, Sur *et al* (2013) reported that dissociated hippocampal cells derived from mouse embryos cultured on peptide amphiphile hydrogels (~7 kPa) showed significantly increased neuronal differentiation, maturation, and synapse density compared with cells cultured on stiffer hydrogels (~25 kPa). MSCs also responded differently to varying mechanical properties: on soft substrates, with moduli comparable to that of the brain (0.1–1 kPa), MSCs preferentially differentiated to neural-like cells; on intermediate stiffness substrates, MSCs differentiated to myogenic cells, and on bone-like stiffness substrates (25–40 kPa), MSCs differentiated to osteogenic cells (Engler *et al*, 2006). Moreover, cell viability was higher on softer hydrogels (Liu *et al*, 2010). Interestingly, Musah *et al* (2012) reported that the viability of human ESCs increased when cultured on stiff poly(acrylamide) hydrogels. These seemingly conflicting results demonstrate that each cell type responds differently to changes in their physical environment, and thereby provide valuable insight into their development, while at the same time informing effective bioengineered materials strategies for tissue repair.

Physical Topographical Cues

The architecture of the native ECM is formed by dynamic cell remodeling, whereby cells can secrete and degrade structural molecules such as hyaluronan to create 3D environments necessary for cellular functions (Kim *et al*, 2011b). The formation of ECM structures, such as fibers, pores, and ridges, provides important structural support for cellular organization, as well as physical cues for cellular interactions (Flemming *et al*, 1999; Hoffman-Kim *et al*, 2010). The size and shape of two-dimensional biomaterial substrates coated with purified ECM molecules has been shown to influence the fate and function of adherent cells such as endothelial cells (Chen *et al*, 1997, 1998; Singhvi *et al*, 1994). For example, relatively large islands of ECM molecules have been reported to promote proliferation, while in stark contrast small islands have been shown to induce apoptosis. Other studies have demonstrated that aligned ECM molecules, as well as the micron-scale topography of fibers or grooves, exert substantial effects on the guidance and orientation of neural cells and their processes (Lietz *et al*, 2006; Rajnicek *et al*, 1997; Schnell *et al*, 2007). This is of particular interest in the development of biomaterial-based scaffolds intended to promote the repair of nervous system tissues, which are highly organized, such as the retina or white matter tracts of the spinal cord (Suzuki and Raisman, 1992). *In vitro* studies have demonstrated the influence of fiber diameter on the orientation of process outgrowth. The greater surface curvature of small-diameter fibers prompted an increasing tendency for neurite outgrowth to follow the longitudinal axis of the fibers: small-caliber fibers promoted greater oriented process growth than large-caliber fibers (Smeal *et al*, 2005; Smeal and Tresco, 2008). Recent advances in

nanotechnology and especially electrospinning have led to an interest in cell–substrate interactions in the sub-micron range (Schnell *et al*, 2007). The ability of fibers with diameters in the sub-micron range to affect the behavior of neural and non-neural cells (Bockelmann *et al*, 2011; Mo *et al*, 2004; Sun *et al*, 2005) has been demonstrated *in vitro*.

Studies into the role of biomaterial topography in the context of regenerative medicine and nerve repair include 3D scaffolds (Bozkurt *et al*, 2009). *In vivo*, Tsai *et al* (2004) demonstrated that poly(2-hydroxyethyl methacrylate-co-methylmethacrylate) hydrogel channels can be used to improve tissue regeneration of transected rat spinal cords. By inserting the transected cord stumps into the hydrogel nerve guidance channels, axonal regeneration was enabled by contact guidance cues while at the same time limiting the growth of scar tissue and maintaining alignment of both the cord stumps and migrating cells. Moore *et al* (2006) have synthesized multi-channelled scaffolds with tunable properties (ie, channel diameter, wall porosity) composed of poly(D,L-lactic-co-glycolic acid) (PLGA), which were designed to enable the use and guidance of multiple cell types within a single scaffold in a spatially defined manner. Such studies have made it increasingly clear that a better understanding of cell–substrate interactions, as well as cell–cell interactions within 3D scaffolds, is required for the optimal design of biomaterial- and cell-based treatment strategies intended to promote functional repair.

CELL DELIVERY

Cell transplantation is a promising therapeutic strategy to replace dead or damaged tissue in the CNS (Figure 3). Three

major challenges need to be overcome to deliver the promise of cell transplantation in regenerative medicine: (1) cell distribution; (2) cell survival/viability; and (3) host-tissue integration. The distribution of exogenously transplanted cells using saline or media is non-ideal, often resulting in aggregated cells (even before injection), thereby reducing cell survival. Cell viability upon transplantation into the host tissue is necessary to permit the transplanted cells to perform their desired functions, such as cytokine and growth factor secretion or cell–cell contact to promote tissue regeneration. Moreover, cells are not easily tracked and are often dispersed and removed by immune cells, disabling host-tissue integration. The inhospitable environment of the injured tissue at the lesion site and the lack of cell adhesion and cell survival factors further limit cell viability. Cell integration with the host tissue must be rapid, but is often limited by chemical and physical barriers. Hydrogels are promising cell delivery vehicles as they allow facile migration of cells and molecular transport of nutrients and oxygen (Orive *et al*, 2009; Pettikiriachchi *et al*, 2010). Moreover, they provide a physical scaffold for the retention of cells at the injection site upon transplantation, thereby overcoming the problem of cell dispersion, and injectable hydrogels facilitate minimally invasive surgery. Taking advantage of the chemistry described in the previous section, the hydrogel can be designed to promote cell survival with the inclusion of pro-survival factors, or to mimic the cell's ECM by incorporating specific chemical cues that direct stem cell fate upon transplantation. This section will focus on recent advances in hydrogel cell delivery vehicles into the brain, spinal cord, and retina in an effort to promote tissue and functional repair after injury or disease.

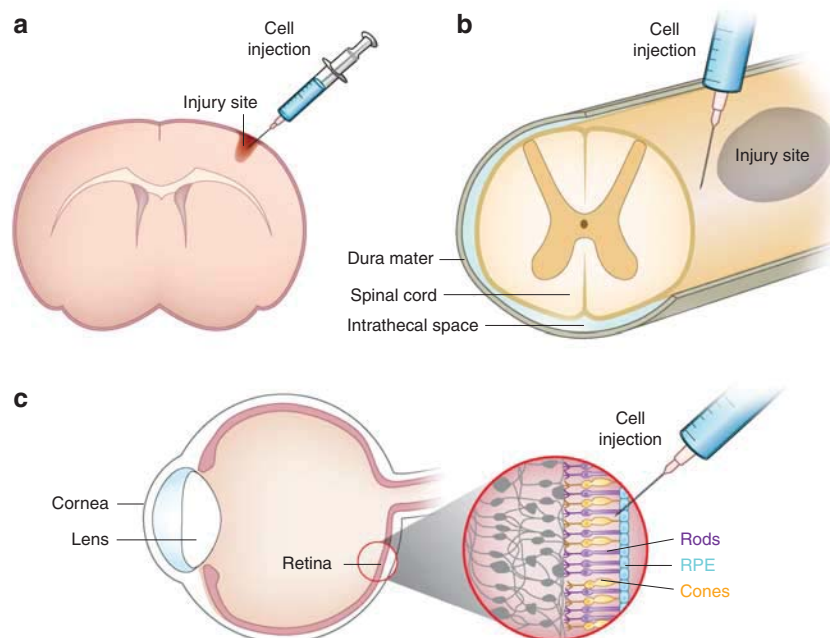


Figure 3. Cell delivery with or without biomaterials to (a) the injured brain, (b) the injured spinal cord and (c) the subretinal space of the retina between the photoreceptors (rods and cones) and the retinal pigmented epithelium (RPE). (b) Image copyright (2005) by Michael Corrin.

Brain

Early studies by Evans and Kaufman (1981) showed that NSPCs transplanted into dysmyelinated *shiverer* mice brains were able to differentiate into myelin basic protein-expressing oligodendrocytes, remyelinate host axons, and decrease tremors in some engrafted animals (Yandava *et al*, 1999). However, low cell survival and integration resulted in high variability in behavioral outcomes (Snyder and Park, 2002). Park *et al* (2002) showed that using a poly(glycolic acid) biopolymer as a delivery vehicle for NSPCs into hypoxia-ischemia-injured mouse brains promoted tissue regeneration by decreasing cavity volume, and enhancing neurite outgrowth and integration of transplanted cells.

Recently, the use of biomaterials that can interact with transplanted cells and the host tissue has been of significant interest. Hyaluronan is a naturally occurring polysaccharide found in the ECM of the central nervous system. It has anti-inflammatory properties, and can interact with various HA-receptors (such as CD44 and hyaluronan-mediated motility receptor) present on different cell types to promote cell adhesion and survival (Jiang *et al*, 2007). Zhong *et al* (2010) used a blend of HA, gelatin, and heparin to deliver NSPCs into stroke-injured mouse brains (see Figure 3a for a schematic representation of cell injections into the injured brain). They observed greater cell survival and attenuated host immune response in the graft site compared with cells delivered in suspension alone. The hydrogel acted as a barrier for infiltrating microglia/macrophages and prevented their migration into the graft (Zhong *et al*, 2010). Similarly, Kauer *et al* (2012) demonstrated that HA-based hydrogels, relative to phosphate buffered saline, increase the cell viability (approximately a three-fold increase from 20 to 60% after 28 days *in vivo*) of mouse NSPCs upon transplantation into mouse brains. These studies underline the benefits (eg, attenuating immune response and promoting cell viability) of using naturally occurring biopolymers as delivery vehicles.

Incorporating bioactive molecules into cell delivery vehicles has been explored to both improve cell survival via enhanced exogenous cell-substrate interactions and promote host-tissue neuroprotection and/or neuroregeneration. Cheng *et al* (2013) developed injectable hydrogels composed of self-assembling peptides for NSPC delivery: a hydrogel consisting of repeating units of the tetrapeptide, Arg-Ala-Asp-Ala, poly(RADA) with a C-terminal IKVAV sequence *vs* saline alone promoted greater tissue repair in the rat brain after a resection-type injury when used to deliver NSPCs. The inclusion of the IKVAV peptide sequence with poly(RADA) significantly increased the differentiation of transplanted NSPCs into neurons compared with poly(RADA) alone. Moreover, transplantation into the rat brain of NSPCs in fibrous poly(ϵ -caprolactone) (PCL) scaffolds immobilized with GDNF significantly enhanced NSPC survival, proliferation, and differentiation into neurons and oligodendrocytes *in vivo* relative to

transplantation of NSPCs in the absence of PCL (Wang *et al*, 2012a).

Vascularization of a transplanted graft is another important consideration for integration into the host tissue. To this end, Bible *et al* (2012) encapsulated VEGF within PLGA biodegradable microparticles coated with fibronectin to enhance NSPC adhesion. The authors demonstrated that release of VEGF recruited host endothelial cells into the graft site, resulting in the formation of primitive neurovascular units in the graft site. Interestingly, Matsuse *et al* (2011) reported that co-delivery of bone marrow-derived MSCs dispersed in collagen sponges with basic fibroblast growth factor (bFGF)-releasing gelatin microspheres improved cell viability, distribution, angiogenesis, and motor recovery following ischemic stroke. Together, these studies demonstrate that biomaterials can increase cell viability after transplantation into the brain, an effect that can be even further increased by incorporating bioactive molecules.

Spinal Cord

Attempts to replace lost cells and create a growth-promoting environment following spinal cord injury have included transplantation into the lesion site of: stem cells, such as NSPCs (Pfeifer *et al*, 2006), ESCs (McDonald *et al*, 1999), and iPSCs (Tsuji *et al*, 2010), peripheral cells (Bunge and Pearce, 2003) such as Schwann cells (Li and Raisman, 1994) and olfactory ensheathing glia (Ramon-Cueto *et al*, 1998), and central glia, such as oligodendrocytes (Nistor *et al*, 2005) and astrocytes (Davies *et al*, 2006). Such cell-based strategies promote some functional improvements in experimental animal models of spinal cord injury, but their mechanism(s) of action remain poorly defined, particularly due to poor cell survival following transplantation (Bradbury and McMahon, 2006). Furthermore, transplanted stem/progenitor cells differentiated uncontrollably, leading to negative side effects, such as allodynia (ie, hypersensitivity to normally non-painful stimuli) (Hofstetter *et al*, 2005). Delivery of the cells within hydrogels aims to improve cell survival. By incorporating peptides and/or growth factors, cell survival and/or controlled differentiation of stem/progenitor cells can be achieved. Although the approaches may ultimately be used for neurodegenerative diseases of the spinal cord, such as amyotrophic lateral sclerosis, we will focus solely on traumatic spinal cord injury in this review.

Itosaka *et al* (2009) found that fibrin-based hydrogels used to transplant MSCs into acute hemisectioned rat spinal cord lesions improved the survival of the transplanted cells and promoted functional recovery compared with saline or single-cell injections 4 weeks after injury. Delivery of oligodendrocyte precursor cells in hydrogels composed of HA and gelatin, crosslinked with PEG, in a demyelination lesion model of the spinal cord resulted in axon remyelination (Li *et al*, 2013); however, identification of transplanted *vs* host cells was ambiguous, and thus the

effect of the hydrogel on the survival of transplanted cells was unclear.

The co-delivery of stem cells with growth factors provides a strategy to control cell fate after transplantation. Johnson *et al* (2010a) transplanted mouse ESC-derived neural progenitor cells (ESC-derived NPCs) within fibrin scaffolds into a sub-acute, hemisectioned rat spinal cord lesion. The scaffolds were functionalized with heparin-binding peptides, which bind heparin and subsequently heparin-binding proteins (ie, PDGF-AA and NT-3) (Johnson *et al*, 2010b). The co-delivery of growth factors with ESC-NPCs significantly improved cell viability and neuronal differentiation after 2 weeks. However, by 8 weeks, the transplanted stem cells had formed tumors, clearly demonstrating the need for transplantation of defined and purified cell populations. An injectable hydrogel composed of HA and methyl cellulose (HAMC, which was first described by Gupta *et al* (2006), was used to successfully deliver both human umbilical derived stem cells and rat NSPCs into clip-compression injured rat spinal cords (Figure 3b) (Caicco *et al*, 2013b; Mothe *et al*, 2013). To promote the differentiation of transplanted rat NSPCs into oligodendrocytes, HAMC was functionalized with recombinant PDGF-A (Tam *et al*, 2012). Sub-acute transplantation of NSPCs in PDGF-functionalized HAMC into spinal cord injured rats, relative to conventional media controls, showed improved functional recovery on the ladder walk test, and increased host oligodendrocytes and host neurons. Furthermore, the combination of NSPCs and PDGF-A functionalized HAMC promoted the tissue sparing around the lesion site and a reduction in lesion volume (Mothe *et al*, 2013).

Synthetic hydrogels, such as poly(2-hydroxypropyl methacrylamide) (PHPMA) functionalized with RGD, have also been investigated for MSC transplantation into the chronic balloon compression injured spinal cord (Hejcl *et al*, 2010). Animals received implants 5 weeks after injury, and some cells were present within the hydrogel after 6 months. Animals that had PHPMA/RGD + MSC transplants showed greater behavioral functional repair compared with animals with injury alone, but not relative to animals that had hydrogel implants alone, obscuring the benefit of the MSCs. The tissue benefits observed in animals transplanted with the PHPMA/RGD + MSCs included: reduced tissue atrophy, migration of astrocytes and Schwann cells into the scaffold, accompanied by axons, and angiogenesis. Other synthetic hydrogels, such as poly(*N*-isopropylacrylamide)-*g*-PEG (PNIPAAm-*g*-PEG) and poly(*N*-isopropylacrylamide)-*g*-methylcellulose (PNIPAAm-*g*-MC) branched copolymers have also been transplanted in hemisection models of spinal cord injury (Conova *et al*, 2011). The authors investigated short survival times (1 and 2 weeks) after acute injections and demonstrated that the hydrogels did not contribute to the inflammatory response, the glial scar or demyelination, and were permissive to axonal growth when supplemented with BDNF. Although transplanted fibroblasts survived when transplanted within PNIPAAm-*g*-PEG,

neither a detailed analysis nor comparison with cell injections without the hydrogel was included. Thus, natural and synthetic polymers, particularly when conjugated to other bioactive molecules, can function as effective cell delivery vehicles into the injured spinal cord.

Hydrogels with unique architectures, such as self-assembling peptide nanofiber scaffolds (SAPNS), have promoted repair in spinal cord lesions (Tysseling-Mattiace *et al*, 2008). The structure of the nanofibers facilitated neurite guidance across the lesion site (Zhan *et al*, 2013). SAPNS were used to transplant Schwann cells and NSPCs into acute lesions of dorsal column transections of the spinal cord (Guo *et al*, 2007). Both the cell types survived up to 6 weeks after transplantation, the longest time point investigated. The combination of hydrogel with Schwann cells promoted the greatest axonal ingrowth of motor and sensory neurons. Furthermore, host cells infiltrated and blood vessels extended into the scaffold. Therefore, cell- and axon guidance in the injured spinal cord can be achieved by controlling the topology of biomaterials.

Interestingly, pre-differentiation of NSPCs to neurons was shown to enhance cell survival and functional repair after transplantation in a full transection spinal cord injury rat model relative to animals receiving *in situ* differentiated NSPCs (Kim *et al*, 2011a). Cells were transplanted using fibrin-filled chitosan hydrogel guidance channels that matched the modulus of spinal cord tissue (Freier *et al*, 2005). NSPCs that had been pre-differentiated to neurons with dibutyryl cyclic AMP (dbcAMP) before transplantation survived significantly (by 80%) better than cells that were differentiated *in situ*, by local, sustained release of dbcAMP. Moreover, synapse formation and behavioral repair were evident only in animals transplanted with dbcAMP pre-differentiated neural cells. These studies demonstrate the importance of the differentiated progeny coupled with the biomaterial for greater survival of transplanted cells.

Retina

The first cell transplantation experiment in the eye was performed in the late 1950s, when Royo and Quay (1959) isolated a whole retinal sheet from a dog fetus, and implanted it in the anterior chamber of the mother's eye. Numerous subsequent studies have since transplanted cells into the retina in an attempt to regenerate damaged photoreceptors (Gouras *et al*, 1984; Gouras *et al*, 1985; Li and Turner, 1988). However, it was later realized that cell transplantation in solution suffered from significant drawbacks such as cell reflux from the injection site, cell aggregation, cell death, and lack of integration into the host tissue (MacLaren *et al*, 2006; Wongpichedchai *et al*, 1992). Interestingly, retinal pigmented epithelia (RPE) and whole-fetal retinal cells that were transplanted as a sheet survived better than cell suspensions (Seiler and Aramant, 1998, 2012; Turner *et al*, 1988) as the continuous adherence to their matrix protected them from anchorage-dependent cell

death, known as anoikis (Taddei *et al*, 2012). In addition, cell polarity was maintained, and greater immune tolerance was observed compared with cells transplanted in suspension (Ghosh *et al*, 2008; Wenkel and Streilein, 2000). Transplanting cells with biomaterials, in a defined cellular microenvironment, is advantageous for graft-cell survival and integration. Materials provide an adhesion surface for the cells, and afford more uniform cell distribution, thereby decreasing cell aggregation (Ballios *et al*, 2010). They can also be used as vehicles for the incorporation of additional chemical factors that can enhance cell survival, differentiation, and integration.

Early studies of cell transplantation into the retina focused on biomaterial biocompatibility. Bhatt *et al* (1994) reported no gross evidence of rejection using sheets of collagen I to transplant human RPE into the rabbit subretinal space. Moreover, transplantation of biodegradable gelatin membranes containing neural retina sheets into the eyes of rabbits did not elicit an inflammatory response (Hsiue *et al*, 2002). Degradation of the biomaterial was also investigated in order to prevent persistent retinal detachment at the transplantation site, which could lead to complications such as photoreceptor degeneration, gliosis, and proliferative vitreoretinopathy (Lewis and Fisher, 2003; Lo *et al*, 2011; Pastor *et al*, 2002). These early reports demonstrated the feasibility of biomaterials for cell transplantation into the retina.

The mechanical properties of biomaterials used for cell transplantation are key to ensure minimal retinal tissue damage after transplantation. For example, Lavik *et al* (2005) demonstrated that scaffolds composed of a blend of PLGA and poly(lactic acid) (PLA), prepared via solid-liquid phase separation, resulted in reduced elastic modulus and increased maximum strain at failure compared with PLA alone (Lavik *et al*, 2005). The reduced elasticity of the scaffolds means that they are more compliant to the sensitive retinal tissue (ie, the scaffolds can more readily alter their conformation to match the structure of the retina), and thus reduces the physical damage to the retina upon transplantation. In addition, the increased maximum strain at failure means that the scaffolds are less likely to break during the transplantation procedure. Mouse retinal progenitor cells (RPCs) were efficiently and uniformly dispersed along the surface of PLGA/PLA scaffolds. RPCs adhered to the scaffolds, differentiated into Müller glia, and were implanted into the rat subretinal space. However, no evidence of photoreceptor or other neuronal differentiation is shown, which would have been preferable for transplantation into animal models of retinal degeneration. Müller glia transplantation has not been shown to have a regenerative effect in the retina, and usually impedes transplanted cell integration in the retina (West *et al*, 2008). Thus, the authors suggest that future studies should also utilize chemical cues delivered in these PLGA/PLA scaffolds to promote differentiation into functional photoreceptors. Using the PLGA/PLA scaffolds, ~50% of transplanted cells were estimated to be alive after 14 days,

which is significantly greater than that achieved by bolus injections, where typically ~1–2% of cells survive (MacLaren *et al*, 2006); however, a direct comparison is difficult because different cells were delivered in these two studies (Lavik *et al*, 2005; MacLaren *et al*, 2006). Importantly, a subsequent study demonstrated that biomaterials increase cell survival after retinal transplantation compared with bolus injections (Tomita *et al*, 2005a). Transplantation of RPCs into the subretinal space with laminin-coated PLGA-PLA scaffolds promoted a 10-fold increase in cell survival over bolus injection. At 2 and 4 weeks after transplantation, RPC differentiation was observed, as evidenced by the expression of neurofilament 200 (NF-200, a neuronal marker), PKC- α (a bipolar cell marker), recoverin and rhodopsin (photoreceptor markers). Some cell migration into the retinal layers was also observed. Probing the mechanism of increased survival conferred by the biomaterial, the authors showed that EGF, which is used in the culture medium of the RPCs, had adsorbed to the biomaterial and may have contributed to the increased survival observed in the biomaterial group. An advantage of this particular approach is that the biomaterial allows for more precise control over the placement of the transplanted cells in the host tissue such that it can be positioned at the site of the degenerated area (for example, in the macula for AMD patients). A potential caveat is that although PLGA-PLA scaffolds promote cell survival and differentiation upon transplantation, the implantation surgery is highly invasive and the biomaterial, although degradable, is still present after 4 weeks, and thus may lead to retinal detachment. Even though the molar mass and shape of the biomaterial and implantation site influence its degradation rate, PLGA is known to be a slowly-degrading material within the eye. For example, PLGA microspheres have been detected in the eye 6 months after transplantation (Giordano *et al*, 1995). These studies demonstrated that cells transplanted with biomaterials showed increased cell survival and integration into the retina; however, there were still shortcomings that needed to be overcome.

A significant obstacle to promoting functional recovery following cell transplantation is the successful migration of cells out of the biomaterial and integration into the host tissue. To enhance cell migration out of the biomaterial, Tucker *et al* (2010) transplanted RPCs using a scaffold consisting of PLGA encapsulated with matrix metalloproteinase-2 (MMP2). MMP2 is known to degrade neurocan, a CSPG, and CD44, a hyaluronan-binding protein (Zhang *et al*, 2007b). Neurocan and CD44 can form fibrous networks in the degenerating retina (and in other CNS regions), and have been shown to impede neurite outgrowth, ultimately causing cell death (Chaitin and Brun-Zinkernagel, 1998; Inatani *et al*, 2000; Krishnamoorthy *et al*, 2000; Moon *et al*, 2003). RPCs that were delivered from PLGA-MMP2 scaffolds exhibited greater migration into the host outer nuclear layer of the retina compared to PLGA-bovine serum albumin (BSA) scaffolds. Furthermore, the

migrating RPCs differentiated into photoreceptors as determined by the expression of the photoreceptor markers recoverin or rhodopsin. Importantly, the presence of MMPs did not negatively affect retinal morphology. This study shows that functionalization of biomaterials with factors that neutralize inhibitory clues of the host micro-environment can improve the integration of RPCs into the retina.

Another technique to promote the survival, migration, and integration of transplanted RPCs into the retina was developed by Tao *et al* (2007), who used microelectromechanical system technology to create ultrathin (6 μm) poly(methyl methacrylate) (PMMA) sheets with or without pores of defined sizes. The survival and proliferation of murine RPCs cultured on porous and non-porous PMMA sheets *in vitro* were comparable to cells cultured on tissue culture polystyrene. Interestingly, RPC transplantation with porous PMMA sheets into the mouse subretinal space demonstrated significantly more surviving cells after 4 weeks compared with non-porous PMMA sheets. The difference was hypothesized to be due to an inferior retention of the RPCs in the non-porous PMMA during the transplantation process, leading to increased cell reflux. Substantive cell migration into the host retina was also observed with the porous scaffolds; migrated donor cells expressed glial fibrillary acidic protein (GFAP), NF-200, or recoverin as evidence of differentiation towards Müller glia, neurons, and photoreceptors, respectively. The retinal detachment created during transplantation appeared to resolve itself within 4 days, possibly due to the thin, porous material used; however, although PMMA is biocompatible and has been used as a lens replacement for many years (DeVore, 1991), it is a stiff, non-degradable material (Yamanaka *et al*, 1985). Therefore, it would either ultimately require a second surgery for its removal or, if left within the eye indefinitely, would likely result in a long-term immune response or other complications associated with the intraocular use of PMMA (Amon and Menapace, 1994; Rowe *et al*, 2004; Tognetto *et al*, 2003).

The use of materials that cause the least amount of damage to the eye during the transplantation procedure is a significant consideration, and has led to the development of injectable biomaterials. All of the aforementioned materials were transplanted in the subretinal space through a 5-mm scleral incision, using forceps, which entails significant trauma to the eye. In an effort to resolve this issue, Redenti *et al* (2009) used microfabricated poly(glycerol sebacate) (PGS) scaffolds that were 45 μm thick with 50 μm diameter pores, and coated them with laminin. Mouse RPCs cultured on PGS showed increased adhesion and proliferation compared with those cultured on glass. This highly elastic material was injected through a 25-gauge needle, requiring only a 1–2 mm scleral incision. (see Figure 3c for a schematic representation of subretinal injections). PGS is biocompatible and degrades within ~ 1 month after subretinal transplantation. Subretinal transplantation of mouse RPCs in PGS showed that mouse RPCs migrated

into the inner and outer nuclear layers of the host retina, with few differentiating into photoreceptor and bipolar cells; however, the efficacy of this matrix for cell delivery *in vivo* is unclear, as no control group consisting of cells in the absence of PGS was included. Interestingly, Ballios *et al* (2010) studied the injectable HAMC hydrogel for RSC transplantation, first showing its benefit *in vitro* and then *in vivo*. Importantly, the HAMC hydrogel was injected through an even finer (than the PGS scaffold), 34 gauge needle with fast gelation after injection. An *in vivo* degradation assay in the subretinal space demonstrated resorption of the gel after 7 days, thereby minimizing risks of adverse retinal detachment while maximizing cell migration and host-tissue integration. After subretinal injection in mice, RPCs delivered in HAMC were evenly distributed along the Bruch's membrane, whereas RPCs delivered in conventional saline controls were aggregated. Importantly, the retinal architecture was not affected by the hydrogel injection. Together these data demonstrate that this is a compelling biomaterial for cell delivery to the retina while obviating the complicated surgery associated with transplanting biomaterial sheets.

BIOMOLECULE DELIVERY TO THE CNS VIA HYDROGEL AND NP VEHICLES

Delivery of bioactive molecules to the CNS is currently being pursued to promote neuroprotection and plasticity, modulate the inflammatory response, and stimulate endogenous stem cells to proliferate and migrate to the injured tissue (Figure 4) (Dibajnia and Morshead, 2013). However, CNS treatment is challenging as many CNS disorders are characterized by a multifactorial pathophysiology (ie, expression of growth inhibitory molecules, genetic alterations, inflammation, altered immunoresponse, excitotoxicity, and oxidative stress) making successful single drug development difficult (Rossi *et al*, 2013). Furthermore, delivery of therapeutics into the CNS is limited by the low permeability of the blood–brain/spinal cord barrier to many of these molecules (Groothuis, 2000; Pardridge, 2012). Efforts to overcome this issue have included using high doses of therapeutics delivered to the intrathecal space of the spinal cord by either bolus injection or prolonged infusion with osmotic minipumps. These techniques are non-ideal, resulting in either uneven, short-lived exposure to the delivered drug (with bolus) or possible infection/blockage (with external minipump/catheter systems). Intraventricular injections into the brain bypass the BBB, but are highly invasive and result in significant tissue damage with cannula insertion through brain tissue. More recent strategies involve chemical or electromagnetic disruption of the barrier coupled with functionalized NPs or liposomes that have limited capacity to cross the blood–brain/spinal cord barrier (Rossi *et al*, 2013).

Biomaterials as drug delivery systems can offer localized and sustained delivery. Furthermore, they provide a

BOX 2 Stem Cells for CNS Repair

Stem cells are defined by two cardinal properties: (I) self-renewal and (II) differentiation into committed progenitors that give rise to mature cells (Becker *et al*, 1963; McCulloch and Till, 2005; Siminovitch *et al*, 1963; Till and McCulloch, 1961). Stem cells have been classified by their developmental potential as totipotent (able to give rise to all embryonic and extra-embryonic cell types, such as the zygote), pluripotent (able to give rise to all cell types within the body of an organism), multipotent (able to give rise to a subset of cell lineages), oligopotent (able to give rise to a few cell lineages), and unipotent (able to contribute only one mature cell type) (Bongso and Richards, 2004). Stem/progenitor cells used for transplantation include:

- *Embryonic stem cells (ESCs)* are pluripotent cells derived from the inner cell mass of the pre-implantation blastocyst (Evans and Kaufman, 1981).
- *Induced pluripotent stem (iPS) cells* are pluripotent cells derived from adult cells that are reverted to an embryonic stage by the introduction of certain transcription factors such as OCT4, SOX2, Klf-4, and c-Myc (Takahashi and Yamanaka, 2006).
- *Neural stem/progenitor cells (NSPCs)* are multipotent cells derived from the CNS that can differentiate into neurons, oligodendrocytes and astrocytes. In the brain, they are located in the lateral walls of the ventricles (subventricular zone, SVZ) (Reynolds and Weiss, 1992) and the dentate gyrus in the hippocampus (subgranular zone) (Clarke and van der Kooy, 2011). In the spinal cord, they are located in the central canal (Weiss *et al*, 1996).
- *Retinal stem cells (RSCs)* are rare multipotent cells that reside in the ciliary epithelium of the adult eye (Tropepe *et al*, 2000). They can give rise to each of the seven different cell types of the retina, as well as retinal pigmented epithelium.
- *Retinal progenitor cells (RPCs)* are oligopotent cells from the retina of newborn mice or human embryos at 10–14 weeks of gestation (Kelley *et al*, 1995; Klassen *et al*, 2001). They differentiate only into certain retinal cell types, such as rod photoreceptors, amacrine and bipolar cells, and can be propagated for a limited number of passages.
- *Mesenchymal stromal cells (MSCs)* are multipotent cells derived from the bone marrow as well as non-marrow (eg, blood and adipose) tissue and differentiate into osteoblasts, chondrocytes and adipocytes (Dominici *et al*, 2006; Friedenstein *et al*, 1970). Although they do not readily differentiate into neural cells, they are easily accessible and have shown to promote recovery in experimental animal models of CNS injury (Hofstetter *et al*, 2002).

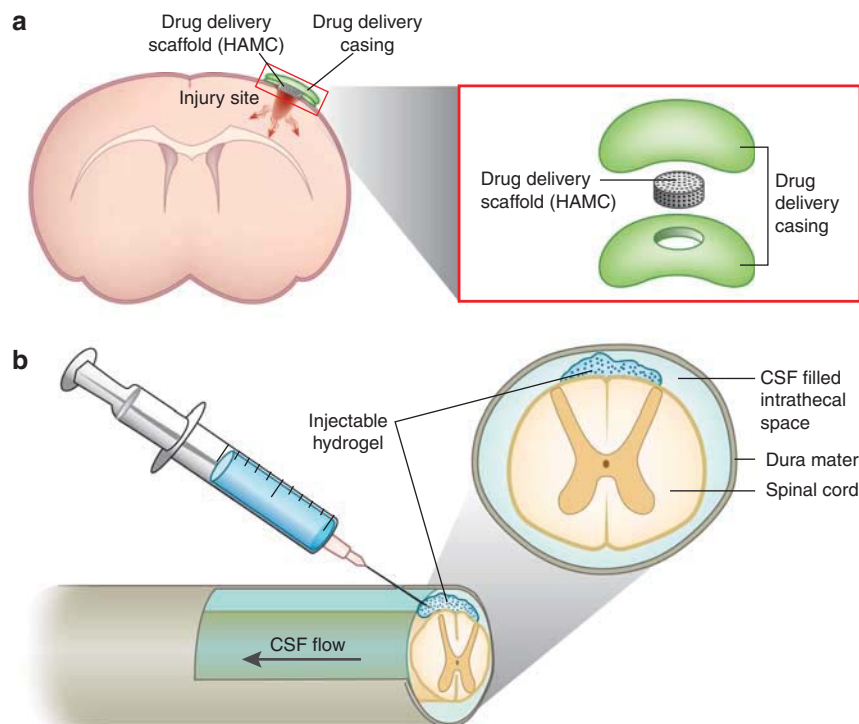


Figure 4. Schematics of hydrogel-based drug delivery system to (a) the injured brain, and (b) the injured spinal cord. (a) The drug-containing hydrogel (HAMC) is placed on top of the cortex, permitting diffusion into the brain. The black arrows in the horizontal cross-section indicate diffusion in all directions. (b) Hydrogel injection into the space between the spinal cord and the dura mater, termed intrathecal space. Fast gelling hydrogels enable local release at the injection site. Figures adapted with permission from (a) Cooke *et al* (2011); copyright 2011 Elsevier. (b) Image copyright (2005) by Michael Corrin.

physical scaffold or depot for sustained release while at the same time protecting the therapeutic biomolecule from degradation *in vivo*. The properties of these biomaterials can be altered to tune the release rate of the biomolecule into the target tissue. Prolonged release is important because it obviates the need for multiple injections to sustain a constant dosage. This section will highlight some of the recent advances using biomaterials to deliver therapeutic molecules to the brain and spinal cord.

Brain

Several growth factors have been shown to promote the proliferation (Erlandsson *et al*, 2011; Popa-Wagner *et al*, 2010; Sun *et al*, 2003; Teramoto *et al*, 2003; Tureyen *et al*, 2005), migration (Kolb *et al*, 2007; Ohab *et al*, 2006; Schabitz *et al*, 2007), and differentiation (Kolb *et al*, 2007; Popa-Wagner *et al*, 2010; Schabitz *et al*, 2007) of endogenous stem cells, and improve functional recovery (Erlandsson *et al*, 2011; Kolb *et al*, 2007; Ohab *et al*, 2006;

BOX 3 Hydrogel Properties Considered Relevant for Drug and Cell Delivery to the CNS

In general, some issues/properties should be considered for developing hydrogel-based drug and cell delivery systems:

- The chemical and mechanical properties of the material, eg, to mimic the extracellular microenvironment in terms of chemical properties, such as growth factors and extracellular matrix proteins, and mechanical properties in terms of stiffness, porosity, and topography.
- The ability to load multiple drugs/biologics and control their release.
- The distribution of cells within the hydrogel, which will influence their survival and capacity to integrate with the host tissue.
- The release kinetics, the control of which allows the appropriate dose of growth factor or drugs to be released over a given period of time, eventually with different kinetic profiles at the same time for one or multiple agents.
- The ability to protect proteins against degradation.
- The biodegradability/bioresorbability of the material.
- The injectability of the delivery system, allowing for minimally invasive surgery.
- The interactions with the host immune system (biocompatibility).
- The non-toxicity of the delivery system and its degradation products.
- The stability/gelation time, the control of which ensures that the biomaterial hydrogel (and either cells or biomolecules encapsulated within) remains at the injection site to achieve local delivery.

Popa-Wagner *et al*, 2010) in experimental animal models. However, some of these studies used either invasive delivery techniques such as intraventricular injections, which can lead to further damage, or systemic injection, which can result in off-target, cytotoxic or non-specific effects due to the very high dosage required to cross the BBB before systemic degradation and circulation.

Efforts to increase the bioavailability of drugs delivered into the brain via systemic delivery have resulted in the use of various drug carriers, such as polymeric NPs, which are described in detail elsewhere (Orive *et al*, 2009). Polymeric NPs comprise natural or synthetic polymers (eg, PLA and PLGA) and are between 10–1000 nm in diameter. These particles enhance drug circulation and can be designed to target and cross (to a limited extent) the BBB following systemic injection. Liu *et al* (2013a, 2013b) recently reported the use of PEG-PLA NPs to deliver the neuroprotective peptide NAP (NAPVSIPQ) into the brain via tail-vein or intranasal injections. To promote NAP transport across the BBB, the authors covalently conjugated a transferrin-mimetic peptide (B6: CGHKAKGPRK) (Liu *et al*, 2013a) or lactoferrin (Liu *et al*, 2013b), a glycoprotein in the transferrin family, to PEG-PLA NPs. Transferrin receptors are located on the surface of endothelial cells of the BBB and are involved in receptor-mediated transcytosis to transport bound-ligands across the BBB. Tail-vein injections of these NPs resulted in greater accumulation of the B6-PEG-PLA and lactoferrin-PEG-PLA NPs in the brain compared with PEG-PLA NPs alone. Behavioral experiments (Morris water maze) showed that animals treated with NAP-laden B6-PEG-PLA or lactoferrin-PEG-PLA NPs preserved hippocampal function in animal models of Alzheimer's disease. These studies show that systemic delivery of therapeutic molecules can be used to permeate the BBB and preserve functional benefits using the appropriately designed carriers.

Nakaguchi *et al* (2012) recently reported local delivery of insulin-like growth factor-1 and hepatocyte growth factor using gelatin microspheres to promote proliferation and

neuronal differentiation of endogenous brain NSPCs (Nakaguchi *et al*, 2012). The microspheres were injected into the striatum of mouse brains to reach the NSPC niche in the subventricular zone (SVZ). Increased neurogenesis (defined by the number of doublecortin-positive (DCX⁺) cells) was observed with growth factor releasing microspheres relative to vehicle controls 7 days post-injection.

An alternative strategy for drug delivery to the NSPC niche was developed by Cooke *et al* (2011), who demonstrated that epi-cortical implants of EGF-containing HAMC hydrogels (Figure 4a) can provide sustained delivery to the SVZ. Furthermore, Cooke *et al* (2011) and Wang *et al* (2012b) reported deep brain tissue penetration of two factors, EGF and EPO, respectively, previously shown to stimulate endogenous stem cells and promote tissue repair in an animal model of stroke (Kolb *et al*, 2007). Interestingly, chemical modification of EGF with PEG enhanced its tissue penetration depth, while preserving its bioactivity, relative to EGF alone (Cooke *et al*, 2011). EGF-PEG induced significantly more Ki67⁺ proliferating cells than EGF or vehicle controls. EPO, similarly delivered from the epi-cortically-placed HAMC hydrogel, was also shown to stimulate the migration of DCX⁺ neuroblasts from the SVZ. Another molecule that has been shown to promote neurogenesis following stroke in animal models is cyclosporine A (CsA) (Erlandsson *et al*, 2011). Recently, Caicco *et al* (2013a) demonstrated sustained local release of CsA to the brain using epi-cortical implants of CsA encapsulated in PLGA microspheres dispersed in HAMC. CsA was shown to be released for 25 days into the brain and to reach the NSPC niche in the SVZ, which is key to stimulating neurogenesis of endogenous NSPCs (Caicco *et al*, 2013a; Erlandsson *et al*, 2011). Together, these studies show that therapeutic molecules can be delivered into the brain using an epi-cortical delivery approach, thereby circumventing the BBB and invasive injections.

Regeneration of injured tissue is critical to functional recovery and this is enhanced by re-vascularization. To

enhance angiogenesis, Zhang *et al* (2007a) encapsulated VEGF₁₆₅ in poly(dimethylsiloxane-tetraethoxysilane) (PDMS-TEOS) that was then injected into a brain cavity. The PDMS-TEOS biomaterial filled the brain cavity and restored the brain shape compared with the distorted shape that remained without biomaterial injection. Furthermore, delivery of VEGF₁₆₅ significantly increased the number of endothelial cells and astrocytes in the scaffold compared with delivery of PDMS-TEOS alone (Zhang *et al*, 2007a). Alginate hydrogels used to deliver VEGF₁₆₅ into stroke-injured rat brains also resulted in significant improvement in locomotor activity compared with vehicle controls (Emerich *et al*, 2010). Interestingly, sustained VEGF₁₆₅ release resulted in significantly better behavioral recovery and reduced lesion volume compared with bolus delivery of VEGF₁₆₅, thereby demonstrating the beneficial effects of sustained delivery strategies.

Spinal Cord

Intrathecal delivery via minipumps has been used as an alternative to systemic and oral administration. Unfortunately, direct injections into the CSF show only a poor distribution of the drug into the spinal cord parenchyma (Rossi *et al*, 2013). Furthermore, possible side effects include obstruction, leakage, breakage and dislodgment of the catheter, hemorrhage, CSF leaks and infections (Belverud *et al*, 2008). More recently, NPs and hydrogels have been investigated as an alternative way to deliver drugs to the injured spinal cord. NPs typically have a short half-life *in vivo*, and are often coated with PEG to increase their half-life in the bloodstream and prevent macrophage uptake. They have been used to deliver methylprednisolone (Kim *et al*, 2009), prostaglandin E1 (Takenaga *et al*, 2010), and mixtures of different neurotrophic factors (BDNF, GDNF, NGF, CNTF, and other peptide fragments) (Menon *et al*, 2012) to the injured spinal cord. However, injected NPs often leave the zone of injection as they are not confined by any support, and easily extravasate into the circulatory system, where they are distributed all over the body, including the liver and spleen (Kim *et al*, 2009). Therefore, local delivery of drugs/biomolecules with hydrogels (Figure 4b) or NPs dispersed in hydrogels have been suggested to provide a targeted therapy that is able to maximize the efficacy of bioactive agents while minimizing their side effects.

Localized drug delivery into the injured spinal cord using biomaterials has focused significantly on axonal regeneration to promote motor function repair. One of the earliest studies investigating the potential of combinations of hydrogels and bioactive molecules was done by Goldsmith and de la Torre (1992) where collagen scaffolds were supplemented with 4-aminopyridine (a potassium channel blocker), laminin, beta-glia maturation factor, or omental lipid angiogenic factor. Implantation of these hydrogels into completely transected spinal cords of cats promoted the growth of dense bundles of neurites into the collagen matrix

and into the distal spinal cord. These fibers showed a maximal outgrowth of 90 mm below the transection site upon addition of 4-aminopyridine into the collagen matrix. Unfortunately behavioral analysis was not included in this investigation.

Jain *et al* (2006) used agarose gels and lipid microtubes to deliver BDNF to dorsal hemisection injuries of the spinal cord. BDNF reduced both the reactivity of astrocytes and the production of CSPGs at the lesion site, leading to more regenerating fibers entering the implanted hydrogel at 6 weeks after injury. However, the functional benefits of this strategy were not described and the complications associated with having to cool the agarose solution during its delivery make this strategy impractical. NT-3, in the same neurotrophic family as BDNF, was delivered via PLA-PEG hydrogels to dorsal hemisection injuries, and detected at the lesion site for up to 14 days (Piantino *et al*, 2006). Remarkably, NT-3-treated animals showed greater axonal regeneration and improved functional recovery relative to vehicle controls. Taylor *et al* (2006) also delivered NT-3, but into a suction ablation model of spinal cord injury, using fibrin hydrogels with a heparin binding system (Johnson *et al*, 2010b). They observed greater axonal ingrowth into the lesion compared with control scaffolds (without NT-3) 9 days after injury. Furthermore, reduced GFAP immunoreactivity was detected at the white matter border of the lesion with fibrin scaffolds compared with saline injections. However, animals treated with or without fibrin scaffolds and NT-3 did not show functional improvement over saline controls.

Experimental treatment strategies to promote neuroprotection and tissue sparing aim to reduce the loss of cells at and around the lesion site. Jimenez Hamann *et al* (2005) injected concentrated collagen solutions into the intrathecal space to deliver EGF and bFGF to the spinal cord after clip compression injury. They observed that EGF diffused deep into the tissue, but the signal was lost after 7 days, whereas bFGF could still be detected adjacent to the hydrogel after 7 days. After 56 days, collagen solutions containing EGF and bFGF improved white matter sparing and showed the lowest cavitation volume and the greatest ependymal cell proliferation relative to controls; despite these promising results, no functional benefits were observed.

The inflammatory response after spinal cord injury is thought to have beneficial and detrimental properties, depending on the type, severity, and time after injury (Chan, 2008; Kwon *et al*, 2004). Several studies have demonstrated that both inhibition and promotion of the inflammatory response can have beneficial effects (Ghirnikar *et al*, 2000; Hauben *et al*, 2000; Rapalino *et al*, 1998; Wamil *et al*, 1998). Kang *et al* (2010) delivered minocycline, an antibiotic, to lateral hemisection injuries via tetronic-oligolactide hydrogels. The implantation of the hydrogel alone allowed blood vessel formation in the lesion cavities, and in combination with minocycline, macrophage infiltration, and CSPG deposition decreased while axon growth through the lesion area increased in a dose-dependent

manner 7 weeks after injury. As no behavioral analysis was reported, it is not clear whether this strategy results in functional improvements.

The two most prominent and extensively investigated regenerative agents are chondroitinase ABC (ChABC), which has been shown to degrade the growth inhibitory CSPGs at the glial scar, and anti-NGO antibodies, which neutralize the myelin-associated inhibitory molecule NGO-A (Chen *et al*, 2000; Zuo *et al*, 1998). ChABC is relatively unstable at 37 °C (Tester *et al*, 2007) and requires sustained local delivery to the spinal cord for efficacy. Thus, hydrogel-based delivery systems must not only achieve desirable sustained release profiles, but also protect ChABC against enzymatic and hydrolytic degradation *in vivo*.

Agarose microtubes were used to deliver ChABC to dorsally hemisectioned spinal cords (Lee *et al*, 2010). ChABC was stabilized with trehalose in the formulation to retain its biological activity. Thermostabilized ChABC remained active at 37 °C *in vitro* for up to 4 weeks using a non-quantitative bioassay. The delivery system reduced CSPG levels at the injury site for up to 6 weeks post-SCI with delivery of the thermostabilized ChABC. Co-delivery of NT-3 with ChABC promoted axonal regeneration and demonstrated some functional benefit at 6 weeks after injury compared with animals receiving agarose gels without NT-3 (Lee *et al*, 2010). In a separate study, Hyatt *et al* (2010) used pre-gelled fibrin hydrogels to stabilize and deliver ChABC to dorsal transection injuries. Compared with simple intraspinal injections of ChABC, the fibrin delivery system contained more bioactive ChABC at the lesion site (as determined by zymography) at 3 weeks after injury and led to a 37% reduction of CSPGs. Recently, an affinity-based controlled release system was designed for ChABC where bioactive release was quantified over a 7-day period (Pakulska *et al*, 2013). These studies demonstrate that biomaterials are able to stabilize ChABC *in vivo* and maintain its bioactivity for a longer period of time compared with simple injections.

Antibodies against the myelin inhibitory molecule NGO-A (ie, anti-NGO-A) have been shown to improve functional recovery in experimental animal models when delivered by an intrathecal catheter (Freund *et al*, 2006; Liebscher *et al*, 2005) and have been studied in a phase I clinical trial (Hawryluk *et al*, 2008). To achieve sustained release, anti-NGO-A is typically delivered by osmotic minipumps; however, as external minipumps are susceptible to infection, patients enrolled in the clinical trial received repeated injections of anti-NGO-A into the intrathecal space. In a complimentary approach, the delivery of an antibody that acts as a competitive antagonist for the native receptor of NGO-A (NGO-66 receptor, NgR) has also been reported to promote axonal regeneration (Wei *et al*, 2010), as activation of NgR by NGO-A inhibits neurite outgrowth (Domeniconi *et al*, 2002; Liu *et al*, 2002). To avoid multiple injections, Wei *et al* (2010) immobilized NgR antibodies (anti-NgR) to HA-poly (L-lysine) hydrogels, which were directly implanted after

lateral hemisection of the spinal cord (Wei *et al*, 2010). Anti-NgR diffusion out of the hydrogel was slow and immunohistochemical analysis showed that most of the NgR antibodies were still present within the scaffold 2 weeks after implantation, thereby competitively inhibiting NgR from activation with NGO-A. However, at 4 weeks, more antibody diffused out of the hydrogel and was detected within the tissue; anti-NgR was still detected in the tissue at 8 weeks, albeit in lower concentrations. The antibodies had no effect on angiogenesis, and blood vessels were found in both hydrogel-antibody and hydrogel vehicle control groups within the lesion site. Both groups showed decreased GFAP immunoreactivity compared with untreated controls, likely due to the anti-inflammatory effects of hyaluronan. Anti-NgR delivery promoted greater axonal regeneration compared with untreated controls, suggesting that the sustained diffusion of anti-NgR from HA hydrogels is a promising strategy to promote regeneration of the injured spinal cord. The challenge of this study is translation to the patient as the hemi-section model is not as clinically relevant as compression models.

FUTURE RESEARCH DIRECTIONS

The use of hydrogels, and especially injectable hydrogels, for local and controlled drug and cell delivery to the CNS is a new and rapidly expanding discipline in the field of regenerative medicine. The injectability and resorbability of the hydrogel obviate the need for both invasive surgical procedures associated with transplants and removal of non-degradable materials. Yet, there is no magic bullet for the treatment of CNS disorders, requiring a combined strategy of biomaterial, cell and biomolecule to achieve therapeutic benefit. The resulting clinical translation of such combination strategies will be inherently more complicated; however, there is precedence in other diseases such as the use of drug eluting stents for occluded blood vessels or antibody-drug conjugates for cancer treatment.

Future hydrogels must provide a delivery system for both sustained biomolecule and cell delivery. Although conceptually attractive, cell transplantation often suffers from low cell survival (Cooke *et al*, 2010). Whether in an immunoprotective barrier (Cheung and Anseth, 2006; Su *et al*, 2010) or an injectable hydrogel, cell survival and integration remain key challenges to the field. Both the biomaterial design and the differentiated cell progeny will influence cell survival and integration. These factors are intimately related as cells need to survive sufficiently long to integrate and at the same time they need to integrate with the host tissue to survive. Thus, host-tissue integration has to occur rapidly after transplantation. This can be promoted with angiogenesis or by overcoming the chemical or physical barriers to cell integration, such as the glial scar, which forms after CNS injury. Each injury type will require a specific, optimized delivery system, with different cell-biomolecule combinations.

Diffusion of bioactive substances is largely dependent on the physical properties of molecule. Modification of EGF with PEG (Cooke *et al*, 2011) has previously been shown to increase its depth of penetration in rodent brains; however, the challenge is to demonstrate that biomolecules can penetrate sufficiently deep into the brain of larger animals after intrathecal or epi-cortical delivery. Furthermore, prolonged exposure of certain molecules may be problematic as this may result in the over-proliferation of normal cells. This illustrates the need to precisely control the kinetics of drug release from polymers, whereby a potential strategy would be to fine tune the interaction between the drug and the biomaterial, such as by using protein binding partners with varying binding affinities to conjugate the drug to the biomaterial (Vulic and Shoichet, 2012). Other disadvantages of current hydrogel delivery systems include the low loading capacity for hydrophobic drugs and the difficulties in controlling the diffusion of small molecules that have low steric hindrances (Perale *et al*, 2012). This can be overcome by either derivatizing drugs with a hydrophilic molecule, such as PEG, or encapsulation in polymeric NPs. The latter approach has also been used to prolong the delivery of protein therapeutics; however, the encapsulation process itself can degrade the protein and the amount of protein/polymer delivered is usually less than 5–10%, resulting in more polymer than therapeutic being delivered.

An appropriate cytoarchitecture may be required for optimal cellular response, but this will inherently complicate the surgical procedure. In addition to chemical and cellular strategies of regeneration, combination strategies with rehabilitation and enriched environment will pave the way of the future, taking advantage of multiple stimuli to regenerate the brain. There is already some evidence of this potential (Garcia-Alias *et al*, 2009; MacLellan *et al*, 2011), which will be expanded upon in the future.

With defined 3D culture systems, where the mechanical, chemical, and biological milieu is controlled, our understanding of the stem cell niche and disease progression will be enhanced and this knowledge should lead to better defined hydrogels for greater success *in vivo* in the future. Research into the development of dynamic, 'smart' materials that can respond to changes in external stimuli such as pH, temperature, and the presence of specific ligands (de Las Heras Alarcon *et al*, 2005; Han *et al*, 2013) is growing, and these new materials provide exciting possibilities to not only study the dynamic nature of cell–cell and cell–matrix interactions, but also for use as stimuli-sensitive drug delivery vehicles. (Roy *et al*, 2010)

Although biomaterials demonstrated beneficial effects in experimental animal models, new biotechnological- and/or chemical processes may need to be developed before clinical translation. For example, the purification of biomaterials is imperative for safe use in human patients, therefore requiring them to be rigorously tested and void of cytotoxic components (eg, endotoxins and toxic reagents used during chemical modifications) (Rietschel *et al*, 1994). In addition, biomaterials that have undergone chemical modifications

(eg, conjugation of bioactive molecules) may require different chemical procedures to produce large quantities of biomaterials and bioactive molecules, as the same chemistry used for small-scale synthesis may be impractical for large-scale production (Milne, 2011; Ranjan *et al*, 2012). Nonetheless, the ability to create reproducible and well-defined biomaterial systems for delivery of therapeutic biomolecules and cells into the CNS in a non-invasive manner should advance translational applications to the clinic.

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The authors acknowledge that a composition of matter patent has been issued for HAMC and additional patents are under review for cell and drug delivery with HAMC.

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