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Injectable hydrogels for central nervous system therapy

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Abstract

Diseases and injuries of the central nervous system (CNS) including those in the brain, spinal cord and retina are devastating because the CNS has limited intrinsic regenerative capacity and currently available therapies are unable to provide significant functional recovery. Several promising therapies have been identified with the goal of restoring at least some of this lost function and include neuroprotective agents to stop or slow cellular degeneration, neurotrophic factors to stimulate cellular growth, neutralizing molecules to overcome the inhibitory environment at the site of injury, and stem cell transplant strategies to replace lost tissue. The delivery of these therapies to the CNS is a challenge because the blood–brain barrier limits the diffusion of molecules into the brain by traditional oral or intravenous routes. Injectable hydrogels have the capacity to overcome the challenges associated with drug delivery to the CNS, by providing a minimally invasive, localized, void-filling platform for therapeutic use. Small molecule or protein drugs can be distributed throughout the hydrogel which then acts as a depot for their sustained release at the injury site. For cell delivery, the hydrogel can reduce cell aggregation and provide an adhesive matrix for improved cell survival and integration. Additionally, by choosing a biodegradable or bioresorbable hydrogel material, the system will eventually be eliminated from the body. This review discusses both natural and synthetic injectable hydrogel materials that have been used for drug or cell delivery to the CNS including hyaluronan, methylcellulose, chitosan, poly(N-isopropylacrylamide) and Matrigel.

(Some figures may appear in colour only in the online journal)

1. Introduction

Central nervous system (CNS) diseases and injuries including those of the brain, spinal cord and retina are some of the most devastating for patients and their families. The organs of the CNS are not only responsible for sensory and motor functions, but also encode our personality and sense of self. Their functional deterioration creates a huge impact on quality of life.

Injury to the CNS can be due to a trauma (e.g., traumatic brain injury, traumatic spinal cord injury, stroke), degeneration (e.g., age-related macular degeneration, multiple sclerosis) or genetic disorder (e.g., Huntington's disease, retinitis pigmentosa), but what all these conditions have in common is cellular degeneration and death [1]. Most mature neurons in the CNS are post-mitotic cells, unable to divide, and their destruction often leaves a patient with permanent functional loss. Regenerative medicine aims to replace or

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regenerate cells, tissue or organs to restore or establish normal function [2]. As such it promises to be a key therapeutic method for CNS injury. Two main approaches of regenerative medicine include: (1) the delivery of new cells and promotion of their survival, differentiation and integration with the host tissue, or (2) delivery of drugs or protein therapeutics to promote endogenous cell stimulation and regeneration.

Using stem cells to replace the lost cells of the CNS is a main research area, as stem cells have the unlimited ability for growth in culture and their progeny have the ability to differentiate into various cell types. Two types of stem cell sources show particular promise in CNS applications: embryonic stem cells [3] and adult neural stem cells [4, 5]. The latter are especially interesting because they circumvent the ethical issues associated with harvesting cells from human embryos. Cell delivery challenges include increasing survival, promoting differentiation to the desired cell type, and promoting integration into the existing cellular architecture. Often, the cell delivery vehicle is influential in all these aspects.

In lieu of implanting new cells, small molecule or protein drugs can induce the injured axons to re-grow or spared axons to sprout and compensate for lost function. Drug therapies can be neurotrophic—directly stimulating growth, neuroprotective—saving spared neurons from degeneration, or neutralizing—mitigating the toxic environment around the injured or diseased site. Examples of neuroprotective therapies and neutralizing molecules include methylprednisolone [6], anti-NOGO-A [7, 8], and chondroitinase ABC [9, 10] for spinal cord injury (SCI), β-secretase inhibitors for Alzheimer's disease [11], and sirtuin 2 (SIRT2) inhibitors for Parkinson's disease [12]. Neurotrophic molecules that directly stimulate cellular regeneration include growth factors such as brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and nerve growth factor (NGF). It is important to note, however, that none of these therapies provides that elusive 'magic bullet', often showing inconsistent results between studies. This is especially apparent with methylprednisolone—although long considered a standard of care for SCI, its utility is currently considered questionable [13].

With increasing knowledge about the pathology of CNS diseases and injuries, gene therapy is another viable alternative for treatment. Short interfering ribonucleic acid (siRNA) can be delivered to knock down expression of a certain gene [14–16], or conversely, deoxyribonucleic acid (DNA) plasmids can be delivered to increase gene expression [17, 18].

Neural degeneration is a complex process that differs between conditions, so the diversity and number of potential drug targets is extensive. Still, the delivery of any of these cells or therapeutic molecules to the injury site is a challenge in itself because the blood–brain barrier limits the transport of most molecules into the CNS. Herein, we will discuss current strategies for drug and cell delivery to the CNS, including the role of injectable hydrogels in overcoming some of the major obstacles still present in this field.

2. Drug delivery to the CNS

2.1. Current CNS drug delivery methods and their shortcomings

Delivery of drugs to the CNS is complicated by the blood–brain barrier (BBB), the blood–spinal cord barrier (BSCB) and the blood–retinal barrier (BRB). The endothelial cells of the BBB are different from those in the periphery due to the presence of tight junctions that limit paracellular transport. Transcellular transport is also limited due to few endocytic vesicles, high metabolic activity and lack of fenestrae [19, 20]. Most classical small molecule pharmacology agents have negligible transport through these barriers, preventing efficient vascular drug delivery to the CNS [21, 22] and thus limiting conventional delivery strategies. This can be overcome either by targeting methods to allow drugs to cross the BBB or direct delivery to the tissue. This review is focused on the latter. For detailed review of targeting methods see references [20, 23]. Ideally, a direct drug delivery strategy would provide localized release to the desired site of action, sustained release at a clinically relevant concentration for a desired length of time, all in a minimally invasive fashion. Additionally, once the required drug delivery has been achieved, the delivery system would biodegrade or be resorbed without eliciting an inflammatory reaction from the body. Combining all of these characteristics into a single drug delivery strategy has proven to be a challenge.

Currently used methods for direct drug delivery to the CNS are bolus injection, and continuous infusion using a catheter/minipump system. Bolus injection into the intrathecal space for delivery to the spinal cord is hindered by continuous cerebrospinal fluid (CSF) flow. The CSF flows at a rate of 0.35 mL min^{-1} [21], dispersing any injected drug throughout the CNS and minimizing local release. Moreover, the entire CSF volume is produced and cleared about every 5 h [21] requiring higher doses and repeated injections. The catheter/minipump system is used for sustained intrathecal or intraventricular drug delivery; however, implantation of the device requires invasive surgery, causing cell and tissue death around the insertion site [24], as well as increased risk of infection from the external minipumps [25]. Catheters are also prone to dislodgement, kinking, tearing and disconnection, affecting as many as 40% of patients who use them [26]. Intraventricular infusion is also hindered by the limited diffusion of drugs from the ventricular surface into the brain parenchyma. The efficacy of diffusion decreases with the square of distance, so a typical small molecule with a diffusion coefficient of 5×10^{-6} cm^2 s^{-1} takes 8 h to diffuse 1 mm [27]. This means that the CSF is turned over and the molecule is cleared before it has time to enter the tissue [21]. For targeting of the retina, topically applied drops do not sufficiently penetrate through the natural protective ocular barriers hence the only approved posterior segment treatments require invasive delivery methods, including intravitreal injections or surgical procedures that pose safety risks such as infection, retinal detachment and vitreous hemorrhage [28].

More recently, biodegradable polymeric implants have been employed as drug depots for sustained delivery [29, 30].

A major drawback is that these pre-formed polymeric implants require invasive surgical techniques for implantation.

Injectable, *in situ* gelling hydrogels provide an alternative to physical implants. Injection through a fine needle is less invasive than implantation and thereby facilitates surgery and recovery. By using a biodegradable polymer that does not persist after drug release, the need for surgical removal is also obviated.

2.2. Injectable hydrogels for drug delivery to the CNS

Hydrogels are physically or chemically crosslinked polymeric materials that contain a high proportion of water (usually >90%). As such, they are highly biocompatible and very popular for tissue regeneration strategies [31]. Because of their porous nature, hydrogels are ideally suited for drug loading, with drug release dependent on the rate of diffusion through the hydrogel matrix. This can be tuned by controlling the crosslink density of the hydrogel, effectively creating a localized depot for sustained drug release [32]. A downside of the high water content is that hydrophilic drugs such as proteins are very soluble and tend to diffuse out of the gel on the order of hours to days. In order to increase delivery time, hydrophilic drugs can be covalently bound to the hydrogel via a cleavable linker. The rate of drug release is then also dependent on the rate of linker cleavage or hydrolysis [33, 34]. Another popular strategy is combining a hydrogel with other drug delivery methods such as liposomes and lipid microtubules [35, 36] or polymeric microspheres [37–40]. The hydrogel keeps the particles in place at the injury site and limits burst release commonly seen with microsphere systems, while the hydrophobic liposomes or microspheres provide extended release times for hydrophilic molecules [41].

Many injectable hydrogels also have the potential to form gels *in situ*. Polymers with a lower critical solution temperature (LCST) below body temperature will gel in response to temperature increase, while other polymers such as alginate or chitosan form gels due to ionic interactions, either through the addition of salts or changes in pH [42]. The addition of a photoinitiator to monomers can also allow for light-induced hydrogel formation [43]. Additionally, by choosing hydrogel materials that are biodegradable or bioresorbable, the delivery system will eventually be eliminated from the body. These materials therefore hold the promise of fulfilling all the characteristics outlined above for an ideal drug delivery system. The following is a review of materials that have been used as injectable hydrogels for drug delivery to the CNS, and some of the applications for which they have been used. It is important to note that this is not an exhaustive review of materials that have been used for drug delivery in general, rather it focuses on injectable hydrogel materials that have been used for delivery of CNS relevant drugs *in vitro* or *in vivo*.

2.2.1. Natural polymers. Natural polymers, such as hyaluronan, fibrin and collagen, are advantageous because they have already been used in clinical applications such as dermal fillers, lubricants, wound sealants and surgical sponges [44, 45]. Other naturally derived polymers, such as agarose and chitosan, have readily available functional groups which facilitate chemical modification. In addition, the gelation of many of the natural polymers is controlled by temperature and/or pH: agarose gels as temperature is decreased whereas methyl cellulose and collagen gel as temperature is increased; chitosan gels with increased pH.

Agarose is a polysaccharide of D-galactose and 3,6-anhydro-L-galactopyranose derived from the cell walls of red algae. It is attractive for drug delivery because it has soft, tissue-like mechanical properties, and can form porous gels at low temperatures [46]. Agarose is heated to solubilize the powder in aqueous solutions and then gels through hydrogen bonding upon cooling. However, unmodified agarose gels very slowly at body temperature [47]. To overcome this limitation, Jain *et al* used an external liquid nitrogen cooling system [46]. Agarose solutions containing BDNF-loaded lipid microtubules were injected into the intrathecal space of a rat with a dorsal over-hemisection injury at T10. The liquid nitrogen cooling allowed the agarose to solidify quickly *in situ*. BDNF delivered in this way was found to reduce the reactivity of the astrocytes and the production of chondroitin sulfate proteoglycans (CSPGs) and enhanced the number of regenerating fibres that entered the hydrogel chondroitinase ABC-loaded lipid microtubules to the injured spinal cord in rats [36, 48]. Although in these studies the spinal cord was exposed during injection of the agarose gel, one can envision a method where the liquid nitrogen is also delivered onto the gel in a minimally invasive manner, such as a fine needle. However, while moderate cooling may be beneficial [49], there is a possibility of harmful side effects from exposure to liquid nitrogen (−196 °C) in these sensitive tissues [50].

Chitosan, produced by the deacetylation of chitin from crustacean shells, is another natural polysaccharide that can potentially serve as an injectable drug depot. Chitosan can form gels by covalent crosslinking with aldehydes such as glutaraldehyde [51] or ionic crosslinking by polyanions such as sodium citrate or sodium tripolyphosphate [52]. Chitosan crosslinked with beta-glycerophosphate (BGP) was used to deliver ellagic acid as a brain cancer therapy [53]. These materials gelled within 3 min at body temperature and showed a linear release of drug for up to 14 days. The ellagic acid was loaded into the gel simply by adding it to the chitosan solution before crosslinking. This method requires only a mildly acidic aqueous solution to dissolve the chitosan making it attractive for proteins that are stable under these acidic conditions but may denature under harsher conditions.

Fibrin is a promising matrix material because of its natural role in wound healing and its current application as a tissue sealant (Tissucol®). Fibrin gels form upon crosslinking of thrombin-activated fibrinogen by Factor XIII in the presence of Ca^{2+}. Interesting work with fibrin matrices for drug release has been done by the Hubbell and Sakiyama-Elbert groups. By incorporating bi-domain peptides into the fibrin structure, drug release was controlled through reversible binding. For example, a peptide containing a heparin binding domain on one terminus was incorporated into the fibrin matrix through a Factor XIII substrate on the other terminus. This peptide

sequesters heparin within the matrix and can slow the release of any heparin binding protein such as fibroblast growth factor (FGF) as they reversibly bind the heparin [54–56]. This system has since been tested for controlled delivery of NGF, NT-3 and BDNF [56]. In addition, this type of matrix can be injected and polymerized *in situ* [57, 58] and has been shown to enhance neural fibre sprouting after subacute SCI in rats [59]. Theoretically, any protein-peptide binding pair with various binding affinities could be used in this system making it tunable and widely applicable.

Collagen is the main component of connective tissue and type I collagen is the most abundant protein in humans [60]. Collagen sponges such as Gelfoam® are not only used clinically as hemostatics, but have also been used for drug delivery applications [61]. Collagen is a prime candidate for an *in situ* gelling material due to its inverse thermal gelling properties; it can be applied as a chilled acidic solution that gels upon injection to body temperature and pH [62]. However, collagen gels alone are quite weak, and are often crosslinked to improve durability. Macaya *et al* showed that genipin was able to effectively create a robust collagen gel *in situ* [63]. Genipin is also 3000 times less toxic than glutaraldehyde and is therefore a more desirable crosslinker. Another study shows a collagen gel stably releasing ciliary neurotrophic factor (CNTF) for up to 12 days *in vitro* improving the survival, growth and proliferation of neural stem/progenitor cells (NSPCs) [64]. Combining the drug release and *in situ* crosslinking strategies for collagen could provide a robust CNS drug delivery device. Complexes of positively charged methylated collagen and DNA have also been suggested as a controlled release gene delivery technology [65].

Yang *et al* studied the biocompatibility of amphiphilic diblock copolypeptide hydrogels made of poly-L-leucine, poly-L-lysine, poly-L-homoarginine and poly-L-glutamate in the CNS [66]. The ratio of hydrophilic to hydrophobic residues was varied to obtain different gelation times. Upon injection into a mouse forebrain these gel deposits were found to elicit similar levels of gliosis, inflammation and toxicity to neurons, myelin and axons as injections of physiological saline. Over time, these gels were also found to exhibit blood vessel in-growth and limited nerve in-growth. Another interesting peptide application is self-assembling peptides (SAP). These molecules form self-assembling nanofibrous scaffolds in response to changes in pH, temperature or salt concentration. When functionalized with specific active sequences such as the laminin-derived peptide isoleucine-lysine-valine-alanine-valine (IKVAV), these structures have been shown to have both histological and functional benefits in rat and mouse models of SCI [67–69].

Hyaluronan (HA) is a popular material for tissue regeneration because it is normally present in high levels in the extracellular matrix of connective, epithelial and neural tissues. HA is known to play roles in cellular processes like cell proliferation, morphogenesis, inflammation and wound repair, and interacts with cells primarily through CD44 and RHAMM surface receptors [70]. However, HA alone does not gel and is rapidly degraded through the action of the enzyme hyaluronidase present throughout the body, and also readily cleared due to its high solubility. Efforts to crosslink HA in order to make it more suitable for drug delivery applications are ongoing [71, 72].

The Shoichet lab has been developing an injectable hydrogel for drug delivery composed of a physical blend of hyaluronan (HA) and methylcellulose (MC), referred to as HAMC. The HA renders the material shear thinning, allowing it to be injected through small gauge needles, while lowering the gelation temperature of the inverse thermal gelling MC, thereby allowing the viscous liquid to gel at body temperature. The material has been shown to be biocompatible, bioresorbable and to attenuate inflammation in the CNS [73, 74]. In fact, injection of HAMC alone in spinal cord injured rats resulted in better re-sealing of the dura than in control animals (figure 1) [74].

This material has been used for the delivery of growth factors to the stroke injured brain as well as the injured spinal cord [73, 75, 76]. Hydrophilic proteins diffuse through the HAMC matrix typically within 24 h. To obtain extended release profile for these therapeutics, they are loaded into PLGA nanospheres which are then dispersed within the HAMC. Although proteins diffuse *in vitro* through the particles and through the gel alone quite quickly, the combination of the two gives a non-intuitive linear release profile with a low burst release [77].

Hydrophobic drugs, such as the vasodilator nimodipine, have been dispersed in their solid form directly in HAMC. MC helps to solubilize hydrophobic drugs from five- to tenfold over their normal solubility in aqueous solution [78]. This allows an extended release profile that can be tailored by changing the size of the solid drug particles.

MC has also been studied on its own. Tate *et al* investigated the utility of MC as an injectable scaffold for the repair of brain defects [79]. MC was found to exhibit low viscosity at 23 °C and to form a soft gel with the addition of salt at 37 °C, ideal for this type of application. A small amount of initial polymer erosion was followed by relative polymer stability over a two-week period. The presence of MC did not alter the size of the injury cavity or change the patterns of gliosis as compared to injured, vehicle-injected rats highlighting its biocompatibility.

2.2.2. Synthetic polymers. The use of synthetic polymers in drug delivery is prevalent as they can be tuned in terms of composition and molar mass. They can also be synthesized to include reactive functional groups for either crosslinking or modification with biomolecules.

Poly(N-isopropylacrylamide) (PNIPAAm) has been widely studied as a temperature responsive drug delivery system [80–82]. Its LCST lies between room temperature and body temperature and thus it is soluble at room temperature, but it gels at body temperature. At physiological temperatures, PNIPAAm homopolymer gels hold little water and show poor elastic recovery, but by combining PNIPAAm with poly(ethylene glycol) (PEG) the mechanical and swelling properties of the polymer can be tailored [83]. PNIPAAm-PEG was used by the Lowman group to deliver BDNF for repair of a partial hemisection SCI model simply by mixing the drug along with the PNIPAAm-PEG at room temperature [84]. This

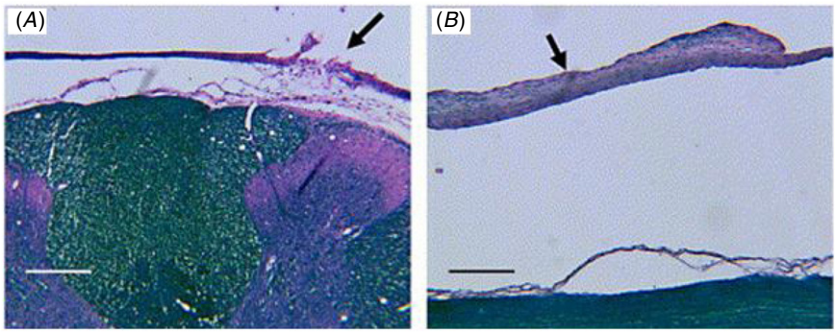

Figure 1. Representative histology sections stained with luxol fast blue and counterstained with hematoxylin and eosin four weeks after intrathecal injection of (*A*) saline and (*B*) hyaluronan/methyl cellulose (HAMC). The dura remains torn after injection of saline whereas it has self-sealed after injection of HAMC. (Arrows indicate the torn and re-sealed dura, respectively.) Scale bar = 200 μm. Reprinted with permission from [74]. Copyright 2006 Elsevier.

group also demonstrated release of bioactive BDNF and NT-3 *in vitro* for a period of 30 days with minimal burst release from the same type of hydrogel [85]. This type of scaffold is also being investigated as a nucleus pulposus replacement after intervertebral disc degeneration [83] as well as a drug delivery vehicle for the retina [86].

While poly(lactic acid) (PLA) is perhaps best known in drug delivery as micro/nanoparticles, it can also be used in hydrogels. PLA-PEG-PLA triblock copolymers were used for delivery of NT-3 to the injured spinal cord in rats. The PLA-PEG-PLA macromer was polymerized *in situ* using a photoinitiator and light where it was shown to deliver NT-3 for a period of two weeks [87]. The same polymer was used for the delivery of BDNF and glial-derived neurotrophic factor (GDNF) to the brain; however in this case, the polymer was pre-formed into 280 μm diameter fibres *ex vivo*. These fibres could then be extruded through a 25 gauge needle into the brain [39].

Pluronic F127 is an ABA block copolymer made up of poly(propylene oxide) and poly(ethylene oxide) that exhibits inverse thermal gelling. Strappe *et al* used 15% Pluronic gels for lentiviral delivery of the green fluorescent protein (GFP) gene to the CNS [88]. No decrease in transduction efficiency was observed with Pluronic compared to traditional transduction and no toxic effects were observed in 293T cells. Stereotactic delivery of viral vector in 15% Pluronic F127 to the thalamic region of the rat brain resulted in the transduction of predominantly astrocytes close to the injection site. However, there was also some tissue damage and an increase in activated macrophages, suggesting limited biocompatibility of this material. Geroski and Edelhauser have shown that this same material can provide localized, sustained delivery of dexamethasone across the human sclera for treatment of retinal diseases [89].

3. Cell delivery to the CNS

3.1. Current cell delivery methods and their advantages/shortcomings

Cell delivery in general relies on transplanted cells either producing a desired therapeutic molecule over a period of time to promote endogenous repair, or replacing lost cells with donor cells that can integrate and function with host tissue.

Biomaterial-based delivery of cell populations may involve their encapsulation within a biocompatible material in an attempt to isolate the transplanted cells from immune attack and prolong their function *in vivo*. Hydrogels have been in use for almost 30 years to encapsulate cells to treat, for example, endocrine disorders such as diabetes [90]. These materials commonly include derivatives of the biopolymer sodium alginate [91–93], or synthetic scaffolds including polyacrylonitrile/polyvinylchloride, polyurethane, polypropylene or poly(2-hydroxyethyl methacrylate) [94–96]. The successful application of cell therapy/transplantation to the damaged CNS in preclinical models and clinical scenarios has been demonstrated in a variety of applications [97, 98]; however, none of these strategies are used routinely clinically. For example, transplantation of fetal nigral tissue into Parkinson's disease models was based on the idea that the missing neurotransmitter, dopamine, would be produced by the cells in the neural grafts [99]. Immortalized cells, such as pheochromocytoma (PC12) cells have been used in encapsulation strategies for the treatment of Parkinson's disease, and baby hamster kidney (BHK) cells engineered to produce NGF have been used for the treatment of Alzheimer's disease [100, 101].

However, limitations to these technologies include diffusion limitations based on material mesh size, which limits overall cell loading [102, 103]. These various strategies and materials are reviewed more extensively elsewhere [104, 105]. This review will focus on cell transplantation to allow cells to integrate and replace the function of lost host cells in the CNS. Currently, the majority of experimental approaches in this field inject donor cells as dissociated single-cell suspensions in buffered saline, media or other aqueous-based solvents. There are, however, a number of biomaterial-based delivery strategies being developed: these include the delivery of cells on implantable biomaterial scaffolds or delivery of cells suspended in injectable polymeric matrices.

3.2. Solid implantable scaffolds for cell delivery (in the CNS)

Physical constructs are often used in cell delivery strategies in order to provide a permissive environment for regeneration

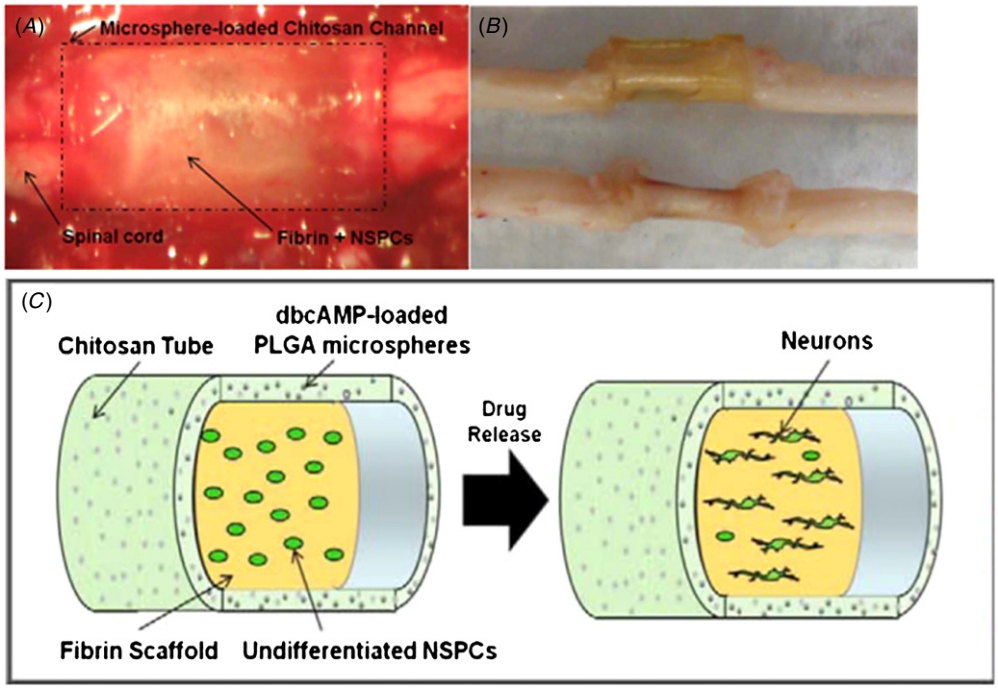

Figure 2. Channel implantation after spinal cord transection facilitates tissue bridging and NSPC survival. (*A*) Photograph of the surgical implantation of fibrin-filled chitosan channels. (*B*) Tissue bridges obtained from animals two weeks after implantation. (*C*) Schematic of the entubulation strategy. NSPCs are seeded on fibrin scaffold within a chitosan channel. Drug-loaded PLGA microspheres release the differentiation factor dibutyryl cyclic-adenosine monophosphate (AMP) in a local and sustained manner, influencing NSPCs to preferentially differentiate into neurons. Reprinted with permission from [116].

in the CNS. These implants have most commonly been applied in the context of transection injury to the spinal cord. When the cord is completely severed, the stumps can be placed in nerve guidance channels analogous to systems used clinically for peripheral nerve repair. There are many physical factors which play a role in material design of these scaffolds, including tube dimensions, wall thickness, porosity and mechanical strength. Matching the specific modulus of the material with the injured tissue at the implant–tissue interface has been shown to be important in avoiding necrosis at this location [106, 107]. Nerve guidance channels have been constructed out of poly(2-hydroxyethyl methacrylate-*co*-methyl methacrylate) (P(HEMA/MMA)) [108]. While P(HEMA/MMA) is biocompatible, it is not biodegradable. As such, investigators have looked at *in vivo* biodegradable materials such as poly(L-lactide) [109], poly(hydroxybutyrate) [110], chitosan [111–113] and collagen [114]. These scaffolds have been used to deliver a variety of cell types including Schwann cells [109, 110, 115], astrocytes [115] and neural stem/progenitor cells [111, 112, 116]. These have been included in the inner lumen of tubes, either adherent to the inner surface or suspended in a hydrogel such as dilute collagen [117], laminin-functionalized agarose [118], or fibrin [116] within the tube (figure 2). Implantable hydrogels can also be used when the cord is partially transected to fill the tissue defect and promote bridging of the gap [119–121].

Recently, the use of implantable scaffolds has been adopted by researchers looking to enhance the efficiency of cell therapy for retinal degeneration. Cell death, leakage and migration from the transplantation site occur with cells delivered as a suspension in saline [122]. Implantable scaffolds provide a substrate on which to seed retinal progenitor cells, isolated from developing retina. These scaffolds have been constructed from materials including poly(L-lactic) acid (PLLA)/PLGA [123], laminin-coated poly(methyl methacrylate) (PMMA) [124], poly(glycerol-sebacate) (PGS) [125, 126], polycaprolactone (PCL) [127], and electrospun PLGA constructs [128]. However, these scaffolds do not match the tissue modulus and may lack the flexibility required for sub-retinal delivery, making the delicate tissue vulnerable to damage from the implant [123]. Matching implant to tissue modulus, particularly in delicate regions of the CNS, is an area of research that merits investigation not only for cell delivery but also drug delivery applications.

3.3. Injectable hydrogels for cell delivery (in the CNS)

There are a number of advantages conferred by using injectable gel systems to deliver cells to the CNS, as opposed to a solid, implantable scaffold. In the CNS, the size and shape of the lesion can vary widely depending on the pathology or site of injury. An injectable hydrogel which can fill an irregular void is desirable. The injectability immediately confers a minimally invasive surgical advantage, particularly when the hydrogel can be tailored to be delivered through small-gauge needles [129]. Minimally invasive procedures in various sites in the CNS, including brain and eye, are generally associated with lower patient morbidity [130, 131]. Furthermore, limiting donor cell aggregation and promoting cell distribution can enhance survival and host integration [132]. Cell death due to

the absence of cell adhesion was described in the early 1990s [133], a condition termed *anoikis*. Proposed mechanisms of the action of *anoikis* are described elsewhere [134]. Therefore, the very presence of a matrix to which cells can adhere during and immediately after transplantation may confer a survival advantage over aqueous-only vehicles.

In general, hydrogels are becoming more widely used with encapsulation of Schwann cells and neural progenitor cells to promote neural regeneration via cell-based trophic support. However, these gels also remain widely investigated as stand-alone materials for neural regeneration applications. For example, hydrogels are under investigation as fillers for nerve conduits composed of natural materials such as collagen (e.g. Neuragen Nerve Guide®) or synthetic materials (e.g. Silastic®). Examples of hydrogels used as fillers include agarose, fibrin and keratin. In the peripheral nervous system, some natural materials appear to promote regeneration simply by providing a physical matrix to allow Schwann cell in-growth and axonal extension [135]. Hydrogel scaffolds of higher mechanical integrity are also promising in neural regeneration through provision of physical guidance cues [108, 136, 137]. Glial and neural migration through hydrogels can be enhanced through delivery of soluble growth factors such as NGF from fibrin [138]. Combinations of matrix-bound ligands and physical guidance cues have also been used to guide neural regeneration [139].

The barriers to cell transplantation identified in the literature can be broadly summarized as issues surrounding cellular distribution, survival and integration in host tissue. Cells transplanted into a host environment can undergo cell death by multiple mechanisms, with low reported cell survival between 0.2% and 10% [140–142]. Differences between groups may result from different transplant techniques [142], injury models [142–144] and host response [145, 146], species of animals, and cell types used [143, 147, 148]. While these factors may differentially affect cell distribution, survival and integration, many groups have reported that increased cell survival correlates with increases in functional recovery assays [149, 150].

Injectable biomaterials, both natural and synthetic, have been tested by researchers *in vitro* and *in vivo* for their efficacy in cell transplantation strategies in the CNS. The *in vitro* evaluation of biomaterial strategies to improve cell transplantation is reviewed elsewhere [151], and this discussion will focus on strategies demonstrating promise as injectables *in vivo*.

3.3.1. Natural polymers. Injectable, space-filling, hydrogels for cell delivery to the brain are generally targeted to the cystic cavity formed following injury resulting from trauma or stroke [79, 152]. Investigators have primarily attempted to use cells injected in Matrigel for transplantation to the injured brain [153, 154]. Matrigel contains extracellular matrix protein components such as collagen and laminin, to which cells can adhere. It also contains several other growth factors including basic fibroblast growth factor (bFGF), epidermal growth factor (EGF) and insulin-like growth factor (IGF). Neural progenitor cells (NPCs) were injected following a middle cerebral artery occlusion in rats, a model of ischemic stroke [154]. The volume of the necrotic infarct cavity decreased when cells were injected in Matrigel versus a suspension in artificial cerebrospinal fluid (aCSF). In addition, the cell survival was significantly better within the Matrigel delivery vehicle. Notwithstanding these positive results, Matrigel is derived from mouse sarcoma, and is highly variable and ill-defined.

Injectable hydrogel strategies for cell delivery in SCI also make use of Matrigel, with effects such as increased transplanted Schwann cell survival to over 36% of donor cells [150]. While other injectable hydrogels composed of laminin/collagen mixtures also supported cell survival (27% of donor cells), culture in MC decreased cell survival (2%) relative to delivery as a suspension in media (14%). The very low survival in MC may reflect the non-cell adhesive nature of this material. However, a strict evaluation of cell survival must rule out the possibility of proliferation of the donor population following transplantation. In this study, cell proliferation accounted for approximately 3%–5% of surviving Schwann cells at the time of evaluation post-transplant. A correlation between angiogenesis and cell survival was observed, and may reflect the important dependence of donor cell survival on the ability to access nutrients and oxygen exchange in host tissue.

Since the original formulation of Matrigel contains growth factors secreted from the mouse sarcoma, another group used growth factor-reduced Matrigel (gfrMG) to investigate the effect of the ECM component of the matrix in isolation [153]. They found that a gfrMG vehicle promoted cell survival, proliferation, migration and neurite outgrowth following transplantation of ES-derived NPCs to the injured brain. Matrigel is a matrix of heterogeneous composition and, in addition to ECM and growth factor components, contains numerous other proteins in small amounts. These remaining factors might also affect cell signalling pathways regulating proliferation and differentiation of transplanted stem cells. Since the exact composition of Matrigel is unknown, it is unsuitable for translation to clinical therapy. Nevertheless, specific matrix components of Matrigel have been shown to improve cell survival after transplant to the brain, including collagen I [155] and a mixture of collagen I and laminin or fibronectin [156, 157].

Of note, mixtures of fibrin and fibronectin show some promise as injectable hydrogels for cell therapy in SCI [58]. In this case, the material was used to promote endogenous cell survival following SCI. Cells delivered in fibrin and fibrin/fibronectin materials showed statistically improved survival and integration with host tissue compared to collagen implants. This demonstrates the importance of selecting a biomaterial that may have beneficial impact on the host tissue, and not only the donor cell population. Bridging implants in SCI have been fabricated from non-ECM natural materials such as chitosan [111, 112, 158] and filled with injectable natural polymers. In a recent study, chitosan tubular structures were designed for *in situ* release of biomolecules to neural precursor cells encapsulated within fibrin gels inside these tubes. When implanted in a severed SCI model, cell survival was high. Interestingly, pre-differentiated neurons survived better and showed some functional benefit relative to

in situ differentiated cells and controls [116]. Pre-differentiated cells are defined as stem cell progeny that are differentiated *in vitro* prior to transplantation. *In situ* differentiated cells are transplanted stem/progenitor cells that undergo differentiation *in vivo*, following transplantation into the host tissue. Cell-adhesive hydrogels have shown promise in these applications, including dilute collagen [117] and laminin-functionalized agarose [118].

Other injectable hydrogels have been investigated for their potential wound healing responses in the CNS. For example, when an injectable blend of HAMC was injected into the intrathecal space, the dura was observed to heal within four weeks compared to a persistent tear following injection of aCSF [74]. The abated inflammatory response in the cord tissue with the application of HAMC is consistent with this improved healing. HA, in particular, has been shown to promote wound healing in other tissues [159, 160], including decreased glial scarring and increased angiogenesis in the brain [161].

As a transplant model, the adult mouse retina is a structure akin to post-natal and adult human retina. A minimally invasive, injectable and bioresorbable blend of HAMC was recently developed for transplantation of adult retinal stem cells (RSCs) into the sub-retinal space of adult mice [132]. This represents the first report of an injectable hydrogel delivery strategy for cellular therapy in the retina. The use of this injectable hydrogel allows for normal RSC survival and proliferation and for continuous integration with retinal pigment epithelium (RPE) over the surface of the retina. Cell survival and distribution were improved relative to traditional saline vehicles (figure 3). This system may prove useful in the treatment of advanced retinal degeneration, where large areas of RPE are lost [162]. Ultimate application of this strategy to the clinic will depend on improved visual function resulting from greater cell survival and host tissue integration.

3.3.2. Synthetic polymers. Although many natural and synthetic polymers have been investigated *in vitro* as potential injectable hydrogels for cell delivery to the brain [151], very few studies have attempted delivery *in vivo*. Additionally, synthetic biomaterials for *in vivo* delivery have been relegated to solid implants made of materials such as polyglycolic acid (PGA) [163] or poly(lactic-co-glycolic acid) (PLGA) scaffold particles [164, 165]. Similarly, few synthetic injectable materials have been utilized for *in vivo* cell transplantation applications for SCI. Schwann cells [110, 115, 166], astrocytes [115] and NSPCs [111, 112] have been included in the inner lumen of tubes either adherent to the inner surface or suspended in a hydrogel within the tube. Synthetic polymers including poly(L-lysine)-coated polycarbonate [115], poly(D,L-lactic) acid [109, 166], and poly(beta-hydroxybutyrate) (PHB) [110] have been used to form the supportive tube for bridging implants.

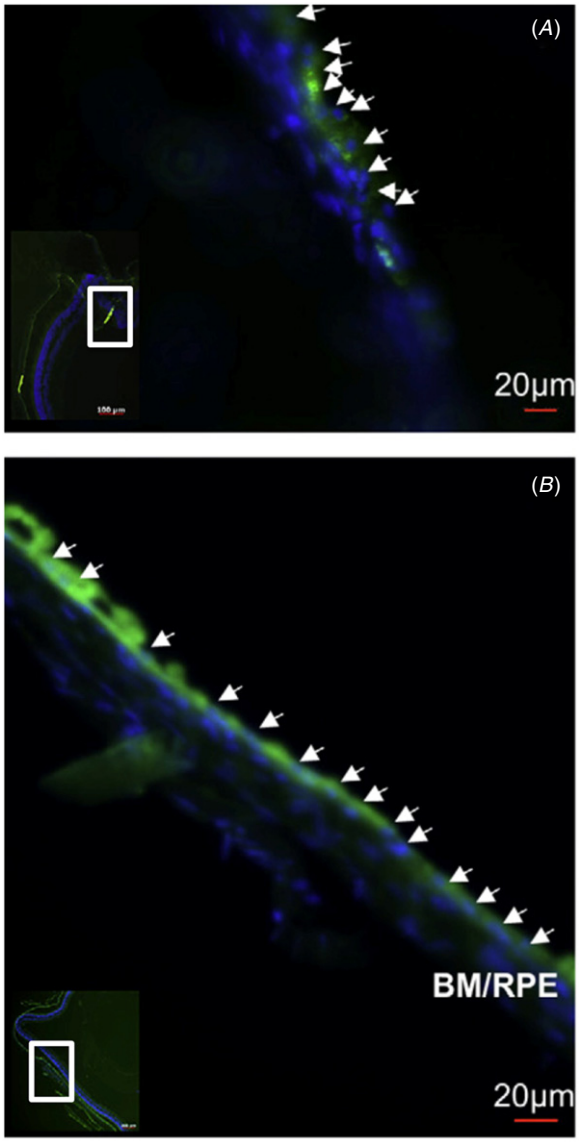

Figure 3. Sub-retinal transplantation of GFP+ retinal stem cells (RSCs) in a physical blend of hyaluronan (HA) and methylcellulose (MC)—HAMC. (*A*) Transplantation of GFP+ RSCs in saline shows non-contiguous cellular integration and localized cellular aggregates (inset, rotated) atop Bruch's membrane (BM), suggestive of aggregation pre- or post-transplantation. (*B*) Transplantation of GFP+ RSCs in HAMC shows contiguous areas of RPE integration over large areas of retina (inset), suggesting HAMC maintains cellular distribution during injection and prevents aggregation pre- or post- transplantation. Arrowheads indicate location of individual nuclei of transplanted cells (Hoechst nuclear stain, blue). Note that the even spacing between arrowheads in (*B*) suggests that these cells are spreading in a monolayer fashion over Bruch's membrane. Scale: 20 μm (inset 100 μm). Reprinted with permission from [132]. Copyright 2010 Elsevier.

4. Future outlook

While injectable hydrogel drug and cell delivery systems describe an important advance, a combination strategy is likely required for therapeutic benefit in CNS injuries [167, 168], and successful hydrogel systems will be those that cater to both drug and cell delivery. An interesting approach is the use of genetically modified cells that secrete biomolecules to promote their own differentiation and integration. For example, NSPCs modified to secrete chondroitinase ABC could be injected into a SCI site. The secreted enzyme would then clear the area of inhibitory proteoglycan components that

would facilitate cell integration. While conceptually attractive, an on-going challenge with cell transplantation is cell survival. Whether in an immunoprotective barrier or an injectable hydrogel, cell survival and integration remain key challenges to the field. Optimization of combinatorial strategies will be required at all levels including developing appropriate cell populations for transplant, finding the most potent, synergistic drug and biomolecule combinations, and matching these with innovative biomaterial vehicles. The complexity and barriers to tissue regeneration make the CNS a challenging tissue target for repair; however, injectable hydrogel strategies for cell delivery can build on the successes in drug delivery to advance translational applications to the clinic.

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