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Review

A hyaluronan/methylcellulose-based hydrogel for local cell and biomolecule delivery to the central nervous system

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ABSTRACT

Regenerative medicine strategies rely on exogenous cell transplantation and/or endogenous cell stimulation. Biomaterials can help to increase the regenerative potential of cells and biomolecules by controlling transplanted cell fate and provide a local, sustained release of biomolecules. In this review, we describe the use of a hyaluronan/methylcellulose (HAMC)-based hydrogel as a delivery vehicle to the brain, spinal cord, and retina to promote cellular survival and tissue repair. We discuss various controlled release strategies to prolong the delivery of factors for neuroprotection. The versatility of this hydrogel for a diversity of applications highlights its potential to enhance cell- and biomolecule-based treatment strategies.

1. Introduction

The central nervous system (CNS) is difficult to regenerate after injury due to the inhibitory microenvironment and glial scar that form. Promoting tissue repair after disease or injury to the CNS is further complicated by the blood-brain barrier (BBB), which limits the diffusion of systemically delivered therapeutics. To overcome this limitation, intrathecal delivery into the spinal cord via mini-pump or repeated bolus injection into the eye have been used to administer treatments (Engman et al., 2011; Tom and Houlé, 2008); yet, these strategies are highly invasive, with repeated bolus injections having the potential for both tissue damage and higher risks of infection and external mini-pumps having higher risks of infection. Several strategies are being pursued to transiently open the BBB (Huang et al., 2017); however, opening the BBB allows cells and biomolecules from the periphery to enter the CNS, and this may further exacerbate injury or degeneration.

Alternative approaches to circumvent the BBB involve the use of biomaterials, such as injectable hydrogels, which can locally sustain the delivery of biomolecules directly to CNS tissue. When designing a biomaterial to carry cells or biomolecules, it is important to consider how the material will affect host tissue response, interact with cells, undergo gelation, and degrade. The hydrogel should gel quickly once injected, to avoid quick clearance by cerebrospinal fluid and limit spread, thereby maintaining local release. The hydrogel should also be minimally swelling to reduce any further tissue damage and be bioresorbable to avoid a second procedure to remove the vehicle.

The Shoichet lab developed a hydrogel composed of hyaluronan and methylcellulose (HAMC) with the above-mentioned design criteria in mind (Gupta et al., 2006). This hydrogel has been widely used in cell and drug therapies for treatment of animal models of ischemic stroke, spinal cord injury, and retinal degeneration (Ballios et al., 2010; Delplace et al., 2019; Führmann et al., 2016; Tuladhar et al., 2015). The hyaluronan (HA) component is shear-thinning, allowing the gel to be injected through fine-gauge needles. The methylcellulose component (MC) is inverse thermal gelling, forming a physically crosslinked hydrogel at the site of injection and at physiological temperatures. The gel is bioresorbable: HA is metabolized by hyaluronidases that cleave N-acetyl-D-glucosamines in the polymer chains; and methylcellulose dissolves due to its weak physical crosslinks (Kang et al., 2008). The gel typically degrades over 14 d in vitro and over 4–7 d in vivo after injection into the brain, spinal cord or eye. The degradation rate is determined by the molar mass, the weight percentage of HA and MC and the disruption of the hydrophobic crosslinks that form between MC polymer chains in the hydrogel (Gupta et al., 2006; Kang et al., 2008).

The HAMC hydrogel has been shown to attenuate the inflammatory response in the CNS. For example, when delivered into a syringomyelia rat model of spinal cord injury, HAMC injected into the rat intrathecal space reduced levels of interleukin-1α and scarring compared to

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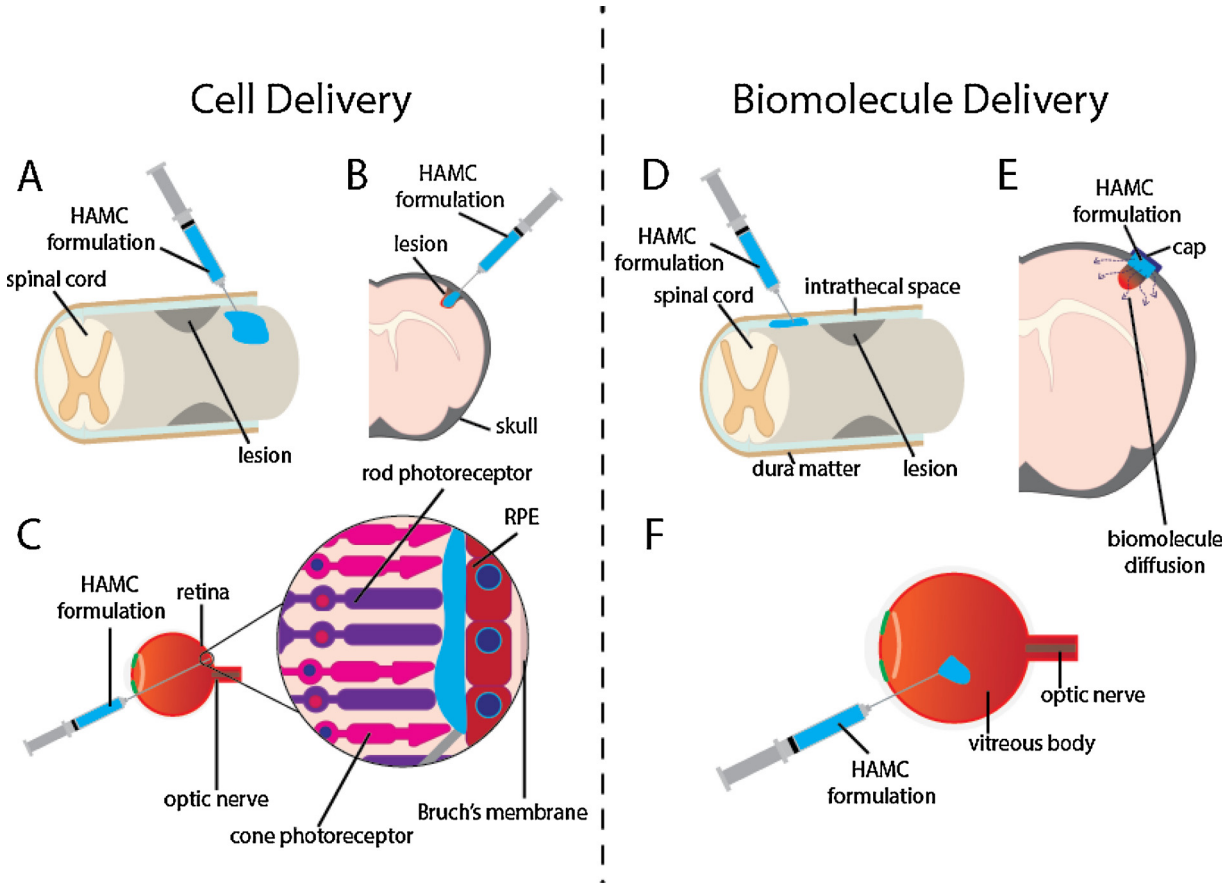

Fig. 1. Cell and biomolecule delivery approaches to the (a) and (d) spinal cord, (b) and (e) brain, and (c) and (f) retina using a hyaluronan/methylcellulose-based hydrogel.

injections of artificial cerebrospinal fluid (Austin et al., 2012a). The enhanced tissue and functional responses in animals that received HAMC injections was attributed to HA, as it was shown to reduce LPS-mediated microglial activation in vitro (Austin et al., 2012b). The anti-inflammatory properties of HA have also been observed in other tissues (Hirabara et al., 2013; Ialenti and Di Rosa, 1994; Masuko et al., 2009; Nakamura et al., 2004; Sunabori et al., 2016).

Since its initial development, HAMC has been used to deliver a number of cell types and biomolecules to the brain, spinal cord, and retina (Fig. 1). This review will detail the development of HAMC as a cell and biomolecule delivery platform.

2. Cell delivery strategies to the central nervous system

Many preclinical studies have shown that injection of exogenous cells into the injured CNS promotes regeneration and repair (Curtis et al., 2018; Mine et al., 2013; Santos-Ferreira et al., 2015; Wu et al., 2018); however, cell survival and integration remain significant challenges.

In retinal degeneration, damage is typically limited to one or two cell types, leaving the rest of the neural circuitry intact (Chichagova et al., 2018). The field of regenerative medicine in the retina has been guided by this central hypothesis that by replacing those cell types that have degenerated, and having them integrate with the existing neural circuitry, vision will be restored. In the case of age-related macular degeneration (AMD), researchers have developed strategies to transplant either retinal pigment epithelium (RPE) or photoreceptors to the subretinal space (Carr et al., 2009; da Cruz et al., 2018; Lamba et al., 2009; Schwartz et al., 2015). A similar strategy has been pursued with retinitis pigmentosa, where degeneration starts with rod photoreceptors (Mandai et al., 2017; Yang et al., 2010). Currently, two different RPE delivery methods are being pursued in clinical trials for treatment of AMD: as suspensions in saline or as grafted sheets. Implanting a monolayer maintains polarity and viability of the RPE cells, but requires a more invasive procedure (Diniz et al., 2013). Injection of cells in saline suspensions is less invasive than a surgical graft, but is compromised by a higher propensity for cell death, cell aggregation, and backflow (Qi et al., 2015; Westenskow et al., 2015). To overcome issues of aggregation in the syringe prior to injection, the syringe is usually agitated during injection. This makes injection into the subretinal space more difficult and can be overcome by first distributing cells in a hydrogel, such as HAMC, which results in an even distribution of cells vs. aggregated cells when injected in saline (Ballios et al., 2010).

Two key challenges of cell transplantation in the CNS, and in most other tissues and organs, are cell survival and cell integration. HAMC had a pro-survival effect on retinal stem cell (RSC)-derived rod photoreceptors (RSC-rods) through a CD44-hyaluronan mediated mechanism (Ballios et al., 2015). Interestingly, HA also had a pro-survival effect on primary mouse rod photoreceptors cultured in vitro where HA activated the mTOR pathway (Mitrousis et al., 2016). Moreover, the inflammatory response to cells injected in HAMC was significantly lower than that of cells injected in saline, quantified by CD68+ staining for macrophages (Ballios et al., 2015).

HAMC also improved the survival and distribution of transplanted neural stem cells (NSCs) that were injected directly into the stroke-injured site of the mouse brain (Ballios et al., 2015). Using the endothelin-1 model of stroke (Tennant and Jones, 2009), NSCs injected in HAMC survived better and resulted in improved locomotor function than those injected in saline. The significant functional recovery seen in the NSCs delivered in HAMC could be attributed to improved cell distribution

and survival, as was observed for RSC-rods delivered to the retina. Most of the surviving NSCs stained positive for glial fibrillary acidic protein, a marker for astrocytes. This is consistent with previous reports where NSC differentiation into astrocytes after transplantation was associated with improved behavioural recovery due to their supportive and protective role in maintaining neuronal homeostasis (Bacigaluppi et al., 2016; Barreto et al., 2011; Smith et al., 2012).

Integration, or the functional synaptic connections between transplanted cells and the host tissue, remains another challenge in the field of cell transplantation (Hippert et al., 2016). To promote integration of photoreceptors, a transient gliatoxin (α-aminoadipic acid, AAA) has been shown to disrupt the outer limiting membrane and promote photoreceptor migration to the outer nuclear layer (Ballios et al., 2015; West et al., 2008). Recent advances in the photoreceptor transplantation field has determined that the conventional method of quantifying cell integration –that is, labeling donor cells with GFP– may be misleading. Multiple groups have now confirmed the occurrence of material exchange, or the transfer of cytoplasmic content from donor to host photoreceptors, which suggests that there is much less cell integration occurring than previously thought (Ortin-Martinez et al., 2017; Pearson et al., 2016; Santos-Ferreira et al., 2016; Singh et al., 2016). While the exact mechanism of material exchange is still being elucidated, when RSC-rods were transplanted into the sub-retinal space of triple knockout blind mice, a 10–15% recovery was achieved in pupillary light response compared to vehicle injected eyes where there was no response (Ballios et al., 2015).

2.1. Peptide modifications in HAMC

Differentiation of endogenous or transplanted neural progenitor cells toward astrocyte or oligodendrocyte fates are promising options for regenerating the CNS. Oligodendrocytes are able to myelinate spared or regenerated axons at the lesion site of a spinal cord injury (Yazdi et al., 2018), while astrocytes can support neuronal survival through functions such as ion buffering and glutamate regulation (Barreto et al., 2011). The fate of transplanted stem or progenitor cells is determined by the growth and differentiation cues present in the recipient environment, and the ability to continue to control cell fate in vivo is vital to improving the efficiency of cell transplantation.

In addition to tuning the stiffness and gelation time of HAMC, HAMC can also be modified with peptides and growth factors to further promote survival and differentiation of transplanted cells. Using bioorthogonal coupling chemistry, Tam et al. (2012) covalently bound GRGDS, a peptide to promote cell adhesion, and platelet derived growth factor A (PDGF-A), a factor to promote oligodendrocyte differentiation, to the same MC chain of HAMC (Fig. 2). Since MC resorbs at a slower rate than HA, the MC was thiolated to immobilize both maleimide GRGDS and maleimide-streptavidin. Biotinylated-PDGF-A was then conjugated to the streptavidin-MC. This dual-modified HAMC-GRGDS/PDGF-A enhanced neural stem and progenitor cell (NSPC) differentiation to oligodendrocytes in vitro compared to unmodified HAMC and HAMC-GRGDS alone (Tam et al., 2012).

When NSPCs were delivered to the rat injured spinal cord using HAMC-PDGF-A, oligodendrocyte differentiation was improved, but not significantly higher than NSPCs delivered in media alone, suggesting that the local environmental cues overwhelmed cell fate (Mothe et al., 2013). While graft survival was poor in both delivery vehicles, rats that received cells in the modified HAMC hydrogel had smaller tissue cavities, indicative of reduced secondary damage, and significantly better recovery in fine motor function when compared to rats that had cells delivered in media controls.

To further investigate the effect of RGD and PDGF-A on cell fate in vivo, the modified hydrogel was used to transplant human oligodendrocyte precursor cells (OPCs) into rats with a moderate, clip compression spinal cord injury (Führmann et al., 2016). Induced pluripotent stem cells were pre-differentiated into the oligodendroglial lineage in order to promote remyelination and delivered one-week post-injury. Significantly more cells survived when delivered in HAMC-GRGDS/PDGF-A compared to cells in media alone. OPCs injected in HAMC-GRGDS/PDGF-A had a much lower propensity to proliferate or form tumours, likely because PDGF-A continued to promote oligodendroglial differentiation in vivo (Führmann et al., 2016). While HAMC-GRGDS/PDGF-A was able to attenuate teratoma formation, this study highlights the importance of cell purity and the need for efficient differentiation protocols and cell sorting methods to eliminate residual pluripotent cells. The animals that received OPCs in HAMC-GRGDS/PDGF-A had smaller tissue cavities at the end of the study period, suggesting tissue regeneration and/or tissue sparing. In addition to this, the same group of animals also demonstrated significantly higher performance in motor function compared to those animals that had cells delivered in media controls.

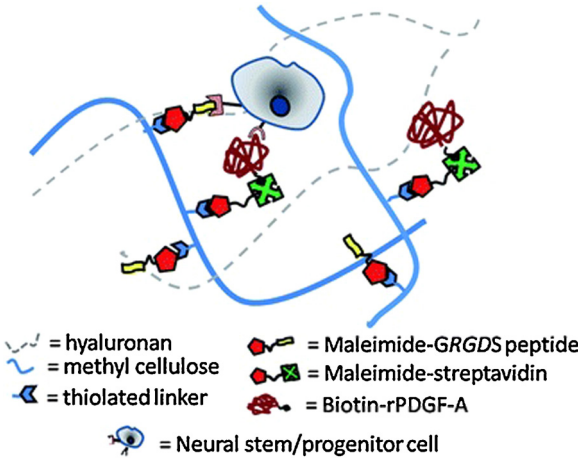

Fig. 2. HAMC was modified with GRGDS and PDGF-A to promote NSPC differentiation into oligodendrocytes (Tam et al., 2012). Reproduced with permission of Royal Society of Chemistry, in the format Journal/magazine via Copyright Clearance Center.

2.2. Cell maturity influences transplantation outcome

Transplantation success is largely driven by the cell population. Fully differentiated cells typically fare poorly, with a higher propensity for apoptosis, whereas more immature cells are at risk of dedifferentiation or uncontrolled proliferation. In the mouse retina, it is well-established that post-mitotic photoreceptor precursors survive better than fully mature photoreceptors (MacLaren et al., 2006). Ballios et al. (2015) found that there was greater cell survival when less mature RSC-rods were transplanted into mice compared to injection of fully mature RSC-rods or undifferentiated RSCs.

Payne et al. investigated how cell maturity influenced transplant survival after stroke with human induced pluripotent stem cell-derived, cortically-specified neuroepithelial progenitor cells (cNEPs). The cNEPs were differentiated for 0, 16 and 32 d, to represent three distinct neuronal populations based on expression of nestin, β−III tubulin, and MAP2 (Payne et al., 2017). These three cell populations were transplanted in HAMC into the endothelin-1 rat model of stroke. Interestingly, the immature (0 and 16 d differentiated) cell populations survived better than the late (32 d differentiated) cells. Cell survival correlated with improved behavioural recovery; yet, surprisingly, injection of HAMC alone also promoted some functional repair, which may be due to the immunomodulatory effects of HAMC (Payne et al., 2018). Unexpectedly, injection of mature 32 d differentiated neurons, suffered significant cell death prior to injection, which resulted in host tissue degeneration that was overcome by neither the viable cell population nor HAMC itself. Transplantation may have selected for the survival of immature cells, as the mid- (16 d) and late- (32 d)

differentiated groups had similar protein expression profiles as the early (0 d) cell population both after injection through a fine gauge needle in vitro and after 56 d in vivo.

3. Local biomolecule delivery strategies to treat diseases and disorders of the CNS

Delivery of bioactive molecules to provide neuroprotection and stimulate tissue repair are of great interest to treat disorders and diseases of the CNS. While many different proteins have been identified as candidate therapeutics, delivery of biomolecules to the CNS is inherently challenging. Biomolecules are often too large to pass the blood-brain, blood-retina, or blood-spinal cord barriers, resulting in low (if any) amounts of therapeutic entering into the CNS. Furthermore, systemic administration can lead to adverse, off-target effects in the body.

HAMC is a versatile drug delivery vehicle enabling several different methods of localized, sustained biomolecule release. Biomolecules have been released from HAMC after being solubilized, incorporated as particulates, or encapsulated in poly(lactic-co-glycolic acid) (PLGA) nanoparticles (Avgoustakis et al., 2002; Farokhzad et al., 2006; Mittal et al., 2007). Furthermore, electrostatic adsorption and affinity interactions have been used to control the release and increase the availability of bioactive proteins from HAMC (Pakulska et al., 2016; Vulic and Shoichet, 2012). In this section, we will highlight different controlled release strategies using HAMC to deliver therapeutic molecules to the brain, retina, and spinal cord.

3.1. Soluble biomolecule delivery

Bolus injection of therapeutics suffer from fast drug clearance and limited bioavailability after being administered to the CNS. Biomolecules can be incorporated as a suspension in HAMC prior to injection to enhance bioavailability and provide localized, sustained release to the CNS. For example, erythropoietin (EPO) was dispersed into HAMC and injected in the intrathecal space in a rodent model of traumatic spinal cord injury (Kang et al., 2008) and to the epicortex in a rodent model of stroke (Wang et al., 2012). EPO has shown to be neuroprotective in ischemic and compressive models of spinal cord injury (Celik et al., 2002; Kaptanoglu et al., 2004) and stimulate the migration and regeneration of neurons (Tsai et al., 2006). In a clip-compression model of severe spinal cord injury, HAMC loaded with a suspension of soluble EPO was injected into the intrathecal space immediately after injury. EPO diffused out of HAMC within the first 2 d and promoted greater neurogenesis in the injured spinal cord when compared to bolus injection (Kang et al., 2008). When HAMC loaded with soluble EPO was administered to surface of the brain in a rodent model of stroke injury, EPO diffused out over the course of 2 d and had significant regenerative tissue benefits (Wang et al., 2012). The EPO drug loaded delivery system reduced the lesion size at the site of injury and increased the number of neuroblasts and mature neurons in the subventricular zone of the brain when the delivery system was administered 11 d post-stroke.

When biomolecules are delivered epicortically or intrathecally, the system is less invasive than direct injection into tissue; however, penetration depth may be limited. To enhance tissue penetration and diffusion into the brain and spinal cord, proteins have been modified with poly(ethylene glycol) (PEG). For example, epidermal growth factor (EGF), which stimulates endogenous NSPCs in the subventricular zone and promotes tissue regeneration (Craig et al., 1996), was covalently modified with PEG to enhance its penetration into the rodent stroke-injured brain, thereby stimulating endogenous neural stem cells (Wang et al., 2011). Similarly, fibroblast growth factor 2 (FGF2) was modified with PEG, loaded into the HAMC hydrogel and injected into the intrathecal space in a rodent model of compressive spinal cord injury (Kang et al., 2010). PEGylated FGF2 showed greater tissue penetration than FGF2 alone and has been shown to promote tissue repair and regeneration through its trophic and angiogenic properties (Rabchevsky et al., 1999). The use of HAMC to localize the therapeutic at the injection site and PEGylation to enhance tissue penetration effectively sustains release for one to two days. To achieve longer durations of release, other strategies such as encapsulation or affinity release are necessary.

3.2. Encapsulation of biomolecules in nanoparticles dispersed within HAMC to control release

Biomolecules that are soluble in HAMC diffuse out of the hydrogel very quickly, typically within 24–48 h. Insoluble molecules dispersed as particulates within HAMC diffuse out more slowly, over the course of several days. Those biomolecules that are first encapsulated in PLGA nanoparticles (or microparticles) prior to dispersion in HAMC diffuse out the slowest, over the course of 1–2 months. These phenomena were demonstrated with the common immunosuppressant, cyclosporin A (CsA) (Caicco et al., 2013). CsA has been shown to be neuroprotective and neuroregenerative by acting directly on the endogenous neural stem/progenitor cells to promote their proliferation and enhance their survival in rodent models of stroke (Hunt et al., 2010). Solubilization of CsA in HAMC yielded rapid release in vitro, with the drug diffusing out of the vehicle over the course of 2 days. Particulate CsA was released at a significantly slower rate, with dissolution-controlled release that was extended for 7–10 d in vitro. Encapsulation of CsA in PLGA microspheres extended release for 21–28 d. Since CsA most effectively stimulates neural stem cells when delivered for a prolonged period of time, the latter PLGA/HAMC composite formulation was investigated in vivo. The drug-loaded composite was injected directly on the uninjured mouse brain cortex where CsA was shown to penetrate the brain tissue to the neural stem cell niche of the subventricular zone for at least 24 d.

To test the tissue benefit of sustained CsA delivery, it was encapsulated in the PLGA/HAMC composite formulation and administered locally to the epicortex vs. systemically in an endothelin-1 stroke injured rat (Tuladhar et al., 2015). Notably, 1000-times more CsA was required to be administered systemically to achieve the same concentration in the brain as the localized, controlled release strategy. Moreover, there was a consistently higher concentration of CsA in the brain utilizing the controlled release strategy at earlier time points. Interestingly, not only was the infarct volume in the brain smaller in animals that received local CsA, but it was also significantly smaller in the groups that received HAMC vehicle alone, demonstrating the tissue benefit of the drug as well as the delivery vehicle itself. While CsA is a potent immunosuppressant, and there may be concerns with its delivery after stroke, the concentration of CsA in the heart, lungs, liver, and spleen was three orders of magnitude lower when delivered locally in HAMC to the brain in comparison to systemic delivery, thereby opening up the opportunity for CsA as a stroke therapy. Indeed, therapeutics that previously demonstrated efficacy, but are not specific to a particular target tissue and could cause systemic toxicity, may now be further pursued utilizing this strategy.

In traumatic spinal cord injury, the primary mechanical injury causes hemorrhage and ischemia, that in turn lead to hypoxia and widespread cell death (Ahuja et al., 2017). Further damage, initiated by a secondary cascade, continues for weeks and thus requires prolonged administration of therapeutics. Importantly, HAMC loaded with PLGA nanoparticles is biocompatible in the intrathecal space after a compressive spinal cord injury in the rat: there were no differences in inflammation, scarring, or locomotor function 28 d after administration relative to controls (Baumann et al., 2010). When FGF2 was encapsulated in PLGA nanoparticles and dispersed in the HAMC hydrogel, release was extended to 18 d (Kang et al., 2013). When this formulation was injected into the intrathecal space after clip compression injury of the rat spinal cord, higher blood vessel density in the dorsal horns post injury was evident, reflecting a tissue benefit.

Co-encapsulation of proteins with other molecules within PLGA nanoparticles enhances loading efficiency and bioactivity of released proteins (Cleland and Jones, 1996; Péan et al., 1999; Rosa et al., 2000). For example, co-encapsulation of platelet-derived growth factor-AA (PDGF-AA) with 400 Da PEG increased the loading efficiency of the PDGF-AA from 49 ± 3% to 60 ± 5% (Elliott Donaghue and Shoichet, 2015). Furthermore, co-encapsulation of biomolecules with other excipient molecules can improve their bioactivity and influence their release from HAMC. Anti-NogoA is an antagonist of the myelin-associated inhibitor NogoA that reduces neurite outgrowth after traumatic spinal cord injury (Stanwick et al., 2012). Co-encapsulation of anti-NogoA with trehalose significantly improved the bioactivity of the released molecule, potentially due to the stabilizing effect of sugars on protein molecules during PLGA nanoparticle formulation (Elliott Donaghue et al., 2016). Co-encapsulation with $MgCO_3$ or $CaCO_3$ has also been useful at neutralizing the acidic byproducts associated with PLGA degradation, which can impact protein activity (Zhu et al., 2000).

HAMC loaded with biomolecules encapsulated in PLGA nanoparticles can provide long-term, localized, sustained release and is more useful in applications that require longer treatment paradigms, such as in the delivery of therapeutic biomolecules for the treatment of spinal cord injury or ischemic stroke. Moreover, the PLGA/HAMC composite is easily injectable, biocompatible and bioresorbable facilitating surgical use.

While the HAMC hydrogel has only utilized PLGA nano- and microparticles, there are other particles that could also be incorporated to provide localized, sustained release of biomolecules. Alginate microspheres, liposomes, and lipid microtubes have all been used to control the release of other therapeutic factors such as bone morphogenetic protein-2 and hepatocyte growth factor (Kolachala et al., 2011; Li et al., 2008; Ribeiro et al., 2004). Loading of these particles into HAMC would localize them at the site of dysfunction or injury and could further improve therapeutic efficacy.

3.3. Electrostatic adsorption of biomolecules on nanoparticles dispersed within a hydrogel to control release

Encapsulation of biomolecules within PLGA nanoparticles is an effective strategy for controlled release; however, the amount of protein encapsulated is often very small, typically accounting for less than 1% by mass (Bao et al., 2006). Moreover, the encapsulation process itself can denature protein therapeutics due to exposure to high shear and organic solvents (Govender et al., 1999).

Fascinatingly, proteins adsorbed onto the surface of PLGA nanoparticles (and dispersed in a hydrogel) share the same release profile and bioactivity as encapsulated proteins (Pakulska et al., 2016). By taking advantage of the electrostatic interactions between positively charged proteins and negatively charged PLGA nanoparticles, protein release was controlled (Fig. 3). Moreover, the release profile and bioactivity of the un-encapsulated, adsorbed proteins matched that of the encapsulated proteins over a 28 d release in vitro. Extended release profiles were obtained for several positively charged proteins – stromal cell-derived factor-1α (SDF), neurotrophin-3 (NT3), and brain-derived neurotrophic factor (BDNF), which all adsorbed onto PLGA nanoparticles loaded into a hydrogel – but not for negatively charged proteins, such as EPO. Release from the nanoparticles was tuned by the number of binding sites (ie. the number or available surface area of nanoparticles) and pH, with faster release at lower pH and slower release from PLGA nanoparticles buffered with $MgCO_3$. The hydrogel created a milieu with which to deliver and localize the nanoparticles, but the nature of the hydrogel - HAMC, crosslinked methylcellulose or even agarose - did not impact the release profiles observed.

The electrostatic interactions between PLGA-carboxylates (i.e., PLGA-CO_2-) and protein ammoniums (i.e., protein-NH_3^+) diminish as PLGA degrades to acidic products. This bulk degradation of PLGA results in a local acidic microenvironment and the protonation of carboxylates to carboxylic acids (i.e. PLGA-CO_2H), thereby reducing the electrostatic interactions between polymeric nanoparticles and proteins and resulting in their release.

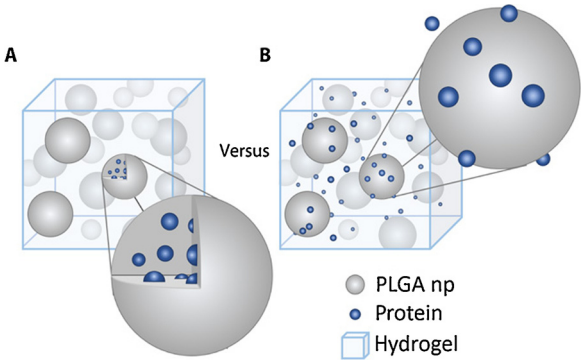

Fig. 3. A) Conventional encapsulation of biomolecules within PLGA nanoparticles dispersed in a hydrogel and B) Electrostatic adsorption of biomolecules onto the surface of PLGA nanoparticles dispersed in a hydrogel. Reprinted/adapted with permission from (Pakulska et al., 2016). © The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. Distributed under a Creative Commons Attribution Non-Commercial License 4.0 (CC BY-NC) http://creativecommons.org/licenses/by-nc/4.0/.

The system was computationally modelled to further investigate the release mechanism. The PLGA nanoparticles were modeled as obstacles in the hydrogel, having short-range attractive interactions with the proteins. Initially, the nanoparticles had an attractive potential that progressively approached zero as the nanoparticles degraded. The model reproduced the experimentally derived release curves and confirmed the hypothesized release mechanism. The computational model can provide insight into biomolecule transport with this delivery system for future applications.

Electrostatic adsorption of proteins onto charged nanoparticles overcomes the major limitations of encapsulation. Higher concentrations of bioactive proteins can be delivered and the system is broadly applicable to net positively-charged proteins. Another system has been developed for net negatively charged proteins, wherein positively charged domains interact with the surface of negatively charged laponite nanoparticles controlling their release from a cryogel (Koshy et al., 2018).

3.4. Affinity-based controlled release of biomolecules from HAMC

Affinity-based controlled release provides an alternate strategy to deliver proteins. It is particularly useful to deliver fragile proteins and has broad applicability. As affinity release does not rely on encapsulation, controlled release can be achieved without the use of additional polymers. Heparin and heparin-binding peptides have previously been used to control the release of therapeutic proteins, but are inherently limited to proteins that bind to heparin (Ho et al., 2009; Lee et al., 2007; Tae et al., 2006). Additionally, collagen matrices have been used for affinity-based controlled release, but this system is also limited to proteins that contain a specific collagen-binding domain (Fan et al., 2010).

A more generalized solution was achieved by the expression of fusion proteins with Src homology 3 (SH3) and modification of the delivery vehicle with SH3 binding peptides (Fig. 4). HAMC was functionalized with short, proline-rich peptides on the methylcellulose chains of the HAMC hydrogel, which have specific, attractive interactions with SH3 that are recombinantly expressed as fusion proteins with the therapeutic protein of interest (Vulic and Shoichet, 2012). This methodology was first demonstrated with the expression of FGF2-SH3 and its release from HAMC modified with SH3 peptide binding partners. By controlling the affinity of the interaction between SH3 and its binding

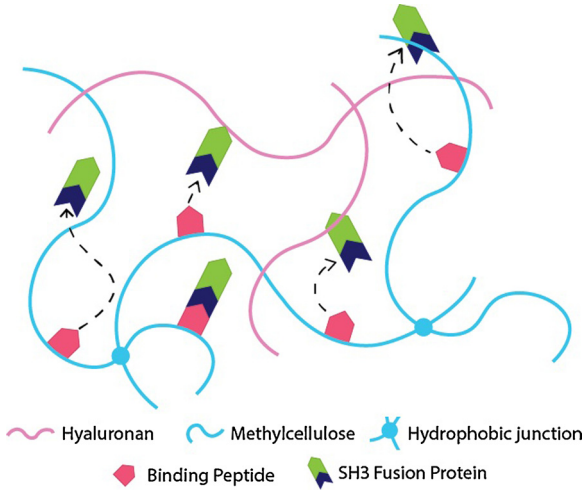

Fig. 4. Affinity-based controlled release system with Src Homology 3 (SH3) fusion proteins and SH3 binding peptides covalently linked to the methylcellulose chains in HAMC.

partners (i.e., weak or strong) and the number of peptide binding partners bound to MC of HAMC, the release of FGF2 was tuned by the series of on-off interactions. This strategy has been demonstrated with several proteins, as detailed below.

Chondroitinase ABC (ChABC) is a potent enzyme that degrades proteoglycans in the glial scar, which form after traumatic injuries in the central nervous system, such as after stroke or spinal cord injury, and hinders tissue regeneration (Fawcett and Asher, 1999; García-Alías et al., 2009). ChABC is a particularly fragile protein that is most effective with local, sustained release and for which conventional encapsulation strategies are ineffective. ChABC-SH3 was expressed as a fusion protein produced by *Escherichia coli* and released from a chemically- and physically-crosslinked methylcellulose hydrogel (Pakulska et al., 2013). Bioactive ChABC-SH3 was released for at least 7 d in vitro and demonstrated both tissue and functional benefit in a rodent model of spinal cord injury over 28 d (Pakulska et al., 2017).

Insulin-like growth factor 1 (IGF1) and ciliary neurotrophic factor (CNTF) have both been shown to be neuroprotective against multiple models of retinal degeneration (Arroba et al., 2011; Lipinski et al., 2015; Ma et al., 2015; Seigel et al., 2006). IGF1 has been shown to promote proliferation and survival in photoreceptors, RPE cells, and retinal ganglion cells through the PI3K/Akt signaling pathway and inhibition of caspase-3, while CNTF acts through the JAK/STAT pathway (Sieving et al., 2006; Wang et al., 2015, 2018; Wen et al., 2012). The controlled release and bioactivity of each of IGF1-SH3 and CNTF-SH3 were demonstrated in vitro from HAMC modified with SH3 peptides (Delplace et al., 2019; Parker et al., 2016). Sustained release of IGF1-SH3 from HAMC-binding peptide protected RPE cells from serum starvation in vitro while sustained release of CNTF-SH3 from HAMC-binding peptide resulted in the characteristic downregulation of visual cycle genes after intravitreal injection into the mouse eye, demonstrating its bioactivity in vivo (Delplace et al., 2019).

To better understand the affinity release system, it was computationally modelled to determine which factors were most important to control the release rate (Vulic et al., 2015). Asymptotic analysis of the release governing equations determined parameters that could be tuned to achieve the desired protein release profile. In one regime, release was governed by fast unbinding dynamics. In a second regime, release was governed by the formation of complexes with binding partners. In both these regimes, diffusion was the rate-limiting step. In a third regime, release is governed by the decomplexation of the bound proteins, which is slower relative to diffusion. The first regime describes the SH3/SH3-binding peptide system, and was validated against previously derived experimental results. It was determined that the strength of the affinity interaction, concentration of ligands, and hydrogel geometry significantly affected the release profile and could be used to further tune the system in the future.

4. Combination strategies to promote tissue and functional repair

Disorders affecting the CNS are extremely complex, and administration of therapeutics targeting multiple pathways have shown significant benefit (Alonso de Leciñana et al., 2006; Karimi-Abdolrezaee et al., 2006; Lu et al., 2004). Co-delivery of multiple biomolecules from HAMC has been pursued to promote tissue and functional repair in animal models of disease. For example, to overcome the inhibitory environment that follows after spinal cord injury while at the same time promoting axonal outgrowth, anti-NogoA was co-delivered with neurotrophin-3 (NT3) from PLGA nanoparticles dispersed in HAMC to the spinal cord in a rodent model of a compressive spinal cord injury (Elliott Donaghue et al., 2016). Increased neurofilament density caudal to the lesion was observed and NT3 was hypothesized to have a stabilizing effect on anti-NogoA. Furthermore, combined delivery of NT3 and anti-NogoA improved the Basso, Beattie, and Bresnahan score of injured animals and induced greater recovery of hindlimb-forelimb coordination. Similarly, to overcome the chondroitin sulfate proteoglycans (CSPGs) in the glial scar that occur after traumatic injury in the CNS while also stimulating endogenous stem cells, ChABC was co-delivered with SDF to the injured spinal cord in a rat model of compressive spinal cord injury (Pakulska et al., 2017). ChABC-SH3 was released from a crosslinked methylcellulose hydrogel functionalized with SH3 binding peptides (Pakulska et al., 2016), while SDF was released from PLGA nanoparticles, by electrostatic interactions, dispersed in the same hydrogel. Their release profiles were independently characterized, with SDF being released over 28 d and ChABC being released over 10 d. The combination of the two therapeutics resulted in functional improvement in spinal cord-injured rats; however, the beneficial effects were attributed to the action of ChABC. This was substantiated by immunohistological analyses of damaged tissue with reduced CSPGs and increased axonal outgrowth observed in both the ChABC and ChABC/SDF groups.

Co-delivery of biomolecules has been effective in rodent models of stroke as well where, for example, the sequential delivery of epidermal growth factor (EGF) followed by EPO) was demonstrated with a local HAMC hydrogel delivery strategy (Kolb et al., 2007). Temporally controlled release of both factors was key to tissue repair, with EGF stimulating the generation of NSPCs and EPO reducing apoptosis of the newly generated cell population. EGF was first modified with PEG to enhance tissue penetration and then encapsulated in PLGA nanoparticles. To delay the release of EPO, it was first encapsulated in PLGA nanoparticles and additionally coated with poly(sebacic acid) (Wang et al., 2013). Both nanoparticles were incorporated into HAMC, and sequential release was demonstrated first in vitro and then in vivo. This system demonstrated the same sequential delivery achieved using a catheter infusion with a mini-pump. When compared to the more invasive mini-pump system, the PLGA/HAMC composite treated animals showed similar tissue repair and neurogenesis, with reduced apoptosis, thereby demonstrating the significant benefit of this local delivery strategy.

Modulation of the degenerating microenvironment using biomolecules can be combined with cell delivery to further promote cell survival and transplantation outcome. For example, ChABC and cNEPs were delivered to the site of injury in a clip compression model of spinal cord injury (Führmann et al., 2018). ChABC-SH3 was incorporated into a MC hydrogel functionalized with binding peptides and injected into the intrathecal space while cNEPs were encapsulated in HAMC and injected directly into healthy tissue rostral and caudal to the injury. Cell transplantation led to reduced cavity volume and co-delivery with ChABC resulted in greater neuronal differentiation of the cNEPs than

delivery of cNEPs alone. The addition of biomolecules can be used to support transplanted cell survival and drive differentiation to desired cell types within the host environment.

Combination strategies are most effective when they are synergistic. The delivery of multiple biomolecules with different controlled release rates provides great flexibility in terms of the mechanisms of action. These strategies can be pursued to directly address the pathophysiology of CNS disease.

5. Future outlook

Injectable hydrogels provide great opportunities in regenerative medicine. The HAMC hydrogel is particularly compelling because it is easily injectable through fine needles and resorbable, thereby enabling minimally invasive surgeries and eliminating the need for gel retrieval. HAMC has significantly improved transplanted cell survival in models of retinal degeneration, stroke, and spinal cord injury. However, cell survival and in vivo differentiation continue to be challenges in the transplantation field. The development of a hydrogel to efficiently direct cell fate in vivo may be a promising avenue to improve cellular therapies in the future. Given the complexity of the CNS and the devastation that occurs after traumatic injury or during neurodegeneration, it is clear that combination strategies are required for successful repair and regeneration.

The HAMC hydrogel delivery system is versatile in terms of what can be delivered and for how long. With the inclusion of PLGA nanoparticles or affinity-based strategies, bioactive release can be controlled for weeks to months. Continued innovations in controlled release strategies will enable the simultaneous co-delivery of multiple molecules at distinct rates. These strategies can be used to stimulate the endogenous cells, enhance the environment in which cells are transplanted or prime the host environment for greater survival of transplanted cells. Co-delivery of cells with biomolecules in gels like HAMC will likely be required for optimal success in tissue regeneration and functional repair.

Competing interests statement

MSS is a co-founder of AmacaThera and holds a composition of matter patent on HAMC.

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