Materials Advances

ROYAL SOCIETY OF CHEMISTRY

REVIEW

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Cite this: *Mater. Adv.*, 2021, 2, 2561

Injectable hydrogels in stroke and spinal cord injury treatment: a review on hydrogel materials, cell–matrix interactions and glial involvement

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Received 24th September 2020,
Accepted 4th March 2021

DOI: 10.1039/d0ma00732c

rsc.li/materials-advances

Central nervous system (CNS) pathologies, such as stroke and spinal cord injury, remain debilitating issues due to the inhibitory environment in the CNS. Many research works have focused on combinatorial therapeutic approaches, such as biomaterial scaffolding, cell transplantation and biomolecule delivery, in the hope of effectively improving functional recovery *in vivo*. Unfortunately, to date, there is still no effective treatment to regain mobility post-injury. In search of better therapeutic strategies, injectable hydrogels are becoming a popular treatment option for CNS diseases due to their tuneable mechanical properties and the minimally invasive nature of administration. Moreover, the ability to encapsulate exogenous cells and therapeutic molecules while providing an environment that is permissive to cells and promote cell survival incentivises the use of injectable hydrogels in CNS disease treatment. In this review, we will discuss the advances that have been achieved in the recent decade in injectable hydrogel systems for tissue regeneration after stroke and spinal cord injuries. In particular, we focus on the cellular response and tissue integration related to these hydrogel systems. We hope to provide useful insights on materials choices for future research work in injectable hydrogels for stroke and spinal cord regeneration.

1. Introduction

Despite advances in medicine and technology, pathologies of the central nervous system (CNS), such as stroke and spinal cord injury (SCI), remain debilitating issues globally. Stroke is one of the main causes of adult disability and the third leading cause of death worldwide.[1] In the next decade, stroke will continue to increase the burden on patients and society.[2] By 2030, approximately 3.4 million Americans are expected to suffer from ischemic stroke, a prevalence increment of 20.5% as compared to 2012.[3] In the case of SCI, it is estimated that over 1 million people suffer from SCI in North America. The lifetime costs for treatment and care range from $1.1 to $4.7 million USD per person, which aggregates a direct cost that exceeds $7 billion per year in the United States alone.[4] To date, there is still no effective treatment for CNS diseases. The inhibitory microenvironment in the CNS makes it difficult for self-regeneration to take place. Hence, in search for better therapeutic treatment options, recent studies have focused on the introduction of tissue scaffolds that mimic the extracellular matrix (ECM) to promote regeneration.[5–7] Correspondingly, a variety of scaffold materials in combination with various biological agents have been developed. These may include exogenous cells, microRNAs and growth factors.[8–15]

Injectable hydrogels have become a popular scaffolding treatment option for CNS diseases. The tunable mechanical properties and the ability for minimally-invasive administration make injectable hydrogels a more attractive option as compared to other traditional scaffolding methods, which require surgical procedures. While there have been many reviews on injectable hydrogels for stroke and SCI treatments, most of them have focused on the design strategies, particularly the physical and chemical properties of various scaffold materials.[5,6,16–20] In this review, the advances of injectable hydrogels and cell transplantation for recovery in CNS diseases will be discussed, focusing on the aspect of cellular response and tissue integration. We then assess the suitability of hydrogel materials in different stages of tissue regeneration *in vitro* and *in vivo*. We hope that this review will aid the future selection of materials and the development of injectable hydrogels for CNS disease treatment.

2. Disease pathogenesis and cellular involvement

2.1. Stroke and SCI pathology

Stroke can be divided into haemorrhagic stroke and ischemic stroke. Haemorrhagic stroke is caused by ruptured cerebral blood

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vessels or abnormal blood vessel structures, while ischemic stroke is a result of interruption in blood supply to the brain. Studies have shown that about 85% of stroke patients suffered from ischemic stroke.[21] Thus, in this review we will mainly discuss studies related to ischemic stroke. Ischemic stroke is mainly due to the blockage of blood vessels in the brain and the inability of the body to establish reperfusion. Subsequent brain damage stems from a complex series of pathological events, such as depolarization, inflammation, and excitotoxicity. These phenomena greatly impair the stability of the blood–brain barrier and activate the release of free radicals and proteases, which not only causes local neuronal cell death, but also further expands the damage.[20] Unlike the other organs and tissues, brain tissues are very sensitive to ischemia. The core of the infarct is usually immediately and irreversibly damaged and the related nerve function is immediately lost. Soon after, the core boundary expands to adjacent tissues, resulting in apoptosis and cell death to the distal end of tissues due to vascular occlusion.[22]

The cause of SCI is commonly due to sudden mechanical impact to the spinal cord parenchyma that results in fracture, contusion, compression or laceration of spinal cord tissue.[23] Following the initial impact on spinal cord tissue, the focal destruction of neural tissue at the lesion triggers a sequence of chronological events that eventually lead to the destruction of neural tracts, also known as secondary injury. Within 15 min post-injury, multiple hemorrhages are often seen in the grey matters where re-perfusion does not usually occur within the first 24 h.[24] Following the initial injury, the loss of blood supply and the lack of self-healing mechanism lead to vascular insult, hemorrhages and ischemia which ultimately result in cell death and necrotic tissues within 24–48 h post-trauma.[23,25] The native microenvironment of the spinal cord inhibits its ability for self-recovery as axonal destruction results in filtration of glial cells and other non-CNS cells to clear up the debris. The debris from myelin and oligodendrocytes form the initial component of glial scar to contain further damage, followed by migration and proliferation of astrocytes that upregulate the production of glial fibrillary acidic protein (GFAP) and form the bulk of non-permissive scar.[26] The clearing of debris and glial scarring form a cystic cavity at the lesion which acts as a wall to prevent axonal regrowth and neurite outgrowth.[6] Over time, continuous apoptosis of oligodendrocytes within the lesion leads to further demyelination of axons and Wallerian degeneration. This further creates an environment that prevents axonal regrowth after SCI.[5]

Injuries in the CNS lead to tricky pathologies as self-regeneration and recovery are discouraged in the native CNS microenvironment and axonal regeneration and functional recovery does not occur actively.[27] Treatments in CNS injuries have focused on restoring secondary injuries and providing structural support and extracellular cues for cell ingrowth and tissue recovery.[28] The use of injectable hydrogel has become an appealing treatment option due to the capability in providing mechanical support as well as flexibility in shapes and material choices. In SCI, the lesion is further complicated by glial scars formed by reactive astrocytes, leading to further inhibitory barrier that prevents recovery. With regard to the administration approaches, hydrogels could be implanted *via* open-wound surgery and injection in the SCI model, but limited to the injection method in the stroke model.

2.2. The roles of glial cells in stroke and spinal cord injury treatment and the potential of cell transplantation

Glial cells in the CNS, mainly astrocytes, microglia and oligodendrocytes and their progenitors, support and enable effective nervous system function. In the healthy CNS, glia aid in the homeostasis of the microenvironment. While remaining relatively inactive in the healthy state, glial cells act to increase inflammatory actions and help modulate the environment in the event of an infection or injury. These cells play crucial roles in tissue regeneration after CNS pathology by regulating inflammation and supporting neuronal growth.[29]

Reactive astrocytes limit the expansion of the lesion by forming glial scar around the lesion and releasing neurotrophins through anti-excitatory toxicity, thereby providing neuroprotection.[30] The views on the effects of glial scar remain controversial. Traditionally, the glial scar is believed to prevent axonal regrowth.[31] However, recent studies have shown that, by preventing glial scar formation, axonal regrowth is reduced *in vivo*, thus suggesting the potential beneficial effects of astrocytes and glial scarring in axonal regeneration.[32] Polarization of microglia can be either pro-inflammatory, which produces cytotoxic factors leading to inflammation and oligodendrocyte apoptosis by releasing pro-inflammatory cytokines, or anti-inflammatory reactions that produce trophic factors to promote axon regeneration.[33,34] In the case of oligodendrocytes, despite the extensive neuronal cell death after CNS injuries, activated oligodendrocyte progenitor cells (OPCs) show significant proliferation and migration to replace lost cells. The interaction with cytokines promotes OPC differentiation into oligodendrocytes to replace dead oligodendrocytes in the lesion and support axon regrowth.[35,36]

Neuron cell death and glial inflammation pose a major challenge in tissue regeneration following CNS injuries. In this regard, cell transplantation has become a popular therapeutic option for the treatment of stroke and SCI due to the potential of (1) directly replacing damaged cells in the lesion, (2) providing neuroprotection to the surviving neuro connective tissues, and (3) providing a supportive cellular growth substrate for axonal regrowth.[37] However, cell transplantation commonly faces major cell death, low cell migration and integration, as well as limited directional guidance of axonal growth.[38,39] In addition, the potential safety risks associated with stem cell transplantation require the development of rigorous protocols to ensure cell homogeneity, quality assurance, and no tumorigenicity.[40]

3. The roles of injectable hydrogels in cellular response and tissue regeneration in stroke and spinal cord injury treatment

To overcome the challenges faced in tissue regeneration within the CNS, combinations of scaffold materials and cellular and

molecular therapies have to be considered according to the type and severity of the injury.[5,41] Injectable hydrogels are widely used to treat pathologies in both hard and soft tissues due to their resemblance to natural ECM structures and minimally-invasive administration. Some common requirements for injectable hydrogels include biocompatibility and non-cytotoxicity. Depending on the types of tissues and application, the rate of degradation, gelation time and mechanical properties of hydrogels need to be carefully considered.[42] In hard tissue applications, such as bones and cartilages, the mechanical properties of hydrogels are one of the main considerations due to the weight bearing nature of these tissues.[43] However, the good mechanical properties of hydrogels are difficult to achieve, particularly up to the mega pascal range, without modifications in functional groups or crosslinking which often use cytotoxic agents that affect the cytocompatibility of the hydrogels.[44] Instead, the viscoelastic nature of injectable hydrogels is more suitable for applications in soft tissues like skin, cardiac tissues and neural tissues.[43]

Injectable hydrogels are viscoelastic materials that are able to fill the irregularly-sized defects in the CNS injuries. They may also facilitate tissue-implant integration and allow modulation of the microenvironment to reduce scarring while promoting regeneration.[45–50] The use of hydrogels provides a more favorable environment by mimicking the natural ECM structure and/or providing suitable stiffness that supports cell attachment and functionality.[51] The mechanical properties of hydrogels should be tuned to closely match the native CNS tissues, which possess a mechanical stiffness of about 100 Pa to 1000 Pa for both brain and spinal cord tissues,[52,53] thus allowing primary cell attachment and differentiation towards a neural lineage. As hydrogels are crosslinked with high porosity, they are ideal carriers for neuroprogenitor cells. The potential of differentiating into functional neurons, astrocytes and oligodendrocytes aids in modulation of the microenvironment at the injury site.[16]

Natural polymers are a popular choice of material for injectable hydrogels due to their resemblance to the native CNS tissues. Many contain intrinsic amino acids that can be readily modified for cell adhesion.[54] Some common materials of choice include collagen, gelatin, hyaluronic acid (HA), chitosan, alginate, agarose and methylcellulose.[10,16,55,56] Such natural polymers have their inherent advantages as they are typically the components of the ECM, thus making them more biologically active in stimulating cellular functions.[57] For example, collagen is known to improve cell migration,[58] HA is known to directly activate intracellular signalling pathways *via* CD44 cell surface receptors[59] and chitosan is known for its antibacterial properties.[60] Combining the unique properties from various natural polymers and their inherent characteristics, such as biocompatibility, non-immunogenicity and non-toxicity, cells and host tissues could potentially have a higher chance of survival and proliferation in these hydrogels.[61]

Self-assembling peptides (SAPs) are amino acid-based molecules that undergo sol–gel transition at neutral pH and ionic concentration to form ECM-like networks.[62] ECM molecules, growth factors and cells are also frequently incorporated to enhance cell attachment, cell migration and tissue regeneration.[6] The most suitable time for regeneration to take place post traumatic CNS injury is at the sub-acute stage, *i.e.*, 7–14 days post injury. This is the period when the initial cascade of inflammation starts to take place and the microenvironment is not too harsh for implantation to take place.[63] A brief summary of the use of injectable hydrogels in CNS disease treatment is shown in Fig. 1A.

3.1. Challenges and advances of injectable hydrogels

Injectable hydrogels are able to form scaffolds *in situ* and fill irregular defects to aid in establishing tissue-implant integration. As injuries to the CNS are typically irregular in shapes, the use of injectable hydrogels eliminates the needs for preform scaffolds that require excising viable tissues to accommodate implantation and reduces the risks of scaffold deformation and compression.[64,65] Scaffold compression may be unfavourable in CNS treatment as it can significantly increase scaffold stiffness beyond theYoung's modulus of the CNS tissues.[66] In addition, when comparing to traditional scaffolding or nerve guides, injectable hydrogels are typically highly porous with a high proportion of water ($>90\%$), making them ideal drugs and cell carriers with a controllable diffusion rate by changing the crosslinking density. A downside would be the high water content that significantly increases the rate of diffusion of hydrophilic drugs out of the hydrogel and could be problematic if prolonged drug release is required.[67] The *in situ* gelation property of injectable hydrogels allows the release of molecules in a controlled fashion and prevents drugs from being washed away by the body fluids from the targeted regions.[68] Lastly, as direct cell transplantation often faced significant cell death, injectable hydrogels are favorable in this context, since these hydrogels can support the survival of exogenous stem cells by allowing modification of scaffold stiffness, incorporation of cell binding cues and inclusion of growth factors.[69–71]

Advances in injectable hydrogels help to solve various clinical challenges in CNS disease treatment. Specifically, injectable hydrogels have received attention as carriers for proteins and drugs. However, initial burst release of loaded proteins and drugs can lead to undesired effects and ineffectiveness of treatment and this has been one of the main challenges for injectable hydrogels.[72] Many approaches have been developed to overcome this problem. For example, the formation of complex networks *via* crosslinking to reduce the burst release of incorporated proteins and drugs,[73,74] ''pendant chain'' system which delays the release by covalently grafting a protein backbone to the hydrogels *via* a cleavable linker,[75] incorporation of proteins and drugs into micro- or nanoparticles to serve as a drug reservoir in the composite hydrogel system,[76] and incorporation of high-affinity ligands to prolong drug release[77] have all been reported. Other enhancements in the hydrogel system were also developed to improve the biological effects. In this respect, ligand-functionalization has been extensively studied to enhance cell infiltration and binding capabilities. Small oligopeptide sequences within the ECM proteins such as RGD peptides were incorporated to enhance

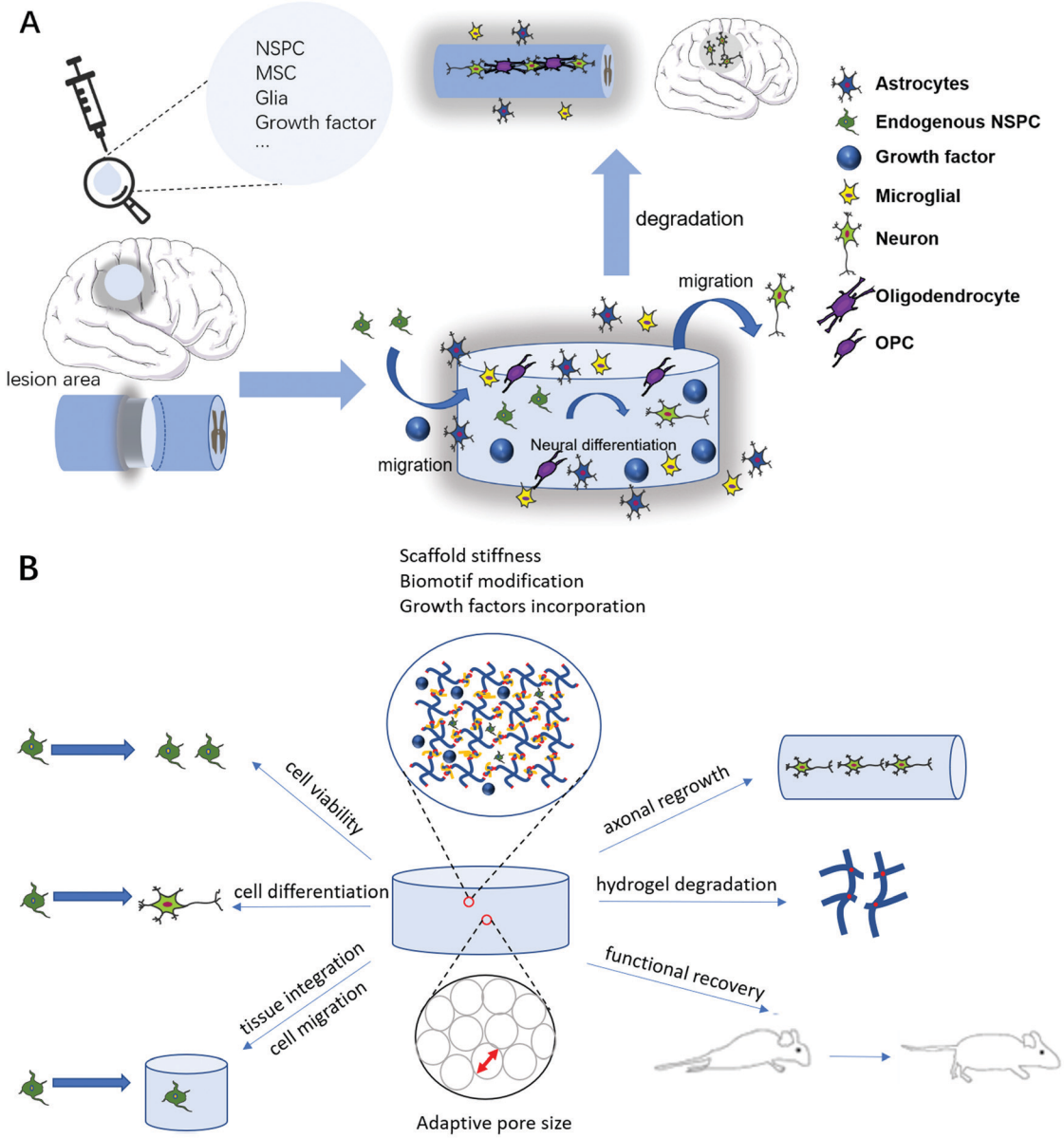

Fig. 1 (A) Administration of injectable hydrogel gelates into the lesion area. The ECM-mimicking network modulates the microenvironment and promotes cell migration and differentiation in the lesion to restore nerve connection. (B) Possible cell behavior considerations of injectable hydrogels.

cell adhesion within the hydrogel.[78] Furthermore, peptide sequences like IKVAV and YIGSR are known to interact with mammalian neurons. Correspondingly, the incorporation of these sequences has been shown to enhance neural differentiation.[79,80]

3.2. Incorporation of exogenous cells in injectable hydrogels

Injectable hydrogels in combination with cell transplantation have been studied extensively in recent years in the hope of improving neural regeneration and functional recovery. Several types of primary cells have been explored. These include neural stem/progenitor cells (NSPCs), Schwann cells (SCs), olfactory-ensheathing cells (OECs), mesenchymal stem cells (MSCs) and human-derived induced pluripotent stem cells (iPSCs).[20,63,81] Among these cell types, NSPCs, MSCs and glial cells are the common cell types to be incorporated within hydrogels for transplantation treatments. In particular, NSPCs can not only differentiate into mature neurons to replace lost cells, but also promote endogenous repair, such as enhancing angiogenesis, providing immunosuppression, and promoting recruitment of endogenous cells.[82–84] MSCs, on the other hand, play a bigger role in contributing to the stimulation of angiogenesis and inhibition of microglial activation by secreting cytokines (IGF-1, VEGF, EGF and FGF) to reduce neuronal death. MSCs could also potentially differentiate towards the neural lineage.[85,86] Glial cell transplantation could also be advantageous due to its potential to directly modulate the environment and meditate myelin regeneration.[87]

Despite the efforts from the past decades, there is no effective CNS disease treatment that results in the restoration

of function. Here, we will look at the recent research studies on injectable hydrogels, including the use of cell transplantation, in the past decade. This ensures that the latest findings on the use of injectable hydrogels are covered. The choices of hydrogel materials will mainly be based on tissue and cellular responses. A brief summary of possible design considerations is shown in Fig. 1B.

3.3. *In vitro* cell viability

Cell transplantation to the CNS often faces the problem of low cell survival rates. In this regard, *in vitro* cell culture studies focusing on evaluating the roles of hydrogels in the regulation of cellular responses have allowed the close examination of the viability of various cell types when delivered within these biomaterials. In particular, cell–matrix interactions are crucial in modulating cellular homeostasis and directing communication to cell cytoskeleton, growth factor receptors and intracellular signalling cascades to ensure cell survival.[88] Table 1 summarizes the *in vitro* studies on cell-incorporated injectable hydrogels and their corresponding cell viability and differentiation outcomes. Previous studies showed that mesenchymal and neural stem cells have the potential to replace lost cells and modulate inflammation and the local wound environment.[89] Both NSPCs and MSCs seem to be able to survive and proliferate well with proper cellular and physical cues. Most studies have shown that these cells are able to survive in their respective hydrogels for more than 2 weeks,[84,85,90–92] suggesting sufficient cell–matrix interactions between the incorporated cells and the hydrogel substrates.

Many materials have been tested for cell survival and proliferation *in vitro*. Among these, we noticed significant improvement in cell proliferation in hyaluronan-, chondroitin sulfate- and collagen-based materials.[55,71,83,84] The findings suggest that natural polymers found abundantly in the body are more suitable for inducing cell proliferation. We believe that the CD44 interaction could be the reason for improvement in cell viability. CD44 is a cell receptor found in various cell types, including neural stem cells and glial cells in the CNS.[93–95] Specifically, CD44 forms receptor–ligand interactions with natural polymers including HA, chondroitin sulfate and collagen.[96–98] The activation of the CD44 ligand binding domain allows binding of activator proteins that in turn triggers downstream signalling pathways, such as the Ras-Raf-MEK-ERK pathway, which leads to cell proliferation.[99] While studies on cell viability *in vivo* typically last for 4–8 weeks, Rouleau and colleagues[92] were able to maintain viable hiPSC-derived neurons and glial cells in the silk fibroin hydrogel for more than 2 years. However, given the fact that the silk fibroin hydrogel showed minimal degradation and maintained its hydrogel network over the long culture duration, such materials may be less ideal for the regeneration and replacement by new native tissues. Some studies only conduct *in vitro* experiments for less than 7 days.[100,101] It could be beneficial to conduct *in vitro* experiments for a longer duration to ensure cell survival and the differential potential *in vivo*, where the environment is harsher.

3.4. *In vitro* cell differentiation

Cell transplantation can modulate the diseased microenvironment by enhancing cell differentiation. Cell differentiation is crucial for the transplanted stem and progenitor cells to differentiate into mature and functional cells. This allows replacement of diseased tissues, promotes tissue regeneration through the secretion of regenerative factors and provides neuroprotection.[102,103] While the effectiveness of cell transplantation depends largely on the number of cells transplanted, undifferentiated cells were shown to minimize cell death as compared to fully differentiated transplanted cells.[104] Previous work done by Payne and colleagues[82] showed that the transplantation of undifferentiated induced pluripotent stem cell-derived neural stem cells (iPSC-NSCs) resulted in greater functional repairs compared to late-differentiated cells which caused more tissue damage due to greater cell death.

From Table 1, some of the studies demonstrated the ability of injectable hydrogels to promote encapsulated cell differentiation to express immature neuronal markers at short time points[105,106] and mature neural markers at longer time points.[90,98] The choice of hydrogel materials seems to be critical in promoting the differentiation of encapsulated cells. SAPs are able to facilitate the differentiation of cells to express mature neuronal or oligodendrocyte markers without further modification of materials.[90,98] Natural materials, on the other hand, often rely on additional modification to enhance cell differentiation *in vitro*. For example, hyaluronic acid-methylcellulose (HA-MC) alone did not enhance the differentiation of NSPCs as compared to cells cultured in neurobasal media.[105] Collagen-only hydrogels worsened the differentiation potential of NSCs to neuronal lineage as compared to cells cultured on poly-D-lysine (PDL)-coated coverslips.[107] *In vitro* cell differentiation was enhanced when these natural polymers were further modified with bioactive motifs (*e.g.* IKVAV, LRE peptides) or incorporation of growth factors (*e.g.* PDGF, VEGF).[108–111] We believe that, while injectable hydrogels made of natural materials are better in promoting cell viability *in vitro*, cell differentiation could be attributed to the presence of bioactive peptide sequences. SAPs are shown to have greater potential in promoting differentiation as they can be readily modified to include bioactive peptide sequences. Natural polymers, however, also achieved improved differentiation outcomes after biomotif modification. Specifically, Silva and colleagues[112] compared the differentiation outcomes between scaffolds containing the bioactive IKVAV sequence, a non-bioactive sequence and a cell suspension with IKVAV soluble peptide. The results demonstrated that physical entrapment of bioactive epitope SAPs was the main reason for improvement in cell differentiation. Moreover, the stiffness of the hydrogels influences not only cell survival, but also the differentiation fate of transplanted cells.[113] For example, silk is a stiff material by nature,[83] thus affecting the differentiation potential of encapsulated neural stem cells. Although there are conflicting reports on cell differentiation potential in silk fibroin hydrogels *in vitro*,[92,114] the high intrinsic stiffness of silk could explain the inconsistency in different studies and the low percentage of

Table 1 Examples of injectable hydrogels in enhancing cell viability and differentiation *in vitro* (papers are only taken from 2010 onwards)

	Injectable polymers	Cell source	Culture conditions	Culture duration	*In vitro* cell viability	*In vitro* differentiation	Ref.
Neural stem/progenitor cells	Collagen/genipin	NSCs	DMEM-LG, 10% FBS, 1% penicillin–streptomycin, N2 supplement, and 20 nm mL^{-1} FGF	10 days	NSC viability reduces significantly at genipin concentrations higher than 0.25 mM after 3 days in culture.		Macaya *et al.* (2011)[55]
	SAP	NSCs	Serum-free basal medium, supplement FGF2 and EGF	12 weeks	Significant improvement in cell viability for cells encapsulated in SAP compared to Matrigel and collagen groups	Significant increase in Tuj-1, GFAP and GalC expression by 12 weeks (~30% Tuj-1, ~25% GFAP and ~25% GalC) in culture compared to days 3 (~5% Tuj-1, <1% GFAP and <1% GalC)	Koutsopoulos *et al.* (2013)[98]
	HA-MC covalently modified with or without recombinant rat platelet-derived growth factor-A (rPDGF-A)	Rat adult brain-derived NSPCs	Neurobasal-A, 2% B27, 1% L-glutamine, 1% penicillin–streptomycin	7 days	Significant improvement in cell viability for NSPCs encapsulated in the hydrogels	RIP (>2-fold increase) compared to HAMC and control groups. Non-significant between HAMC and control groups	Mothe *et al.* (2013)[105]
	HA modified with PLGA microspheres containing VEGF and Ang1	NSCs derived from mouse forebrain	DMEM/F12	5 days	NSCs were seeded and viable on the HA hydrogel with PLGA microspheres		Ju *et al.* (2014)[101]
	Silk fibroin hydrogel functionalized with IKVAV peptide	hESC-derived NSCs	(DMEM)/F12 medium, supplemented with 2% StemPro Neural Supplement and 2 mM Glutamax, bFGF (20 ng ml^{-1}) and EGF (20 ng ml^{-1})	7 days	Silk-IKVAV group showed the higher fluorescence intensity of encapsulated cells than that of the unmodified silk group, reflecting greater cell viability	β III-tubulin (3%). MAP2 (1.8%)	Sun *et al.* (2017)[114]
	HA-based self-polymerizing hydrogel	Human iPSC-derived NSPCs	DMEM F12 supplemented with L-glutamine, nonessential amino acids, penicillin–streptomycin, and 10 ng ml^{-1} basic FGF	7 days	Significant increase in NSPC proliferation in gels with an elastic modulus of 350 Pa		Moshayedi *et al.* (2016)[83]
	Gelatin methacrylate hydrogel (GelMA) for encapsulation of iPSC-derived NSCs. Cells were cultured for 7 days	Human iPSC-derived NSCs	1 : 1 mix of neurobasal medium and MEM/F12 supplemented with N2, B27, 1 × Glutamax and penicillin/streptomycin	7 days	Cell viability was found to be significantly higher in soft-GelMA at both days 3 and 7. Encapsulated cells in soft-GelMA showed elongation compared to rounded cells in medium- and stiff-GelMA	Significant increase in the level of Tuj-1 in soft-GelMA compared to both stiff- (7.6-fold) and medium-GelMA (3.3-fold) at day 3	Fan *et al.* (2018)[115]
	Collagen-HA-Laminin based hydrogel	Rat spinal cord derived NPCs	DMEM/F12, 1% N2, 1% penicillin/streptomycin	5 days		NG2: Col-HA-Lam (66.8%), Col-HA (40.1%), Col-Lam (24.1%)	Geissler *et al.* (2018)[107]
	Thiolated HA (HA-SH) modified with RGD/YIGSR/IKVAV	hESC-NSPC	hESC serum-free media, DMEM/F12, 1× StemPro neural supplement, 2 mM Glutamax, 0.1× antibiotic/antimycotic, 20 ng mL^{-1} bFGF, 20 ng mL^{-1} EGF	>70 days	Significantly higher viable cells in hydrogels with RGD than in hydrogels without RGD at day 70	Significant increase in olig2, β III-tubulin and OCX in 3D hydrogel culture compared to 2D culture Significant reduction in GFAP in 3D hydrogel culture compared to 2D culture	Seidlits *et al.* (2019)[71]
	Chondroitin-4-sulfate A (CS-A) hydrogel	Human iPSC-derived NSPCs	DMEM/F12, 45% Neurobasal, 0.5% N2 supplement, 1% B27	2 weeks	Total number of survival neurons was nearly tripled compared to the non-encapsulated NPCs		McCrary *et al.* (2020)[84]
	HA matrix modified with laminin-derived IKVAV and Leu-Arg-Glu (LRE) peptide and	hiPSC	Supplement, 1% GlutaMAX, 1% nonessential amino acids, 0.1 mM β-mercaptoethanol (β-ME; Sigma Aldrich), 100 U mL^{-1} penicillin/streptomycin	2 weeks	The inclusion of IKVAV and LRE and/or GPQGIWGQ in the matrix did not significantly affect cell number in the matrices at any	$Tuj\text{-}1^{+}$ cells were found in all HA groups: (1) HA difunctionalised with azide and thiol reactive groups (DIFF-HA), (2) HA modified with IKVAV and LRE peptides	Perera *et al.* (2020)[106]

Table 1 (*continued*)

	Injectable polymers	Cell source	Culture conditions	Culture duration	*In vitro* cell viability	*In vitro* differentiation	Ref.
	GPQGIWGQ cross-linker (PEP + MMP-HA)		(Sigma Aldrich), 5% knockout serum replacement		studied time point based on DNA content	(PEP + HA), (3) DIFF + MMP + HA and (4) PEP + MMP-HA. Only PEP + MMP-HA group was found to have significant increase in neurite length.	
	Silk fibroin	hiPSC-derived NSPC	50% F12/DMEM, 50% Neurobasal medium, 1% Glutamax, 1% non-essential amino acids, 0.5% N2 supplement, 1% B27 supplement, 1% penicillin/streptomycin and 20 ng mL^{-1} FGF-2	>2 years	DAPI counts maintain throughout the culture duration.		Rouleau *et al.* (2020)[92]
Mesenchymal stem cells	Gelatin polymer hydrogel	Mouse BMSCs	DMEM, 10% FBS, 1% penicillin–streptomycin	2 weeks	The BMSCs proliferated while maintaining their original morphology in the gels		Osanai *et al.* (2010)[85]
	Collagen/genipin	Porcine MSCs	DMEM-LG, 10% FBS, 1% penicillin–streptomycin	10 days	Significant increase in DNA content (7-fold increase) for MSCs in collagen/0.25 mM genipin gels from day 1 to day 7		Macaya *et al.* (2011)[55]
	HA-MC hydrogels	Human umbilical tissue-derived cells (hUTC)	Dulbecco's modified Eagle medium, Penicillin–streptomycin, GlutaMAX™	3 days	Out of 4 blends of hydrogel (0.5/0.5, 0.75/0.75, 1.0/1.0 and 1.0/0.75), only a significant decrease in encapsulated cells was found in the 1.0/1.0 blend		Caicco *et al.* (2012)[100]
	Self-assembling bone marrow homing peptide ($BMHP_1$)	Human endometrial-derived stromal cells (hEnSC)	NPBM, bFGF, EGF, NSF-1, BDNF (50 ng/ml), 1% antibiotic, 2 nM glutamine	21 days	hEnSC viability significantly improved when encapsulated in $BMHP_1$	Neural-like differentiation was observed for hEnSCs encapsulated into $BMPH_1$ 14 days post incubation Significant fold increment in Tuj-1, NF, MAP2 and Bcl2 expression 21 days post incubation	Tavakol *et al.* (2016)[90]
Glial cells	0.5%/0.5% w/v HA-MC hydrogels modified with RGD peptide and PDGF-A	hiPSC-OPC	OPC media	7 days	Significant increase in viable cells in HAMC-RGD (~60%) compared to HAMC (~25%) and TCP group (~20%).		Führmann *et al.* (2016)[10]
Primary cells	Crosslinked sericin hydrogel (GSH)	Cortical neurons from rat pup	DMEM, supplemented with 2% B27, 1% glutamine and 1% penicillin/streptomycin	20 days	Primary cells effectively attached and grew on the GSH. The hydrogel promotes axon extension and branching of primary neurons.		Wang *et al.* (2015)[91]

neural differentiation observed by Sun and colleagues. On the other hand, Fan and colleagues[115] tested gelatin methacrylate hydrogels (GelMA) of different stiffnesses using a compression test. Specifically, three different stiffnesses of hydrogels were tested, including soft (~680 Pa), medium (~1230 Pa) and hard (~2030 Pa), and the group concluded significant improvements in both *in vitro* cell viability and differentiation with soft-GelMA gels. The measured stiffness of the soft hydrogel matches the stiffness of the CNS tissues,[52] which explains the findings from the study.

3.5. *In vivo* tissue integration and cell migration

Tissue integration can be defined as the physical, biological and mechanical connection of the interface between the implanted materials and the surrounding native tissues.[116] Poor tissue integration of the scaffold is often one of the factors contributing to poor cell survival and tissue regeneration *in vivo.* It often leads to the formation of fibrous scarring and cystic cavity which obstructs potential neuronal ingrowth.[64,117] Proper integration between the tissue and scaffold often results in a high degree of cell infiltration to modulate the microenvironment in the lesion, given that the architectures of the scaffold are permissive to cell infiltration.[118] In addition, tissue integration is also related to supporting axonal growth by facilitating endogenous cell infiltration and migration and deposition of laminin.[119] Endothelial cell infiltration and the formation of blood vessels as blood flow are also essential to sustain the growth of regenerating axons and the surrounding tissues.[84]

In the local inhibitory microenvironment of the CNS, differentiation of transplanted stem cells into neurons for long-term survival and tissue integration is particularly challenging. As shown in Table 2, scaffolds that exhibit good tissue integration and promote cell migration typically result in reduced presence of reactive astrocytes ($GFAP^+$) and inflammatory cells ($IBA\text{-}1^+$). Further examination also confirms the ability of neurons to infiltrate into the hydrogel (NF^+, $NeuN^+$, DCX^+, $Tuj\text{-}1^+$, β III $Tubulin^+$, *etc.*). We believe that these injectable hydrogels do provide sufficient tissue integration as evident from the reduced inflammation and enhanced cell migration and differentiation.

Overall, there seems to be no distinctively ''perfect'' material that promotes tissue integration and cell migration. In fact, many studies have shown evidence of improvement in scaffold–tissue connection and cell infiltration regardless of the choice of materials. Improvement in tissue–scaffold integration can be indicated by (1) a reduction in tissue scarring,[101,120,121] (2) a reduction in cystic cavity,[119,122] and (3) infiltration of endothelial cells.[84,123,124] While natural polymers and ECM-mimicking SAPs can be modified to improve implant integration into native tissues, it is worthwhile to note that other than ensuring good integration to the tissues surrounding the lesion, the pore size of the scaffold should also be carefully considered to ensure that the internal architecture of the hydrogel is conducive to cell infiltration.[5] Furthermore, while scaffolds are usually made of hydrophilic materials or coupled with hydrophilic antigens to improve tissue and cell attachment, the stiffness of the scaffold is also an important parameter to consider for proper tissue integration and promoting cell migration. As studied by Lam and colleagues,[9] HA hydrogels with two different stiffnesses were examined and significantly higher $GFAP^+$ signals were found in the animals that were implanted with the stiffer scaffold. This potentially leads to larger cystic cavity and reduced cell migration.

3.6. *In vivo* axonal regrowth

One of the main objectives in CNS therapy is to promote axonal regrowth and to reconnect neuronal network. Axonal regeneration is one important factor influencing tissue recovery by sprouting uninjured axons and eventually leading to functional recovery.[125] The use of injectable hydrogels could enhance axon regeneration from the corticospinal tract (CST).[6] Cell transplantation also facilitates the regeneration by either the differentiation of exogenous transplanted cells[9] or facilitating the differentiation of endogenous neurons.[13] Since injured axons in the CNS do not actively regenerate, the amount and extent of axonal regrowth within the scaffold are important parameters to be considered for potential functional recovery. From Table 2, most groups deployed the strategy of attracting the migration of endogenous NSPCs into the lesion and promoting their proliferation and differentiation into neurons. Although neuron markers like DCX and Tuj-1 could sufficiently prove the capability of these hydrogels in promoting neural-like proliferation and differentiation, most studies do not provide evidence of mature neuron differentiation. On the other hand, mature neuron markers (MAP2, NeuN, NF, *etc.*) could be better choices as indicators of growing mature neurons that are associated with axonal sprouting.[126] This is because axonal sprouting is known for strengthening existing connections and facilitating new synaptic connection across the lesion, which are essential to restore proper signal transmission.[127]

While most growth factor-incorporated scaffolds seemed to be more effective in promoting axonal regrowth and tissue regeneration, we identify three materials with significant improvement in axon regeneration marked by the expression of mature neuron markers: SAP,[128] HA-MC[10,129] and heparin-poloxamer (HP).[130] The SAP was made of laminin-derived IKVAV,[128] HA-MC, was modified with the RGD sequence and growth factors were incorporated in both HA-MC and HP hydrogels.[10,130] These modifications facilitated attachment and promoted the proliferation of cells to modulate the microenvironment that is conducive to axonal regrowth. The incorporation of OPCs in the hydrogel also potentiates the differentiation into oligodendrocytes.[10] We believe that these materials, with proper modifications that facilitate cell infiltration and differentiation, could effectively promote axonal regeneration and preserve the integrity of newly formed axons. In addition, BDA labelling done by Liu's group[120] (SAP hydrogel) and Wang's group[130] (HP hydrogel) confirmed the connection of neurons between newly formed axons and those in native tissues, which could potentiate functional recovery.

Table 2 Examples of injectable hydrogels in promoting tissue regeneration *in vivo* and functional recovery (papers are only taken from 2010 onwards)

	Injectable polymers	Cell source	Implantation site	Implant duration	Tissue integration and cell migration	Axonal regrowth	Scaffold degradation	Functional recovery	Ref.
Neural stem/progenitor cells	HA hydrogel matrix modified with RGD	hiPSC-NPC	1.5 mm anterior, 2 mm lateral to the bregma	1 week	Significant reduction in IBA-1$^+$ signals in the cell + hydrogel group compared to cell only and control groups	Significant increase in the DCX$^+$ signal in the cell + hydrogel group compared to cell only and control groups			Lam *et al.* (2014)[9]
	Self-assembling laminin-derived epitope hydrogel	hiPSC-NSC	0.5 mm and 2.0 mm anterior, 2.5 mm lateral to bregma, and 1.5 mm below the dura	36 weeks		Grafted cells were observed around the hydrogels in the host tissue which were NeuN$^+$. Significant increment in NeuN$^+$ cells compared to cell-only group		Significant functional improvement in forelimb sensorimotor function test in the cell + hydrogel group over cell only and control groups over 9 months post-injury	Somaa *et al.* (2017)[128]
	HA-MC hydrogels	hiPSC-derived cortically-specified neuroepithelial progenitor cells (cMEP)	Two locations corresponding to cortical lesion sites: AP 0.0 mm, ML 3.0 mm; and AP 2.3 mm, ML 3.0 mm	50 days		No significant difference in Sox2, DCX, βIII-tubulin and NeuN signals among the early-, mid-, and late-differentiated cell groups 50 days post-transplantation		Comparing to day 4: Early-differentiated cNEPs: Significant improvement at day 42 and 56; Mid-differentiated cNEPs: Significant improvement at day 56 only Late-differentiated cNEPs: Non-significance at both days 42 and 56	Payne *et al.* (2019)[82]
	Chondroitin sulfate-A hydrogel	hiPSC-NSPCs	Intracranial transplantations (2 μL, 100,000 cells μL^{-1}) were performed into the stroke core region	2 weeks	Significant increase in the number of microvessels (Glut1, SMA, BrdU) in the stroke core region following treatment with hydrogel + cell compared to cell or hydrogel only groups 2 weeks post-transplantation			Significant forelimb sensorimotor function test in the cell + hydrogel group over 2 weeks compared to the hydrogel only group and control	Mccrary *et al.* (2020)[84]

Table 2 (*continued*)

	Injectable polymers	Cell source	Implantation site	Implant duration	Tissue integration and cell migration	Axonal regrowth	Scaffold degradation	Functional recovery	Ref.
Mesenchymal stem cells	Poly(*N*-isopropylacrylamide) (PNIPAAm), crosslinked with either PEG or MC	Rat skin fibroblasts (RSFs)	C4-5 hemisection or L4-L5 hemisection	2 weeks	Hydrogels allow cell migration from the host tissue into the scaffold. IBA-1 staining indicates no increase in inflammatory response compared to commercial Gelfoam matrix	RT-97 staining observed in both PNIPAAm-PEG and PNIPAAm-MC 2 weeks post-implantation CGRP staining indicates that axons are grown from the dorsal root into the lesion	PNIPAAm-PEG is non-degradable. PNIPAAm-MC's degradability was not tested		Conova *et al.* (2011)[8]
Glial Cells	0.5%/0.5% w/v HA-MC hydrogels modified with RGD peptide and PDGF-A	hiPSC-OPC	1 week after clip compression at T2	8 weeks	The HAMC-RGD/PDGF-A group showed significant reduction in cystic cavity compared to the cell transplantation only group and control Limited glial scarring observed in the HAMC-RGD/PDGF-A group	NF^+ axons were observed to be able to extend processes into the graft		Non-significant improvement over the control group throughout 9 weeks post-injury (except week 8)	Führmann *et al.* (2016)[10]
Acellular	Fibrin/fibronectin (FB/FN) hydrogel		T7–T9 hemisection	4 weeks	FB and FB/FN hydrogels generally integrated to the spinal cord with little to no cavities, while the FN hydrogel showed large cavities at the tissue–implant interface 4 weeks post-surgery	Significantly higher $N52^+$ signal at 4 weeks for FB/FN (∼12%) compared to FB (∼7%), but non-significant compared to FN (∼10%)			King *et al.* (2010)[119]
	HA-MC hydrogel incorporating erythropoietin		A disk with 2 mm opening was fixed over the burr hole with bone glue. Hydrogels were injected into the hole such that the gel is in direct contact with the brain cortical surface	11 days	Significant reduction in the $GFAP^+$ signal in the ipsilateral cortex ventral to the lesion site in the HAMC group at both 4 d and 11 d post-injury compared to the non-treated group	HA-MC incorporating erythropoietin group significantly increased the number of DCX+ positive cells compared with the no treatment or HAMC group at 4d and 11d post implantation (2-fold increase) HA-MC incorporating erythropoietin enhances the number of NeuN+	PNIPAAM is non-degradable		Wang *et al.* (2012)[129]

Table 2 (*continued*)

	Injectable polymers	Cell source	Implantation site	Implant duration	Tissue integration and cell migration	Axonal regrowth	Scaffold degradation	Functional recovery	Ref.
						mature neurons in the injured area by more than 4-fold at day 4 and more than 10-fold at day 11			
	Free GDNF loaded or GDNF microsphere loaded alginate/fibrinogen hydrogel		T9-T10 hemisection	12 weeks		NF^+ signal was significantly higher in the free GDNF group compared to the microsphere-loaded GDNF group and non-treated group 6 weeks post-surgery NF^+ signal was significantly higher in the free GDNF group compared to the non-treated group but non-significant against the microsphere-loaded group 3 months post-surgery $GAP43^+$ signal was significantly higher in the microsphere-loaded group compared to the free GDNF group and non-treated group both 6 weeks and 3 months post-surgery		The free GDNF group achieves superior results in hindlimb paw angle and left hind paw intensity using Catwalk™ compared to the microsphere-loaded group and non-treated group	Ansorena *et al.* (2013)[56]
	VEGF loaded alginate/fibrinogen hydrogel		T9–10 hemisection	4 weeks	Significant increase in endothelial cell infiltration ($RECA^+$) in hydrogels loaded with VEGF 4 weeks post-surgery	Significantly higher neural cells (βIII $tubulin^+$) in the VEGF loaded hydrogel group (∼16%) compared to the control group (∼11%) but non-significant against the hydrogel only group (∼13%) 4 weeks post-surgery Significantly higher neurite growth ($GAP43^+$) in the VEGF loaded hydrogel group (∼14%) against hydrogel			des Rieux *et al.* (2014)[123]

Table 2 (*continued*)

Injectable polymers	Cell source	Implantation site	Implant duration	Tissue integration and cell migration	Axonal regrowth	Scaffold degradation	Functional recovery	Ref.
					only (~9%) and control groups (~3%)			
SAP hydrogel made of $K_2(QL)_6K_2$ (QL6)		1 day after T6-7 clip injury	8 weeks	Significant reduction in both $GFAP^+$ and IBA-1^+ signals in the lesion are in the QL6 group compared to control 8 weeks post-injury	BDA retrograde labeling showed significant increment in the density of the BDA-labeled fibers in the QL6 group compared to the control group 8 weeks post-SCI at 2 mm, 4 mm and 8 mm rostral to the lesion center	The scaffold was found to be almost fully degraded *in vivo* at 8 weeks	Significant overall improvement in the BBB locomotor score over the control group	Liu *et al.* (2013)[120]
HA based hydrogel modified with anti-Nogo receptor antibody and poly-L-lysine incorporated VEGF and Ang1 implantation		1.5 mm × 1.5 mm × 1.0 mm cortical cavity	10 weeks	Significant reduction in IBA-1^+ and $GFAP^+$ 2 weeks post-injection compared to the non-treatment group			After implantation of the HA hydrogel together with PLGA microspheres, a significant improvement in forelimb sensorimotor functional test was observed at 6 weeks compared to the HA hydrogel only group	Ju *et al.* (2014)[101]
HA-MC hydrogels with cyclosporine-A		Brain's cortical surface	2 weeks		HA-MC treatment with cyclosporine-A significantly enhanced the number of proliferating cells ($Ki67^+$), with a 1.32-fold increase compared to untreated group and a 1.11-fold increase compared to HA-MC only group after 7-day post-implantation. However, DCX staining showed non-significance among groups			Tuladhar *et al.* (2015)[11]
Single walled carbon nanotube-functionalised poly(*N*-isopropylacrylamide) (SWNT-PNIPAAM)		C7 1 mm × 1 mm × 1 mm incision	8 weeks	Migrated neuron observed in the injury site with reduction in cyst size and tissue scarring compared	β III-tubulin staining showed infiltration of axons into the scaffold in SWNT-PNIPAAM group			Sang *et al.* (2016)[133]

Table 2 (*continued*)

	Injectable polymers	Cell source	Implantation site	Implant duration	Tissue integration and cell migration	Axonal regrowth	Scaffold degradation	Functional recovery	Ref.
					to the PNIPAAM only group and non-treated sham group				
	Porcine-derived UBM-ECM hydrogel		Ventral posterior region of the cavity	12 weeks		Infiltration of DCX^+ cells is evident in the peri-infarct area, as well as within the degrading ECM hydrogel 1 day post-injury Neovascularization (RECA) was observed in 3 mg mL^{-1} and 4 mg mL^{-1} hydrogel groups, but not in the 8 mg mL^{-1} group	Decrease of approximately 32% of ECM hydrogel volume over 12 weeks post-implantation and the lesion volume was reduced by 28%	Non-significant functional recovery	Ghuman *et al.* (2017)[124]
	Imidazole-poly(organophosphazenes) (I-5) hydrogel		T10-11 1 week after 200 kDyns contusion	8 weeks	I-5 injection resulted in an almost complete disappearance of cystic cavity 4 weeks post-injection, while an increase in cystic cavity size was observed in the control group	A substantial number of NF^+ axons observed in the matrix. Significant increase in 5-HT^+ axons 8 weeks post-surgerySignificant increment in Glut-1^+, DCX^+ and $Ki67^+$ in the peri-infract area Significant improvement in MBP^+, $MAP2^+$, and NF^+ signals in the aFGF-HP group 28 days post-surgery. Anterograde BDA staining 5 mm caudal to the injury suggested significant axonal regeneration into the lesion in the aFGF-HP group 28 days post-surgery	Rapid degradation of hydrogel *in vitro* with 7 days	Significant improvement in the BBB score at 6, 7 and 8 weeks post-surgery	Hong *et al.* (2017)[122]
	HA-based microporous annealed particle (MAP) hydrogel		Stereotaxic coordinates 0.26 mm AP, 3 mm ML and 1 mm DV	10 days	Significant reduction in both $GFAP^+$ and IBA-1^+ in the MAP group				Nih *et al.* (2017)[121]
	aFGF-loaded heparin-poloxamer hydrogel		T9 after moderate crushing injury	4 weeks	Significant reduction in both			Significant improvement in	Wang *et al.* (2017)[130]

Table 2 (*continued*)

Injectable polymers	Cell source	Implantation site	Implant duration	Tissue integration and cell migration	Axonal regrowth	Scaffold degradation	Functional recovery	Ref.
		using a vascular clip		glial scar thickness and glial scar volume in the aFGF-HP group 28 days post-surgery.			the BBB score and inclined plane core for the aFGF-HP group at 3, 7, 14 and 28 days post-surgery.	
Decellularized optimized acellular nerve-derived hydrogel (iOA)		C3/4 after 200 kDynes contusion	8 weeks		Significant increase in %NF^+ coverage at the distal area in the iOA group compared to control 8 weeks post-surgery, but non-significant throughout the lesion	Rapid degradation of hydrogel	Non-significant functional outcome	Cornelison *et al.* (2018)[134]
Glycosaminoglycan-based hybrid hydrogel encapsulating SDF-1α and bFGF		2 mm lateral to the midline of a 3 mm by 5 mm craniotomy window and 1.5 mm anterior to the Bregma	5 weeks		DCX^+ signal increased 4-fold and $Ki767^+$ signal increased 3-fold 21 days post-implantation		A significant recovery of forelimb movements could be seen clearly in the hydrogel group throughout 4 weeks post-implantation compared to the PBS group	Jian *et al.* (2018)[13]
Porcine-derived urinary bladder matrix (UBM)-ECM hydrogel		Ventral posterior region of the cavity	90 days		Significant increment in $NeuN^+$ cells in 3 mg mL^{-1} and 4 mg mL^{-1} hydrogel groups compared to the 8 mg mL^{-1} hydrogel group	Approximately 80% of the low-strength ECM hydrogel was degraded within 14 days of implantation and the density of nerve cells in ischemia cavity gradually increased, whereas only 32% of the high-intensity ECM hydrogel resorbed by 90 days		Ghuman *et al.* (2018)[135]

Ang1, Angiogenesis marker; Bcl2, Antiapoptotic marker; GFAP, Astrocyte marker; GAD, Axons originating from GABAergic interneurons; Glut1, Brain endothelial cell marker; RIP, Early oligodendrocyte marker; BrdU; RECA, Endothelial cell marker; GAP43, Growth neurite marker; DCX, Immature neuron marker; MAP2; N52; NeuN; NF; RT-97, Mature neuron marker; IBA-1, Microglial marker; SMA, Muscular artery marker; MBP, Myelin protein marker; Tuj-1; β III-tubulin, Neuron-specific marker; Sox2, Neural stem cell marker; GalC; NG2; Olig2, Oligodendrocyte marker; Ki67, Proliferating cell marker; CGRP, Sensory axon marker; 5-HT, Serotonergic neuron marker.

3.7. Scaffold degradation

The degradation rate of an injectable scaffold plays an important role in tissue regeneration. Fast scaffold degradation potentially leads to implant failure as the void space suppresses the formation of mechanically competent tissues to support the growth and development of newly formed cells and tissues.[131,132] In contrast, slow scaffold degradation impedes the growth of new axons and tissues.[6] With the implantation time being prolonged, an excessively large volume of hydrogel will reduce the invasion of migrating cells and the potential recovery could be sub-optimal. One important consideration for hydrogel degradation is to anticipate the rate of degradation with respect to axonal regrowth.

From Table 2, the non-degradable poly(*N*-isopropylacrylamide) (PNIPAAm) scaffold could provide permanent support for regeneration,[8,133] but it could potentially prevent tissue regrowth. On the other hand, the rapid degradation of the decellularized optimized acellular nerve-derived hydrogel (iOA)[134] resulted in thick and dense GFAP at the scaffold–tissue interface. We believe that the lack of overall neurite ingrowth and functional recovery could be due to insufficient early mechanical support. In the early stage of hydrogel scaffold implantation, there will be continuous cell migration, including macrophages, NSPCs, astrocytes and OPC, where the migration of macrophages accelerates the biodegradation of the scaffold.[135] Loss of mechanical support leads to failure in the cell–ECM interaction and the following signal transduction to promote cell development and growth, hence the decline in the potential regrowth of native tissues to serve as the structural support to continue assisting in cell and tissue regeneration. In this regard, Cornelison *et al.*[134] suggested a duration that is required for the onset of axon regeneration, which is typically between 4 and 6 weeks post-injury. However, we observed robust NF infiltration into fiber–hydrogel scaffolds as early as one week post-injury.[136] Significant improvement in axonal regrowth was also found in other studies with the hydrogel degradation rate being faster than this proposed period.[122,135] These observations could be attributed to the hydrogel materials that were used and the corresponding affinity of cells towards these materials, which in turn facilitated cell–matrix interactions to promote tissue regrowth. Along this line, studies have also shown that hydrogel degradation plays a significant role in influencing cell behavior and differentiation.[137] For example, MSC spreading and survival have been shown to be directly correlated to the hydrogel degradation rate.[138] Therefore, the degradation rate of hydrogels should be tailored to the hydrogel materials used and controlled to promote cell survival and differentiation behavior.

3.8. Functional recovery

One of the ultimate goals of CNS treatment is to restore both sensory and motor functional capabilities. Axonal regeneration has been shown to be closely related to functional recovery in CNS diseases. Angiogenesis and recruitment of cells for neurogenesis to the injured areas are equally crucial.[1,83,139,140] While axonal regrowth into the lesion is encouraging, functional recovery requires reconnection of neurons between rostral and caudal sides.[123] From Table 2, the discoveries from various research groups seem to suggest that significant axonal regrowth does not always equate to functional recovery. For example, HA-based hydrogels were observed to promote axonal regrowth in the previous sections.[10,129] However, not all studies showed the expected functional recovery. One possible reason could be the differentiation nature of transplanted cells. Führmann *et al.*[10] suggested that only a portion of transplanted cells would differentiate into mature and functional oligodendrocytes and myelinated axons. Payne *et al.*[82] compared the differentiation stages of NSPCs in transplantation and found out that late-differentiated cells were less effective in promoting functional recovery. Perhaps the maturity of transplanted cells should also be an important consideration, given that matured cells are more sensitive to the injection transplantation process. In addition, the type of transplanted cell also plays a role in either supporting the growth of endogenous tissues or integrating with the host tissues.[141] Embryonic stem cells (ESCs) are known to be capable of differentiating into nearly all cell phenotypes. Advances in genetic modification allow directed differentiation of ESCs to the desired cell types, such as neurons and oligodendrocytes for CNS repair.[142] MSCs are used in cell transplantation in the CNS for both neuroprotection and neuroregeneration strategies, where cells are used to either protect parenchymal cells in the lesion or promote axonal regeneration and sprouting. MSCs are able to secrete pro-survival growth factors (*e.g.* BDNF, VEGF, FGF2, *etc.*) and have the potential to be differentiated into neurons.[143] The use of iPSCs has gained popularity in recent years due to the ability to reprogram to xeno-free and genetically stable cells to potentiate recovery in the CNS such as neuroprotection, modulation and regeneration. The ability to be derived into different neural lineages, for example, neural network reconstruction by iPSC-derived neural cells, axon remyelination by oligodendrocytes, and neurotrophic factors secreted by neural cells, shows promising therapeutic effects for cell transplantation.[144]

As a result of CNS injury, trophic factors are downregulated.[145] Many groups have incorporated growth and trophic factors into hydrogels to help stimulate neural regeneration and growth. Both Ansorena *et al.*[56] and des Rieux *et al.*[123] studied growth factor-loaded alginate/fibrinogen hydrogels, but the latter were unable to achieve improved functional recovery. This could be due to the insufficient amount of growth factors incorporated into the hydrogels to support axonal growth. Lutton *et al.*[146] also suggested that the sole effect of VEGF might not be sufficient for modulating regeneration in the complex CNS environment and would require a combination of effects from other growth or trophic factors. Scaffolds that promoted decent animal behaviour outcomes typically had a degradation rate of no less than 4 weeks and were almost fully degraded at about 8 weeks post-injury. The fast rate of hydrogel degradation might be a possible reason for poor functional recovery in some studies as fast degradation of hydrogels failed to provide sufficient support for growing axons.[134]

There are many factors contributing to functional recovery after CNS injuries. Although studies show conflicting findings on the correlation between axonal regeneration and functional recovery, reconnection of the neural network is still important as neural signal conduction is crucial for recovery of movements. Axonal regeneration is affected by the hydrogel material used, the maturity and types of transplanted cells, the involvement of hydrogel modification and growth factors, and hydrogel degradation. Recent studies have also focused on the involvement of glial cells in axonal regeneration and functional recovery. For example, studies have suggested that astrocyte recruitment to the lesion site may have a permissive role in axonal growth instead of only forming an inhibitory barrier[147,148] and demonstrated the ability for inflammation regulation and minimizing cellular degeneration.[149,150] OPC proliferation, oligodendrocyte differentiation and maturation are also important to promote axonal remyelination.[151]

4. Summary and suggestions for future works

The treatments for CNS diseases are limited. The prognosis of patients is often poor and this could seriously reduce their quality of life. Stem cell transplantation has registered several clinical studies by protecting and repairing CNS injuries. The transplanted stem cells can aid in repairing damaged nerves and also play a beneficial role through immune regulation or regulation of endogenous regeneration. However, cell transplantation faces minimal success at clinical stage mainly to due poor survivability and poor tissue integration of transplanted cells.[17]

From the studies in the recent years, HA-based hydrogels seem to be more popular options in CNS disease treatments. Since the first report of the study involving HA coupled with methyl cellulose,[152] hyaluronan-based hydrogels have been widely used in the animal models for the treatments of CNS diseases owing to its injectability and thermoresponsiveness. HA also helps to attenuate inflammatory response and promote endogenous axonal regrowth in the body.[11] Nevertheless, regardless of hydrogel materials, recent CNS disease treatments seem to be focusing on eliciting endogenous neurogenesis, probably due to the concerns in immune response due to exogenous cell transplantation and the possibility of teratoma formation. Although exogeneous cell incorporation in injectable hydrogels has become an increasingly popular option, many studies only focused on the potential and differentiation outcomes of cell encapsulation within injectable hydrogels *in vitro*, but did not proceed with cell transplantation *in vivo* and only focused on the effects of acellular hydrogels and encapsulated molecules on tissue regeneration. It is difficult to determine the best scaffold material with the most effective therapeutic outcomes. For example, certain materials are more effective in promoting cell survival (*e.g.* natural materials) and certain materials promote cell differentiation (*e.g.* SAPs). ECM modifications and biomolecule incorporation are also effective in enhancing the therapeutic effects of injectable hydrogels. The choice of hydrogel materials could, therefore, be based on the desired outcome of the studies. We are hopeful that the information provided in this review could offer insights that would be useful for the future design of injectable hydrogels.

To ensure that research on injectable hydrogels is clinically relevant, functional recovery should be the focus of the studies. This is because the end goal for any treatment options is to improve and restore the functional capabilities of patients.[153] The CNS functions are so diverse and complex that it is not possible to only use a single assessment method to cover all aspects of functions. This includes sensory, locomotor, and cognitive functions such as learning, memory and problem solving.[154] Sensory recovery and feedback are important in controlling motor functions, while motor recovery is responsible for initiating muscle fibers to achieve limb movements.[155] Common sensory tests include Von Frey Hair (VFH) microfilament tactile sensory test[156] and heat and cold sensation tests.[157,158] Locomotor tests, on the other hand, include Basso, Beattie and Bresnahan (BBB) scale,[159] open field tests[160–163] and swim tests.[164] As symptoms of anxiety are common post-stroke and SCI, open field tests that make use of rodents' behavior to stay at the corners under stress or anxiety are also commonly used.[155,165] Cognition assessment is crucial for neurological functions including memory and emotions. This is especially critical after stroke as patients often suffer from cognitive impairments.[166] Some common methods to assess memory in animals include Morris water maze test (Morris, 1981),[167] Y-maze test,[168] novel object recognition/location test[169] and radial-arm test.[170] The key for choosing the appropriate functional assessment scale should depend on the surgical models used and knowing the advantages and limitations of different systems to choose the most appropriate assessment methods.

Instead of discovering new material combinations for CNS disease treatment, the focus of future research may shift more from the development of new materials to understanding the mechanisms behind the tissue response towards the hydrogels and axon reconnection leading to functional recovery after hydrogel implantation. While animal models are essential to examine the effectiveness of injectable hydrogels *in vivo*, alternatives, such as *ex vivo* or organ-on-a-chip models, may be useful platforms to assess the effectiveness of hydrogel materials. *Ex vivo* models, organotypic slice culture (OSC), for example, can serve as alternatives to assess the post-injury interaction of cells within the spinal cord. OSC is able to mimic the post-injury environment by recapitulating elements of glial scar. This could be useful in tackling the issues of glial scarring in spinal cord repair.[171] Neural system-on-a-chip is a scaffold-based *in vitro* system for studies of complex physiological tissue interactions on a functional and customizable substrate. The system may be useful as a platform to study the nerve injury model since it allows direct observation of the states of injury and regeneration. It also potentially allows the studies of higher order neural anatomies.[172] With these platforms, we could possibly achieve direct observation of cellular response towards

various scaffold materials within a shorter period of time and with reproducibility. This could ease the efforts of testing scaffolds *in vivo* and cut down on the costs of animal studies. The limitations of these platforms could be the inability to assess inflammatory response and interaction between the immune system and the injury lesion. Additionally, functional studies can only be carried out with *in vivo* models.

Despite some limitations in current studies on *in situ* injectable hydrogels, some groups have managed to obtain promising axonal recovery and functional improvement. This provides incentives for continuing the development of injectable hydrogels in CNS injury treatment. With increasing understanding on the physiologies of CNS, there is hope that we can engineer optimal injectable hydrogels that are useful and beneficial to clinical applications.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the National Research Foundation, Singapore, under its Intra-CREATE Thematic Grant Programme (NRF2019-THE002-0001) and the MOE Tier 1 grants (RG38/19 and RG37/20).

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