Design of Injectable Materials to Improve Stem Cell Transplantation

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Abstract Stem cell-based therapies are steadily gaining traction for regenerative medicine approaches to treating disease and injury throughout the body. While a significant body of work has shown success in preclinical studies, results often fail to translate in clinical settings. One potential cause is the massive transplanted cell death that occurs post-injection, preventing functional integration with host tissue. Therefore, current research is focusing on developing injectable hydrogel materials to protect cells during delivery and to stimulate endogenous regeneration through interactions of transplanted cells and host tissue. This review explores the design of targeted injectable hydrogel systems for improving the therapeutic potential of stem cells across a variety of tissue engineering applications with a focus on hydrogel materials that have progressed to the stage of preclinical testing.

Keywords Injectable hydrogels · Stem cells · Regenerative medicine · Cell survival · Functional recovery

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Introduction

One of the largest challenges facing stem cell-derived therapies is the survival and engraftment of transplanted cells into host tissue. In general, only approximately 1–20 % of transplanted cells survive, significantly limiting their therapeutic potential [1–5]. While many stem cell sources (adult, embryonic, induced pluripotent) are being investigated for regenerative applications, all share universal challenges that occur at each stage of transplantation. It is useful to divide an injectable hydrogel therapy into the following phases: pre-injection, injection, acute post-injection, and long-term survival and function, in order to identify the distinct challenges and potential solutions to maintaining cell survival (Table 1). While hydrogel design strategies can help mitigate the challenges of each phase of transplantation, no single material currently exists that addresses all of these issues simultaneously.

In the first section of this review, we will describe each of the transplant phases individually with a focus on which hydrogel design approaches can be used to promote cell viability during that specific phase. The relative priority ranking of the various cell survival challenges will be different for each clinical indication and site of transplantation. No single hydrogel formulation will be optimal for all stem cell transplantation therapies. In order to select an appropriate injectable material for a specific application, one must first carefully assess and prioritize the specific challenges and requirements for that particular tissue. Thus, in the second section of this review, we will focus on applying the hydrogel design strategies discussed in the first section to develop therapies for specific tissue applications, with an emphasis on hydrogels that have been evaluated in preclinical models.

Table 1 Challenges that reduce stem cell survival during transplantation and biomaterials strategies to address these challenges

Transplant phases	Challenges to stem cell survival	Hydrogel design approaches	References
Pre-injection	Prolonged exposure to cell encapsulation reagents	Cell-compatible chemical crosslinking Dual barrel syringe	[6–8]
	Prolonged exposure to non-physiological encapsulation conditions	Cell-compatible physical crosslinking Dual barrel syringe	[6–8, 9••, 10••, 11]
Injection	Cell damage during injection	Plug flow injectable hydrogels Microbead/capsule vehicles	[9••, 10••, 11–17, 18•, 19–24]
Acute post-injection	Cell dispersal from target area	Rapid gelation Rapid self-healing	[25–32]
	Anoikis	Ligand presentation from synthetic matrices Decellularized matrices	[4, 25, 33–43]
	Hypoxia	Oxygen delivery Growth factor delivery Gene delivery	[44–50]
	Poor nutrient transport	Macroporous hydrogels Adaptable hydrogels Hydrogel degradation Pro-angiogenic materials Pre-vascularized materials	[51–53, 54•, 55, 56]
	Immune response	Immunomodulation	[57–64]
Long-term survival and function	Limited cell migration	Macroporous hydrogels Ligand presentation Adaptable hydrogels Hydrogel degradation	[41, 53, 54•, 55, 56, 65, 66•, 67, 68]
	Reduced/inappropriate cell secretome	Mechanical properties Growth factor delivery Gene delivery	[69–72]
	Poor cell differentiation	Mechanical properties Growth factor delivery Gene delivery Ligand presentation	[55, 56, 66•, 67–69, 72–74]

General Hydrogel Design Approaches to Address Cell Viability Challenges at Each Stage of Transplantation

Pre-injection Phase

While most hydrogel designs have focused on cell-material interactions that occur after injection, recently, more studies have begun to also consider how cells interact with the material prior to and during the injection process. Even the choice of the hydrogel crosslinking mechanism, chemical or physical, can influence cell survival in the syringe pre-injection. For chemical gels, crosslinking involves the formation of irreversible, covalent bonds between polymer chains using chemical crosslinking reagents. Physical hydrogels, on the other hand, form through temporary, reversible associations between chains, including hydrogen and ionic bonds [75–78]. Both crosslinking methods can be potentially detrimental to cell survival. For example, many chemical crosslinking reactions often have cross-reactivity with biomolecules presented on the cell surface or utilize reagents that have some degree of cytotoxicity prior to crosslinking. For physically crosslinked hydrogels, frequently an external trigger is used to induce gelation (e.g., changing solvent pH or ionic strength), which exposes cells to non-physiological conditions [79]. As long-term exposure to these crosslinking mechanisms can decrease cell viability, one way to overcome this concern is the use of dual barrel syringes to isolate cells from the potentially cytotoxic component until immediately before delivery [6–8, 80, 81]. Unfortunately, these crosslinking strategies can be difficult to reproducibly control in a clinical setting, as each injection site has its own local microenvironment that can impact crosslinking kinetics. Current focus has shifted to a new strategy in which cells are pre-encapsulated in injectable hydrogels that are already in the gel phase ex vivo. Such hydrogels contain reversible crosslinks, including the use of dynamic covalent bonds or supramolecular assembly, that allow them to shear thin upon application of shear force to enable

injection of the gel through syringe needles and catheters [9••, 12–14, 82].

Injection Phase

Studies suggest that cells exposed to syringe needle flow experience mechanical shear and extensional forces that can damage the cell membrane. Interestingly, this effect is exacerbated when cells are injected in low viscosity solutions such as saline or cell culture medium [15, 83]. Recent work has demonstrated that some injectable hydrogels can protect cells from membrane damage; however, this effect seems to be limited to weak gels only (<100 Pa), although the exact rheological requirements are still unknown [9••, 10••, 11, 16, 17, 83–85]. Data suggests that mechanical protection is likely due to hydrogel "plug flow," where shear-thinning at the walls forms a lubricating layer that enables the rest of the gel and encapsulated cells to slip through the needle relatively undeformed [12, 15, 18•, 19, 85]. An alternative strategy is the encapsulation of individual cells (or small clusters of cells) into microbeads that are able to protect cells from the extensional forces that can damage cell membranes [20–24].

Acute Post-injection Phase

Achieving cell retention at the target site, and thus therapeutic efficacy, requires rapid gelation of the pre-polymer solution or rapid self-healing of an injectable gel. If the gelation is too slow, the cells can become dispersed. The gelation kinetics must be carefully tuned, though, because if gelation is too rapid, it can clog the needle [4, 25, 33, 34, 79]. A variety of strategies have been developed for different crosslinking mechanisms. For example, for supramolecular gels such as MAX peptide gels, the kinetics of assembly can be tuned by altering the association energy of the self-assembling components [26, 27]. For ionic crosslinking, an interesting strategy is the controlled release of the crosslinking reagent, Ca^{2+}, into alginate [28]. Finally, external triggers, such as light-activated crosslinking of diacrylate and methacrylate-modified hydrogels, can also be used to initiate the crosslinking process and maintain cell retention in the target area [29–32].

In the acute post-injection phase, transplanted cells can be confronted with a host of survival challenges including hypoxia, lack of nutrients, and lack of a three-dimensional supporting matrix. Depending on the specific clinical application, the relative importance of these different challenges can vary dramatically. Thus, biomaterials designed for different potential therapies often focus on addressing different challenges. For example, transplanted cells that are delivered into ischemic tissue face the acute challenges of hypoxia and lack of nutrient delivery. To combat hypoxia, the material can be engineered to release oxygen directly [44]. Alternatively, the material can deliver factors that assist cells in surviving hypoxia, such as HIF-1α [45, 46, 86, 87]. A longer-term strategy is to design the material to promote vascularization in situ; for example, through the delivery of VEGF to induce angiogenesis from neighboring blood vessels [47–50]. While this serves the dual purpose of providing both oxygen and nutrients to the target site, the process of angiogenesis typically requires days to weeks. An alternative strategy to enhance the rate of vascularization is to deliver "pre-vascularized" biomaterials to the target site [88], although this strategy is usually incompatible with injectable material delivery. In the absence of an integrated vasculature structure, nutrient transport can still be promoted through the use of macroporous materials that contain interconnected channels to permit rapid diffusion [51–53]. For example, one recently reported system used an injectable composite of two different materials with very different biodegradation rates. As the rapidly degrading component (i.e., the sacrificial porogen) breaks down, a series of large pores are left behind. These large pores not only facilitate the diffusion of nutrients but they also were found to promote the migration of transplanted cells [54•].

Another common challenge at many transplantation sites is the lack of a healthy extracellular matrix. This is especially problematic for adherent cell types that undergo anoikis without proper matrix signaling cues. To combat this challenge, recent work has investigated the use of decellularized matrix, either purified or as complex mixtures, for cell delivery [4, 25]. Injectable materials that utilize decellularized matrix offer plenty of cell-binding sites necessary to anchor transplanted cells and prevent apoptosis. Furthermore, decellularized matrix can be harvested from the donor tissue of interest providing appropriate, tissue-specific biochemical cues necessary for transplanted cell engraftment and function [4, 25, 35, 36]. Alternatively, synthetic scaffolds can be decorated with known matrix ligands to elicit specific interactions with cell-surface receptors [37–43].

The immune and inflammatory response after tissue injury results in a harsh environment that transplanted cells must overcome to survive. In order to achieve this, materials that were once thought to be "bioinert" can be used to modulate the immune system [57]. An exciting recent advance within biomaterials design is the use of elements to stimulate or suppress the immune system. These design elements include release of soluble cytokines [58, 59], delivery of immuno-engineered cells, and presentation of immunomodulatory peptide sequences [60, 61]. A thorough discussion of this topic is beyond the scope of this review, and the interested reader is pointed to several recent excellent reviews on immunomodulatory biomaterials [49, 57, 62–64].

Long-Term Survival and Function Phase

Transplanted stem cells have different mechanisms through which they can have therapeutic effects. For some clinical

applications, stem cells are thought to directly participate in the formation and function of new tissue. In other applications, the primary therapeutic function is thought to be indirect through the secretion of pro-regenerative factors, which act on the host tissue. Thus, depending on the underlying therapeutic mechanism, the biomaterial can be designed to enhance the cellular function. A variety of biomaterial properties are known to influence cell differentiation and maturation including mechanical properties such as stiffness and stress relaxation rate [65, 66•, 73, 89], presentation of biochemical ligands [90–92], delivery of soluble factors or morphogens [93, 94], and material degradation [55, 56, 67, 68]. Each combination of stem cell type and tissue type will likely require different optimal biomaterial properties to influence differentiation, and the underlying mechanisms governing these interactions are only just beginning to be elucidated. Much of the work to date on biomaterials-guided differentiation has focused on studies of bone marrow-derived stem cells in an in vitro setting, and it remains to be seen if these insights hold true for other cell types and if they can be translated to in vivo applications. Even less work has been done to identify which biomaterials cues influence the stem cell secretome, although early work is promising [69–71]. For example, in vitro studies suggest that biomaterial mechanical properties can modulate the pro-angiogenic secretome of mesenchymal stem cells [71].

Developing mechanistic causal relationships between biomaterial parameters and stem cell function (either differentiation or secretion) is challenging for two main reasons. First, stem cells are simultaneously receiving multiple input signals from the matrix where the signal transduction pathways that propagate and amplify these signals have many points of crosstalk, resulting in non-linear relationships. For example, cells may have a different sensitivity to a range of material stiffness depending on the density of biochemical ligands that is presented [66•]. Second, manipulating one biomaterial property often has the unintended consequence of also changing several other biomaterial properties. For example, a common technique to increase biomaterial stiffness is to increase the crosslinking density, but this is usually accompanied by a decrease in biodegradation rate and a decrease in the diffusion rate of paracrine secreted signals [67, 68, 72]. Thus, studies in the area of biomaterials-guided differentiation and secretion require careful design to tease apart the intersecting mechanistic relationships. This area of research is likely to continue to expand for the next several decades.

Current research aims to address these issues, but there is no one hydrogel formula that is able solve all of the challenges stem cells face during the transplantation process. A single material property has the ability to impact several different challenges. For example, different hydrogel mechanical properties may be appropriate for different phases of the transplantation process (Fig. 1). While a weak hydrogel may be optimal for shielding cells from forces exerted during injection, the

mechanics may prove insufficient for long-term cell retention and function. Furthermore, these properties are highly dependent on specific applications, and thus potential materials must be tunable in order to be optimized for a given therapy. In the next section, we will highlight injectable hydrogel design strategies based on *tissue-specific* needs and applications. In particular, we will place an emphasis on those materials evaluated in preclinical models.

Specific Hydrogel Design Choices for Specific Tissue Applications

Cardiovascular Stem Cell Transplantation Therapies

Stem cell therapies have been studied extensively in cardiovascular applications such as myocardial infarction (MI) and peripheral arterial disease (PAD) [95]. Researchers have attempted to offset the irreversible cell death from ischemia that occurs in the myocardium during MI or endothelium in PAD through the introduction of stem cells into the injury site in hopes of replacing lost cells and/or encouraging native tissue remodeling through the secretion of regenerative growth factors [96–98].

The cardiac tissue environment includes several cell types including cardiomyocytes, pacemaking cells, fibroblasts, and endothelial cells, as well as extracellular matrix (ECM) proteins such as collagen, fibronectin, hyaluronic acid, and proteoglycans [99]. Collagen, the most common component of cardiac ECM, forms fibrils that contribute to the mechanical properties of the heart with an approximate physiological stiffness of ~10–20 kPa [74]. While it is unclear if an optimal injectable material would have mechanical properties that match this physiological stiffness or would be weaker or stiffer, it is clear that cells sense and respond to matrix material properties. For example, functional output of embryonic and neonatal cardiomyocytes (CMs) or hiPSC-derived CMs in vitro depends heavily on substrate mechanical stiffness, with increased electrical output and contractile beating observed on 8–14 kPa substrates [100, 101]. Thus, any material used to improve stem cell-derived therapies for cardiovascular tissue must be designed with these mechanical properties taken into consideration.

Alginate has been used as an injectable, naturally occurring biomaterial to deliver stem cells for cardiac tissue regeneration [102, 103]. Alginate is a polysaccharide from seaweed that crosslinks when exposed to calcium ions, making it an ideal injectable material as gel formation will not occur until it comes into contact with physiological calcium. This would prevent clogging in the long catheters used in cardiac injection methods. Since alginate is a non-fouling and non-adhesive biomaterial, functionalization with cell-adhesive domains must take place to encourage cell attachment and matrix
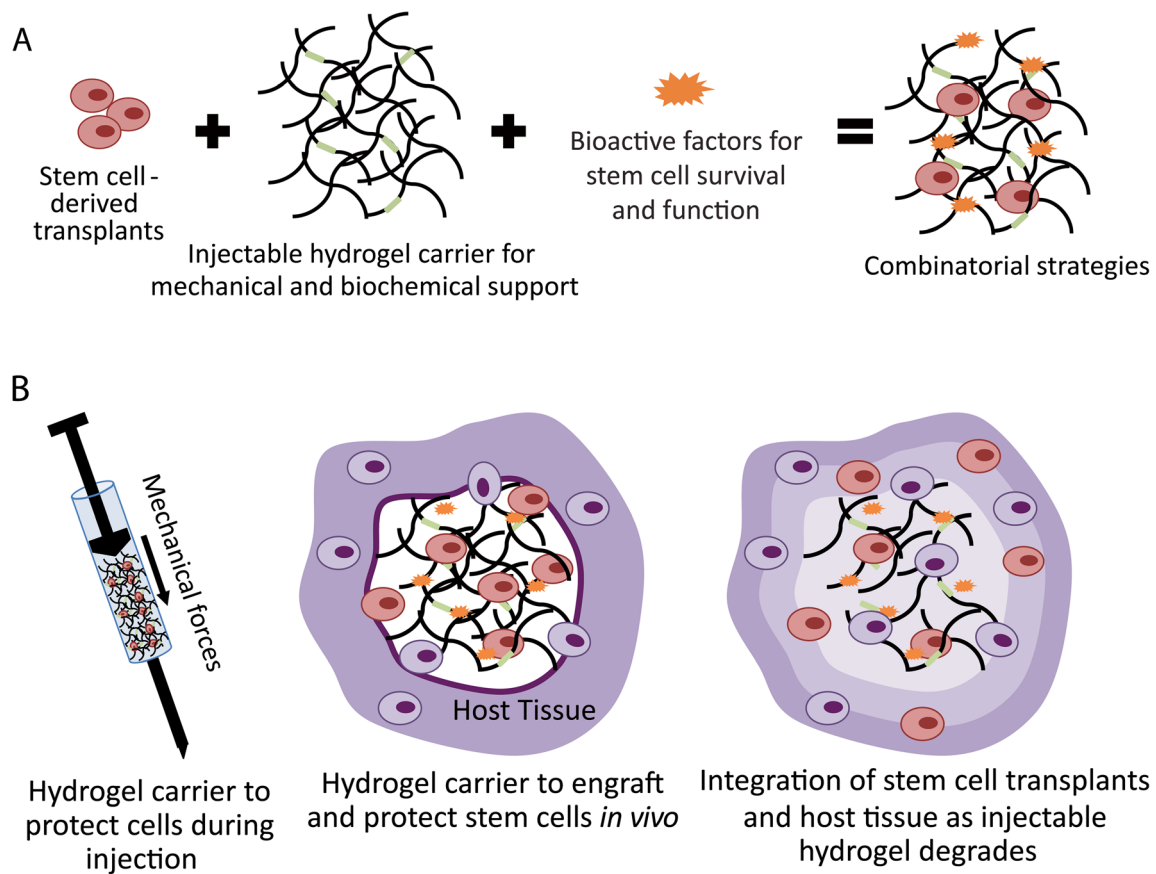
Fig. 1 Design of injectable hydrogel delivery platforms for improved stem cell-derived therapeutics. **a** Combinatorial regenerative medicine strategies often include encapsulation of stem cell-derived transplants within injectable hydrogels designed to provide cell appropriate mechanical support and biochemical cues along with co-encapsulation of bioactive factors. **b** The design of injectable hydrogels must consider four separate phases of hydrogel use. In the first and second, some injectable hydrogels can protect cells during the potentially harmful pre-injection and injection processes, which exposes cells to a variety of crosslinking mechanisms and mechanical forces. Third, some injectable hydrogels can improve acute cell survival and functionality by providing appropriate mechanical and biochemical matrix cues along with soluble bioactive factors. Fourth, carefully developed injectable materials can promote grafted cell function within host tissue as it degrades.

remodeling. Alginate, modified with the cell-binding ligand RGD found in collagen and fibronectin, has also improved human mesenchymal stem cell (hMSC) retention from 9 % in saline controls to 60 % in a direct injection model [104]. Further studies have shown that the concentration of alginate, and thus mechanical stiffness, also plays a significant role in cell retention. MSCs transplanted in a 2 % alginate solution (~2 kPa) were found to be retained and survive 4× higher than that of cells transplanted with saline or even 1 % alginate (~700 Pa) [105•].

Whereas alginate crosslinks in the presence of Ca^{2+}, other materials can form hydrogels at physiological temperatures making them potential injectable hydrogel cell carriers. The study by Roche et al. also examined the use of a thermosensitive, injectable chitosan/β-glycerophosphate (β-GP) gel as the hMSC vehicle and found cell retention also significantly improved to 50 %. Chitosan has also been used as a cell carrier for brown adipose derived stem cells (ASCs) in myocardial repair, with a reported 70 % increase in cell retention as well as improved angiogenesis and preserved

heart function [104]. Unfortunately, chitosan/β-GP and other thermosensitive hydrogels may prove difficult for use in non-direct injection methods that make use of catheters. While catheter delivery of transplanted cells is less invasive than direct injection, it requires the cell/gel mixture to travel a long distance through the body, which can potentially result in early gelation and failure to inject into the damaged tissue [106]. Gelfoam, an FDA-approved, gelatin-based gel, has also been shown to successfully transplant MSCs as a heat-sensitive injectable material [107]. As an alternative to naturally occurring biomaterials, Xia et al. designed a synthetic, injectable, thermosensitive copolymer composed of poly *N*-isopropylacrylamide (PNIPAM)/acrylic acid/2-hydroxylethyl methacrylate-poly(ε-caprolactone) and functionalized with collagen I to deliver hMSCs to an infarcted heart. Cell retention within the heart was 4× higher with the injectable hydrogel compared to cells alone and this corresponded with increased heart function, increased angiogenesis, and decreased fibrous scarring [108]. As with other injectable, thermosensitive composites, the copolymer suffers from the

potential to gel in catheters, but may prove a more useful tool for direct myocardial injection therapies. Another interesting synthetic injectable material is self-assembling nanofibers, which have been used in mini-pig models to improve bone marrow-mononuclear cell retention 10-fold in treating MI and improving both diastolic and systolic functional outcomes [30].

In treating PAD, transplanted MSCs have been used to produce pro-angiogenic factors needed for regeneration and are more commonly delivered systemically [109]. However, embryonic derived stem cell (ESC) and induced pluripotent derived stem cell (iPSC)-derived endothelial cells (EC) have been used to improve endothelialization and vascular regeneration of the occluded arteries in ischemic tissue through intramuscular injection [110–112]. Unfortunately, cell survival after intramuscular transplantation is poor due to the immunoreactive, ischemic, and necrotic host environment. The Heilshorn group showed the use of a weak, shear-thinning, protein-based hydrogel with cell-adhesive domains improved iPSC-EC viability during the injection process by protecting the cells from mechanical forces within the needle [9••]. Furthermore, incorporation of vascular endothelial growth factor (VEGF) into the hydrogel cell carrier improved muscle regeneration while minimizing inflammation and necrosis. Finally, collagen, which gels at body temperature, has been used to deliver BMSCs intramuscularly in a PAD model. Improved angiogenesis and hind limb perfusion was observed with an increase in local blood vessel density [113]. Xu et al. designed a synthetic, injectable hydrogel with a PNIPAM backbone that exhibited strong mechanical properties (~17 kPa) when raised to body temperature. The incorporation of the pro-survival factor basic fibroblast growth factor (bFGF) with the hydrogel improved MSC survival after intramuscular injection, as well as increased blood vessel density, limb perfusion, and muscle diameter [114].

Cartilage Stem Cell Transplantation Therapies

Cartilage degeneration occurs through the breakdown of the connective tissue that covers bones at joints, particularly in joints at the knees, elbows, and spine. This can result from diseases such as osteoarthritis, mechanical wear, crystal formation from gout, diabetes, and rheumatoid arthritis [115]. Current stem cell therapies used to treat cartilage degeneration include the use of MSCs and ASCs differentiated into chondrocytes, the main cellular component of cartilage, in order to replace lost cells [116]. Unfortunately, many of these cells die within this avascular environment [117].

While chondrocytes make up the main cellular component of cartilage tissue, the main structural component is composed of ECM proteins including collagens I and II and a significant fraction of proteoglycans. Cartilage tissue must have significant mechanical properties to withstand the high forces that occur in joints as they minimize the friction between connected bones [118]. Therefore, any injectable material intended for long-term presence in the joint must also be able to withstand these mechanical forces. In addition, the ideal material would provide pro-survival cues, which might include native-like protein and proteoglycan content, in order to encourage transplanted cell survival and integration, as well as promote endogenous tissue remodeling.

Photopolymerizing hydrogels have shown promise for delivery of stem cells in cartilage regeneration work [119]. Studies have shown advances in the use of chitosan-based injectable hydrogels for improving ASC and human synovial MSC survival in articular cartilage regeneration. This group has developed a methacrylated-chitosan-based material (MeCG) that allows for injection followed by photopolymerization in situ under visible blue light. To specify this material for cartilage applications, the group modified the hydrogel by conjugating transforming growth factor-β (TGF-β) and incorporating collagen II and the proteoglycan, chondroitin sulfate (CS). This material improved chondrogenic differentiation as well as improved cartilage ECM deposition in a rat chondral defect model [120•]. This material may be an improvement upon other pure mammalian-based materials such as collagen or CS alone, which would be rapidly degraded by the body [121]. Similar visible light-crosslinking hydrogels have been investigated, in which methacrylated-gelatin hydrogels used to inject MSCs showed strong mechanical properties (~30 kPa) at physiological conditions and strong integration with native cartilage compared to cells delivered in faster degrading agarose [30]. Finally, MSCs have been delivered via a cartilage-specific, hydrogel carrier system composed of a UV-crosslinking, synthetic polymer base (poly(ethylene oxide) diacrylate) incorporating hyaluronic acid and TGF-β. This system has demonstrated successful in vitro differentiation of MSCs into chondrocytes and generation of cartilage-like tissue when injected subcutaneously and transdermally UV-crosslinked [31, 32]. The design of this system using the proteoglycan hyaluronic acid improved the viscosity of the solution, preventing dispersion of the injected MSCs and improving cartilage formation [31].

Thermosensitive hydrogels have also been utilized for increasing stem cell efficacy in cartilage repair [32]. Thermoreversable chitosan/β-GP/hydroxyethyl cellulose hydrogels were shown to support human and mouse MSC survival and proliferation, while further incorporation of TGF-β3 improved chondrogenic differentiation [122•]. Similarly, chitosan-poly(vinyl alcohol) copolymer hydrogels used to deliver MSCs showed significant regeneration of rabbit articular cartilage defects, particularly when TGF-β was introduced through MSC adenoviral overexpression [32].

These studies highlight current methods in improving the regenerative potential of stem cell-based therapies for

cartilage repair with encouraging preclinical results. Many more studies have made strides in developing promising, novel injectable hydrogel systems for cartilage applications; however, these materials have not yet progressed to preclinical experiments and have only been shown effective in in vitro models [123–126].

Nervous System Stem Cell Transplantation Therapies

Diseases and injuries to the nervous system impact a significant portion of the population with devastating implications. While much of the underlying etymology behind neurological diseases such as Parkinson's and Alzheimer's is unknown, each results in irrevocable loss of specific neuron populations. In addition, injury and ischemia to the spinal cord (SCI) and brain results in significant neural cell death, leading to substantially diminished movement and sensation as well as impaired mental and cognitive function [93, 127–129]. Stem cell-derived therapies are currently being investigated in numerous neurological-based clinical trials looking to either replace lost neuron populations or provide supporting glial cell types, such as oligodendrocytes and astrocytes [127, 130]. Unfortunately, despite promising preclinical data, no stem cell-based therapies have been able to move to the market or clinic, often due to failure to show improvement in humans and limited cell characterization [131]. The neural microenvironment post injury can be incredibly cytotoxic due to ischemia, presence of inhibitory myelin debris, and release of excitotoxic molecules [130]. Furthermore, neural cells tend to be very sensitive to handling, thus the injection process itself may be decreasing cell survival.

Therefore, in order to improve transplanted cell survival, engraftment, and integration with host tissue, researchers have designed injectable hydrogel systems that are specifically tailored to improve neural and glial cell phenotypic function within injured nervous tissue. The neural tissue environment has several unique characteristics that need to be taken into account when developing therapeutic strategies. Unlike musculoskeletal or connective tissues, neural tissues are mechanically very compliant, with stiffness ranging from 100 to 1000 Pa [73, 132, 133]. This property of the in vivo matrix appears to translate to preferred in vitro substrates. For example, stem cells cultured on softer substrates tend to differentiate down a neural lineage compared to cells on stiffer substrates, and primary neurons respond to more compliant materials by producing longer neurite extensions [38, 40, 73, 134]. Therefore, using hydrogel carriers that are significantly stiffer than native neural tissue would likely limit integration of transplanted cells due to both failure of transplanted cells to differentiate into appropriate cell types in the stiff matrix as well as failure to promote host cell penetration and remodeling. ECM proteins expressed in neural tissue are primarily laminins, collagens, and fibronectin [135]. Laminin contains two cell binding sites, IKVAV and YIGSR, that neurons have an affinity for, with IKVAV promoting significant neurite outgrowth and YISGR promoting neural cell attachment and survival [38, 136, 137]. Neurons are also dependent on several soluble signaling factors for survival and regeneration including neurotrophin-3 (NT3), glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), and platelet-derived growth factor-A (PDGF-A) [138, 139]. Many groups have therefore attempted to incorporate these factors into injectable hydrogels to improve functional recovery after disease or injury to the nervous system.

In part due to their mechanically compliant properties, naturally derived biomaterials have been historically favored in the development of injectable hydrogel carriers for neural applications. For example, fibrin hydrogels have been used to deliver ESC-derived neural progenitors for treating SCI [138, 139]. Fibrin hydrogel properties can be easily tuned to mimic native environmental properties simply by altering the concentration of fibrinogen. Fibrin delivery also improved neural progenitor cell (NPC) survival and influenced differentiation into neural phenotypes when modified with a growth factor delivery system [138]. Sustained delivery of GDNF, NT3, PDGF-A, as well as other growth factors from injectable fibrin scaffolds with mouse NPCs impacted astroglial scar formation and macrophage response, as well as improved neuronal differentiation [139]. Matrigel, another naturally derived material that is rich in laminin and collagen, has also been investigated for cell delivery, as it can be injected due to its thermal gelation property. In vitro, Matrigel is used extensively to support stem cell survival, proliferation, and differentiation, especially into neural lineages. In an ischemic stroke model, delivery of ESC-derived NPCs with Matrigel significantly improved cell survival and outcomes in sensorimotor and cognitive function [140]. Furthermore, Matrigel (growth factor reduced) improved ESC-derived NPC survival compared to artificial cerebrospinal fluid and increased dopaminergic neuron differentiation for treating Parkinson's disease [141]. The authors suggested that the use of Matrigel suppressed the normal immune response to grafted cells, thus increasing the number of dopaminergic neurons, rather than the material itself inducing differentiation. Unfortunately, Matrigel is derived from mouse sarcoma and cannot be used in clinical applications.

Hyaluronan or hyaluronic acid (HA) is commonly found in the nervous system and therefore is a promising material for delivering cells to neural tissues [142]. The Shoichet group has designed and extensively studied hyaluronic acid-methylcellulose (HAMC) hydrogels for cell delivery and have found encouraging results for SCI and stroke therapies [142, 143, 144••]. This family of hydrogels can be tuned to match native neural mechanical properties and has been shown to reduce scarring and inflammation in

SCI [145]. In addition, these hydrogels modified with recombinant PDGF-A led to increased survival of transplanted adult NPCs and improved differentiation into oligodendrocytes after SCI. This combination therapy led to increased graft survival, improved host tissue sparing, and decreased SCI pathology, which correlated with increased behavioral recovery [146]. HAMC similarly led to increased oligodendrocyte differentiation of transplanted iPSC-derived oligodendrocyte progenitor cells, but most importantly, it attenuated teratoma formation compared to cells delivered in saline alone [144••]. Degradable HA hydrogels modified with the cell-binding domain RGD have also been used to deliver iPSC-derived NPCs for treating stroke. While the hydrogels did not improve cell survival post-cerebral injection compared to cells alone, differentiation of iPSC-NPCs into doublecortin positive neuroblasts was significantly increased with HA delivery [147, 148]. Another HA variant, modified with heparin sulfate and collagen, has similarly been tested as an ESC-derived NPC delivery vehicle for ischemic stroke therapy. The use of the support matrix increased transplanted cell survival and decreased microglia response. Unfortunately, while cell survival increased 2-fold, it only increased from 300 to 600 cells out of the original 100,000 cells transplanted [149]. Lastly, HA carriers modified with poly-L-lysine (PLL) for enhanced cell attachment were shown to improve transplanted BMSC survival and differentiation in a thoracic SCI model. Compared to cells transplanted alone, cells with HA-PLL gels led to improved hind limb locomotion, a result rarely seen with MSC therapies alone in SCI [150].

Other biomaterials that are naturally derived or bio-inspired have shown promise in enhancing the therapeutic potential of stem cells for treatment of neural injury and disease. For example, the self-assembling peptide K2(QL)6K2 (QL6) has previously been shown to reduce the associated pathology observed after SCI with decreased inflammation, glial scarring, and cell death [151]. Therefore, Iwasaki et al. probed the synergistic effect of QL6 transplanted in succession with adult NPCs in a cervical compression SCI. QL6 did not statistically improve NPC survival in vivo (0.25 % for cells only versus 0.62 % for cells and QL6), yet the addition of the self-assembling peptide led to decreased microglia activation and gliosis and increased motoneuron and neuron sparring resulting in improved forelimb function [2]. It is of note that the NPCs were not embedded within the QL6 solution; instead, the treatments were injected separately, and thus it is uncertain whether the peptide would improve cell survival to greater effect if they were co-delivered. Composite scaffolds made of poly(lactic acid) nanofibers embedded in an injectable xyloglucan hydrogel improved the survival and reinnervation of dopamine progenitor cells in Parkinson's debilitated mice, with significant improvement observed when the scaffolds were optimized with GDNF and BDNF co-delivery [152].

Osteoinductive Stem Cell Transplantation Therapies

Due to our increasingly aging population, bone-related diseases and injuries are on the rise with an increasing need for bone grafting technologies. An extensive amount of research is being undertaken to replace lost bone cells and encourage native tissue regeneration using stem cell-derived technologies [153, 154]. There are currently dozens of clinical trials probing the use of stem cells, primarily MSCs, for bone regeneration applications in a variety of indications including cancer, osteonecrosis, pseudo-/osteoarthritis, fractures, periodontal disease, and spinal fusions [154, 155]. The goal of stem cell-derived therapies is to initiate new tissue remodeling and growth with cohesive integration of grafts and host tissue allowing for proper movement and function. Unfortunately, lack of nutrients from blood supply and poor mechanical support can lead to graft failure to integrate and potential morbidity [154]. In addition, while cell-seeded hydrogels have been investigated broadly for bone tissue engineering, a non-invasive, injectable cell carrier system is needed in order to deliver cells to difficult-to-reach and non-uniform injury sites.

In response, researchers are developing a variety of supporting scaffolding systems to facilitate engraftment of transplanted cells with host tissue and differentiation into osteogenic phenotypes. Key matrix properties of bone that must be considered include their protein and mineral composition and their structural and mechanical strength properties. Bone is characterized as cortical and trabecular bone, with cortical bone being dense, compact tissue and trabecular bone being spongey and porous. On the ultrastructural level, bone is composed of compacted collagen fibrils that are mineralized with hydroxyapatite ($Ca_5(PO_4)_3$) microparticles. Cortical and trabecular bone stiffness can range between 100 and 2000 MPa, with substrate stiffness in the 100 kPa range supporting enhanced osteogenic differentiation of MSCs in vitro [73, 156].

Many groups have investigated the use of PNIPAM, a thermosensitive polymer, for injectable cell delivery in bone applications. PNIPAM is a synthetic polymer that can be tuned to match the mechanical stiffness of bone once injected at body temperature [157, 158•, 159]. Watson et al. demonstrated that decorating PNIPAM with pendant phosphate groups improved biodegradation and biointegration of the hydrogel with host tissue, as well as support enhanced mineralization, MSC differentiation, and bone formation in a rat cranial defect model [159]. Further work has shown promising results with MSC delivery using PNIPAM hydrogels either grafted with gelatin or incorporating gelatin microspheres [157, 158•]. Gelatin, which is denatured collagen, is an

excellent source of natively relevant bioactive adhesion sites for both transplanted and host cells. The use of gelatin microspheres within injectable PNIPAM and encapsulated MSCs led to increased direct bone-to-hydrogel contact, cell infiltration, and osteoid formation [158•]. In addition, direct grafting of gelatin to PNIPAM increased the rate of new bone formation with extensive graft integration into the host tissue [157, 158•, 159].

Alginate has also been investigated as a stem cell carrier for bone regeneration applications. Functionalizing alginate with the RGD cell-binding domain for co-delivery of MSCs with bone morphogenetic protein-2 (BMP-2) led to increased mineralization in vitro and increased bone formation in vivo in a critically sized femoral defect model [160]. Alginate hydrogels have also been shown to support hMSC migration and osteodifferentiation when chemically modified with osteoinductive growth peptides [161]. Unfortunately, this work was only performed in vitro, and efficacy in vivo has yet to be evaluated. Indeed, there are dozens of promising new injectable hydrogel stem cell carrier technologies being investigated for bone regeneration, including Pluronic F127, chitosan/collagen/β-GP, calcium phosphate cement, and several synthetic polymers, yet they have only been tested in vitro or in non-bone defect, subcutaneous in vivo models [161–165].

Other Targets for Injectable Stem Cell Therapeutics

Recent advances in stem cell biology have opened new doors in developing therapies for less high profile diseases and organ systems than those discussed above. Accordingly, with the increased attention given to stem cell-derived treatments for other applications, interesting new methods to improve their efficacy have arisen using injectable, combinatorial hydrogel strategies. For example, in treating retinal degenerative diseases, stem cell therapies routinely fail to survive and integrate. Recent research has shown, however, that encapsulation of retinal progenitor/stem cells (RPCs) in hyaluronan-methyl cellulose (HAMC) hydrogels supports robust survival and proliferation. Most importantly, when transplanting cells in vivo, the use of the HAMC hydrogel improved RPC distribution through the impacted area compared to saline, as well as improved grafted retinal rod survival and functional visual integration [142, 166]. Engineering new muscle through regenerative medicine strategies has also shown promise through enhanced delivery and survival of MSCs, muscle stem cells, and skeletal muscle satellite cells using thermosensitive, injectable hydrogels such as collagen/chitosan/βGP [167], composite synthetic polymers PNIPAM/acrylic acid/2-hydroxyethyl methacrylate-oligomers [168], small intestinal submucosa [169], and fibrin [22]. Wound healing has also been a targeted research area for potential stem cell-derived therapies. Delivery of hASCs for full-thickness skin wounds using injectable gelatin microspheres was shown to significantly improve the wound healing rate, stem cell retention, and growth factor secretion levels compared to delivery of cells alone [170]. Similar functional results in wound healing have been observed when BMSCs were delivered using cell-protective alginate beads within injectable hydrophobic poly(ether urethane) hydrogels compared to implanting pre-formed scaffolds [171•] or transplanting cells within gelatin-poly(ethylene glycol) hydrogels [172]. Lastly, chitosan-based polymers have been shown to improve iPSC-derived hepatocyte (iPSC-Heps) survival and integration for liver tissue engineering as well as ASCs for acute kidney failure. Carboxymethyl-hexanoyl chitosan hydrogels were shown to successfully engraft iPSC-Heps through direct intrahepatic delivery and to reduce necrotic tissue area and to improve liver function [173••]. Thermosensitive chitosan chloride hydrogels were capable of supporting enhanced ASC survival and proliferation in an acute kidney injury model, as well as reducing host renal cell apoptosis and improving microvessel density and renal function [174]. While research in these areas is limited to only a handful of studies in each case, promising in vitro and in vivo data suggest combining stem cell-derived therapies with injectable hydrogels can significantly improve their therapeutic potential.

Conclusion

Throughout these different areas of regenerative medicine applications, a common theme has emerged indicating that stem cells hold great potential for pronounced therapeutic benefits. Unfortunately, harsh conditions after injury and disease, as well as the delivery process itself, can significantly hinder the functional impact of transplanted cells. Therefore, delivering cells in carefully designed, cell-protective and cell-supporting injectable hydrogels may significantly enhance therapeutic efficacy for several different regenerative medicine applications. Looking forward, these injectable materials are expected to improve the rate of clinical translation for stem cell-derived therapies by increasing grafted cell survival and functionality.

Compliance with Ethical Standards

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