Biomaterial Strategies to Bolster Neural Stem Cell-Mediated Repair of the Central Nervous System

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Keywords

Neural stem cells · Biomaterials · Regenerative medicine

Abstract

Stem cell therapies have the potential to not only repair, but to regenerate tissue of the central nervous system (CNS). Recent studies demonstrate that transplanted stem cells can differentiate into neurons and integrate with the intact circuitry after traumatic injury. Unfortunately, the positive findings described in rodent models have not been replicated in clinical trials, where the burden to maintain the cell viability necessary for tissue repair becomes more challenging. Low transplant survival remains the greatest barrier to stem cell-mediated repair of the CNS, often with fewer than 1–2% of the transplanted cells remaining after 1 week. Strategic transplantation parameters, such as injection location, cell concentration, and transplant timing achieve only modest improvements in stem cell transplant survival and appear inconsistent across studies. Biomaterials provide researchers with a means to significantly improve stem cell transplant survival through two mechanisms: (1) a vehicle to deliver and protect the stem cells and (2) a substrate to control the cytotoxic injury environment. These biomaterial strategies

can alleviate cell death associated with delivery to the injury and can be used to limit cell death after transplantation by limiting cell exposure to cytotoxic signals. Moreover, it is likely that control of the injury environment with biomaterials will lead to a more reliable support for transplanted cell populations. This review will highlight the challenges associated with cell delivery in the CNS and the advances in biomaterial development and deployment for stem cell therapies necessary to bolster stem cell-mediated repair.

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Introduction

Damage to the central nervous system (CNS) can lead to cognitive, motor, and sensory dysfunction, as well as impaired function of major organs. Differences in structure, cellular composition, endogenous electrical currents, and biochemical gradients exist both within and across the brain and spinal cord, making it challenging to study and understand post-injury pathophysiology [Yoon et al., 2017; Xuan et al., 2019; Uchida et al., 2020]. Despite the differences that exist within and across the brain and spinal cord, there are similarities that can be

Fig. 1. Cell transplant survival is highly dependent on several parameters that need to be considered when working with stem cell-based therapies. Prior to transplantation, cell expansion and subsequent dissociation into single cell suspensions can stress the cells, resulting in a decrease in viability (0). Once loaded into a syringe, forces (F) experienced during injection (1) and a lack of sites for cell attachment (2) can lead to further cell death. After injection, the transplanted cells are subjected to a highly inflammatory microenvironment, becoming the target of a biochemical assault

from pro-inflammatory cytokines and reactive oxygen species (3). Transplanted cells may also experience immune cell-mediated removal by infiltrating lymphocytes and macrophages (4). Altogether, these forces and environmental-based stressors can result in a precipitous drop in transplant survival to 0.1–8% survival, severely limiting their regenerative potential. The number associated with transplant challenge (1–4) has been superimposed on the timeline to indicate the presumed contribution to cell death if that challenge is not addressed.

observed in these tissues following injury to the CNS. The primary injury results in loss of neurons and glia, as well as injury to the vasculature supplying the tissue. Following primary injury, the body attempts to clear debris and prevent infection through mobilization of neutrophils, microglia, and macrophages to the injury. The local immune response leads to increased release of pro-inflammatory factors, resulting in further tissue damage, known as the secondary injury. As part of this response, astrocytes and fibroblasts deposit extracellular matrix (ECM) and align to create barriers known as the glial and fibrotic scars, respectively, to prevent further inflammation-mediated damage, but these barriers also limit axonal elongation through the injury. Furthermore, intact axon tracts retract and demyelinate further from the injury site, making it more challenging to regenerate the lost tracts.

There are currently several tissue engineering approaches that seek to address specific facets of the CNS injury pathophysiology [Dumont et al., 2016], as well as many cell-based approaches to promote tissue plasticity and repair [Ruff et al., 2012]. Neural stem cells (NSCs) offer one promising means to address both the post-CNS injury pathophysiology and promotion of tissue plasticity and regeneration. NSCs are able to proliferate and differentiate, leading to repopulation of the damaged tissue with CNS cells [Tetzlaff et al., 2011; Iyer et al., 2017; Bruggeman et al., 2019], aiding with tissue repair. Support from endogenous cells is also enhanced by transplanted NSCs that are capable of secreting neurotrophic factors and ECM components [Hawryluk et al., 2012; Laterza et al., 2013; Willis et al., 2020] necessary for local cell recruitment and repair. NSCs can also modulate pro-inflammatory immune cells toward more pro-regenerative pheno-

Table 1. Biomaterials used for cell delivery to the CNS can impact several transplant outcomes, including survival, attachment, and differentiation

Biomaterial	Cell type	Injury model	Survival	Attachment	Differentiation	Reference
SHIELD	Schwann cells	C5 contusion SCI – rat	✓	✓		[Marquardt et al., 2020]
PEG tubes	Spinal progenitors	C5 hemisection SCI – mouse	✓	✓	✓	[Ciciriello et al., 2020]
Injectable peripheral nerve matrix	Schwann cells	T8 contusion SCI – rat	✓			[Cerqueira et al., 2018]
Collagen scaffold + EGFR-Fab	NSCs	T8 transection SCI – rat		✓	✓	[Xu et al., 2017]
PLGA scaffold	Spinal progenitors	T9/T10 hemisection SCI – mouse	✓	✓	✓	[Dumont et al., 2018a]
RADA ₁₆ -IKVAV	NSCs	TBI – rat	✓	✓		[Cheng et al., 2013]
Alginate	Bone marrow stromal cells	C5 transection SCI – rat	✓			[Gunther et al., 2015]
Polyurethane	NSCs	TBI – zebrafish	✓		✓	[Hsieh et al., 2015]
Hyaluronic acid-acrylate	iPSC-NSCs	Stroke – rat	✓		✓	[Lam et al., 2014]
Collagen HA laminin	NPCs	C4 contusion SCI – rats			✓	[Geissler et al., 2018]
poly(sebacoyl diglyceride)- IKVAVs scaffold	NSCs	T10 transection SCI – rat	✓	✓		[Gong et al., 2020]
Fibrous PLGA-PEG	iNSCs	T10/T11 transection SCI – rat	✓			[Liu et al., 2015]
QL6 self-assembling particles	NPCs	C6 contusion SCI – rat	✓			[Zweckberger et al., 2016]
GelMA	iNSCs	T9/10 transection SCI – mouse	✓		✓	[Fan et al., 2018]
PLL-xyloglucan + GDNF	NPCs	Parkinson's disease – mouse	✓			[Wang et al., 2016]
Chondroitin sulfate methacrylate	NSCs	T10 contusion SCI – rat			✓	[Liu et al., 2019]
poly(2-hydroxyethyl methacrylate) hydrogel	Spinal progenitors	T8 hemisection SCI – rat	✓			[Ruzicka et al., 2013]
QL6 self-assembling particles	NPCs	C6 contusion SCI – rat	✓		✓	[Iwasaki et al., 2014]
Polyglycolic acid	NPCs	T10-T11 hemisection SCI – rat	✓			[Shin et al., 2018]
HAMC-rPDGF-A	NSCs	T2 contusion SCI – rat	✓		✓	[Mothe et al., 2013]
HA	iPSC-NSCs	Stroke – mouse	✓			[Moshayedi et al., 2016]
HAMC-RGD/PDGF-A	OPCs	T2 contusion SCI – rat	✓	✓	✓	[Führmann et al., 2016]
Fibrin + growth factors	NSCs	T3 transection SCI – rat	✓		✓	[Lu et al., 2012]
Fibrin-heparin + NT3, PDGF, GDNF	NPCs	T2 contusion SCI – rat	✓			[McCreedy et al., 2014]
Collagen scaffold + paclitaxel	NSCs	T8 transection SCI – rat			✓	[Li et al., 2018]

Traumatic injury and disease models were both included in the assessment of biomaterials combined with cell types, which are denoted as indicated in their respective study. NSCs, neural stem cells; NPCs, neural progenitor cells; iPSC, induced pluripotent stem cells; SCI, spinal cord injury; TBI, traumatic brain injury; T, thoracic; C, cervical.

types [Bonnamain et al., 2012; Nazmi et al., 2014; Assinck et al., 2017] and remediate excitotoxicity [Assinck et al., 2017]. It is important to note that the benefits attributed to NSCs vary depending on NSC source, isolation and culture methods, donor age, transplantation conditions, and survival capacity [Tetzlaff et al., 2011; Iyer et al., 2017]. In the context of this review, we will use the term NSC to refer to both neural stem and progenitor cell populations isolated from the brain or spinal cord, as well as NSC-like cells derived from induced pluripotent stem cells and embryonic stem cells. A recent review on the techniques for deriving each of these cell populations can be found elsewhere [Tang et al., 2017].

The therapeutic benefits of NSCs are limited by their ability to survive transplantation within the inflammatory injury milieu, as detailed in Figure 1. Specifically, NSC survival is predicated on the transplantation method, transplantation timing, cell sourcing and expansion, and the engraftment microenvironment [Tetzlaff et al., 2011; Iyer et

al., 2017]. Location of implantation, cell dose density, and timing are also important influencers of cell survival [Piltti et al., 2013a, 2013b, 2015]. Biomaterials can increase NSC transplant survival and engraftment beyond the traditional means of improving NSC survival outlined above. Indeed, several naturally occurring and synthetic biomaterial options for co-transplantation with NSCs have previously been reviewed [Cooke et al., 2010; Iyer et al., 2017], and we have compiled a list of cell-biomaterial strategies for CNS applications in Table 1. In this review, we will evaluate biomaterial properties that impact NSC survival and engraftment directly, as well as indirectly through environmental remediation with different CNS injury models. Additionally, we later look to expand on cell-biomaterial therapies by reviewing current literature relevant for combinatorial therapies that build on prior biomaterial systems. This section is intended to propose promising technology that could be expanded into larger platforms, relevant for improving stem cell survival and tissue regeneration.

Fig. 2. Biomaterials are an advantageous base for tissue engineering platforms as they are easily modifiable to obtain properties desirable for the required application. Implant mechanical properties can be modified to be similar to those of native tissues. Prior to implantation, biomaterial implants can be functionalized with various surface residues that can encourage cell adhesion upon implantation, facilitating cell infiltration and tissue repopulation. Tissue repopulation and regeneration rates can also be matched with tunable biomaterial degradation rates. Furthermore, biomaterials can encapsulate or serve as a vehicle for cell transplants, improving their microenvironment. In the injury site, scar attenuation has been observed as the biomaterial implant can provide support for previously open cysts. A biomaterial implant also can serve as a depot for local release of a loaded therapeutic factor that can further modulate the injury environment.

Biomaterials for NSC Delivery and Engraftment

NSC transplantations are heavily influenced by their local microenvironment, and biomaterial strategies present an easy method for modulation. Biomaterial interventions are advantageous as they often have easily tunable properties, as outlined in Figure 2, that can improve transplantation conditions. Moreover, biomaterials can provide either a vehicle or medium for transplantation that is not present when cells are transplanted alone. This distinction is important, and in several instances of combined biomaterial and NSC strategies, controls for either the biomaterial or the NSCs alone were not present, thus making comparisons between strategies difficult. These studies were still included; however, we denote controls in the text to prevent readers from making assumptions on the effects of the biomaterial and/or NSC alone.

Overcoming Cell Delivery Challenges

The primary method for cell transplantation is syringe-based injection of the cells at the site of interest. Preparation of the cells prior to transplantation can lead to environmental stressors such as altered gas levels, reduced nutrient availability, and residual dissociation products if preparing single cell suspensions. Cells are then additionally subjected to physical stressors when loaded into the injection syringe. During injection, cells undergo three major mechanical stressors: pressure difference across the cell, shear forces from linear shear flow,

and stretching from extensional flow [Aguado et al., 2012]. These conditions can rupture cell membranes, significantly reducing the number of viable cells during transplantation.

Biomaterials that mitigate external forces on cells can reduce force-induced cell death by redistributing shear forces. Load redistribution can be achieved through storage modulus modifications, which describes the ability of a viscoelastic material to store energy elastically under oscillatory strain, and shear-thinning property modifications, which are characterized by a decrease in viscosity when shear force is applied. Strategies to tune these viscoelastic properties and engineer protective biomaterials for cell encapsulation have been reviewed elsewhere [Madl and Heilshorn, 2018]. Alginate hydrogels with varying storage moduli (0.33–58.1 Pa) were used to observe the effect on the transplantation of different cell populations including human umbilical vein endothelial cells, human adipose stem cells, rat mesenchymal stem cells, and mouse NSCs [Aguado et al., 2012]. Hydrogels with a storage modulus of 30 Pa resulted in the highest cell viability across all tested cell populations in an in vitro cell injection model. Extensional flow, which causes stretching and deformation as the cells move from the larger diameter of the syringe to the smaller diameter of the needle, was found to be lowest in alginate gels with a 30-Pa storage modulus compared to gels with lower storage moduli. When injected, the hydrogel underwent shear-banding, where the layer of the alginate hydrogel

near the wall of the needle shear-thins and acts as a lubricant, while the rest of the hydrogel on the inner layers held its form, thus reducing the apparent stress experienced by the suspended cells [Aguado et al., 2012].

Shear-thinning hydrogels reduce the shear force that the cells experience, which can contribute to a significant loss of viable transplanted cells. Marquardt et al. [2020] built upon prior protective hydrogel strategies [Cai et al., 2015], developing a shear-thinning hydrogel for injectable encapsulation and long-term delivery (SHIELD) that protected Schwann cells during injection and prevented cell reflux, where cells flow out of the injury through the injection site after needle removal. The SHIELD gel included an engineered protein (C7), proline-rich 8-arm polyethylene glycol (PEG), and a thermoresponsive molecule, poly(N-isopropylacrylamide), allowing for gel deformation under injection forces and reformation after injection. Schwann cells encapsulated in the SHIELD hydrogel and injected into a cervical contusion spinal cord injury (SCI) model experienced a 7.4-fold increase in Schwann cell viability compared to cells injected in saline at 48 h post-transplant and 12-fold increase in Schwann cell viability at 4 weeks post-transplant. Increased Schwann cell survival with SHIELD hydrogel use additionally resulted in greater functional recovery compared to Schwann cells in saline [Marquardt et al., 2020]. While Schwann cells, not stem cells, were used in this study, they experience many of the same viability problems in transplantation as stem cells, rendering the SHIELD hydrogel a highly promising biomaterial delivery system that can be applied to NSC transplantations. Developing a biomaterial that is supportive and protective of NSCs during transplantation is crucial for allowing NSCs to maximize their therapeutic potential.

Supporting Cell Adhesion

Once transplanted into the injury, surviving cells face a new set of challenges for their long-term survival. Cells transplanted in media or saline lack physical support, leading to apoptosis in the absence of cell adhesion in a process known as anoikis [Cooke et al., 2010; Mitrousis et al., 2018]. Rho and ROCK signaling inhibitors reduced anoikis in NSCs transplanted into an uninjured mouse brain [Koyanagi et al., 2008], suggesting possible pharmaceutical options to circumvent anoikis. Biomaterials can also reduce anoikis by providing a substrate for transplanted cells to attach to, thereby limiting anoikis-attributable cell death due to lack of support [Marquardt and Heilshorn, 2016]. Biomaterials can be functionalized with ECM proteins, ECM-based peptide motifs, or other cell adhesion molecules to facilitate cell attachment. Several

ECM proteins and peptides within biomaterials bind to the $\beta 1$ integrin on NSCs, leading to an upregulation of MAPK signaling, activating survival and proliferation pathways [Stukel and Willits, 2015].

Hydrogels like the self-assembling peptide, RADA₁₆, have shape-memory properties where they can organize into a pre-programmed shape or morphology, even after shear-thinning. RADA₁₆ also includes the laminin-based IKVAV peptide motif for encapsulated NSC attachment [Cheng et al., 2013]. Cells suspended in RADA₁₆ were injected into a traumatic brain injury model in rats, and immunohistochemical analysis showed significant proliferation and greater neuronal differentiation compared to the hydrogel without IKVAV. RADA₁₆ gels can also be functionalized with FGL, a motif from neural cell adhesion molecule (NCAM), to serve as a neuroprotective hydrogel in NSC-biomaterial implants [Wang et al., 2015]. A similar approach was used to deliver oligodendrocyte progenitor cells (OPCs) [Führmann et al., 2016] or NSCs [Mothe et al., 2013] in media or a hyaluronic acid/methyl cellulose (HAMC) copolymer hydrogel functionalized with the fibronectin-based peptide, RGD, and platelet derived growth factor (PDGF). Cell survival and migration were increased in both OPCs and NSCs in the HAMC-RGD/PDGF hydrogel compared to the cells transplanted in media alone. Other peptides derived from NCAM are also being investigated to selectively limit adhesion to CNS-derived cells and to facilitate increased neuronal differentiation of stem cells [Xu et al., 2014]. Another approach employed is the use of antibodies and antigen binding factors to bind to epidermal growth factor receptors (EGFRs) expressed on the surface of NSCs. A scaffold functionalized with an EGFR antibody was designed to promote NSC adhesion to the scaffold and direct neuronal differentiation. Both NSCs and neurons were increased in the EGFR-functionalized scaffold compared to the scaffold without the EGFR antibody [Xu et al., 2017]. Biomaterials as a vehicle for cell transplantation can significantly limit anoikis, thereby improving survival, but they can also be used to provide greater control over the cell populations that attach to the substrate and to guide stem cell differentiation. These combined benefits of biomaterial adhesion sites for both transplanted and endogenous cell populations can work synergistically to improve the regenerative potential of transplanted cells within the biomaterial.

Mimicking Biomechanical Properties

Biomaterial mechanical properties are not just important for injection, but also need to be designed for the

specific tissue and cell types of interest. Healthy and diseased tissues in the CNS have a broad range of stiffnesses that can significantly alter cell phenotypes, and this is reflected with biomaterial-cell transplants [Barnes et al., 2017]. In particular, biomaterial stiffness can impact NSC proliferation and differentiation [Stukel and Willits, 2015, 2018]. Biomaterial stiffness can also impact the pathophysiology that limits tissue repair after CNS injury, including cell infiltration, inflammation, scar formation, and cavitation [Dumont et al., 2015, 2016]. As the injury pathophysiology can directly impact NSC survival, proliferation, and fate, it is thought that biomaterial regulation of these processes can indirectly impact NSCs and their responses.

In the context of CNS repair, it is important that the biomaterial storage modulus is comparable to that of native brain or spinal cord tissue (0.5–10 kPa) [Stukel and Willits, 2015, 2018; Karimi et al., 2017]. NSCs encapsulated in a polyurethane (PU) thermoresponsive hydrogel with varying storage moduli (30% PU1: 4,000 Pa, 25% PU1: 1,100 Pa, 30% PU2: 2,400 Pa, and 25% PU2: 680 Pa) were transplanted into zebrafish embryos after traumatic brain injury [Hsieh et al., 2015]. NSCs suspended in the 25% PU2 hydrogels that had a storage modulus of ~680 Pa led to increased proliferation and neuronal differentiation of the transplanted NSCs, while the other PU hydrogels with greater storage moduli led to greater glial differentiation. The 25% PU2 hydrogel also increased functional recovery measured by the spontaneous coiling rate of the embryos 24 h post fertilization [Hsieh et al., 2015].

Biomaterials with optimized biomimetic mechanical properties can also help modulate scarring in the injury [Moshayedi et al., 2016]. Hyaluronic acid (HA) hydrogels with storage moduli of 100, 350, and 1,000 Pa were injected into a stroke cavity. HA hydrogels with 100 Pa storage modulus had the least hydrogel volume remaining 2 weeks post-injection and had the largest infarct area compared to all other tested conditions. Conversely, HA hydrogels with 350 and 1,000 Pa storage moduli had a significant volume remaining 2 weeks post-injection at the stroke site; however, the 350 Pa hydrogel resulted in the smallest remaining infarct area, suggesting improved tissue sparing and ingrowth. The 350 Pa hydrogel also improved NSC survival, proliferation, and differentiation in a cell adhesion molecule and conjugated growth factor-dependent manner. Interestingly, conditions that fostered the greatest survival and proliferation led to increased astrocytic differentiation among the transplanted cells [Moshayedi et al., 2016]. Similarly, a reduction in

scarring, increased tissue sparing, increased stem cell survival, and improved neuronal differentiation have been found with low storage modulus gelatin methacrylate [Fan et al., 2018], collagen HA laminin [Geissler et al., 2018], and PEG [Ciciriello et al., 2020] hydrogels when transplanted after SCI.

Mechanical properties are not solely limited to material stiffness, but are also informed by the micro- and macro-structure of an implant. Biomaterial pore size and density can reduce the bulk mechanical properties of an implant to more closely mimic that of the CNS tissue, as observed previously with PEG hydrogel tubes [Dumont et al., 2019]. The resulting mechanical shift and presence of a physical space for cell infiltration has been well documented to impact inflammation after injury in several tissue types [Madden et al., 2010; Underwood et al., 2011], including the CNS, where porosity also limits scar formation and cavitation [De Laporte et al., 2009; Dumont et al., 2016; Margul et al., 2016; Nih et al., 2017, 2018; Park et al., 2018a]. Pores within scaffolds can also provide a space for cell loading and delivery to the injured CNS [Dumont et al., 2018a] or provide a depot for delayed stem cell transplantation [Ciciriello et al., 2020] following biomaterial-mediated remediation of the injury site.

Locally, pore size and interconnectivity have significant effects on cell adhesion, phenotype, proliferation, differentiation, and nutrient supply. Biomaterials with smaller pore sizes and greater porosity have more surface area for cell adhesion, a cell process necessary for survival, proliferation, and differentiation. Pore size can also impact cell phenotype for infiltrating immune populations. Pore sizes of 30–40 μm lead to a shift in macrophage phenotype toward a pro-regenerative expression profile, while also decreasing the formation of foreign body giant cells and increasing angiogenesis [Madden et al., 2010; Underwood et al., 2011]. Pore size is also an important determinant of oxygen and nutrient penetration through a biomaterial. Low porosity can limit the amount of oxygen and nutrients within a space, which will limit cell proliferation and ingrowth in vivo [O'Brien et al., 2005; Loh and Choong, 2013]. Interconnectivity of a porous biomaterial allows for improved cell infiltration within the scaffold, and for nutrients and oxygen to permeate throughout. Interconnected pore spacing is also relevant for vessel infiltration that will be necessary for long-term nutrient supply to the area. Interconnected nodes should be approximately 60 μm apart to support vascularization of the biomaterial [Sieminski and Gooch, 2000]. Ultimately, the pore size, density, and interconnectivity will impact bulk mechanical properties that must be balanced

with the local impacts of porosity, namely nutrient transport and cellular responses to facilitate enhanced transplant survival and regeneration. Furthermore, porous biomaterials can serve as a delivery platform for lentiviral-mediated overexpression of a protein of interest [Park et al., 2018a; Smith et al., 2020]. This viral depot can combinatorially work with cell delivery to improve NSC transplantation strategies. Inclusion of biomimetic mechanical properties for biomaterials is becoming increasingly important to target both the endogenous tissue response and to provide a suitable microenvironment for NSCs that can drive cell survival and neuronal differentiation.

Combinatorial Strategies to Support Stem Cell-Mediated Repair

Biomaterial-based technologies have advanced significantly in their regenerative abilities, and have become ubiquitous throughout almost all of tissue engineering. Often times, they serve as platforms for therapeutic delivery with highly tunable properties that are advantageous for controlling local release of growth factors and drugs. Additionally, they can serve as a platform for delivering stem cells by offering numerous protective roles to increase survival, as highlighted throughout this review. With that in mind, therapeutic and cell delivery biomaterial platforms are often thought of as two independent strategies. Individually, both demonstrate success in their therapeutic goals, but combining with other strategies could result in further synergistic gains to fulfill the need for improved transplant survival and regeneration. Altogether, incorporating seemingly independent approaches into one technique will result in a robust stem cell delivery platform that has the opportunity to be broadly applicable beyond CNS repair.

Engineering Vascular Support

Stem cell differentiation and behavior is highly dependent on the local microenvironment, known as the stem cell niche [Morrison and Spradling, 2008; Ferraro et al., 2010]. It is common for stem cell niches throughout the body to include high-density vascular networks, as they are responsible for delivering oxygen and nutrients necessary for differentiation and proliferation [Morrison and Spradling, 2008; Putnam, 2014]. Endothelial cells (ECs) are an important structural component, and the primary cell type, of blood vessels that also play an important role in angiogenesis. In the CNS, the subventricular zone

(SVZ) is one example where NSCs are in close contact with ECs. It has been shown that neurogenesis of SVZ NSCs is directly dependent on their distance away from resident vasculature [Shen et al., 2008; Tavazoie et al., 2008]. Furthermore, co-culturing ECs with cerebral cortex NSCs from embryonic day 10–11 (E10–11) in vitro in two-dimensional (2D) transwell plates increases NSC self-renewal and neurogenesis [Shen et al., 2004; Sun et al., 2010]. Other in vitro models attempting to recreate the NSC niche using either static or fluid flow 2D cell culture setups have shown that signaling molecules released from cerebral microvascular ECs further support E10–11 and adult SVZ NSC differentiation and fate [Shen et al., 2004; Dumont et al., 2017, 2018b]. Conversely, postnatal day 1 (P1) [Li et al., 2006] and E14 [Roitbak et al., 2008] NSCs also offer a protective role for ECs when in co-cultures against ischemic conditions with HIF-1 α and VEGF signaling, further highlighting the close relationship between the two cell types. Moreover, co-transplantation of bovine pulmonary microvascular ECs and cortex-derived ischemia-induced adult NSCs has been shown to increase NSC survival at 5 days and 28 days after ischemic stroke [Nakagomi et al., 2009]. Of the surviving NSCs and their progeny, approximately 3% gave rise to NeuN⁺ neurons, a relatively low percentage, but significantly more than NSCs transplanted alone. Applying the advantages of in vitro co-culturing, niche modeling, and early co-transplantation studies to hydrogel-based cell transplantations could present an effective strategy for improving NSC-mediated regeneration. While there is currently minimal work with in vivo co-transplantations in hydrogel networks, feasibility of NSC/EC co-spheroids encapsulated into an injectable gelatin-based hydrogel loaded with human mesenchymal stem cells (hMSCs) and implanted into a Sprague-Dawley rats [Han et al., 2019] and with GFP NSCs from P1 transgenic GFP mice and brain ECs into a macroporous polylysine-PEG hydrogel [Ford et al., 2006] demonstrated improved angiogenesis. Advantages from an NSC standpoint were not investigated in either study, but it is not unreasonable to think that the NSCs would benefit from improved vascular formation as the local NSC niche is regulated by distribution of signals present in blood circulation [Goldberg and Hirschi, 2009; Otsuki and Brand, 2017; Karakatsani et al., 2019].

Acellular alternatives to establish a vascularized, niche-like implantation site that could be used for NSC delivery have been explored with poly(lactic-co-glycolic acid) (PLGA) [Bible et al., 2012], HA [Nih et al., 2017], and HA-heparin [Nih et al., 2018] microparticle networks that release VEGF. Each study demonstrated robust re-

vascularization; however, vessel patency was significantly improved with HA-heparin microgels. In particular the density of the VEGF within the microgel network was an important design characteristic with many of the particles devoid of VEGF and a subset of particles loaded with a high VEGF density [Nih et al., 2018]. Without the careful design of VEGF loading parameterization, it was found that high-density microvascular structures were present, but resulted in increased inflammation and astrogliosis [Bible et al., 2012], rather than the increased tissue repair and functional recovery observed in the VEGF density-controlled HA-heparin biomaterial [Nih et al., 2018]. Only one of these studies explored their biomaterial for NSC transplantation; however, due to hypervascularization and increased inflammation, there was an increase in astrocyte differentiation [Bible et al., 2012]. In each of these studies a network of biomaterial microspheres was loaded into the injury, thus generating a porous biomaterial network. As described earlier, the porosity of the biomaterial will impact nutrient transport, vascular cell infiltration, and vessel formation [Loh and Choong, 2013]. Future studies should look to combine the benefit of angiogenic materials to generate an environment that is supportive of subsequent NSC transplantation.

Mediators of Transplant Differentiation

Biomaterial composition and release of bioactive molecules, such as neurotrophins, readily impact NSC differentiation, as reviewed elsewhere [Wang and Sakiyama-Elbert, 2019]. The effects of these factors on NSC differentiation can be challenging to parse from proliferation and survival, as differentiation is not possible without survival. Aside from biochemical cues, researchers have also tried to control the resulting NSC progeny through cell sorting for desirable progenitor or precursor populations [Hu et al., 2012; Sher et al., 2012; Wang et al., 2013; Vadivelu et al., 2015; Butenschon et al., 2016], delivering micro-tubule stabilizing drugs [Li et al., 2018], and pre-conditioning NSC populations with support cells known to increase neurogenesis [Lowry et al., 2008]. It is unclear if pre-conditioning or selection of NSCs has an impact on differentiation, or if the injury site is primed to promote survival of select NSC phenotypes. This dichotomy between increased survival and ensuring desirable cell phenotypes is best observed from a study using HA hydrogels with varying concentrations of brain-derived neurotrophic factor (BDNF), and bone morphogenic factor-4 (BMP-4) [Moshayedi et al., 2016]. In this study, the HA hydrogel with elevated BMP-4 promoted increased survival of transplanted cells; however, it resulted in increased astro-

glial differentiation. HA with lower BMP-4 concentrations and elevated BDNF resulted in lower survival, but within the surviving population, there was increased neuronal differentiation, suggesting an inverse relationship between survival rates and tissue repopulation. It has been suggested that this inverse relationship mimics brain development during which some neurons are selectively culled. As neurons are formed, they begin to elongate axons and are highly susceptible to apoptosis [Dekkers et al., 2013]. During this process, caspases responsible for triggering apoptosis can also produce mitogens that stimulate NSC proliferation [Bergmann and Steller, 2010]. One way to address survival and differentiation challenges is to co-deliver neural progenitors with V2a spinal interneurons to the injured spinal cord, which improves transplant integration with intact circuitry leading to improve functional outcomes [Zholudeva et al., 2018]. More research is needed to explore transplantation phenotypes and differentiation into specific neuron populations. Technologies to evaluate integration of cell transplants with intact circuitry are also advancing [Adler et al., 2017; Anzalone et al., 2018] and provide researchers with more opportunities to validate functionality of differentiated cell transplants.

Immune-Modulating Therapeutics

Design of hydrogels for local drug release has been investigated heavily for many tissue engineering applications [Sharpe et al., 2014; Li and Mooney, 2016], including CNS injuries [Piantino et al., 2006; Kang et al., 2009; Nih et al., 2018]. Without physical and/or biochemical support, stem cell transplant viability remains low, often times below 5% 1 week post-transplantation in immunocompetent rodents [Parr et al., 2008; Tetzlaff et al., 2011; Cusimano et al., 2012; Mothe et al., 2013; Butenschon et al., 2016; Iyer et al., 2017]. Hydrogels alone have some capacity for immunomodulation [Singh and Peppas, 2014; Adu-Berchie and Mooney, 2020]. For example, in SCI models, both fibrin and high-molecular-weight HA-based hydrogel implants can inhibit glial scar formation when implanted immediately after injury [Taylor et al., 2006; Khaing et al., 2011; Zuidema et al., 2018], while HA can additionally reduce astrocyte proliferation through direct binding to cell surface receptors, like CD44, thus limiting inflammation [Back et al., 2005].

Biomaterial-mediated immune modulation can be further enhanced with sustained therapeutic release, whereby a local injury microenvironment can be significantly altered. With that in mind, immune modulation timing post-SCI is highly dependent on the desired target,

as different circulating cells and inflammatory mediators peak in concentration in the hours, days, and weeks post-injury [Donnelly and Popovich, 2008]. The dichotomy of damage versus recovery must be considered when deciding delivery timing for immune-modulating therapies, but it is important to note that full ablation of immune populations is often detrimental as inflammatory cells and mediators have regulatory roles in neuroplasticity [Schwab et al., 2014]. Timing delivery to maximize regulatory roles while limiting damage due to sustained exposure is crucial, further complicating immune modulation. A common target for immune modulation is infiltrating macrophages, as they are a well-studied cell population prevalent in many injury paradigms. Macrophages and microglia, the resident CNS macrophage, exist on a nuanced spectrum of phenotypes that are often simplified to being pro-inflammatory (M1) or pro-regenerative (M2) [Mosser and Edwards, 2008; Novak and Koh, 2013]. Microglia often accumulate quickly (hours) after injury, as they are resident to the CNS, and sustain their presence into the chronic window while macrophages often peak in concentration at 1–2 weeks with a second wave of macrophage infiltration reported to occur at 8 weeks, well into the chronic injury [Donnelly and Popovich, 2008; Beck et al., 2010; Dumont et al., 2016]. Anti-inflammatory factors, including those from the interleukin (IL) family of cytokines including IL-4, IL-10, IL-13, and IL-33, are important for polarization to the M2 phenotype [Gadani et al., 2015; Liu et al., 2016; Lobo-Silva et al., 2016; Margul et al., 2016; Mori et al., 2016; Park et al., 2018a, 2018b; Ciciriello et al., 2020; Smith et al., 2020]. Slow release of exogenous IL-10 from a biomaterial significantly improves its retention at the injury, lasting across acute to chronic time scales, compared to bolus IL-10 injections, and further reduces M1-like presence, resulting in a more pro-regenerative site [Boehler et al., 2014; Gower et al., 2014; Hellenbrand et al., 2019]. Similarly, immune-suppressing corticosteroids, like methylprednisolone, can be encapsulated into injectable biomaterials and slowly released over the course of days post-injury [Pritchard et al., 2011; Slotkin et al., 2016], but their broad-acting immune suppression has led to controversy over their actual therapeutic effect compared to potential complications [Cheung et al., 2015; Fehlings et al., 2017].

There has been little investigation into combining stem cells, hydrogels, and immune-modulating therapeutic release as a combination strategy, but their independent successes highlight the potential synergistic reparative effects possible when combining together. With a more hospitable transplantation niche, cell engraftment

and survival will improve, resulting in improved regeneration. RNAi knockdown of lipocalin 2 [Braga et al., 2020] or tumor necrosis factor alpha (TNF- α) antagonist-mediated suppression [Wang et al., 2014] delivered immediately after injury in the absence of a biomaterial have both increased NSC survival and engraftment, suggesting the combination of anti-inflammatory therapeutics combined with the benefits of a biomaterial could further improve NSC survival and regeneration. Feasibility of controlling both cell and therapeutic delivery from a biomaterial has been demonstrated outside of the nervous system. While not a hydrogel, a combination therapy using a porous PLGA scaffold to co-deliver islets with IL-33 improved graft survival improving exogenous regeneration while also stimulating endogenous cell populations [Liu et al., 2018]. In situ bone regeneration was also observed when combining injectable gelatin-based hydrogels encapsulated with both MSCs and a small hydrophobic drug called icaritin [Feng et al., 2019].

Nanoparticle Therapeutics to Mitigate Inflammation

Nanoparticle delivery is taken advantage of throughout tissue engineering [Hasan et al., 2018], with a large number of strategies focused on administration of a therapeutic payload [De Jong and Borm, 2008; Patra et al., 2018]. Alternatively, nanoparticles have also been used to modulate immune responses post-injury. Post-administration, polymeric nanoparticles are taken up by infiltrating immune cells and have the ability to reprogram their responses and traffic them away from the injury, effectively attenuating their response. Negative surface-charged PLGA nanoparticles bind to circulating immune cells and traffic them to the spleen in numerous CNS injury models [Getts et al., 2012, 2015; Getts, 2014; Park et al., 2019]. Similarly, Papa et al. [2013, 2014] have shown that PEGylated poly(methyl methacrylate) (PMMA) and poly- ϵ -caprolactone nanoparticles were both internalized by pro-inflammatory macrophages and microglia when delivered in a thoracic SCI model. Mice with PLGA bridges implanted within the injured spinal cord that receive daily doses of intravenous PLGA nanoparticles in the first 5 days post-injury were observed to have increased tissue sparing and increased locomotor recovery compared to mice receiving the implant alone [Park et al., 2019]. The combined use of biomaterial approaches in the SCI model suggests that there is a synergy between the materials targeting different aspects of injury pathophysiology. The addition of NSCs could increase tissue regeneration, while the nanoparticles would further mitigate local inflammation that restricts NSC survival to 5% when

transplanted within the PLGA bridges without nanoparticles [Dumont et al., 2018a]. Additionally, nanoparticles can be encapsulated into hydrogels for controlled local delivery to an injury model [Papa et al., 2013, 2014; Mauri et al., 2018; Dannert et al., 2019], limiting off-target accumulation. Poly acrylic acid hydrogels can be functionalized with tunable pore sizes for controlling encapsulated PMMA nanoparticle release [Rossi et al., 2013]. Co-encapsulating nanoparticles into a hydrogel with stem cells could be a combinatorially beneficial tissue engineering strategy. A functionalized hydrogel containing both stem cells and nanoparticles could be implanted into an injury site, where the smaller nanoparticles would have a quick, tunable release [Wang et al., 2020] to reprogram pro-inflammatory cytokines that would normally limit transplant survival [Park et al., 2019]. With the immune response attenuated and shifted away from the transplants, more surviving cells would be able to proliferate and differentiate within the hydrogel and eventually repopulate lost or damaged tissue.

Functionalized nanoparticles can also be used to sequester pro-inflammatory cytokines and chemokines themselves, rather than reprogram immune cells. Post-injury, cytokines are involved in a feedback loop where as they are produced, infiltrating leukocytes sense their presence and thus contribute to further inflammation [Rahman et al., 2018]. Disrupting this cycle by decreasing pro-inflammatory cytokine concentrations at the injury could be beneficial from a tissue regeneration standpoint. A comparable strategy is utilized in viral applications where nanotherapeutics can capture foreign antigens and effectively sequestering them away from damaging their targets on the cell and tissue level [Eyckerman et al., 2016]. This targeting strategy can be applied to cytokines through delivery of cytokine-neutralizing antibodies and glycosaminoglycans (GAG) [Zhang et al., 2020]. Antibody-mediated targeting of TNF- α is the basis for blockbuster drug Humira (adalimumab), used for treating various types of arthritis and gastrointestinal diseases. The FDA has approved numerous antibodies that specifically target a wide number of cytokines other than TNF- α including IL-1 β , IL-17, IL-23, and interferon- γ [Lai and Dong, 2016; Zhang et al., 2020]. Antibody binding is specific enough to limit off-target binding, and once bound, they prevent their target from propagating the immune response as they can no longer bind to infiltrating leukocytes. These antibodies can be conjugated to biomaterials and implanted and delivered locally or nanoparticles and delivered intravenously [Friedrich et al., 2014; Lima et al., 2018]. Similarly, GAGs can be conjugated to biomaterial

or nanoparticle surfaces to interfere with chemokine binding, thus reducing leukocyte recruitment. GAGs are normally present on immune cell surfaces, and help with coordinating immune response, but introducing exogenous GAGs on a biomaterial implant can effectively distract chemokines from binding to their intended targets [Thompson et al., 2017].

Conductive Biomaterials

The NSC niche is a complex microenvironment that directly effects NSC differentiation and maturation based on a number of physical and chemical signals [Conover and Notti, 2008; Decimo et al., 2012]. As described previously, proximity to vasculature and ECs plays a large role in directing endogenous NSC fate. Additionally, electrical signals within the niche can significantly alter differentiation and proliferation, as reviewed previously [Bertucci et al., 2019]. Electrical conditions have been controlled in ex vivo NSC culture, demonstrating that stimulation guides migration, promotes differentiation, and improves viability [Li et al., 2008; Kobelt et al., 2014; Du et al., 2018; Zhu et al., 2019]. Applying this strategy when transplanting cells into a CNS injury model could improve their survival and facilitate improved regeneration. Numerous biomaterials have been developed to have electrically conductive properties [Song and George, 2017; Boni et al., 2018; Vandghanooni and Eskandani, 2019]. Polypyrrole-based neural conduits can direct current electric fields and stimulate guided axonal growth [Lee et al., 2009; Durgam et al., 2010; Nguyen et al., 2014]. Other materials including polyaniline, poly (3,4-ethylenedioxythiophene), and indium phosphide have been used similarly as guidance conduits and doped hydrogels [Boni et al., 2018]. These conduits or doped hydrogels can be used as a cell transplantation platform with potential to improve regenerative outcomes. Combining the benefits that NSCs receive from electrical stimulation with the regenerative and guidance properties of electrically active biomaterials could lead to improved transplant survival, resulting in more robust NSC neural lineage commitment.

Conclusion

Substantial strides have been made to understand cell delivery requirements and develop new biomaterials that offer support and protection for transplanted stem cells. Biomaterials offer a therapeutically promising platform on their own, but also have the potential for further en-

hancement of desired transplant outcomes through integration of additional therapeutics, including cell adhesion peptides, trophic cues, biophysical cues, pathophysiology modifying therapies, or dual biomaterial approaches as discussed in the combinatorial section of this review. A well choreographed treatment regime that integrates these different strategies to treat specific hurdles will likely be needed for any biomaterial-stem cell treatment that moves to the clinic. The next major challenge for this area of research will likely be improving our understanding with regards to the necessary timing for administering particular aspects of a treatment strategy. Moreover, the complexity of the injury microenvironment and the variability of NSC phenotypes needs further investigation to maximize the regenerative potential of transplanted stem cells.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

Funding was provided by the University of Miami Frost Institute of Chemistry and Molecular Sciences Junior Faculty Research Award.

Author Contributions

G.T., A.J.C., and C.M.D. contributed to all aspects of manuscript preparation.

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