

To Serve and Protect: Hydrogels to Improve Stem Cell-Based Therapies

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Harsh environments within damaged and diseased tissues and limited retention and survival of injected stem cells pose major challenges for stem cell therapeutics today. Here, we discuss promising hydrogel-based strategies for improving engraftment and viability of transplanted stem cells and stimulating recruitment of endogenous stem cells for repair.

There is great promise for stem cell-based therapeutics for the treatment of a myriad of diseases and injuries, and substantial investment has been made over the past decade in the search for new therapies. A wide variety of stem cells have been pursued toward this end, including mesenchymal stem cells (MSCs), neural stem cells (NSCs), and pluripotent stem cells, and a detailed review of clinical investigations in this area has been recently reported (Trounson and McDonald, 2015). Stem cell applications have been as varied as treatments for Parkinson's disease, macular degeneration, osteoarthritis and disc degeneration, and heart failure, and such stem cell therapies have generally been considered safe in past trials. Indeed, recent clinical trials using MSCs suggest a limited host response, even when the implanted cells are of allogeneic origin. While such trials generally report some level of success, improvements have mostly been transient and, at least for MSCs, are commonly attributed to trophic factors that modulate immune or inflammatory responses or repair processes (e.g., angiogenesis) that preserve some tissue function. It is clear that success will rely on our ability to recruit or position cells where desired and control their survival, fate, and function in the days and weeks that follow treatment.

In most clinical trials, stem cells are directly injected into a tissue via a syringe or catheter and without any carrier. This process leads to limited initial and sustained stem cell engraftment at the

desired location due to leakage of the cell suspension during injection, as well as potentially reduced viability due to cell damage incurred during the injection process, which can be attributed to the shock of transferring cells out of standard cell culture or to the damaging local microenvironment after injection. Although it depends on the mode of delivery and application, often fewer than 5% of injected cells persist at the site of injection within days of transplantation. Since function within the wound site is correlated with the number of cells retained after injection, improving viable cell engraftment is of utmost importance.

Synthetic and Natural Hydrogels Can Improve Retention and Viability of Injected Stem Cells

One approach that has been taken to improve these outcomes is the use of biomaterial carriers that help to retain injected cells and provide a microenvironment that supports cell viability and function. Hydrogels are a class of biomaterials that are composed of water-swollen polymeric networks, ranging from natural (e.g., fibrin, alginate) to synthetic [e.g., poly(ethylene glycol) or PEG] components. These materials are highly hydrated, can be designed to incorporate a range of biophysical and biochemical cues, and may be designed to be injectable through minimally invasive techniques via syringes and catheters. Injectable hydrogels include those that are assembled and undergo a shear-thinning process during injection to permit flow or those that undergo gelation during and

immediately after the injection process (e.g., two-component systems, photoinitiated, and self-assembly).

In the simplest concept, hydrogels can help retain cells after initial injection at the tissue site of interest. The hydrogel may increase the viscosity of the injected solution and/or act as a mechanical barrier to position cells where desired and provide a site for cell attachment. When designing the appropriate hydrogel for this application, it is essential that the gelation be compatible with cells and limit potentially toxic steps (e.g., chemical initiators, temperature, and shear forces). For this reason, natural hydrogels are often used that assemble through simple changes in salt concentration (e.g., calcium) or pH; however, numerous synthetic materials are being engineered with the appropriate consideration to cell compatibility and with more diverse properties than can be found in natural materials.

Beyond simple initial retention, hydrogels can be designed to provide protection to the injected cells and shield them from an immediate assault, and thus improve their viability in the treated tissue. This need arises from the potentially "hostile" environment of injured or diseased tissues that can deprive cells of nutrients and in some cases are rich with toxins; however, this is dependent on the specific environment (e.g., wound site) and cell type (e.g., MSCs) of interest. Hydrogels pre-loaded with factors such as oxygen or even matrix building blocks (e.g., sugars) may provide fuel for injected cells immediately

post-implantation, until such time that a more “healthy” environment is restored. Hydrogels can also provide a nurturing environment to offset and even overturn the signals from the unhealthy tissue to improve stem cell integration and functional repair. For instance, hydrogels whose backbones have been engineered to possess protease cleavable elements can serve as a “sink,” focusing activity of endogenous proteases in the wound environment on the hydrogel rather than on the native tissue itself (Purcell et al., 2014).

Due to their biocompatibility, non-thrombogenic nature, and resemblance to the extracellular matrix (ECM), alginate hydrogels have been investigated for stem cell delivery in a range of applications where gelation occurs with the introduction of a divalent cation that crosslinks anionic alginate chains. For example, alginate has been examined for the intramyocardial delivery of MSCs, in combination with a synthetic PEG patch. Transthoracic echocardiography and cardiac magnetic resonance imaging showed significantly improved cardiac function, reduced scar size, and increased microvascular density with MSC hydrogel delivery when compared to MSC injection alone, which was attributed to greater cell retention (Levit et al., 2013).

The design of novel self-assembling hydrogels inspired by the natural ECM is another approach to enhance cell survival and integration post-injection. This can be introduced with self-assembling peptides that form nanofibers or through the engineering of polypeptides with specific interactions for hydrogel assembly. For example, recombinant protein polymers that form Mixing-Induced Two-Component Hydrogels (MITCH) have been studied as vehicles to deliver adipose-derived stem cells (ADSCs) (Parisi-Amon et al., 2013). Critical properties of these hydrogels include a cytocompatible embedding protocol, requiring the simple mixing of two liquid components for assembly into a compliant hydrogel, as well as shear-thinning and self-healing thixotropic properties that allow delivery using narrow conduits (e.g., a syringe needle or a catheter). Indeed, subcutaneous delivery of ADSCs using MITCH resulted in improved survival, retention, and matrix deposition within the host when compared to cells alone or delivery using collagen or alginate hydrogels.

Such polypeptide-based hydrogels have been further engineered to introduce a secondary crosslinking to not only provide protection during injection but also promote long-term retention and viability in vivo through extended material presence. This system was termed Shear-thinning Hydrogel for Injectable Encapsulation and Long-term Delivery (SHIELD) and involved the formation of an initial PEG and polypeptide network that was further stabilized via the thermal transition of poly(N-isopropylacrylamide) chains (Cai et al., 2015). The hydrogel improved initial protection of ADSCs when compared to saline alone and the secondary crosslinking improved cell retention over several weeks after injection subcutaneously. These positive findings were attributed to a potential decrease in cell apoptosis, decreased migration from the injection site, and/or enhanced cell proliferation. This example illustrates the potential to engineer hydrogel design (e.g., prolonged stability) to enhance stem cell injection outcomes.

Arguably, hydrogels should be tailored to the specific tissue and injury of interest. While studies have demonstrated that transplanting NSCs can improve functional behavior in stroke models, survival rates of NSCs post-injection are quite low (<10%), potentially due to the inflammatory environment and limited blood supply at the injury site. Hyaluronic acid (HA) has been investigated as a hydrogel for neural applications due to its extensive hydration and presence as a matrix molecule in neural tissues. Recently, injectable hydrogels combining HA and methylcellulose were developed to have minimal swelling, undergo rapid gelation, and be bioresorbable and to attenuate the inflammatory response in the central nervous system. This combination hydrogel was examined for the delivery of NSCs to non-injured and stroke-injured adult mouse brains. A pro-survival effect of the hydrogel was observed through CD44 (receptor for HA) interactions in conjunction with better cell distribution throughout the injection site, resulting in significant functional motor recovery (Ballios et al., 2015). Thus, the engineering of specific hydrogel features such as biological and chemical functionality can improve stem cell delivery outcomes.

Finally, hydrogels may control stem cell fate and function through biophysical and biochemical signals, which has been reviewed in detail elsewhere (Guvendiren and Burdick, 2013). Hydrogel-based signals may include features such as topography, degradation, mechanics, and adhesion, which have all been shown to influence stem cell differentiation in 3D microenvironments. These design features may control in vivo outcomes beyond stem cell commitment to fate and may lead to directed paracrine signaling to influence outcomes such as inflammation and angiogenesis.

Hydrogels Can Enhance Endogenous Stem Cell Function via Direct Delivery of Signaling Molecules

In addition to the direct delivery of stem cells, hydrogels can act to deliver therapeutics at the site of tissue injury to (1) recruit endogenous cells for repair, (2) help protect cells by altering the inflammatory environment, and (3) stimulate cell function in vivo. This is an alternative to the delivery of such therapeutic molecules through cell secretion, including with engineered cells transfected to express specific molecules. Molecule delivery via hydrogels is mediated primarily through diffusion, which is controlled through hydrogel design. More specifically, diffusion and release of molecules to the environment is mediated through material crosslink density and swelling, affinity between the molecule and the polymer (i.e., association constants), and hydrogel degradation mode (e.g., hydrolysis, enzymatic) and rate. Thus, there are numerous design considerations that allow the production of hydrogels with diverse kinetics of molecule release.

Regarding stem cell homing, new therapies are being developed to recruit endogenous cells for tissue repair through delivery of exogenous signals (reviewed by Ko et al., 2013). Often, such regulatory molecules (e.g., chemokines) are elevated in response to injury and are released to the peripheral circulation to mobilize bone marrow cells (BMCs) to mediate repair. For example, stromal cell-derived factor-1 α (SDF-1 α) along with its receptor (CXCR4) are critical regulators of cell homing to the bone marrow, but they also orchestrate BMC mobilization into

the peripheral circulation and local engraftment into tissues. Thus, SDF-1 α is a target molecule for delivery from hydrogels to recruit cells to enhance the repair process. Not only do hydrogels permit the sustained presence of such molecules, but they also allow localized presentation within a target tissue of interest.

As an example, studies have shown that endogenous cell homing (and therefore repair) through myocardial delivery of SDF-1 α increases vascular progenitor cell markers in the heart, stimulates angiogenesis, and attenuates global ventricular remodeling and that knockout of the SDF-1 α /CXCR4 axis leads to deficient hematopoiesis, cardiogenesis, and vasculogenesis. Specifically, the SDF-1 α /CXCR4 axis at the site of injury homes numerous cells, such as BMCs, endothelial progenitor cells (EPCs), MSCs, and endogenous cardiac stem cells, each playing an important role in repair. SDF-1 α /CXCR4 binding activates numerous signaling pathways, including MAPK and Akt (associated with cell survival and proliferation) and VEGF (associated with vasculogenesis and angiogenesis). Due to the unique biological activity of SDF-1 α , delivery from hydrogels can enhance the period of stem cell recruitment to the myocardium. In a recent study, an engineered analog of SDF-1 α was delivered directly to infarcted myocardial tissue using an HA-based hydrogel (MacArthur et al., 2013). The chemokine was sustained for nearly 4 weeks in the tissue, enhanced EPC chemotaxis, and improved vascularity, ventricular geometry, and cardiac function (e.g., ejection fraction, cardiac output, and contractility).

Likewise, the ECM represents a source of various signals (e.g., matrix molecules and sequestration sites for growth factors and cytokines) for application in stem cell therapeutics, particularly as stem cells are controlled through their local niche. ECM-based materials are fabricated by cell

removal with detergents and various treatments that can control the retention of growth factors and cytokines, and they can be processed as implantable structures or injectable hydrogels (Badylak et al., 2009). The obtained ECM is reflective of the tissue it is derived from and thus reflects extensive potential diversity across tissue type and source. ECM molecules remain that can be degraded by local proteases (such as matrix metalloproteinases upregulated after injury), and their degradation products can themselves act as chemotactic signals to recruit endogenous cells. Features of native ECM can likewise be engineered into hydrogels, in the form of protease cleavable epitopes in the hydrogel backbone. These engineered hydrogels then serve, in bulk, as a local sink for activated inflammatory mediators that would otherwise compromise injected stem cells and degrade native tissue at the wound site. Similarly, these materials can be programmed to respond to the inflammatory environment by virtue of biofactor release that is mediated by protease degradation of the hydrogel, affording a great deal of design flexibility and “on-demand” release of factors (Purcell et al., 2014).

Future Considerations in Hydrogel Design

Although these examples represent but a few notable advances in the use of hydrogels for stem cell-based therapies, hydrogel design is changing continuously as new synthesis and processing techniques are developed. There are now numerous dynamic hydrogels that go beyond simple degradation and can respond to the environment or to external triggers (e.g., light) to alter cellular interactions. These dynamic properties can respond to the local environment, including biological signals and mechanics, to provide stem cells the appropriate environment or deliver therapeutics at different stages of repair. The ultimate hydrogel design will permit

injectability for minimally invasive implantation, protection of cells during initial stages to enhance viability and engraftment, and promotion of differentiated function and implant remodeling in a controlled manner that reflects the repair environment, while being permissive to both repair and regeneration. These novel materials, tuned to promote stem cell viability and function, may markedly improve the efficacy of such procedures, unlocking the potential of stem cell-based therapies for various diseases.

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