Molecular Therapy
Review

It's All in the Delivery: Designing Hydrogels for Cell and Non-viral Gene Therapies

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Hydrogels provide a regenerative medicine platform with their ability to create an environment that supports transplanted or endogenous infiltrating cells and enables these cells to restore or replace the function of tissues lost to disease or trauma. Furthermore, these systems have been employed as delivery vehicles for therapeutic genes, which can direct and/or enhance the function of the transplanted or endogenous cells. Herein, we review recent advances in the development of hydrogels for cell and non-viral gene delivery through understanding the design parameters, including both physical and biological components, on promoting transgene expression, cell engraftment, and ultimately cell function. Furthermore, this review identifies emerging opportunities for combining cell and gene delivery approaches to overcome challenges to the field.

Tissue regeneration following disease or injury may require exogenous inputs to augment natural healing programs and suppress inhibitory processes. Given the essential role that the extracellular matrix (ECM) has in maintaining the physiological stability of the microenvironment and guiding tissue-specific function, biomaterial scaffolds have been designed to mimic this environment in an effort to promote tissue regeneration and to serve as vehicles for cell transplantation to promote survival, differentiation, and engraftment. Hydrogels, a class of biomaterial scaffolds, are highly hydrated crosslinked polymer networks that have been constructed from a wide range of both naturally and synthetically derived polymers.[1–4] Several key characteristics of hydrogels make them particularly well suited for mimicking the ECM, namely their biocompatibility;[5–7] their permeability to oxygen, nutrient growth factors, and metabolic waste;[8,9] their tunable mechanical properties;[10–12] and their tissue-like viscoelastic characteristics.[13,14]

Whereas hydrogel designs largely depend on the location of implantation (e.g., tissue type to be repaired or type of stem cell to be delivered), several design parameters can be manipulated to mimic the native ECM and consequently function to promote new tissue formation and stem cell engraftment (Figure 1).[15,16] On the most fundamental level, hydrogels should define a 3D space for tissue formation, as the 3D architecture can support the infiltration and assembly of host cells into structures and induce gene expression programs associated with normal growth or development. Cell seeding and infiltration of the hydrogel scaffold can be facilitated by micron-scale porosity and/or cell-mediated degradation cues introduced into the hydrogel design. Furthermore, ECM-derived adhesion peptides, protein fragments, or native proteins can promote cell adhesion, leading to a number of specific cell processes, such as vascularization, bone regeneration, or the creation of a tissue-specific niche.[17,18] Trophic factors may also be necessary within the environment to drive cellular responses, leading to stem cell differentiation and tissue formation. Although localized and sustained release of trophic factors from biomaterials has been a research focus for nearly 20 years,[19,20] substantial challenges remain regarding formulations that retain activity without developing immune responses[21] or unintended side effects due to high doses. Alternatively, the delivery of gene therapy vectors encoding for trophic factors or other tissue inductive factors may avoid some of the challenges associated with protein delivery while providing the opportunity for sustained and localized availability of the factor. Finally, tissue engineering and regenerative medicine may be nearing a tipping point based on recent investments by industry and an increasing number of clinical trials. For translational purposes, the design approach should consider manufacturing issues, such as availability, reproducibility, and processing strategies, and generally the issues that will be needed for US Food and Drug Administration (FDA) regulatory approval and commercial viability.

This review will describe recent advances in the design of hydrogels as vehicles for cell and non-viral gene delivery to promote tissue regeneration. We discuss the hydrogel design parameters that are available to enhance transplanted cell survival and appropriate interactions with the host tissue. Additionally, we discuss the challenges and progress in the incorporation of non-viral vectors within hydrogels to induce the expression through bioactive cues that promote tissue repair. The manuscript focuses on non-viral approaches due to the greater flexibility in vector design, and interested readers can find excellent reviews on viral vector delivery from biomaterials.[22,23]

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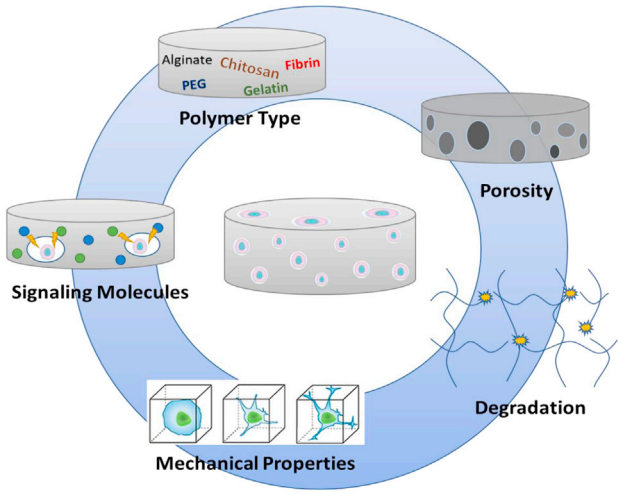

Figure 1. Design Considerations
The hydrogel design for delivery in tissue engineering applications is dictated by the biocompatible polymer type, porosity, mechanical properties, degradability, and signaling factors in the microenvironment.

Hydrogels as Vehicles for Cell Delivery

The delivery of cells has most typically involved the injection of high-density cell suspensions into the target diseased or injured site. However, such direct cell injection methods often have a poor therapeutic response due to a rapid decrease in cell viability, low or modest engraftment of transplanted cells, and limited control over cell fate due to the local environment.[24–26] The delivery of transplanted cells in a scaffold comprised of biocompatible materials addresses these limitations by initially providing protection to the transplanted cells that can enhance survival and prolong retention at the site.[27] Moreover, hydrogels often have physical properties that are similar to the native ECM, such as their mechanical properties and water content.[28] This mimicry of the niche environment can have a considerable impact on cell fate by influencing the morphology, viability, differentiation, and function.[26,29] The two main strategies in utilizing hydrogels for stem cell transplantation are non-integrating approaches that isolate cells from the host tissue through encapsulation within the hydrogel and integrating approaches that allow for the transplanted cells to directly contact host tissue either immediately through a microporous design or over time through biodegradation.[3,30,31] Several examples of hydrogel systems designed for delivering cells in a range of regenerative medicine applications are listed in Table 1. The number of systems investigated in pre-clinical models is large and cannot be listed exhaustively; thus, we have focused on relatively recent publications that are approaching large animal or clinical translation. This section will discuss considerations in these hydrogel designs related to the survival of transplanted cells as well as their ultimate function.

Cell Delivery in Encapsulating Hydrogels

The key role of an encapsulation device is to create an environment that allows for normal cell function, while acting as an immune-regulatory barrier through isolation or modulation of the local area for better survival of the transplanted cells.[32–41] This function can be manipulated by the gelation process, the hydrogel structure, as well as material composition.[30] A common encapsulation approach is illustrated by the TheraCyte device, which has a porous vascularizing outer membrane that promotes tissue integration and an inner impermeable membrane that protects the transplanted allogeneic islets.[42,43] Neonatal pancreatic tissue was implanted in non-obese diabetic mice, survived, and had a response to glucose levels for at least 50 days.[44] Although this original device was not successful in clinical trials, the general strategy has evolved over the course of several companies, including Living Cell Technologies, Beta Logics, Viacyte, and Encaptra. This Encaptra device consists of a single membrane that is immunoisolating while permitting oxygen and nutrients to pass. Viacyte is currently carrying out a phase I/II clinical trial using this device with stem-cell-derived cell sources to assess the safety and efficacy in humans.[45] Other encapsulation devices that have reached clinical trials have been recently reviewed in detail.[46] Whereas these devices provide a clinically translational design for encapsulation delivery, hydrogels provide the same opportunity to overcome barriers, like immune cell infiltration, plus enhanced transport and more tunable properties. In a hydrogel, adhesion sites and biomechanical properties can be manipulated within the gel to enhance cell viability and therapeutic efficacy. Hydrogels are now being developed that utilize the foundational delivery approach provided by the TheraCyte design while offering tunable properties for not only the exterior but the interior of the device to enhance cell motility, viability, and function.

Alginate is a natural polymer derived from algae that has been extensively investigated for cell encapsulation due to its biocompatibility, low toxicity, relatively low cost, and mild gelation by addition of divalent cations, such as Ca^{2+}.[47–49] Alginate can also be modified to improve cell attachment and motility. A double-layered alginate hydrogel system consisting of matrix-metalloproteinases and Arg-Gly-Asp (RGD) peptide in the inner layer was designed to allow transplanted stem cells to proliferate and mobilize to the outer layer following the inflammatory storm caused from surgery.[50] Following transplantation of neural stem cells (NSCs) into a rat brain trauma model, the double-layered alginate hydrogel promoted survival and differentiation of the NSCs. This overall approach focused on NSCs, which have a reduced risk of teratoma formation compared to human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), but the design could be easily adapted to other types of transplanted cells. Alginate-based biomaterials have had great success in rodent models; however, the translations to larger animal models, such as monkeys and humans, have not been immediate successes.[51] Although there were no detectable inflammatory responses in human blood,[52–55] the limited efficacy of two clinical transplantations of human islets in barium-alginate and calcium and barium-alginate spheres has been partially attributed to a foreign body response after transplantation.[56–58] Recently, fibrosis has been reported to be reduced or eliminated based on the diameter of the spheres.[38] Alternatively, alginate has been functionalized with a range of chemical groups in order to screen for chemistries that would avoid

Table 1. Cell Delivery Applications in Natural and Synthetic Hydrogels

	Hydrogel Material(s)	Cells Delivered	Delivery Strategy	Target Application	Ref.
Encapsulated hydrogels	alginate	pancreatic islets	laparoscopic implant of microcapsules	diabetes	184
		adipose-derived and mesenchymal stem cells	injection co-delivered with BMP-2	bone regeneration	185
		CAR-programmed T cells	implanted into the peritoneal or tumor resection cavity	cancer	186
	polyethylene glycol and alginate	ovarian follicles	encapsulated scaffold	ovarian function	177
Tissue-integrating microporous hydrogels	gelatin	adipose-derived stromal cells	microporous microribbon hydrogel injected into cranial defect	bone regeneration	76
	collagen	autogenous chondrocytes	porous scaffold matrix	cartilage repair	82
	alginate	mesenchymal stem cells	injectable, void-forming microporous hydrogel	bone regeneration	178
Biodegradable hydrogels	collagen	iPSC-derived hepatocytes and endothelial cells	encapsulated polyelectrolyte fiber scaffold transplanted into liver	liver tissue regeneration	180
		neonatal astroglial cells	transplanted gel into lesion of spinal cord	spinal cord regeneration	181
	hyaluronic acid	cardiac progenitor cells	subcutaneous injection	angiogenesis	182
		neural progenitor cells	injection into stroke cavity	neural regeneration from stroke	183
	gelatin	cardiac-derived stem cells	intra-myocardial injection with the controlled release of bFGF	cardiac regeneration	172
	polyethylene glycol with degradable peptide crosslinker	adipose-derived stem cells	*in situ* injection with encapsulated siRNA	bone regeneration	160
		iPSC-derived endothelial cells	injection co-delivered with VEGF	muscle repair	173
	fibronectin and agarose	cardiac stem cells	*in situ* injection	cardiac regeneration	174
	fibrin	human embryonic stem cells	epicardial delivery of encapsulating gel	cardiac regeneration	175
		adipose-derived mesenchymal stromal cells	patch applied to surface of skin	wound healing	187

a fibrotic response.[39,40] Vegas et al.[41] recently identified chemically modified alginates, such as triazole-thiomorphiline dioxide (TMTD), as hydrogels that resisted fibrosis around the implant in both rodents and non-human primates. The TMTD alginate hydrogel was then used to transplant hESC-derived β cells into immune-competent streptozotocin (STZ)-treated C57BL/6J diabetic mice. The hydrogel showed no observable foreign body response and supported the engraftment and long-term glycemic correction (174 days with the mice still euglycemic at the end of the experiment) from hESC-derived β cells in immune-competent mice.[43] These results lay the groundwork for studies in autoimmune animal models and future human studies using hydrogel formulations that overcome the immunological barrier inhibiting long-term cell function.

Materials derived from natural materials have had a long history as hydrogels; however, synthetic polymers have become a popular substitute because they provide a more clinically translatable model and more reproducible properties. For these purposes, non-degradable polyethylene glycol (PEG) hydrogels have been widely used for encapsulation. Also, PEG's tunable viscoelastic properties provide a tissue-like permeable membrane with minimal inflammatory response. By applying a conformal coating around islets consisting of PEG and Matrigel, Manzoli et al.[59] demonstrated a strategy for long-term reversal of diabetes with allogeneic islets transplanted in the epididymal fat pad in mice (Figure 2). The incorporation of Matrigel into the PEG coating provided important ECM interactions while keeping the permselectivity low, which resulted in a lack of immune cell penetration and T cell allogeneic priming. In addition to biocompatibility, PEG-based hydrogels are also utilized for their structural support[60] and ease of functionalization.[61] Studies focused on artificial ovarian tissue delivery have shown encapsulated immature ovarian follicles in PEG-RGD hydrogels can enhance primordial follicle development and graft survival compared to non-encapsulated follicles.[62] After a subcutaneous transplant of encapsulated ovarian tissue, ovariectomized adult mice showed restoration of the estrous cycle within two weeks. In contrast to islets, ovarian follicles are avascular and relatively resistant to hypoxia, allowing them to maximize the benefits of immunoisolation methods.

For cells that are more dependent on graft vascularization, a PEG hydrogel was designed using lithography techniques in order to develop an encapsulating gel that had microchannels for vascularization (Figure 3). This encapsulating strategy combines encapsulation with printing systems to generate microchannels in pre-defined regions

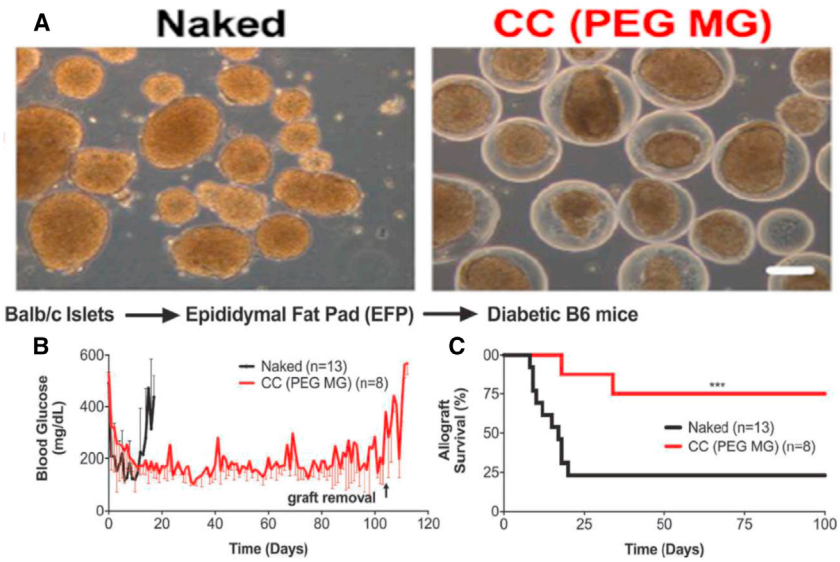

Figure 2. PEG-MAL Matrigel Conformed Coated Islets Transplanted in the EFP Site Reverse Diabetes Long-Term in Murine Allografts without Immunosuppression

(A) Phase contrast (scale bar, 100 μm) images of naked and PEG-Matrigel conformal coated (CC (PEG MG)) islets from Lewis rats. (B and C) Blood glucose of recipient mice (B) and survival (C) of 750–1,000 islet equivalent (IEQ) naked (black; n = 13) or CC (PEG MG) (red; n = 8) islets from BALB/c mice transplanted into fully major histocompatibility complex (MHC)-mismatched chemically induced diabetic B6 mice in the epididymal fat pad using fibrin scaffolds without any immunosuppression.[190] (***p < .001.)

of the nano-porous hydrogel.[63] The scaffold architecture presented here can be designed to improve the transport of nutrients and oxygen to encapsulated cells. Furthermore, the network of larger sized channels could facilitate the invasion of the host vasculature after implantation or be employed for pre-vascularization *in vitro*. Their studies revealed that transplanted islets in these gels had tissue and vascular in growth within the microchannels, which promoted normoglycemia after transplantation and sustained glucose control over the two-month period of study until removal of the device. Collectively, numerous cell encapsulation systems are being developed for the treatment of various diseases. Current advances in material design, immunomodulation, and encapsulation strategies will be critical in addressing the many challenges that are involved in transitioning these cell-based therapies to the clinic.

Cell Delivery with Tissue-Integrating Hydrogels

Hydrogels are also formulated into either a microporous structure or are made degradable in order to facilitate integration with the host tissue (Figure 4). The integration with the host tissue can be advantageous for vascularization to provide nutrient transport necessary for survival and appropriate cell-cell contact that can direct differentiation.[64–66]

Microporous Hydrogels for Cell Delivery. Scaffolds with an interconnected microporous structure can be seeded with cells, and upon transplantation, host cells can infiltrate for integration with the transplanted cells.[67] The microporous structure allows for nutrient transport and waste removal, while also providing a substantial surface for cell adhesion and space for tissue growth.[68,69] Compared to encapsulated designs, porous hydrogels can encourage cell migration and cell-cell interactions, which play an important role in regulating cell proliferation, differentiation, and the organization of some engineered tissues (e.g., cartilage and liver).[58,70] These cellular activities can be inhibited or delayed when cells are encapsulated into gel scaffolds because they are entrapped in the 3D polymer networks. As a result, the incorporation of cell living spaces that are larger than tens of microns in diameter in gel constructs can support cell spreading, migration, proliferation, and then vascularization for the establishing access to nutrients and other systemic cues.[71]

Microporous hydrogels have been developed with many materials for *in vitro* and *in vivo* use through a variety of methods, including sphere templating,[72–76] lyophilization,[77–79] and porogen leaching.[80–83] A challenge that comes with the prefabricated design of microporous scaffolds is uniformly distributing cells throughout the scaffold to promote effective regeneration of highly intricate tissues. Whereas encapsulation strategies allow for cells to be uniformly distributed during initial fabrication, microporous hydrogel designs with a tunnel-like network could diminish the ability to achieve heterogeneous cell distribution. Several seeding techniques have been established to improve uniform cell distribution, but many are lengthy and restrict clinical applicability.[84,85] The most common method for seeding microporous scaffolds is static seeding, in which a concentrated cell suspension is passively introduced on a scaffold. This technique, however, has several limitations that result in low seeding efficiency and poor cell penetration.[86] To improve cell seeding efficiency, Tokatlian et al.[87] utilized a two-phase hydrogel technique where μ-pore 3.5% hyaluronic (HA) hydrogels were formed and then seeded with mouse mesenchymal stem cells (MSCs) mixed into a thin 2.5% nano-pore HA-matrix metalloproteinase (MMP) hydrogel precursor solution (Figure 5). Due to the fluid nature of the precursor solution initially at room temperature, the gel solution flows throughout the pores of the porous hydrogel and distributes the cells uniformly. In sharp contrast to the classical approach of seeding cells on a preformed hydrogel, 3D bioprinting can simultaneously seed cells while fabricating the hydrogel to produce highly organized cellular constructs.[67] This strategy helps to overcome obstacles derived from low cell densities, uncontrollable seeding positions, and a heterogeneous distribution of cells throughout the scaffold. Kolesky et al.[68] recently demonstrated a new bioprinting method using cell-laden GelMA inks as a bioprintable bulk matrix to build vascularized heterogeneous tissue

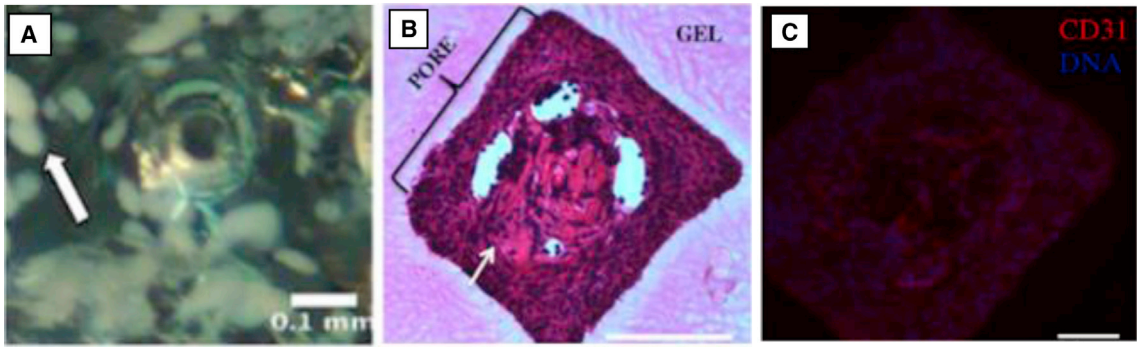

Figure 3. Macroencapsulating PEG Hydrogel Devices with Microchannels
(A) Encapsulated islets surrounding a microchannel in a hydrogel. Islets appear opaque, and a white arrow indicates a representative islet. (B) Cellular ingrowth (indicated by a white arrow) occurred within microchannel regions of hydrogels relative to the surrounding hydrogel. (C) CD31-positive cells are present in the microchannels with a nuclear counterstain. The scale bars represent 100 μm.[84]

constructs. These 3D microengineered systems consisting of vasculature, multiple types of cells, and ECM are able to generate an environment for cell adhesion, remodeling, and migration.

A common caveat of the microporous design is that these scaffolds are formed into a specific shape prior to implantation, which limits the ability to target a specific site.[69] Interestingly, granular materials can be used to generate injectable yet porous materials by injecting microscale hydrogel particles and having them assemble into a bulk granular material. The first example of this approach was termed microporous annealed particle (MAP) scaffolds, where microscale hydrogel spherical beads are injected into a cavity and subsequently annealed to each other to form a bulk gel with the space between the beads serving as pores in the gel. Whereas the void fraction in MAP scaffolds is limited to approximately 20% rather than 80% for other porous hydrogels,[88] MAP scaffolds have a continuous micron-sized porous structure that allows both transplanted cells and surrounding host tissue to infiltrate the scaffold without the need for material degradation. In addition, MAP gels have the ability to conform to the wound shape and promote integration to form cellular structures within days after injection.[89] As a result, MAP hydrogel injection into skin and brain wounds have shown lower inflammation at the wound sites.[89–92] The microporous injectable hydrogel design could expand the applicability of microporous hydrogels through its injectable nature. Recently, another example of granular hydrogels utilizes particle jamming to avoid the need for particle annealing.[74] This work utilizes the phenomenon that jammed granular material behaves as a solid.

Similarly, a cytocompatible fabrication process was recently developed for generating microporous hydrogels with encapsulated cells using gelatin as a leachable porogen.[93] The hydrogel was designed with a wide range of porosities and pore sizes by crosslinking oligo(poly[ethylene glycol]fumarate) in the presence of MSCs and varying sizes of gelatin microspheres. Encapsulated MSCs exhibited high viability immediately following the fabrication process, and culture of cell-laden hydrogels revealed improved cell viability with increasing porosity. An alternative technology is based on using microribbon (μRB)-like gelatin as scaffolds, in which the ribbons are building blocks forming a macroporous structure.[76] Their results suggested that enhancing cell survival and proliferation using a μRBs microporous design further promoted the paracrine-signaling effects of adipose-derived stem cells for stimulating endogenous bone repair. A commercially translated microporous design has been utilized for NeoCart (Histogenics, Waltham, MA, USA), in which autologous chondrocytes are cultured on porous bovine type 1 collagen scaffolds (Figure 6) for the repair of cartilage defects in the adult knee.[78] Whereas the 3D matrix bears load, its open structure allows for influx of MSCs, which ideally differentiate into chondrogenic lineage.[79] A FDA phase II trial comparing NeoCart to microfracture showed significantly better results in all clinical outcome measures in the NeoCart-treated patients.[82] Histogenics recently completed patient enrollment of its NeoCart Phase 3 clinical trial in accordance with the Special Protocol Assessment (SPA) agreement with the FDA. They expect to report ongoing studies next year, followed by a potential Biologics License Application (BLA) filing.[83] The efficacy of these novel design methods for microporous hydrogels demonstrates the promise of cell-based therapies for enhanced long-term tissue regeneration outcomes.

Degradable Hydrogels for Cell Delivery. Encapsulating hydrogels that are degradable can initially function as an immunoisolation barrier similar to the encapsulating hydrogels of section Cell Delivery in Encapsulating Hydrogels, yet their degradability over time can allow for improved cell infiltration and integration with the host. Hydrogel degradation can lead to changes in mechanics and swelling over time, which will in turn affect cell behavior.[94] By utilizing degradable reagents (i.e., peptides, ligands, or proteases) to form unstable bonds, hydrogels can undergo degradation through hydrolytic or enzymatic mechanisms.[95] A common principle is that the degradation rate of the hydrogel should match the rate at which the tissue grows or infiltration is desired. Recently, Lima et al.[96] used biodegradable alginate to fabricate beads encapsulating rat MSCs and fibronectin and implanted the particles in a calvarial bone defect in order to evaluate

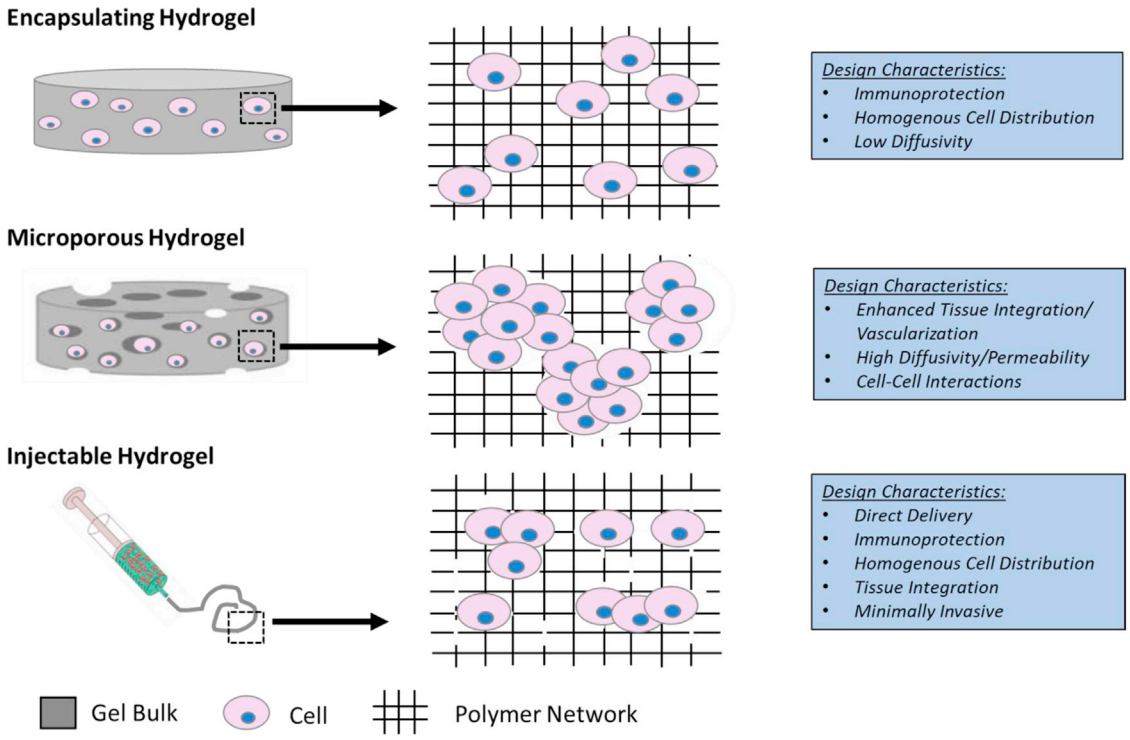

Figure 4. Schematic Representation and Design Characteristics of Cell-Laden Hydrogels

their potential for bone tissue regeneration.[97] The hydrogel's rate of degradation could be controlled to permit accelerated bone tissue growth while preventing cell loss and any toxic exchange of molecules with the surrounding environment.

Many natural polymers are biodegradable, yet their utility in tissue engineering applications can be constrained by the intrinsic properties of the materials. In contrast, synthetic polymers provide an opportunity to control degradation through well-defined mechanisms, such as controlling the crosslinking density of such segments.[98,99] For example, the hydrogel can be crosslinked by reacting the backbone polymer with a peptide sequence that can be degraded by specific cell-secreted proteases endogenous to the wound healing microenvironment, such as matrix metalloproteinases.[100–104] In a recent study, PEG gels co-encapsulating chondrocytes and MSCs were crosslinked with an MMP-degradable peptide in order to match the resorption of the scaffold with the rate of matrix production by cells during cartilage repair.[105] Relative to non-degradable hydrogels, those that allowed for cell-mediated degradation showed significantly increased GAG and collagen deposition, which are key markers for chondrogenesis.[106] Another design consideration utilizing degradative properties evaluated a two-component synthetic PEG hydrogel macrodevice for the delivery of islets to an extrahepatic transplant site (Figure 7). The hydrogel consists of an inner layer crosslinked with a non-degradable PEG dithiol and a degradable outer layer crosslinked with a proteolytically sensitive peptide to enhance localized vascularization. Encapsulated islets demonstrated high viability within the device, and implementation of a vasculogenic, degradable hydrogel layer increased the vascular density around the transplant site. Whereas normoglycemia was not achieved with the device, suggesting that parameters like islet load require further optimization, the results highlight the benefit of degradable interfaces for the promotion of engraftment.

Another application for biodegradable hydrogels is as injectables, which have an unparalleled advantage for delivering cells to specific sites with minimally invasive procedures by undergoing gelation *in vivo*.[107] Injectable hydrogel polymerization can occur as a response to temperature or pH change, ionic cross-linking, solvent exchange or crystallization, or simply thickening upon removal of the injection shear.[93,108] One of the most common polymers used for these biomedical applications is poly (N-isopropylacrylamide) (PNIPAM) due to its lower critical solution temperature being very close to body temperature.[109,110] A recent study evaluated PNIPAM-containing hydrogels used as carriers for intramyocardial delivery of brown-adipose-derived stem cells in rats with myocardial infarction.[111] The hydrogel displayed rapid, subphysiological phase transition temperatures and was capable of noninvasively delivering a liquid suspension of cells that gels *in situ* forming a cell-loaded scaffold, essentially isolating treatment to the injection site. In addition, engraftment around the transplanted cells was significantly enhanced (Figure 8) and therapeutic efficacies were augmented in the myocardial

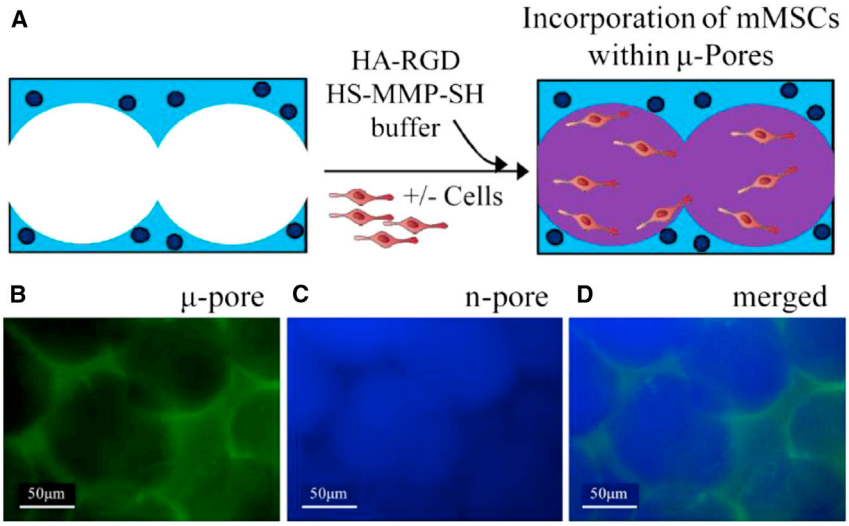

Figure 5. Enhanced Cell Seeding within Porous HA Hydrogel Using a Two-Phase Hydrogel Technique
(A) To effectively seed cells and allow for rapid cell spreading, cells were seeded within the pores of a 3.5% μ-pore HA gel directly with a soft 2.5% HA gel. (B and C) To visualize each phase separately, the μ-pore phase was stained with fluorescein isothiocyanate (FITC) (B), and the inner, n-pore phase was stained with Alexa Fluor 350 (C). (D) Merged fluorescence image of a two-phase hydrogel made using 100 μm beads.[98]

infarction. This research provides new treatment opportunities for diseases like cardiac ischemia, which have seen limited therapeutic success in part due to poor targeting. Gaffey et al.[112] addressed this concern by developing an injectable, shear-thinning HA hydrogel that delivers endothelial progenitor cells to ischemic myocardium. The hydrogel was designed to flow through a syringe with the application of shear force and then re-assemble at the injection site. *In vivo* improvements from this delivery strategy included enhanced cell retention and vasculogenesis, limited adverse remodeling, and improved cardiac function.

These injectable biodegradable hydrogels may also be modified with trophic factors to enhance cell survival or function. Vascular endothelial growth factor (VEGF) has been incorporated into degradable PEG hydrogels encapsulating islets in order to promote localized vascularization.[97] Transplantation of the *in situ*-forming injectable hydrogel at an extrahepatic site supported engraftment and reversal of diabetes, which was not achieved without VEGF. Xu et al.[113] applied a similar approach by using a basic fibroblast growth factor (bFGF) release system to increase MSC survival in a thermoresponsive, biodegradable hydrogel. The incorporation of the pro-survival factor bFGF within the hydrogel improved MSC viability after intramuscular injection, as well as increased blood vessel density, limb perfusion, and muscle diameter. Whereas the functionalization of these hydrogels enhances both oxygen and nutrients to the target site, the hydrogel's ability to promote neovascularization is still being investigated because the process of angiogenesis typically requires days to weeks. These recent studies demonstrate the critical role that local degradation seems to play within tissue engineering constructs.

Hydrogels have received increasing interest as a leading candidate for engineered tissue scaffolds due to their superior biocompatibility and inherent similarity to the natural ECM, in addition to their conducive framework for cellular proliferation and survival. These recent advances discussed above highlight important considerations for designing hydrogels for effective cell delivery. Encapsulation approaches can potentially eliminate the barriers preventing transplantation of xenogeneic or stem-cell-derived allogeneic cells. However, hypoxia can occur at the core of the gel and limit the duration that these cells function. Meanwhile, hydrogels that promote tissue integration through microporous structures or biodegradation are challenged by the immune response but can enhance vascularization and *in vivo* tissue growth. Also, incorporating therapeutic molecules and particles into these hydrogels is another method to guide transplanted cell behavior and function. With continued research in these areas focused on incorporating the benefits of both approaches, hydrogel-based tissue engineering will continue to make advances toward clinical restoration of tissue function.

Hydrogel-Mediated Gene Delivery in Regenerative Medicine

Cell delivery directly provides the cells necessary for tissue formation; however, the function of these cells may need to be augmented by the incorporation of additional factors that promote pro-regenerative processes. The delivery of proteins has been employed to promote repair mechanisms; however, to date, protein delivery for tissue regeneration applications has seen limited success despite several clinical trials, where most success has been observed with decellularized matrices. Therefore, alternative approaches to induce gene expression programs associated with normal growth or development are needed. One such approach is to target gene expression directly through the delivery of genes. In this approach, genes encoding for key transcription, trophic, or growth factors would be delivered from the scaffold to transfect transplanted or infiltrating host cells, which would in turn express the delivered protein to stimulate pro-regenerative behaviors. This strategy has the potential to provide more control over the duration of expression, the activity of the delivery protein, and the delivery of multiple signals from the same hydrogel. Consequently, these benefits can be applied to overcome obstacles that limit the therapeutic efficacy of cell-based therapies, like sufficient vascularization or immune rejection. Furthermore, the use of non-viral approaches avoids safety concerns surrounding the use of viral vectors, including immunogenicity associated with repeated injections and insertional mutagenesis.[114,115] However, it has been difficult to translate non-viral vectors to the clinical stage, mainly

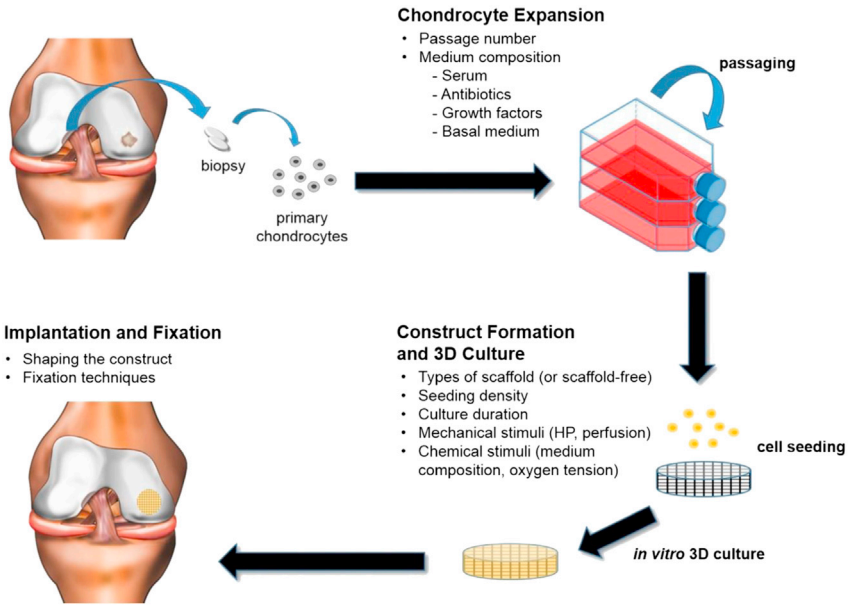

Figure 6. Tissue Engineering Strategies Used in Current Clinical Products
A schematic of the classical tissue engineering paradigm used for the fabrication of the reviewed cartilage products.[108]

due to the primary challenge of achieving sufficient transgene expression to elicit a significant therapeutic response.

Local gene delivery through implantable or injectable hydrogels has been investigated in two different manners—either as a depot that houses plasmid DNA for sustained release into surrounding tissue[102] or as a DNA-loaded biomimetic scaffold that encourages cellular infiltration into the scaffold. In this second approach, cells infiltrate and degrade the scaffold, leading to transfection and local expression of the therapeutic gene. In general, the same scaffold design principles used for cell delivery can apply for gene delivery. In addition, the development of DNA-loaded hydrogels must take into account not only what gene is being delivered but also the plasmid components, hydrogel properties that affect DNA loading and release, and the interactions between cells and the scaffold that affect gene transfer, with the ultimate goal of promoting effective transgene expression and, consequently, a significant therapeutic benefit.

Delivering Pro-regenerative Genes to Enhance Therapeutic Outcomes

The selection of the transgene to be delivered is highly dependent on the target regenerative process. From the first report of scaffold-mediated non-viral gene delivery *in vivo* in 1996 to date, most reported regenerative medicine strategies have delivered genes encoding for native-form secreted growth factors that are functional in the extracellular microenvironment, primarily targeting vascularization and bone formation (Table 2).

In delivering genes (e.g., VEGF) to promote vascularization in the site of scaffold implantation, researchers have evaluated changes not only in vessel count but also in indicators of vessel maturation, such as vessel thickness, length, and the presence of smooth muscle cells around endothelial structures.[103,116] Such strategies are motivated by the key role vascularization plays in repair processes, such as cutaneous wound healing and cardiac repair, in order to regenerate functional tissue. Studies investigating the application of gene-loaded scaffold implants in bone defects have evaluated the extent of cell infiltration and observed new bone formation in the implantation site.[104,105]

Although some of these studies have reported significant therapeutic responses, much room for improvement remains regarding transgene selection as well as of transgene optimization via engineering to enhance its potency to allow for sufficient potential for clinical translation. One less explored strategy is to deliver genes encoding for proteins other than growth factors; in fact, out of these studies, all but one have delivered genes encoding for growth factors from hydrogels.[103] This study delivered 10 μg of a gene encoding for a hypoxia-insensitive variant of the transcription factor hypoxia-inducible factor 1-alpha (HIF-1α) as opposed to its native form from a fibrin matrix administered to a murine cutaneous wound healing model (Figure 9). The authors observed increased levels of angiogenesis and more mature vessel formation as compared to the delivery of 1.25 μg of the VEGF-A_{165} protein per wound, demonstrating the potential to enhance the potency of therapeutic genes through engineering and judicious selection of gene targets.[117] Given the ease of diffusion of growth factors away from the target site, more investigation is suggested in delivering genes encoding for proteins that are active intracellularly in order to enhance the potency of the delivered payload. Expanding the range of genes for consideration may eventually lead to the development of a therapy that is therapeutically promising and clinically relevant.

In addition to the target gene itself, other elements in the expression cassette, such as promoters and post-regulatory elements, can also control the transgene expression profile and therapeutic response. The magnitude of transgene expression from various constitutive and inducible promoters can vary widely both in comparison to one another and in different cell lines, suggesting that much attention should be placed in promoter selection.[118] Attention to these differences is needed in *in vivo* scaffold-mediated gene delivery applications as well, as demonstrated by a study that compared the ubiquitin C (UbC) and cytomegalovirus (CMV) promoters in driving transgene expression from a scaffold *in vivo*.[119,120] In particular, although the CMV promoter is a commonly used constitutive promoter in

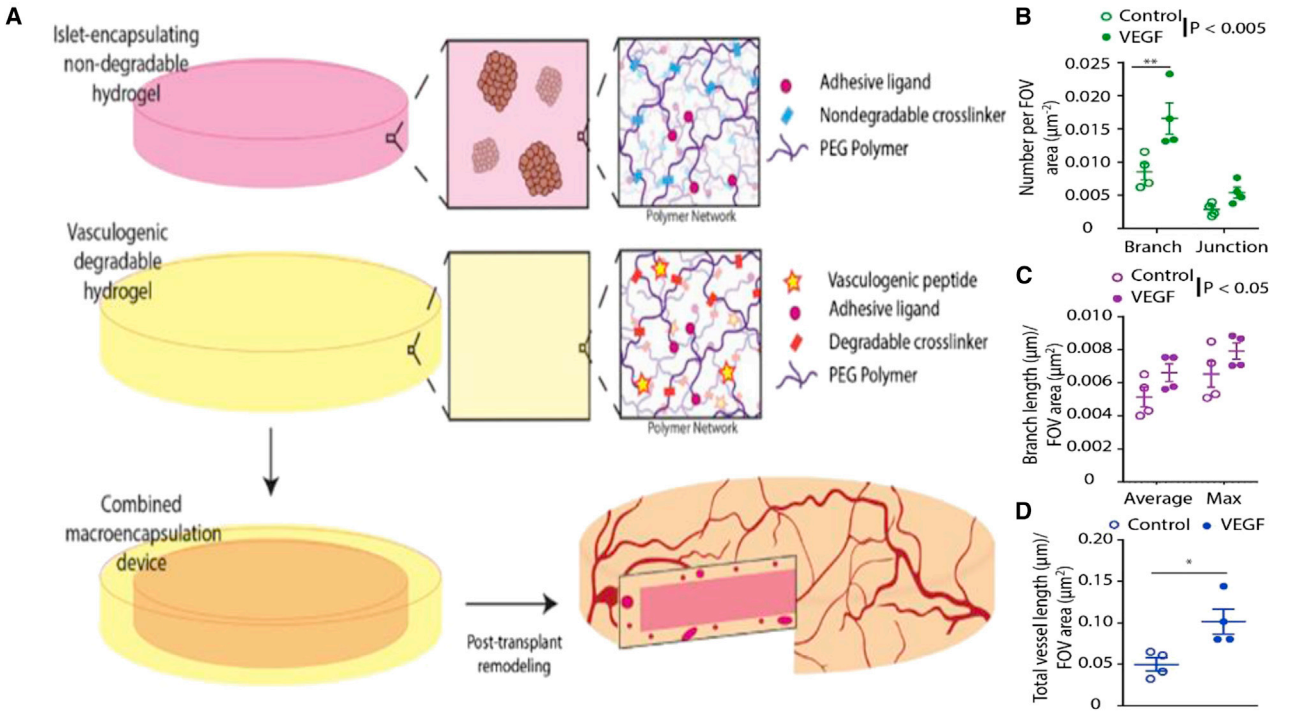

Figure 7. Poly(ethylene glycol)-Based Hydrogel Designed to Encapsulate Islets Shows Vascular Remodeling in the Omentum with Vasculogenic Layer
(A) Schematic for synthetic hydrogel macroencapsulation device design. A non-degradable synthetic hydrogel disk is surrounded by a degradable, vasculogenic hydrogel that remodels to promote device vascularization post-transplantation. (B–D) Surface vascularization was characterized and quantified for number of (B) vessel junctions and branches, (C) average and maximum branch length, and (D) total overall vessel length per field of view (FOV) (n = 4/condition; FOV = 5–8/n). *p < 0.05; **p < 0.005.[105] Error bars, SEM.

mammalian gene expression, this study showed that replacing it with a UbC promoter resulted in higher and more sustained local transgene expression from DNA-loaded scaffolds implanted in mouse intraperitoneal fat, and expression in the CMV condition quickly decreased. Although many promoters of viral origin, such as CMV, exhibit strong initial transgene expression, they can also exhibit methylation, resulting in transcriptional silencing over time in mammalian systems.[121] Furthermore, the development of hypoxia-inducible promoters to control gene expression in ischemic environments is particularly relevant to tissue regeneration applications.[122,123] Cell- or tissue-specific promoters may also be considered, especially with respect to the types of cells infiltrating the scaffold or being transplanted with the scaffold. Lastly, post-regulatory elements, such as woodchuck hepatitis virus (WHP) posttranscriptional regulatory element (WPRE), can also be added to the expression cassette to enhance mRNA stabilization to result in enhanced and longer expression; however, this effect is strongly dependent on the promoter and cell line used.[124,125] Future studies may be performed to test other plasmid components in order to further enhance transgene expression levels to achieve clinical potential.

Another source of transcriptional silencing in transgene expression in mammalian systems due to DNA methylation is the presence of elements of bacterial origin, namely CpG motifs in commonly used plasmid vectors. The use of minicircle vectors, which are circular DNA molecules free of any sequences of prokaryotic origin and typically only consist of the expression cassette, instead of conventional plasmids eschews the risk of immunogenic responses to material of bacterial origin, resulting in reduced inflammation and the avoidance of transgene silencing through methylation of bacterial motifs.[126–129] Whereas minicircles are more complex to produce, especially on a larger scale,[130] preliminary studies administering minicircle DNA have demonstrated the potential of this application to enhance and prolong transgene expression *in vivo*.[131–134] In the first study, intramyocardial and intramuscular injection of minicircle encoding for a marker gene resulted in local expression at least two orders of magnitude higher than a plasmid injection. In addition, minicircle administration was also compared to administration of an adeno-associated virus (AAV); whereas a first intramuscular injection of AAV resulted in higher expression than the minicircle injection, a repeat minicircle injection 28 days later resulted in identically strong expression and a repeat AAV injection resulted in no expression, a response attributed to both a cellular and humoral immune response against the virus (Figure 10).[131,134] This result demonstrates the potential of minicircle vectors to drive expression levels that are even higher than viral administrations. Furthermore, minicircle delivery of the HIF-1α gene via injection into the site of a myocardial infarction resulted in significantly

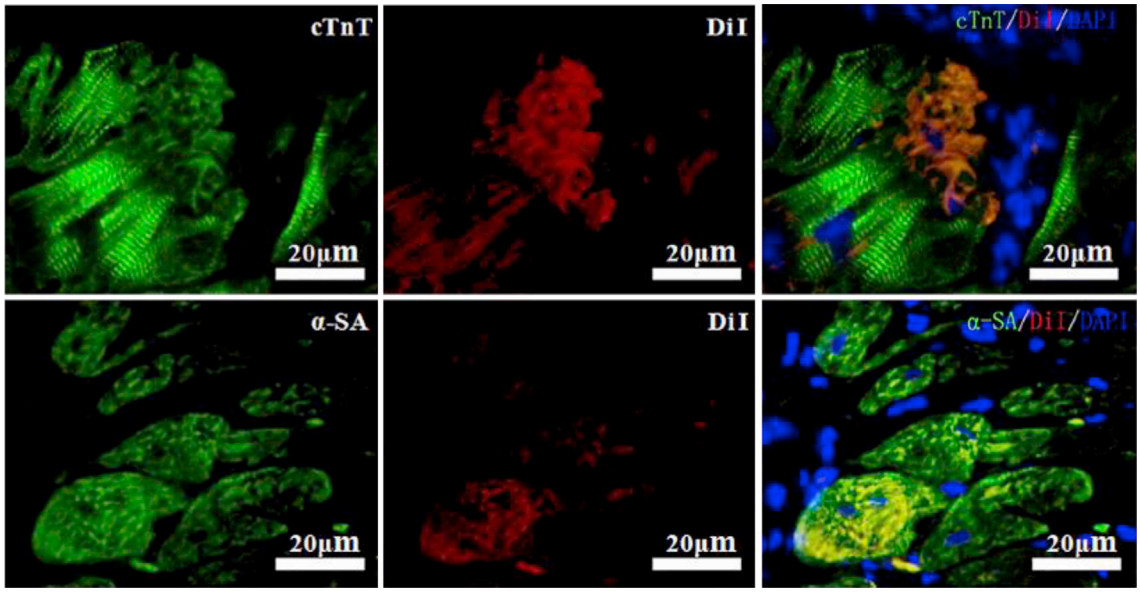

Figure 8. Cardiac Differentiation of Brown Adipose-Derived Stem Cells in Myocardial Environment

4 weeks after transplantation, immunostaining against cardiac markers was performed on cardiac sections. Colocalization of DiI and cardiac proteins (cTnT and a-SA) was observed, indicating differentiation of transplanted cells toward cardiac lineages.[191]

greater improvement of ejection fraction compared to plasmid delivery.[134] In the second study, implanting poly(lactic-co-glycolic-acid) (PLGA) scaffolds loaded with minicircle delivering the BMP-2 gene enhanced bone repair in a murine calvarial defect model compared to a control condition delivering a marker gene; however, no comparison to an analogous plasmid delivery system was reported.[117]

Gene Carrier Considerations and Loading of DNA Therapeutics into Hydrogels

Many DNA delivery systems have been developed for systemic administration, including delivery of naked DNA or packaging the DNA using a gene carrier into a nanostructure. Such carriers may include cationic polymers that interact with the negatively charged DNA to form nanoparticles called polyplexes, lipids to form liposomes or lipoplexes, or niosomes to form nioplexes, all of which generally significantly enhance the uptake of DNA compared to using naked DNA, resulting in greater transgene expression. For a more thorough survey of advances in developing non-viral gene carriers, we direct readers to a recent review.[135,136] However, delivering DNA as a therapeutic from hydrogel scaffolds for regenerative medicine applications concerns the local, not systemic, delivery of this therapeutic, and the design of such strategies present their own challenges and constraints. One key parameter in facilitating cell access to encapsulated DNA in the hydrogel is the cell-mediated degradation of the hydrogel by both infiltrating and transplanted cells, which may be necessary for tissue growth and also serves to release and allow access to the loaded DNA. In fact, transgene expression in cells cultured in a hydrogel scaffold has been shown to be dependent on the rate of hydrogel degradation.[137]

Challenges of loading DNA into hydrogel scaffolds are largely dependent on the delivery vehicle and physical nature of the final delivery formulation. Below, we describe unique challenges posed by the loading of naked and condensed plasmids into hydrogel scaffolds.

Naked Plasmid. The incorporation of naked plasmid is relatively straightforward, as the plasmid can be directly encapsulated within the hydrogel matrix during synthesis or *in situ* injection. Some of the earliest scaffolds developed for gene delivery, termed "gene-activated matrices," combined plasmid solution at high concentrations (about 1 mg DNA per scaffold) with neutralized collagen before freezing and lyophilization to form a DNA-loaded collagen scaffold.[138] The plasmid is relatively robust, and these naked encapsulation approaches have resulted in a positive therapeutic effect *in vivo*, though large quantities of naked DNA are required to observe gene transfer *in vivo*. However, there are several limitations that have limited the translation potential of naked DNA-loaded scaffolds. First, bacterial unmethylated CpG motifs present in naked plasmids are recognized by Toll-like receptor-9, resulting in a strong immunogenic response.[139,140] Given that large quantities of DNA are needed to achieve high transgene expression, this immune response is problematic. Furthermore, naked plasmids are easily degraded by serum nucleases, limiting the long-term efficacy of the delivered plasmid.[141] Lastly, naked plasmids do not result in visible transfection *in vitro*, leaving all testing to be done *in vivo*.

Condensed Plasmid Using Cationic Polymer- and Lipid-Based Gene Carriers. In an effort to overcome the limitations associated with naked DNA, plasmids are commonly condensed with gene carriers into nanoparticles that result in enhanced transgene expression due

Table 2. Inductive Hydrogel Scaffold-Mediated Delivery of Therapeutic Genes *In Vivo*

Gene Delivered	Year	Scaffold Material	Delivery Strategy	Target	Ref.
Bone morphogenetic protein (BMP)-4 and parathyroid hormone	1996	collagen	naked DNA	bone regeneration	188
Parathyroid hormone	1999	collagen	naked DNA	bone regeneration	138
Hypoxia-inducible factor-1α (HIF-1α)	2006	fibrin	DNA/poly-L-lysine (PLL)	vessel formation	117
Vascular endothelial growth factor (VEGF)	2009	collagen	DNA/trimethyl chitosan (TMC)	vessel formation	147
VEGF	2010	collagen-chitosan	DNA/TMC	vessel formation	153
Fibroblast growth factor-2, BMP-2	2012	gelatin, collagen	DNA/PEI-linoleic acid	bone regeneration	189
Platelet-derived growth factor (PDGF)	2014	collagen	DNA/PEI	bone regeneration	146
VEGF	2014	gelatin	DNA/PEI-graphene oxide	vessel formation (cardiac repair)	125
VEGF	2014	HA	DNA/PEI	vessel formation (wound healing)	75
VEGF	2015	HA	DNA/PEI	vessel formation (wound healing)	76

to facilitated transport, protect the DNA from serum nucleases,[141] and can transfect cells both *in vitro* and *in vivo*. However, the materials used to condense DNA are typically highly positively charged, resulting in nanosized aggregates that are prone to aggregation, especially at the concentrations required for incorporation into a hydrogel. DNA can be complexed with cationic polymers to form polyplexes or lipid-based polymers to form lipoplexes or nioplexes. There are two general methods to incorporate condensed DNA into hydrogel scaffolds: surface coating and encapsulation (Figure 11).

Surface coating of condensed DNA relies on electrostatic interactions between the polyplexes and the scaffold material. Such methods often involve incubating a porous scaffold in a solution rich in condensed DNA before washing the scaffold to remove unbound particles[142–145] or hydrating a lyophilized hydrogel in a condensed DNA solution.[146–149] Via the first method, Saul et al.[138] reported a maximal loading capacity of approximately 44 μg complexed DNA per 8-mm diameter by 2-mm thickness porous fibrin gel.[142] The load amount varies with the DNA:PEI ratio used to form the polyplexes, which controls the surface charge of the polyplexes. In addition, this loading capacity is double that achieved through surface coating with a non-complexed plasmid solution, suggesting the important role of charge in these interactions.[142] In a separate study, collagen scaffolds loaded with surface-coated polyplexes encoding for platelet-derived growth factor (PDGF)-B were implanted in a rat calvarial defect model; this implantation resulted in significantly higher bone formation than empty defects and control scaffolds without polyplexes.[132,133]

Condensed DNA can also be encapsulated within hydrogels by mixing the condensed DNA into the gel precursor solution before crosslinking. The Shea lab has encapsulated lipoplexes in PEG and fibrin hydrogels for *in vitro* cell culture[137,150] and coated PLGA scaffolds with lipoplexes for *in vivo* delivery to a spinal cord injury site.[151] More recently, nioplexes, which are similar vesicular particles formed from an aminolipid and a non-ionic surfactant complexed with a nucleic acid, have been encapsulated in methylcellulose and carrageenan hydrogels, demonstrating 80% release of nioplexes over 30 hr.[152] Released nioplexes were able to transfect plated HeLa cells to induce gene silencing upon delivery of anti-sense oligodeoxynucleotides and transgene expression upon delivery of pGFP plasmid. Another recent study used EDC/n-hydroxysuccinimide (NHS) coupling to functionalize low-molecular-weight PEI to graphene oxide nanosheets, which were then complexed with DNA encoding for VEGF to form nanoparticles with DNA and encapsulated in a methacrylated gelatin hydrogel. This treatment improved cardiac outcomes, such as a significant decrease in scar area and an increase in ejection fraction, after implanting in a myocardial infarction model compared to a control hydrogel containing only DNA (Figure 12).[153]

However, encapsulation poses an obstacle due to the tendency of polyplexes, lipoplexes, and nioplexes to aggregate at higher concentrations as they are incorporated into hydrogels; as a result, many studies have only been able to load lower concentrations of condensed DNA.[117,154–157] PEGylating the cationic polymer to reduce surface zeta potential of polyplexes has been demonstrated as one way to decrease aggregation of polyplexes in hyaluronic acid hydrogels, though at the expense of gene transfer efficiency.[144] The Segura lab has also previously developed an approach for concentrated loading of polyplexes called caged nanoparticle encapsulation (CnE), in which polyplexes are formed at low concentrations but lyophilized in the presence of agarose and sucrose to mitigate these charge-based interactions and to preserve particle integrity during the lyophilization before resuspending in low volumes of buffer.[156,158] This technique has been used to incorporate polyplexes into porous hyaluronic acid hydrogels produced using a sphere templating method and tested in a mouse wound healing model for *in vivo* gene transfer. However, although expression of a marker gene was observed, there was no significant difference in angiogenic outcome as measured by density and thickness of vessels within the hydrogel with the delivery of the VEGF

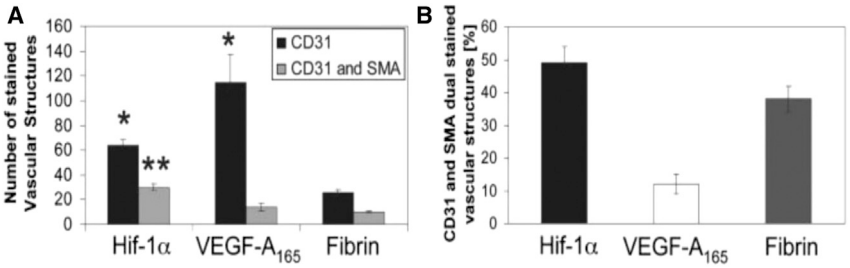

Figure 9. *In Vivo* Assessment of Angiogenesis 7 Days after Treatment with Fibrin Matrices Loaded with the HIF-1α Variant Gene, VEGF-A_{165} Protein, or Nothing
(A) Quantification of CD31-positive and CD31 and smooth muscle actin (SMA) dual-positive vascular structures 7 days after implantation of matrices. (B) Percent of CD31-positive vascular structures, which are also SMA-positive.[117] (*$p < 0.05$ for values different from fibrin [CD31]; **$p < 0.05$ for values different from fibrin [CD31 and SMA].)

gene as compared to a non-therapeutic gene, suggesting still insufficient levels of local transgene expression.[75,76] Similarly, trehalose, another saccharide, can also be used in the lyophilization process to preserve the stability and activity of polyplexes.[159]

Furthermore, condensed DNA encapsulated within hydrogels primarily relies on the gradual degradation of the hydrogel to enable DNA release and exposure to cells, resulting in transgene expression that can be orders of magnitude lower than when compared to surface-coated methods due to lower immediate availability of DNA to cells. This has been observed in two studies that loaded polyplexes via surface-coating and encapsulation methods into porous fibrin and hyaluronic acid scaffolds.[142,144] The transgene expression from the encapsulation method can be improved by tuning the hydrogel formulation to be more prone to cell-mediated degradation. However, using these two methods to load DNA simultaneously may result in the delivery and expression of transgenes following two different profiles, which may be advantageous in circumstances when different expression profiles are needed.

Material Considerations in Incorporating Genes into Cell-Laden Hydrogels for Delivery

Cell interactions with the composition and mechanical properties of the matrix substrate are critical design parameters for regenerative medicine and have been shown to modulate gene transfer, presenting opportunities to design scaffolds that enhance transfection and regeneration. Many of the studies described in this section have reported effects in one or a few cell lines, but such effects may still be cell line dependent. Also, the ideas presented in this section may be beneficial in enhancing gene transfer from hydrogel scaffolds both in co-delivered cells in the scaffold as well as infiltrating cells.

Substrate stiffness contributes to modulating proliferation, migration, and differentiation of cells yet also plays an important role in modulating gene transfer, with transgene expression increasing as a function of substrate stiffness in 2D cell culture.[160] However, in 3D culture of encapsulated mouse MSCs and polyplexes in hyaluronic acid hydrogels, the inverse result was observed, with transgene expression increasing with decreasing hydrogel stiffness.[161] This difference in outcomes is likely due to the dependence of cell proliferation and migration as well as polyplex release on the degradability of the hydrogel. This finding suggests that the efforts in designing biomaterials for gene delivery purposes should also take stiffness into consideration. In addition, surface charge and chemistry influence transfection efficiency, with murine NIH/3T3 fibroblasts cultured on charged hydrophilic surfaces with carboxyl groups exhibiting increased transfection and cells on uncharged methyl-coated surfaces showing decreased transfection.[140] Lastly, topography was also found to influence lipoplex-mediated transfection efficiency, with human MSCs cultured on nanopillar-coated surfaces observing highest level of transfection compared to blank and micropillar-coated surfaces.[162]

Matrix composition can directly determine the efficiency of gene transfer through pathways related to integrin-mediated cell adhesion. For cells seeded in two dimensions, increasing concentrations of the RGD cell adhesion peptide leads to increases in transgene transfer and expression, and an increase in the physical spacing of the ligand on a substrate resulted in decreased transgene expression.[163] However, in cells cultured in three dimensions in a nonporous hyaluronic acid hydrogel, an intermediate RGD concentration of 100 μM and clustering ratio of 0.4 mmol RGD/mmol HA resulted in the highest levels of transfection.[161] However, these effects on both cell behavior and transfection efficiency are specific to cell type and require optimization for the transplanted cell type or for the expected range of cell types of infiltrating cells. Different ECM components as substrates also have varying effects on gene transfer. For example, in mouse MSCs administered a bolus polyplex transfection, collagen-I-coated surfaces inhibit gene transfer, although fibronectin-coated surfaces enhance gene transfer, and different ECM substrates trigger different internalization pathways by which polyplexes are trafficked into the cell.[164] A separate study showed that mouse fibroblasts exhibited increased gene transfer on surfaces coated with fibronectin over collagen in surface-mediated transfection in which polyplexes were coated on the substrate, but the inverse effect was found upon bolus polyplex administration.[144] Cell culture on surfaces coated with the $\alpha_5\beta_1$ integrin-binding domain of fibronectin resulted in significantly enhanced expression due to polyplex-mediated transfection compared to cell culture on surfaces coated with a polymer of similar charge but lacking in cell-binding domains, suggesting that the bioactive integrin-binding function of ECM components are at least partially responsible for such effects.[165]

A better understanding of how cell-hydrogel interactions affect gene transfer may lend insight on how to design more effective gene delivery methods. A study has elucidated the mechanisms by which cytoskeletal dynamics and RhoGTPases control gene transfer and

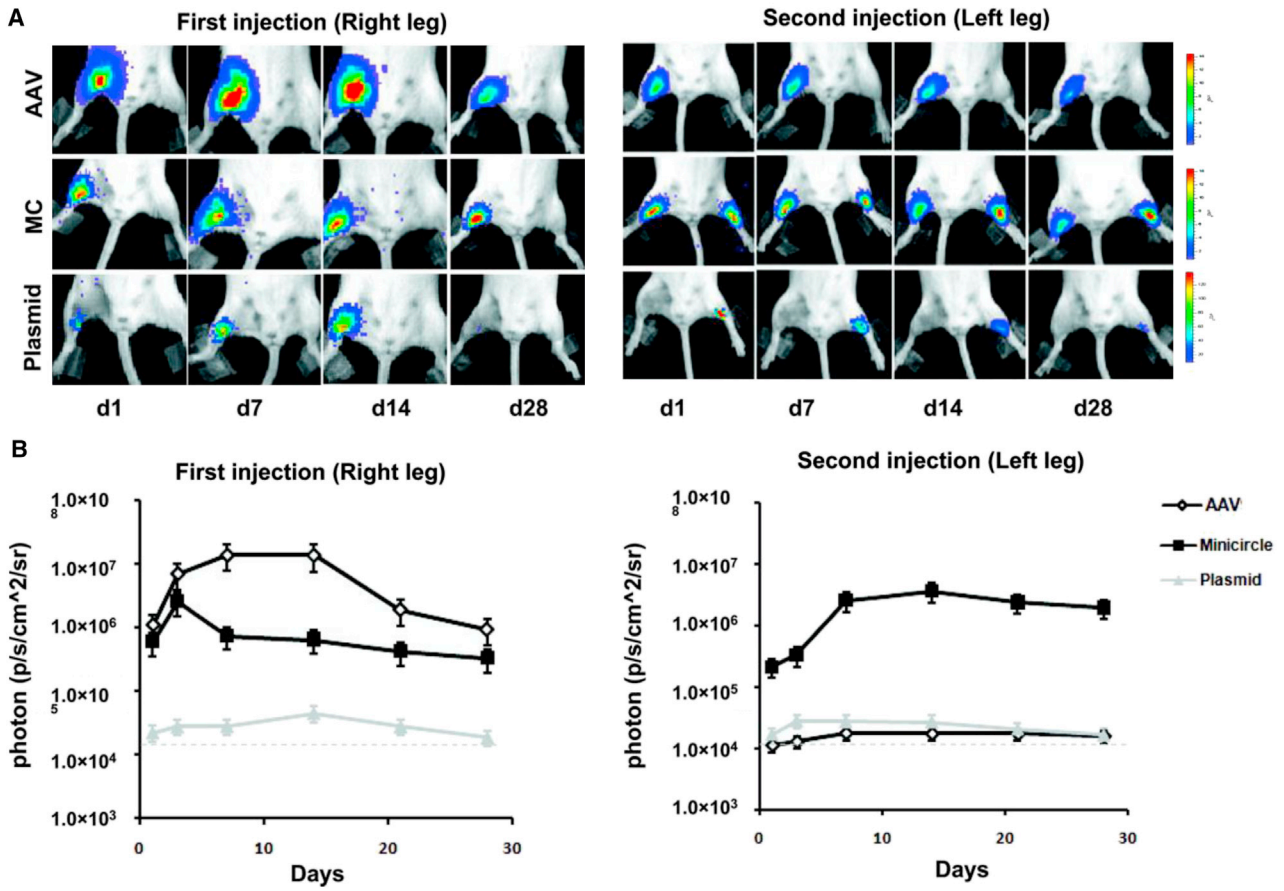

Figure 10. Comparison of Minicircle, Plasmid, and Viral-Mediated Transgene Expression

(A) Representative BLI images of animals injected with AAV, minicircle (MC), and regular plasmid in the right leg (first injection), followed by the left leg 28 days later (second injection). As expected, AAV expression is more robust compared to MC and plasmids initially. However, after repeat injection, AAV expression is not detected in the contralateral leg because of host-mediated humoral immune response. Color scale bar values are expressed as photons per second per square centimeter per steradian (p/s/cm^2/sr). (B) Graphical representation of longitudinal bioluminescence imaging (BLI) after first and second injections in all 3 groups. Note that day 28 of second injection in left leg would represent day 56 of first injection in right leg in the same animal.[134] Error bars, SEM.

trafficking of polyplexes; introducing bioactive signals to manipulate these pathways may be a method of enhancing gene transfer.[166] For example, in a separate study, the inhibition of polo-like kinase-1 (PLK1) was identified as a means of enhancing gene transfer.[167] Microarray analysis has also been used to reveal genes that are upregulated in transfected cells as compared to non-transfected cells using both polyplexes and lipoplexes; notable targets include genes involved in integrin-mediated signaling, cytoskeletal mechanics, and membrane trafficking.[168–170] However, it is important to note that different mechanisms govern gene transfer in 2D culture versus in 3D hydrogel-supported cell culture, suggesting the importance of more thorough studies in studying how a particular scaffold design influences gene transfer through cell-matrix interactions.[171] Also, gene transfer may occur via different mechanisms, depending on the cell type analyzed.

Integrating genes into cell-laden hydrogels to drive differentiation is another promising strategy for enhancing tissue regeneration. Research focused on integration of cell delivery and gene delivery using hydrogels may assist in directing the maturation of those cells if the DNA encodes for a factor that promotes a certain cell lineage.[172] In the field of bone tissue engineering, transforming growth factor-beta 3 and bone morphogenetic protein 2 (BMP-2) genes were complexed with nanohydroxyapatite and, along with MSCs, encapsulated in alginate hydrogels. These dual-delivery hydrogels were shown to direct the differentiation of the stem cells down the chondrogenic lineage to produce cartilage.[173] Wegman et al.[158] investigated this combinatorial approach by encapsulating the BMP-2 gene in alginate hydrogels along with goat multipotent stromal cells. After implantation in a goat bone model, they demonstrated enhanced osteogenic differentiation and bone formation.[174] A challenge with the dual delivery of plasmid DNA and cells includes potential integration of genes into the host genome.[175] The Alsberg lab has demonstrated the promise of a controlled, sustained delivery of small interfering RNA (siRNA) or microRNA (miRNA) with encapsulated MSCs

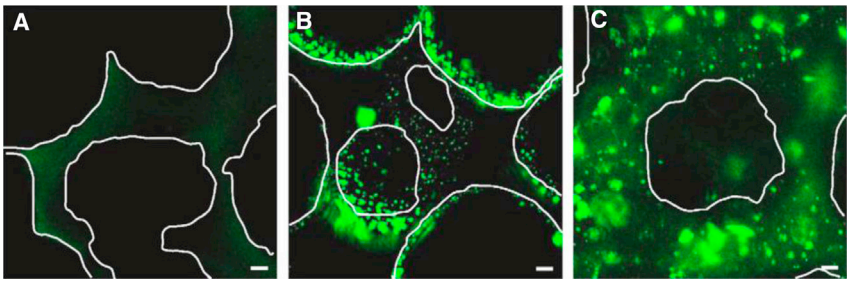

Figure 11. Loading of Porous Scaffolds with Polyplexes by Surface Coating or Encapsulation
Fluorescent microscopy images of porous fibrin scaffolds without polyplexes (A), scaffolds surface coated with polyplexes (B), and scaffolds encapsulated with polyplexes (C). White lines indicate pore boundaries.[142] Scale bars indicate 20 μm.

that downregulate gene expression as an effective alternative tool to drive osteogenesis.[50] In addition, the hydrogel's tunable design provides controllable swelling and degradation properties that can be used for prolonged delivery of gene therapies in order to extend the regulation of the transplanted cell behavior.

Conclusions

With recent advances in design, hydrogel properties, such as porosity, cell-mediated degradability, and tethered bioactive cues, not only provide structural support for infiltrating and delivering cells but also influence and direct cell-cell interactions, proliferation, migration, and differentiation. A hydrogel delivery system no longer represents only a mere static structural scaffold for cells but rather a dynamic and versatile environment. Furthermore, therapeutics, such as genes, can be loaded into hydrogels to induce sustained transgene expression of therapeutic genes in infiltrating and transplanted cells, and hydrogel properties can be modulated to tune the extent of this transgene expression. However, several challenges remain in controlling the dynamics of the hydrogel in the complexity of the tissue environment in healthy and diseased states. Currently, no universal material fulfills all the mechanical needs to improve cell survival and functionality during the different phases of transplantation and tissue regeneration. Although some material mechanical properties, such as stiffness, may be optimal for enhanced long-term retention and differentiation, these same mechanical properties may inhibit the progression of earlier regenerative processes. Regulating the cellular processes and guiding the development of new tissue growth in parallel with the dynamic changes of the tissue environment would be pivotal in

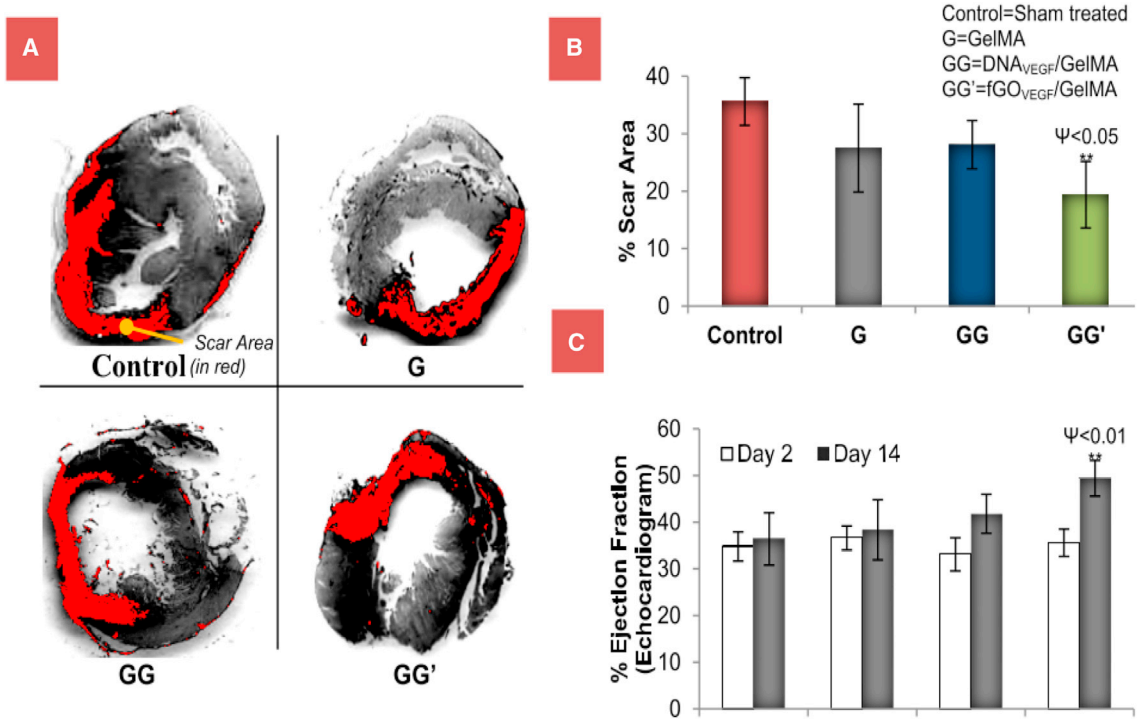

Figure 12. *In Vivo* Assessment of Scar Area and Cardiac Function as Outcomes of Infarcted Hearts Treated with Hydrogels Loaded with Graphene Oxide-PEI-DNA (GG') Compared to Hydrogels Loaded with Naked DNA Only (GG) and Gel Only (G)
(A) Images of left ventricle myocardial sections stained with Sirius red to show cardiac fibrosis. (B) Determination of scar area of the left ventricle. (C) Echocardiographic assessment of cardiac function through monitoring heart ejection fraction.[153] Error bars, SD. (**p < 0.01 for values different from control.)

determining the outcome of the regenerative therapy. A number of hydrogel designs are being investigated to achieve this control as well, such as self-assembling peptides and peptide amphiphiles that provide for controllable gelation, degradation, and presentation of cell adhesion motifs.[162,176]

A promising future research direction is the development of layered biomaterials that can recapitulate the zonal organization of native tissue in order to fulfill multiple mechanical requirements throughout the multiple stages of transplantation. A layered hydrogel consisting of different ECM proteins as well as mechanical properties could offer better delivery properties to direct cell fate and regulate processes such as wound healing and angiogenesis.[163] MSCs and chondrocytes encapsulated in multi-layered scaffolds with spatially varying matrix compositions and mechanical cues have demonstrated promising results in zonal-specific differentiation.[177,178] Another useful functionality that can be incorporated is the ability to increase or decrease hydrogel stiffness after initial crosslinking and implantation. This has been achieved through stimulus-triggered secondary chemistries between functional groups or by incorporating a secondary reaction mechanism that occurs at a much larger timescale than the first primary crosslinking mechanism.[179–183] The stiffness of the hydrogel can therefore be made time dependent and can be tuned to the different stages of the regenerative process.

Recent efforts to study the host response to implanted biomaterials can use these insights to design materials that can proactively direct the immune response upon implantation to be favorable to the regenerative process and the survival and functionality of any co-transplanted cells in the material. Examples include the tuning of material properties, which can increase the presence of pro-remodeling versus pro-inflammatory phenotypes of macrophages in implant site and can also control transplanted stem or progenitor cell behavior and differentiation.[169] The delivery of genes encoding for anti-inflammatory cytokines from scaffolds represents another strategy to promote tissue repair by decreasing expression of pro-inflammatory genes and enhancing expression of pro-regenerative genes.[170] Finally, with emerging technologies, such as CRISPR, transplanted cells can undergo genetic engineering to regulate the immune response as a means to prevent or avoid rejection.[171]

In summary, hydrogels offer great promise in designing suitable environments that provide not only structural support for cells and new tissue growth but also control over gene delivery to promote differentiation and improve therapeutic outcomes. As these hydrogel design considerations continue to be investigated, this system could provide a promising platform for dual delivery of genes and cells for a wide range of applications in tissue engineering.

CONFLICTS OF INTEREST

The authors confirm that there are no known conflicts of interest associated with this publication.

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