SPECIAL FOCUS: RNA THERAPEUTICS FOR TISSUE ENGINEERING*

Messenger RNA Delivery for Tissue Engineering and Regenerative Medicine Applications

Siddharth Patel, MEng,^{1,**} Avathamsa Athirasala, MSE,^{2,**} Paula P. Menezes, MS,^{2,3,**} N. Ashwanikumar, PhD,¹ Ting Zou, MS,^{2,4} Gaurav Sahay, PhD,^{1,5} and Luiz E. Bertassoni, DDS, PhD^{2,5,6}

The ability to control cellular processes and precisely direct cellular reprogramming has revolutionized regenerative medicine. Recent advances in *in vitro* transcribed (IVT) mRNA technology with chemical modifications have led to development of methods that control spatiotemporal gene expression. Additionally, there is a current thrust toward the development of safe, integration-free approaches to gene therapy for translational purposes. In this review, we describe strategies of synthetic IVT mRNA modifications and nonviral technologies for intracellular delivery. We provide insights into the current tissue engineering approaches that use a hydrogel scaffold with genetic material. Furthermore, we discuss the transformative potential of novel mRNA formulations that when embedded in hydrogels can trigger controlled genetic manipulation to regenerate tissues and organs *in vitro* and *in vivo*. The role of mRNA delivery in vascularization, cytoprotection, and Cas9-mediated xenotransplantation is additionally highlighted. Harmonizing mRNA delivery vehicle interactions with polymeric scaffolds can be used to present genetic cues that lead to precise command over cellular reprogramming, differentiation, and secretome activity of stem cells—an ultimate goal for tissue engineering.

Keywords: mRNA delivery, gene therapy, cell reprogramming, tissue engineering, lipid nanoparticles, hydrogels

Introduction

THE MULTIDISCIPLINARY FIELD of tissue engineering and regenerative medicine combines technologies derived from chemistry, material sciences, stem cell biology, and increasingly more frequently, genetic engineering; all with the ultimate goal of developing functional and clinically useful biological tissue substitutes. In recent years, substantial strides have been made in regenerative medicine through the application of strategies that seek to utilize the therapeutic capacity of stem cells and their inherent regenerative capacity upon tissue injury.¹ In addition to their ability to differentiate into multiple cell lineages in response to signaling factors,² the stem cell secretome, which comprises a broad array of cytokines, chemokines, and growth factors,³ has been increasingly acknowledged for its role in repair and regeneration, making

stem cells uniquely suitable for regenerative applications. Harnessing these functions by directing stem cell fate, function, and phenotype, and controlling their natural signaling pathways, can further extend our control over the inherent regenerative capabilities of these systems. In this review, we describe recent examples of how both endogenous and exogenous cell behavior can be engineered for regenerative purposes by the use of nucleic acid therapeutics to either trigger (DNA, messenger RNA) or suppress (small interfering RNA, micro RNA [miRNA], small hairpin RNA [shRNA], antisense oligonucleotides, genome editing) the expression of specific genes and transcription factors, with particular attention to the potential and current use of messenger RNA (mRNA) on various regenerative applications.

mRNA has recently garnered much attention for its potential for gene transfer applications.⁴ This turn of events

¹Department of Pharmaceutical Sciences, College of Pharmacy, Collaborative Life Science Building, Oregon State University, Portland, Oregon.

²Division of Biomaterials and Biomechanics, Department of Restorative Dentistry, School of Dentistry, Oregon Health and Science University, Portland, Oregon.

³Postgraduate Program in Health Sciences, Department of Pharmacy, Federal University of Sergipe, Aracaju, Sergipe, Brazil.

⁴Endodontology, Faculty of Dentistry, The University of Hong Kong, Pokfulam, Hong Kong, China.

⁵Department of Biomedical Engineering, Collaborative Life Science Building, Oregon Health and Science University, Portland, Oregon.

⁶Center for Regenerative Medicine, Oregon Health and Science University, Portland, Oregon.

**These authors contributed equally to this work.

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TABLE 1. CHARACTERISTICS OF DNA AND mRNA

DNA	RNA
Slow and long-lasting expression	Rapid and transient expression
Risk of genomic integration	No risk of genomic integration
Needs to reach the nucleus for effective transfection	Needs to reach the cytoplasm for effective transfection
Difficulty in traversing nuclear barrier results in lower efficiency	Does not need to cross nuclear membrane since translation machinery is found in the cytoplasm
Difficult to transfect nondividing cells	Can transfect nondividing cells
Consists of promotor region, initiator, coding region, termination sequence	Consists of 5' cap, 3' and 5' UTRs, coding region, Poly(A) tail
Delivery based on viral and nonviral methods	Delivery mostly based on nonviral methods

followed improvements in mRNA synthesis and stability, which have allowed its use in a broad range of applications surpassing the potential of DNA-based applications. DNA, utilizing viral (lentivirus, adenovirus, adenoassociated virus, etc.) and nonviral delivery approaches (physical and chemical methods), has been widely employed in tissue engineering since the past two decades.⁵⁻⁹ However, its clinical translation has been hindered due to low efficiency,^{10,11} risk of insertional mutagenesis,¹²⁻¹⁴ as well as unpredictable and slow expression kinetics (Table 1). Moreover, despite the high efficacy of viral vectors, the inherent cytotoxicity and immunogenicity of viral vectors cause further hurdles for successful clinical application of DNA-based gene delivery. Another limitation of viral vectors is their inability to package larger nucleic acids. Therapeutic delivery of mRNA, on the other hand, possesses virtually no risk of genomic integration.¹⁵ Since mRNA does not require nuclear entry (a significant barrier to successful gene transfer using DNA) and needs only to reach the translational machinery in the cytosol for expression of its protein product, it has demonstrated a higher transfection efficiency than DNA-based gene delivery^{10,16,17} and can transfect nondividing cells^{10,11} (Table 1). mRNA transfection is characterized by rapid, transient, and predictable protein expression kinetics allowing greater temporal regulation over protein production (Table 1). As a consequence, mRNA, almost exclusively exploiting nonviral delivery techniques, has been employed in cancer immunotherapy,¹⁸⁻²¹ vaccine development,²²⁻²⁶ protein replacement therapy,²⁷⁻²⁹ cellular reprogramming,^{2,16,30-32} and genome engineering.^{33,34} While still in its infancy, mRNA applications in tissue engineering present significant potential, where traditional DNA-based therapies have failed due to inherent cellular limitations. Especially, the temporal control over expression of key signaling molecules or transcription factors can be used to direct cellular reprogramming and stem cell fate, reminiscent of physiological conditions. It is likely that different RNA molecules, including mRNA, siRNA, shRNA, can be delivered in a controlled fashion that orchestrate tissue regeneration but have the capacity to “switch off” due their transient nature, thus preventing any off-target effects. In this scenario, considering that there is limited literature^{35,36} specifically related to this subject, the present review will summarize the recent advances on the mRNA application in tissue engineering, emphasizing findings that contribute to the better understanding of the molecular mechanisms underlying the regenerating abilities of stem cells and thus potentially impacting the field of regenerative medicine.

***In Vitro* Transcribed mRNA**

Despite the advent of mRNA delivery in the 1970s by polycations³⁷ and liposomes,³⁸ the instability (average half-life of 7h)³⁹ and innate immunogenicity⁴⁰⁻⁴⁵ of mRNA had rendered it unsuitable for therapeutic applications. Significant advances in decoding of mRNA biology have now allowed us to upgrade methods of *in vitro*-transcribed (IVT) mRNA synthesis by incorporating various chemical modifications to improve IVT mRNA pharmacokinetics and pharmacology.

mRNA serves as a template for protein synthesis and is made up of four ribonucleoside bases: adenosine, guanosine, cytidine, and uridine, linked through a phosphate group. With an average size of approximately 2000 bases, this single-stranded molecule can be subdivided into five domains - the 7-methylguanosine cap (m7G) at the 5' end, 5' untranslated region (5' UTR), coding sequence known as the open reading frame, 3' untranslated region (3' UTR), and 3' poly(A) tail consisting of 50–250 adenosine residues (Fig. 1).⁴⁶ Modifications to the 5' cap, poly(A) tail, and 5' and 3' UTRs have been explored for modulation of IVT mRNA immunogenicity and stability.⁴⁷

The 5' cap and the poly(A) tail are critical in the protection of the transcript. Apart from playing a role in regulation of nuclear export of mRNA, the 5' 7-methylguanylate cap protects against degradation by exonucleases and facilitates the initiation of translation through the binding of eukaryotic initiation factor 4E.⁴⁸ Common *in vitro* transcription techniques can result in significant fraction of the caps being introduced in reverse orientation, preventing binding of translation factors.⁴⁹ In response to this phenomenon, mRNA is now synthesized with antireverse cap analogs, which have greatly enhanced translational efficiency.^{50,51} Efficient mRNA capping is an active area of investigation and novel capping analogs have been reported that further enhance the efficiency of mRNA translation (Fig. 2). The poly(A) tail aids in nuclear export of mRNA, promotes translation through interactions with translation initiation factors, and regulates mRNA stability and half-life by preventing degradation by nucleases.⁴⁶ In IVT mRNA, it is elongated either through enzymatic polyadenylation using recombinant poly(A) polymerase or by directly encoding for the poly(A) nucleotides within the DNA template. It has been reported that a poly(A) tail of about 120 nucleotides is necessary for optimal inhibition of mRNA degradation.⁵²

The 5' and 3' UTRs present additional avenues for modifications. The two regions play a role in mRNA

FIG. 1. Native mRNA structure. The key structural domains of mRNA are the 5' cap (also known as the 7-methylguanosine cap), the 5' and 3' UTRs, the coding sequence and the poly(A) tail. Reproduced with permission from³⁵ Copyright Springer Nature Publishing Group 2017. UTR, untranslated region. Color images available online at www.liebertpub.com/tea

translation and stability through the interaction of 5' UTR with translation machinery and the 3' UTR serving as a binding site for microRNA (miRNA) and mRNA decay-promoting proteins.⁵³ A frequent approach for increasing the half-life of IVT mRNA is selecting UTR domains from human mRNAs with higher stability (e.g., human α and β -globin) and incorporating them into the 3' UTR of IVT mRNA.⁵²

The mammalian immune system is well adapted for detecting and eliminating exogenous RNA, recognizing it as a viral or viroid challenge. RNA is identified through pattern recognition receptors (PRRs), Toll-like receptors (TLRs), and nucleotide-binding oligomerization domain-like receptors (NLRs), possessed by immune cells such as macrophages and dendritic cells.^{40,41,44,45} Binding of these receptors triggers an inflammatory cascade, which results in RNA degradation, representing an important consideration for potential application in regenerative medicine. Nonimmune cells also detect exogenous RNA through PRRs, such as the retinoic acid-inducible gene 1 (RIG-1) receptor family to induce innate immune responses.^{42,43} Several chemical base modifications have been examined to restrict immune activation by IVT-mRNA.^{54–56} This process involves the substitution of a base (e.g., uridine) with the equivalent modified base (e.g., 5-methyluracil) in the entire sequence (Fig. 3). The base modifications that have been explored include, but are not limited to, 5-methylcytosine, pseudouracil, and 5-methoxyuracil.^{16,57,58} Moreover, the altered versions of ribose sugar moiety have also been reported with methyl and fluoro substitutions at the C-2 position (Fig. 3). Since base modification is a naturally occurring posttranscriptional event in eukaryotes, these chemical modifications do not inhibit the endogenous ribosomal IVT mRNA translation,^{59,60} and have represented important improvements on

the half-life and stability of mRNA for intracellular delivery and their potential use to stimulate the regenerative capacity of stem cells *in vitro* and *in vivo*. It should be noted that the efficacy of the enhancements conferred by chemical modulation of IVT mRNA seems to depend on the type and method of modification, cell type, and cell differentiation state. Moreover, it is believed that there is still much to be discovered concerning mRNA interactions with the myriad extracellular and intracellular proteins and the full breadth of possible modulations in mRNA engineering is yet to be realized. All of these aspects represent important areas of future study that remain to be explored.

mRNA delivery

The chemical modification of IVT mRNA has greatly improved its efficacy, and despite the wide variety of both membrane disruption-mediated and carrier-mediated delivery strategies, both using physical and chemical methods, intracellular mRNA delivery remains a challenge. It is known that exposure to serum and intracellular nucleases, in addition to immune cells, reduces the half-life of mRNA.^{40,42,61–64} Moreover, the high-molecular-weight and negative charge density of mRNA prevents it from effectively entering cells, due to steric inhibition to diffusion and electrostatic repulsion from the anionic cell membrane. Various delivery technologies have been utilized to overcome one or more of these barriers for intracellular delivery. Originally developed for DNA, siRNA, and miRNA delivery, these technologies are now being explored for mRNA delivery. In this study, we will selectively highlight physical and chemical methods that have been reported to circumvent these barriers. We encourage the reader to refer

FIG. 2. Structures of standard capping (m^7GpppG) and ARCA. In case of ARCA analogs unidirectional transcription ensures translational efficacy. The different capping R groups were reported in the literature at the 3' carbon of ribose in m^7G , which includes (1–3) [with methoxy substitution⁵⁰] and (4–6) [recently reported with azide functionality for bioorthogonal binding⁴⁷]. ARCA, anti-reverse capping analogs. Color images available online at www.liebertpub.com/tea

FIG. 3. Structural features of different sugar and base modifications to enhance translational efficacy of mRNA with reduced immunogenicity. Color images available online at www.liebertpub.com/tea

to a recent review on the topic⁶⁵ for more in-depth details of the described methods.

Physical methods. Physical modes of gene transfer have been developed to achieve direct transfection of cells *in vitro* and *ex vivo*. Electroporation is the most popular of these methods demonstrating high efficiency in direct delivery of mRNA into the cytosol.^{27,66–69} This is achieved through the application of electrical pulses to cells to permeabilize the cell membrane for the entry of mRNA. *In vivo* electroporation of mRNA encoding the neurogenic factor, *Myt1l* was recently used to drive the transformation of neural stem cells into proliferative progenitors.¹⁷ Another technique used for cytosolic mRNA delivery utilizes a “gene gun”, wherein heavy metal particles (e.g., gold) surface coated with mRNA are propelled at high velocities into the cell using pressurized gas.^{70,71} Microinjection, yet another physical method, makes use of a microneedle to inject mRNA into individual cells.^{68,69} The low throughput of this technique makes it extremely labor intensive and hence, has only seen sporadic use.⁶⁸ Sonoporation, using ultrasound along with microbubbles, has also been used to deliver mRNA to dendritic cells *ex vivo*.²¹

Collectively, these techniques have been used primarily for application in cancer immunotherapy,^{21,66} and to a lesser extent in protein replacement therapy,^{27,71} cellular reprogramming,^{72,73} and gene editing,^{68,69} where the latter two have special relevance for the field of tissue engineering. These methods deliver mRNA directly to the cytosol, avoiding the need for endosomal escape and immune activation. The efficacy of physical methods at mRNA transfection is clearly demonstrated by their popularity in *ex vivo* applications, such as cancer immunotherapy,^{66,67,74} gene editing,^{57,68,75,76} and tissue regeneration.^{17,27,66} However, these methods remain expensive, invasive (for instance, extraction of blood), and require extensive optimization.⁷⁷

Chemical methods. The limitations of physical methods have lead to the development of chemical methods for delivery of nucleic acids. Today, chemical techniques, with their high versatility, low cost, and ease of use, have been broadly evaluated for *in vitro*, *ex vivo*, and *in vivo* delivery of nucleic acids. Furthermore, the true advantage lies in their potency to deliver nucleic acids *in vivo*. Parenteral delivery of nucleic acids is faced with additional challenges such as degradation of nucleic acids by immune cells^{40,45} and serum nucleases,^{63,64} and the need for cellular uptake and endosome escape to achieve successful transfection.^{78,79} These problems have been addressed using nanoparticles made up of cationic molecules such as polycations, cationic or ionizable lipids, and lipid-like molecules (Fig. 4). These cationic molecules bind electrostatically to the anionic phosphates on the mRNA backbone leading to efficient packaging of mRNA into nanoparticles. Evasion of immune recognition is accomplished by shielding the nanoparticles using inert materials, such as polyethylene glycol (PEG).⁸⁰ Cellular uptake is achieved through endocytosis following receptor-mediated, electrostatic or hydrophobic binding of nanoparticles to the cell surface followed by internalization.⁸¹ Extensive effort has been put into utilizing ligands (such as transferrin, folic acid, RGD peptide, etc.) toward cell surface receptors to increase specificity and efficien-

cy.^{78,81} A major consideration in design of nanocarriers is their ability to induce endosome escape. Two mechanisms are accepted as potential hypotheses for endosome escape of nanoparticle–proton sponge effect⁸² and membrane destabilization.⁸³ Additional amplification of transfection can be attained through the use of endosomal escape agents, such as chloroquine,⁸⁴ guanabenz,⁸⁵ endosomal escape peptides (such as melittin, muramyl dipeptide, etc.),^{84,86} and leukotriene inhibitors (e.g., MK571, Pranlukast, Zafirlukast).⁸⁶ The flexibility in nanoparticle design and size of mRNA, low cost, ease of scale-up, limited toxicity, and low immunogenicity has made chemical modes the preferred choice for mRNA delivery. Selected examples are provided below.

Polycations. Polycations utilize electrostatic interactions to encapsulate nucleic acids ensuring serum stability. The cationic charge of the nanoparticles thus formed also assists in internalization of these polyplexes (complex of polycation and nucleic acid) into cells. One of the first polymers to be investigated for nucleic acid delivery was Poly-L-lysine (PLL)^{87,88} (Fig. 4A). While PLL was proficient at encapsulating nucleotides, it was unable to induce endosomal escape due to its poor buffering capacity resulting in low efficiency of gene transfer. A new polymer, polyethylenimine (PEI) (Fig. 4A), was later developed to have a high charge density and superior buffering capacity leading to higher efficacy. PEI remains the most explored polymeric delivery material for nucleic acid delivery.^{84,89} PEI grafted to graphene oxide was recently used to complex and deliver mRNA encoding *Oct4*, *Sox2*, *c-Myc* and *Klf4* (Yamanaka factors) for generation of “footprint-free” (gene integration-free) induced pluripotent stem cells (iPSCs) from somatic cells.⁹⁰ Polyaspartate (poly-Asp) (Fig. 4A) is another broadly investigated polycation for nucleotide delivery. Variations of polyaspartate such as poly-Asp(DET), poly-Asp(TET), poly-Asp(TEP), poly-Asp(PEH) have been successfully used for mRNA delivery.^{91,92} To further add to the versatility and utility of these polymers, block copolymers of PLL, PEI, and poly-Asp with PEG have been developed to form “nanomicelles” for enhanced *in vivo* delivery.^{93–95} PEG-polyAsp(DET) and PEG-polyAsp(TET) nanomicelles carrying cartilage anabolic transcription factor, runt-related transcription factor 1 (*RUNX1*) mRNA have shown promising results in treatment of animal models of osteoarthritis.⁹⁶ Poly beta amino esters (PBAEs),^{97,98} and polypeptoids^{92,99} are other class of polycations, which have evolved in design to improve efficacy and reduce toxicity.

Lipoplexes and lipid nanoparticles. Lipoplexes exploit the mechanism of electrostatic complexation to package nucleic into nanoparticles using cationic lipids (e.g., 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTAP), (Fig. 4B) and/or an accessory neutral lipid (such as 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) (Fig. 4B). Notable cationic lipoplex formulations explored for *in vitro*, *ex vivo*, and *in vivo* delivery of mRNA include DOTAP:DOPE, Lipofectamine, TransIT, MegaFectin, and Stemfect.^{28,89} iPSCs have been successfully generated from fibroblasts and subsequently, differentiated

into terminally differentiated myogenic cells using synthetic, IVT mRNA transfected through Lipofectamine and TransIT cationic lipid delivery systems.¹⁶ Further optimization of formulations containing cationic lipids led to the development of highly modular lipid-based nanocarriers known as lipid nanoparticles (LNPs). Most commonly, LNPs are composed of combinations of a cationic lipid, a structural lipid, cholesterol, and PEG-lipid, mixed together in precise ratios. The presence of ‘helper’ lipids (structural lipids, PEG-lipids, and cholesterol) confers stability as well as protection from opsonization and nonspecific uptake by mononuclear phagocyte system resulting in improved circulation half-life and efficacy.^{100,101} Another method employed calcium phosphate to assist in packaging of mRNA within lipid-based carriers.¹⁰² At the cellular level, LNPs are trafficked through the endolysosomal pathway with endosomal escape taking place through membrane destabilization initiated by electrostatic interactions between cationic lipids of the LNPs and anionic lipids of the endosomal membrane. Although, the use of cationic lipids in lipoplexes and LNPs offers high transfection *in vitro*, their toxicity and immunogenicity has led to setbacks for *in vivo* translation.^{103–105}

Over the years, better understanding of the necessities in the delivery process has allowed the development of potent synthetic lipids leading to higher efficacy and lower toxicity.¹⁰⁶ The greatest advancements for *in vivo* delivery of nucleic acids have come in the form of ionizable lipids (e.g., C12–200, TT3, DLin-MC3-DMA, OF-2, Fig. 4B) as replacements for cationic lipids in LNPs. LNPs with ionizable lipids have experienced the most success among nanoparticle gene delivery systems, showing positive results in clinical trials for RNA therapeutics.¹⁰⁷ Patisiran, an LNP

containing siRNA against transthyretin, successfully completed phase 3 clinical trials, making it the first to reach this stage. Several LNP formulations are currently being tested for mRNA delivery in various preclinical and clinical studies, including infectious disease vaccinations and cancer immunotherapy.^{24,108–110}

These chemical methods have evolved to enhance *in vitro* and *in vivo* transfer of mRNA by effectively complexing it to prevent degradation and facilitate intracellular delivery by triggering endocytosis-mediated cellular entry and endosomal escape. Thus, nanoparticle-based mRNA delivery would enable highly efficient, transient *in situ* manipulation of cells and tissues. Furthermore, they can be designed to target specific cell types *in situ* or in a multicell coculture. The highly modular nature of nanoparticles enables various strategies for use of mRNA in tissue engineering, cellular reprogramming, and regenerative medicine.

Hydrogel Scaffolds in Tissue Engineering and mRNA Delivery

A noteworthy aspect that requires special attention regarding the use of mRNA for tissue engineering is that, different from many RNAi strategies where nucleic acids are used for downregulation of intracellular mechanisms, mRNA in tissue engineering is primarily intended to promote tissue formation or certain aspects of cellular response, such as differentiation, reprogramming, and protein secretion (Fig. 5). Given the 3D architectural complexity of tissues in the body, such an mRNA-guided response, ideally needs to be controlled in three dimensions and in a site-specific manner, thus giving rise to 3D multitypic tissues with spatially defined functions and organization, such as

FIG. 5. Schematic showing modes of applications of mRNA delivery in tissue engineering. mRNA in lipid nanoparticles can be encapsulated in hydrogels and other biomaterials (both with cells or without) to spatially and temporally regulate gene transfer. Another potential route of application is *in vivo*, either systemically or through direct injection at the site of regeneration or repair. These methods can be utilized to induce pluripotency in reprogrammed somatic cells, direct differentiation, or stimulate therapeutic function through secretion of chemokines and cytokines (secretome) for regenerative applications. Color images available online at www.liebertpub.com/tea

those in the body. Therefore, local and sustained delivery of mRNA is a challenge that becomes particularly relevant for tissue engineering strategies. Effectively, this means that much of the optimization that has been performed in the field of mRNA complexation,⁶⁵ and bolus intravascular mRNA delivery using nanoparticles,^{79,111} become a stepping stone for future developments on controllable biomaterial “depots” that are compatible with such delivery vehicles. Moreover, in addition to being compatible with these nanodelivery vehicles, such biomaterials need to be compatible with the cells that are being used for new tissue formation. Importantly, the far majority of tissue engineering strategies in the past decade have used cell-laden biomaterials that attempt to replicate the native microenvironment that cells are exposed to in the body,¹¹² which makes these challenges especially more complicated.

For the most part, challenges associated with design and fabrication of complex tissues in the laboratory, a field that has emerged under the broad definition of “biomanufacturing”,¹¹³ have used various types of scaffold materials. These have generally been based either on solid slow degrading or nondegrading systems (i.e., bioceramics, metallic implants, etc.), or on biodegradable polymeric materials, out of which hydrogels have been the ones with far greater attention in tissue engineering. Given the inherent compatibility of hydrogel scaffolds with mRNA nanoparticles, lipoplexes and polyplexes, this review will cover primarily aspects that relate to polymer hydrogels. The reader is encouraged to refer to recent comprehensive reviews on other materials for nonviral intracellular delivery of mRNA in recent publication.¹¹⁴

By definition, hydrogels are three-dimensional networks of polymers that exhibit the ability to swell and retain large amounts of water within their structure. We have recently published a comprehensive review of their recent use in tissue engineering for various applications, and the reader is encouraged to refer to this publication for details.¹¹² Natural hydrogels are engineered based on naturally occurring proteins (e.g., collagen, gelatin, and fibrin), and polysaccharides (e.g., alginate chitosan, hyaluronic acid, dextran), whereas synthetic hydrogels are made from synthetic polymers, such as poly (acrylic acid), PEG, poly (vinyl alcohol), polyacrylamide, and other polypeptides.¹¹⁵ Much of the excitement surrounding polymer hydrogels in tissue engineering stem from their ability to be tailored for specific applications, which can be achieved by carefully tuning their biodegradability, physical and mechanical properties, injectability, and microstructure. This can be achieved either by controlling their polymer to water ratio, or their degree of polymerization upon gelation.^{112,113} Additionally, hydrogels can be engineered to be responsive to specific stimuli, such as enzymatic activity,^{116,117} pH,^{118,119} temperature,^{120–122} light,^{123–125} and electricity.^{126,127} These different components of customization render hydrogels with improved pharmacokinetic and pharmacodynamic properties, and add to their inherent capacity for overcoming problems such as off-target nucleic acid accumulation.¹²⁷ For these reasons hydrogels represent highly desirable materials.

Similar to other aspects of genetic manipulation discussed above, DNA and RNAi delivery using hydrogels have dominated the field for quite some time,¹²⁸ and the use of mRNA for regeneration has only recently begun to received closer attention. This has been primarily previewed by a sharp in-

crease in industry investments in and around mRNA-based therapy, which jumped from nearly nothing in 2011 to close to \$2 billion in 2016.¹²⁹ Of note, like in all other nucleic acid-based therapies, mRNA is a large polyanion that does not readily cross nonpolar cellular and tissue barriers. Therefore, the potential interactions occurring between the mRNA delivery vehicles with hydrogels is a point that requires careful attention. In addition to their composition, crosslinking density and delivery method (injectability, *in situ* polymerization, etc.), which are challenges that are somewhat common to other drug delivery strategies, the electrostatic interactions between hydrogels and mRNA nanoparticles is a factor that needs to be carefully considered.

Given the polyanionic character of mRNA, and cationic property of most nanoparticles used for their delivery, hydrogels that are electrostatically inert have been proposed as improved materials for mRNA-based tissue engineering, these include primarily PEG-based hydrogels and dextran, which have received more attention.¹³⁰ Cationic hydrogels, however, such as gels composed of PEI, chitosan, poly-amidoamine, PBAEs, and poly L-lysine, have been shown to confer advantages to nucleic acid delivery, including mRNA.¹²⁸ This is not only because cationic polymers have the ability to self assemble into hydrogels themselves,^{122,131} but also because their positive charge distribution enables sustained release of sequestered nucleic acid cargo due to the net negative charge of mRNA and other nucleic acid nanoparticles.

An important disadvantage of these materials for tissue engineering, however, is that cationic polymers have been known to activate apoptotic signaling pathways¹³² and to induce formation of nanopores on the cell membrane,^{133,134} which can lead to cytotoxic effects despite the enhanced transfection outcomes. Moreover, hydrogels derived from these polymers, by themselves, lack cell adhesive ligands (RGD, MMP) that are known to regulate important mechanisms of cell response and differentiation. Therefore, future work should seek to design and develop less cytotoxic cationic hydrogels that are compatible with cell loading, existing biofabrication methods, while still offering favorable electrostatic interactions with polyplexes, lipoplexes, and naked nucleic acids. Current research has paid particular attention to the development and modification of PEI with various additives to improve their cytotoxicity,^{135,136} although modification of PEI and other cationic hydrogels for improved mRNA delivery, specifically, has remained relatively stagnant in recent years. Lastly, while cationic polymers have been linked to a more sustained delivery of genetic cargo, negatively charged nanoparticles or mRNA complexes may be more readily repelled in anionic hydrogels,¹²⁸ which is likely to enhance their immediate delivery in areas where fast release is desired, such as for rapid vascularization of engineered tissues or wound healing.

Despite being an area of certain interest in future, relatively few hydrogels have been evaluated as biomaterials for mRNA delivery. The far majority of the work performed using hydrogels has been on RNAi strategies, and the reader is encouraged to refer to a recent review on the use of hydrogels for RNAi therapies.¹²⁸ Of relevance, it has been demonstrated that nucleic acid delivery in 2D and 3D hydrogels can activate sharply different signaling pathways, although the targets may be similar.¹³⁷ Krebs *et al.*

compared the ability of the released nucleic acid to knock-down the expression of GFP in cells that constitutively express this protein among three different types of cell-laden hydrogel scaffolds: calcium crosslinked alginate, photocrosslinked alginate, and collagen. The results showed that alginate hydrogels resulted in rapid release of nucleic acid cargo over 1 week, whereas collagen had more prolonged and controlled release over a period of 2 weeks, where the differences were postulated to result from the electrostatic interactions between the gel matrix and the RNA cargo. Cell-laden PEI/dextran hydrogels that had complete degradation over 17 days demonstrated 80% efficiency in GFP expression knockdown when siRNA was released from the hydrogel matrix.¹³⁰ A subsequent report utilizing siRNA for osteogenic differentiation in cell-laden PEG hydrogels utilized a thiol-acrylate reaction for *in situ* hydrogel gelation, showing a marked increase of cell differentiation upon release of RNA-interfering molecules in a sustained and controlled manner over the course of 3–6 weeks.¹³⁸ Spatially controlled release of nucleic acid in hydrogels was also shown in methacrylated dextran hydrogels loaded with RNA/PEI polyplexes using a syringe pump, demonstrating that localized and site-specific cell response can be achieved through the controlled gradient formation of the hydrogel scaffold. Recent preliminary experiments from our group using gelatin methacryloyl hydrogels photopolymerized using a visible LED light and loaded with dental stem cells (stem cells from apical papilla–SCAP) showed effective intracellular delivery of mRNA for expression of luciferase and GFP, where the mRNA release was directly proportional to the hydrogel concentration, or crosslinking density over a period of 48 h (Fig. 6). An important observation that requires attention is that multiple groups have reported that directly adapting DNA or siRNA methods for mRNA delivery can be ineffective,¹³⁹ which encourages future studies on the specific use of hydrogels for mRNA delivery in tissue engineering.

In summary, the vast number of tools available for the development and tuning of hydrogel scaffolds that has been

generated in the past two decades in the field of tissue engineering will be important to develop improved methods for mRNA delivery. Although the field of genetic manipulation for tissue engineering has been largely skewed toward the use of DNA delivery and RNAi in recent years, there is growing evidence of the ability of mRNA to address important challenges in regenerative medicine. The ideal combination of scaffold-guided cell response with specific mRNA delivery vehicles remains to be determined. Nanoparticle/scaffold composite materials may possess more favorable properties for tissue engineering than a single system by itself, and this is certainly an area that deserves to be explored for future regenerative applications.

mRNA Applications in Tissue Engineering

mRNA delivery for iPSC reprogramming

The ability to derive pluripotent stem cells from somatic cells through the induction of the Yamanaka reprogramming factors (*Oct 4*, *SOX 2*, *KLF 4* and *c-Myc*)^{140,141} has uncovered an autologous source of stem cells for regenerative medicine, without the ethical issues associated with human embryonic stem cells, or the logistics challenges associated with primary mesenchymal stem cell (MSC) isolation. Cell reprogramming was originally achieved through viral transduction and integration of these reprogramming factors into the genome of host cells. This approach, however, precludes their use for therapeutic applications. Since continuous ectopic expression of these reprogramming factors is both unnecessary^{142,143} and undesirable,^{144,145} transient transfection strategies involving plasmid vectors,^{146,147} adenovirus,¹⁴⁸ DNA transposons,^{149–151} or excisable lentiviral vectors,¹⁵² have been employed to induce pluripotency, which have considerably reduced the risk of genomic modification or insertional mutagenesis. However, a consequent reduction in transfection efficiency and levels of expression has been observed using these methods, highlighting an area where mRNA transfection might be of interest. Although, the Sendai virus, which is a nonintegrating vector, provided a high

FIG. 6. Schematic representation of mRNA delivery of luciferase-encoding mRNA LNP encapsulated in cell-laden photocrosslinked GelMA hydrogel. (A) LNPs have restricted access to cells in the pores of the gel, which (B) upon enzymatic degradation of MMP sequence facilitates diffusion of mRNA through the gel matrix reaching more cells and (C) enhancing luciferase expression after 48 h. Around 5% GelMA has greater porosity than 10% GelMA, leading to approximately twice as much mRNA-driven luciferase expression of stem cells from the dental apical papilla (SCAP). GelMA, gelatin methacryloyl; LNP, lipid nanoparticle. Color images available online at www.liebertpub.com/tea

degree of transfection and cytosolic expression, it required elaborate treatment steps to expunge the virus from the cells.^{152–156} Other studies have used recombinant proteins, modified to enhance cell penetration,^{157–159} or nanoparticles¹⁶⁰ to deliver reprogramming transcription factors into cells. Again, the cost of production and purification of recombinant proteins does not justify the low expression and pluripotency induction obtained by these methods. Therefore, mRNA intracellular delivery for somatic cell reprogramming has been an emerging area of interest in the tissue engineering community, since, in principle, it should enable more straightforward protein translation and reprogramming efficiency.

Synthetic mRNA was first used by Warren *et al.* to direct reprogramming by inducing transgene expression of *Oct4*, *SOX2*, *KLF4*, *c-Myc*, and *Lin-28* in fibroblasts. Phosphatase treatment of mRNA and substitution of ribonucleoside bases, 5-methylcytidine and pseudouridine for cytidine and uridine, respectively, enabled relatively high doses of transfection without generating host immune response and cytotoxicity, and cells transfected with modified mRNA showed high dose-dependent transgene expression.¹⁶ The

use of feeder cells and supplementation of medium with the interferon inhibitor B18R further diminished toxic effects of the transfection process. The protocol required periodic, multiple transfections for sustained expression of reprogramming factors over the 2-week duration necessary to induce pluripotency owing to the transient nature of mRNA translation kinetics and degradation, with transgene expression peaking at 24 h.¹⁶¹ While the time and labor-intensive nature of this method may be considered a disadvantage—and can be remedied through the use of scaffold-mediated mRNA/nanoparticle release kinetics, as discussed above—the process also renders flexibility in the stoichiometric combination and temporal expression of the transcription factors,¹⁶² which is expedient. In fact, a dose-ramping regimen developed to adjust mRNA dose to cell proliferation rates resulted in a significant reduction in apoptosis caused by stress during standard reprogramming protocols.¹⁶³ A subsequent publication by Warren *et al.* also described the use of a variant of *Oct4* with a *myoD* trans-activation domain that facilitated more efficient and faster induction of pluripotency (Fig. 7), in addition to other

FIG. 7. iPSC colonies derived from fibroblasts reprogrammed with mRNA cocktail containing modified MyoD and subsequent directed differentiation, reproduced from¹⁶¹ with permission. (A–D) Immunostaining of pluripotent colonies showing expression of nuclear and cell-surface markers. Immunofluorescence images revealing expression of (E) neuroectoderm markers, (F) endodermal marker Sox17, and (G) α -actinin immunostaining of cardiomyocytes derived by directed differentiation of the same iPSC clone. (Scale bars: 100 μ m). iPSC, induced pluripotent stem cell. Color images available online at www.liebertpub.com/tea

improvements to culture conditions, such as 5% O₂, addition of valproic acid to medium,¹⁶¹ and a more recent study that incorporated two more reprogramming factors (*Lin28* and *Nanog*), in addition to the *Oct4* variant, saw additional improvement in reprogramming under hypoxic conditions.¹⁶⁴ Other combinations of reprogramming factor with unmodified mRNA, and in the absence of interferon inhibitors, have also since been employed to induce pluripotency in fibroblasts¹⁶⁵ and the iPSCs obtained in this manner could successfully be differentiated to multiple lineages,^{16,32} but mRNA reprogramming has yet to be applied to other cell types and different media conditions. A recent comparative study conducted on the efficacy of mRNA reprogramming showed highest efficiency among nonintegrating reprogramming methods with epigenetics, homogeneity, and pluripotency markers comparable to lenti-derived iPSCs, although successful mRNA reprogramming could not be achieved in blood cells.¹⁶⁶

As the field of tissue engineering becomes more reliant on the use of reprogrammed cells for cell therapy and regeneration, improvements in the methods of cell reprogramming through nucleic acid delivery are likely to become more relevant. Although both the reprogramming efficiency and mRNA delivery still need to be significantly improved for ultimate clinical use, the efforts expended on phase I/II clinical trials using naked, chemically modified, or protamine-complexed mRNA show great promise for the use of mRNA-guided reprogramming in future regenerative efforts.^{99,167,168}

mRNA-directed cell differentiation

Stem cell differentiation occurs during development of an organism to produce new specialized cells, and also

in adults to replace cells that are lost.¹⁶⁹ Increased understanding of the specific signaling mechanisms that control stem cell fate and lineage commitment through high-throughput DNA and proteomic analyses of the changes in gene expression during self renewal and differentiation of stem cells has enabled directed differentiation of stem cells, particularly in the absence of tissue-specific cues.¹⁷⁰ Extracellular cytokine and growth factor-defined protocols for differentiation are largely inefficient and present a high degree of variability. For many years now, both viral^{171,172} and nonviral¹⁷³ gene delivery methods have been used for transfer of lineage-specifying transcription factors to direct differentiation of stem cells to desired lineages in a controlled manner. Again, these methods entail varying levels of genomic integration, risk of immunogenic response, and possibly tumorigenesis, thus limiting their translational appeal. The aforementioned progress in reprogramming fibroblasts to pluripotent states through synthetic modified mRNA-derived transgene expression of transcription factors suggests that this strategy may be feasibly applied to direct differentiation in stem cells. Following this line of thinking, a proof of concept study achieved terminal myogenic differentiation of mRNA iPSCs to myoblasts by subsequent transfection with modified mRNA encoding *MyoD* (Fig. 8)¹⁶ in time scales comparable to other transfection methods.¹⁷⁴ Two other studies have reported transdifferentiation of fibroblasts to myoblasts by mRNA-driven exogenous expression of *MyoD1* in murine and human models opening up the possibility of other phenotypic conversions using this method.^{164,175} In summary, current research suggests that transgene expression of lineage-specific transcription factors to direct differentiation in stem cells or promoting transdifferentiation of somatic cells through ectopic expression of

FIG. 8. (A) Microscopy images showing keratinocytes transfected with unmodified mRNA (No Mods) (B) 5-methyl-cytosine modified (5mC) (C) Pseudouridine modified (Psi) (D) 5mC + Psi modified-RNA encoding GFP. (E) Immunostaining for the muscle-specific proteins, myogenin (green) and myosin heavy chain (MyHC - red), in murine C3H/10T1/2 cell cultures with (F) mRNA encoding MYOD. Reproduced from³² with permission. Color images available online at www.liebertpub.com/tea

molecular switches, eliminates heterogeneity caused by defined media formulations, allowing reproducibility of differentiation protocols for regenerative applications with quantitative control over expression levels.

mRNA delivery to control stem cell secretome

In addition to their multipotent differentiation potential, the regenerative capacity of stem cells is attributed to their robust paracrine response to tissue injury. In particular, the MSC secretome has been found to be pivotal in modulating immune/inflammatory response, regulating angiogenesis, and maintaining a cytoprotective effect on local cells through both soluble factors and horizontal gene transfer by extracellular microvesicles and exosomes, in response to stress-derived signaling.³ High-throughput analyses using Liquid Chromatography with Tandem Mass Spectrometry Detection (LC-MS/MS), antibody arrays and bioinformatics have identified over 200 proteins as part of the MSC secretome.¹⁷⁶ While preconditioning and molecular stimulation protocols have been developed to stimulate the secretory function of stem cells for therapeutic applications, genetic manipulation through overexpression of selected genes such as *Akt*, and its target genes *IGF-1*, *VEGF*, *GATA-4* and *SDF-1* produced a sustained secretory response.^{177–181} Other studies have identified regulators of the stem cell secretome, such as calcium/calmodulin-dependent protein kinase kinase-1 (CAMKK1)¹⁸² and the activated form of HIF1 α ,¹⁸³ overexpression of which resulted in stimulation of the secretory function in MSCs through the Akt/PI3K pathway. There is also evidence for the involvement of PI3K, ERK1/2, p38 MAPK, and JAK/STAT signaling cascades in the regulation of stem cell secretory actions.^{184–186} While controlled genetic manipulation of the stem cell secretory function would require a deeper mechanistic understanding of the specific function of each of these transcription factors and the response they generate, currently, overexpression of individual growth factors, such as VEGF, PDGF-BB, BDNF, with well-characterized roles in angiogenesis and neuroprotection in transplanted stem cells have found use in recovery and regeneration of tissues, particularly for cardiovascular and neuroregenerative applications.^{187–189} These strategies, in addition to the concerns associated with traditional gene transfer methods, may warrant undesirable side effects as a result of prolonged expression of paracrine factors,^{190,191} which can be avoided by mRNA-driven therapeutics.

VEGF-A, one of the crucial factors in angiogenic signaling, was stably and transiently expressed at physiologically relevant doses in multipotent human embryonic stem cell-derived heart progenitors using chemically modified mRNA encoding *VEGF-A*, and stable accumulation of secreted protein occurred for up to 3 days after transfection. While local administration of modified *VEGF* mRNA after myocardial infarct showed promise in preventing scar formation and engendering regenerative effects through targeted, localized signaling,³¹ both secretory therapeutic functions as well as vasculogenic lineage specification to an endothelial phenotype was achieved in *VEGF*-engineered progenitor cells *in vitro* and *in vivo*¹⁹² evidencing complete functional potency of the translated protein. Based on these studies, in 2017 major pharma-

ceutical companies (i.e., AstraZeneca-Moderna) announced a phase I clinical trial using mRNA *VEGF-A* formulations to investigate the angiogenesis in cardiometabolic disease patients. The outcome expected by this study consists in the formation of more blood vessels and enhanced blood supply providing a unique regenerative treatment option for cardiac patients.¹¹⁰ Cotransfection of multiple signaling factors of P-selectin glycoprotein ligand-1, Sialyl-Lewis^x, and Interleukin-10 in MSCs was successfully performed, which provoked an immunomodulatory response *in vitro*¹⁹³ and *in vivo*¹⁹⁴ as yet another example of enhanced and targeted therapeutic function of mRNA-engineered stem cells.

Another interesting application has been proposed for stem cells in regenerative medicine involving the use of microvesicles and exosomes shed by these stem cells as vehicles of genetic material, following their intrinsic function of transporting proteins beyond cell membranes.^{195,196} Their innate stability against enzymatic degradation, long half-life, and membrane penetrability add to their appeal and can potentiate cell-free, tissue-specific gene modification strategies for regenerative applications.¹⁹⁷ Microvesicles can be modified to be enriched with specific proteins for transfer to target cells by genetic engineering of host stem cells for overexpression of these proteins, as a therapeutic or regenerative strategy that surpasses delivery and protein stability limitations faced by other drug or nucleic acid delivery methods.^{198,199} In this perspective, microvesicles derived from stem cells/precursors, may activate the cell organization capillary-like structures and trigger angiogenesis through horizontal transfer of mRNA or protein delivery that may contribute to tissue regeneration.^{200,201} However, more extensive biological characterization of microvesicles will need to be performed before they can be adopted for translational purposes in regenerative medicine.

mRNA applications for cytoprotection and angiogenesis

In regenerative processes, proteins act as important signaling molecules to control cell response on regeneration of damaged tissue. However, proteins have a low stability when delivered *in vivo* and may denature if directly submitted to the blood stream. To overcome these limitations, mRNAs can be delivered to site-specific locations, then internalized by cells, and activate the cell translation process to produce the target proteins required for tissue repair.³⁶

Huang *et al.*, demonstrated the effectiveness of modified *IGF-1* mRNA delivered intramyocardially during myocardial infarction in impeding hypoxia-induced apoptosis by supplementing cell-secreted and cell-associated IGF-1.¹⁹⁹ Cells induced to express IGF-1 transiently increases phosphorylation of Akt and ERK, as well as activation of HIF1 α , which are known transcription factors for signaling. Margariti *et al.* described the mechanism of derivation of endothelial and stem cells from a single source of partially reprogrammed fibroblasts (4 days of induction of OSKM factors). Treatment of these cells with VEGF before use for regeneration, showed efficient derivation of endothelial cells, which when seeded on decellularized vessel scaffolds showed high reendothelialization potential.²⁰² This method, which according to the authors,

can be accomplished through either DNA or mRNA delivery, offers a nontumorigenic source of personalized cell therapy for treatment of ischemic tissues as well as engineered tissues, and can be applied for the derivation of other cell types that are not easily procurable.^{203–205}

Cas mRNA delivery for xenotransplantation

Xenotransplanted organs or cells have become increasingly popular as therapeutic solutions to overcome shortages in allotransplants. Recent examples of decellularized xenotransplanted organs have contributed to the development of newer strategies of regenerative medicine.^{206–209} The development of molecular tools, such as zinc finger nucleases, transcription activator-like effector nucleases, and CRISPR-Cas systems, has gone a long way toward achieving suppression of immunogenic responses and prevention of infection transfer thus providing long-term protection to xenografts against rejection mechanisms of the recipient organism.

In addition to the inherent ease of handling and efficiency of CRISPR/Cas systems, the development of chimeric single-guide RNAs (sgRNA) for targeted editing^{210,211} has further improved the specificity and efficiency²¹² of this approach and has been widely used for gene editing in cells that can be more effectively used for regenerative approaches,^{213–215} as well as in creation of transgenic animals with desired knockout genes^{216,217} for mechanistic studies. Moreover, CRISPR/Cas systems involving sgRNA can target multiple genomic loci enabling creation of multitransgenic organisms.²¹⁸ For example, Zhou *et al.*, demonstrated the multiplexable nature of the CRISPR/Cas9 mRNA system by targeting one exon of five genes (*B2m*, *Il2rg*, *Prf1*, *Prkdc*, and *Rag1*) in mice zygotes and successfully achieved various immunodeficient strains of mice with different combinations of knockout genes with upto quintuple deletions of all five genes, which could be transmitted through the germline to offsprings.²¹⁹ The incorporation of these methods in creating multimodified transgenic pigs for xenotransplantation with knockout of xenoantigens, such as *GGTA1*, *CMAH*, and *B4GalNT2* and integration of human protective genes (*CD46*, *CD55*, *CD59*), has created a safe, ethically viable, and potentially unlimited source of cells and organs for xenotransplantation,²²⁰ which may form the next stage of implanatable regenerative strategies with more efficient translational outcomes. While transgenic animals are typically created through somatic cell nuclear transfer, the procedure is inefficient and prone to causing phenotypic abnormalities in the animals.²²¹ However, the use of RNA-guided nucleases and Cas nuclease mRNA enables circumvention of multiple steps wherein the two components could be directly injected into the cytoplasm of zygotes,²²² mitigating the low efficiencies and technical challenges associated with somatic cell nuclear transfer has been used to create knockout animals in various species.^{222–225} The advantage of Cas mRNA-mediated gene silencing methods is in the transient nature of expression of the nuclease, enabling efficient on-target genome editing, while curtailing off-target editing due to extended nuclease exposure.^{226,227} However, contradictory results were obtained by Petersen *et al.* when cytoplasmic injection of CRISPR/Cas9 DNA

vectors targeting α 1,3-galactosyltransferase (*GGTA-1*), a major gene responsible for hyperacute rejection in pig-to-primate xenotransplants, resulted in higher efficiency of knockout and lower mosaicism in porcine cells²²⁸ than when nonviral delivery of Cas9 mRNA was used.²¹³ Similarly, high degree of mosaicism was reported by Liang *et al.* in human tripronuclear zygotes using the CRISPR/Cas9 system suggesting that a further examination of delivery methods, nuclease concentrations, and intracellular mechanisms will be required to enhance fidelity and efficiency of these tools for successful clinical adaptation.

Challenges and Perspectives

The field of tissue engineering has evolved dramatically since its inception in the late 90s. Current methods of biofabrication for direct engineering of tissues and organs have represented important strides toward clinical application. Similarly, the developments of advanced *in vitro* tools for drug discovery and disease models, such as integrated organs-on-a-chip,^{229,230} and organoid engineering,^{231,232} have drastically changed the landscape of research on regenerative medicine. The development of improved methods of genetic manipulation of stem cells for regenerative applications certainly represents another facet of the next generation of tissue engineering methods. Borrowing tools from basic biology and metabolomics,^{233,234} proteomics,²³⁵ and material sciences/materialomics,²³⁶ we envision that the generation of libraries^{111,114} of polymers will enable the expedited determination of the ideal combinations of nanoparticle carriers, RNA cargos, and hydrogel scaffolds for controlled cell response. Existing microarray technologies may be of interest in the development of these databases of materials and biological systems to better determine how they interact with one another for ultimate use.

Another area that is likely to receive increasing attention is the integration of genetic modifiers, such as mRNA, RNAi, and DNA, in controllable materials for biomanufacturing. As we hinted above, the complexity of native tissues in the body gives rise to the need for biofabrication methods that can precisely control the position, size, and morphology of each individual component making up complex tissues and organs. Therefore, it is unlikely that this is a situation where a single nanoparticle/mRNA will be able to allow for regeneration/engineering of an entire complex tissue or organ. Most likely the combination of multiple nucleic acids and cell-laden hydrogels engineered through biofabrication methods will bring us one step closer to the controlled formation of functional tissues and organs. In this regard, manufacturing tools such as 3D bioprinting,^{237,238} hydrogel microfabrication,^{112,239} and microfluidics²⁴⁰ will become increasingly important, especially when utilized with the right cells and scaffold materials. Moreover, the ability of controlling the genetic composition of individual cells *in vitro* and *in vivo* will illuminate the range of possibilities that remain poorly explored, including *in situ* reprogramming of stem cells after cell-laden hydrogel implantation, *in situ* genetic manipulation of delivered cells as a means of immunomodulation, controlled genetic vaccines, and other areas that are still in their infancy.

Conclusions

A new generation of synthetic nucleic acids is finding favor for gene modification in a wide variety of applications by enabling controlled transgene expression without risk of insertional mutagenesis or genome modification. Innovative approaches for *in vitro* transcription and modifications to mRNA chemistry, as well as delivery mechanisms have curtailed immunogenicity and improved transfection efficiencies. Additional endeavors to study and optimize scaffold-carrier interactions can add an additional level of spatiotemporal control over transgene expression while allowing integration of mRNA-mediated gene transfer with tissue engineering strategies to precisely modulate cell behavior and function for regenerative applications. Furthermore, the ability to induce pluripotency in somatic cells through mRNA reprogramming has afforded an increasing source of multipotent cells, and the same technology can be feasibly applied to efficiently induce differentiation to desired lineages in a reproducible manner, or to stimulate stem cell secretory functions. The prospect of integration of biofabrication techniques with optimized scaffold carrier interactions and release kinetics for delivery of multiple gene modifiers could represent an important addition to the expanding field of tissue engineering.

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Disclosure Statement

The authors declare no conflicts of interest.

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Address correspondence to:
Luiz E. Bertassoni, DDS, PhD
Division of Biomaterials and Biomechanics
Department of Restorative Dentistry
School of Dentistry
Oregon Health and Science University
Portland, OR 97201

E-mail: bertasso@ohsu.edu

Gaurav Sahay, PhD
Department of Pharmaceutical Sciences
College of Pharmacy
Collaborative Life Science Building
Oregon State University
Portland, OR 97201

E-mail: sahay@ohsu.edu

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