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Submitted for publication July 18, 2011.

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Abbreviations and Acronyms

PD = polydopamine

PEG = poly(ethylene glycol)

PLG = poly(lactide-co-glycolide)

PS = phosphatidylserine

Biomaterial Scaffolds for Controlled, Localized Gene Delivery of Regenerative Factors

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Significance: Biomaterials play central roles in tissue regeneration by maintaining a space for tissue growth and facilitating its integration with the host. The regenerative capacity of materials can be enhanced through delivery of factors that promote tissue formation. Gene delivery is a versatile strategy to obtain sustained production of tissue inductive factors. Biomaterial scaffolds capable of gene delivery have been shown to induce transgene expression and tissue growth.

Critical Issues: The widespread application of biomaterial scaffold systems requires identifying the design principles for the material and vectors that modulate transgene expression temporally and spatially. These technologies and others will ultimately enable spatial and temporal control over expression to recreate the cellular organization and gene expression required for formation of complex tissues.

Recent Advances: The design parameters for the biomaterials and vectors that modulate the extent and duration of transgene expression and the distribution of transgene-expressing cells within and around the injury are emerging. The cellular interactions with the biomaterial, such as adhesion or migration rate, can influence expression. Furthermore, modulating the interaction between the vector and biomaterial can control vector release while minimizing the exposure to harsh processing conditions.

Future Directions: Biomaterial scaffolds that deliver genes encoding for regenerative factors may provide a platform for regenerating complex tissues such as skin, blood vessels, and nerves. Biomaterials capable of localized gene delivery can synergistically target multiple cell processes and will have application to the regeneration of many tissues, with great promise for clinical therapies.

SCOPE

BIOMATERIALS PLAY A central role in tissue regeneration for wound healing by serving to create and maintain a space for tissue growth and supporting cell adhesion and migration that results in integration with the host.¹ The regenerative capacity of these materials can be enhanced through localized delivery of factors such as cytokines or growth factors that can induce tissue formation. Gene delivery is a versatile strategy to obtain sustained production of these

proteins within the wound, as cells expressing the transgene, which are termed transfected or transduced for nonviral and viral vectors, respectively, can function as local bioreactors for the protein of interest. For gene therapy vectors, the physical properties are largely independent of the nucleic acid sequence; thus, various sequences or combination of sequences can be delivered without modifying the delivery vehicle. Vectors may be plasmids, a circular strand of DNA, which are relatively stable and can be

delivered directly from the material, or consist of nucleic acids packaged into viral or nonviral systems that generally have increased efficacy relative to naked plasmid.^{2,3} Biomaterial scaffolds can be loaded with these vectors and engineered to release their payload in a controlled manner serving to maintain an elevated concentration of vector locally, which increases the probability of cellular internalization. In this article, we describe cutting-edge strategies for delivering gene therapy vectors from biomaterial scaffolds and their utility in regenerative medicine.

TRANSLATIONAL RELEVANCE

Barriers to gene delivery include vector transport to the cell surface, internalization, and trafficking to the nucleus to initiate expression of the transgene. Biomaterial scaffolds delivering vectors are primarily targeting mass transport limitations; however, the scaffold design may influence other processes, such as internalization pathways.⁴ Cells within and adjacent to the material typically express the transgene, and the context in which this expression occurs can influence regeneration. The design of the scaffold, including degradation rate, porosity, and architecture, influences the rate of cell ingrowth and the identity of infiltrating cells, which impact the efficiency by which the transgene is expressed. The target articles discussed herein have begun to refine the design criteria for the vector and the scaffold, which will lead to more efficient and controlled delivery systems that are necessary to direct formation of functional tissues for a wide range of applications.

Several of the key issues to be addressed for the material, vector, and implant site include (i) the extent and duration of transgene expression, (ii) the identity of cells infiltrating the scaffold and expressing the transgene, (iii) the number of cells within and adjacent to the implant that express the transgene, (iv) the impact of the material and vector on the foreign body response, and (v) the appropriate factors to express to maximize tissue formation. Fundamental studies at the interface of gene therapy and tissue engineering can address these issues and develop this technology for ultimate use in therapeutic applications.

CLINICAL RELEVANCE

Wound healing requires complex temporal and spatial patterns in the expression of various cytokines and growth factors within the local microenvironment,⁵ which can direct cells into spatially complex arrangements that guide their differenti-

ation and function. Treatments designed to foster the complex wound healing process have had moderate success,^{6,7} likely because of their inability to establish the necessary temporal and spatial patterns in the availability of the regenerative factors.^{8,9} The application of gene delivery from biomaterial scaffolds may provide the technology necessary to effectively recreate these patterns in gene expression and cellular organization that can promote the formation of complex tissues, such as skin, blood vessels, and nerves.

EXPERIMENTAL MODEL OR MATERIAL: ADVANTAGES AND LIMITATIONS

Biomaterial scaffolds, fabricated as either microporous structures or hydrogels, provide a support for tissue growth, and delivery of genes encoding for inductive factors can significantly enhance their bioactivity. Microporous scaffolds and hydrogels have distinct physical properties and their selection depends upon the intended use, and their varied properties influence the mechanisms for delivering the vectors and the expression profile.^{10,11} The delivery strategies for vectors can generally be categorized as (i) encapsulation or (ii) immobilization. For the encapsulation strategy, the vector is encapsulated into the scaffold during fabrication and must remain stable throughout scaffold processing. Alternatively, vectors are immobilized to the scaffold after fabrication, which avoids exposing the vector to processing steps that may reduce its activity. Both strategies serve to maintain an elevated local concentration of the vector, which increases the probability of cellular internalization and transgene expression, while limiting off-target delivery.

Naked plasmids can be encapsulated or immobilized to biomaterials due to their relative stability compared to viral vectors.¹² However, relatively large quantities must be delivered due to their relative inefficient delivery. Plasmids can also be complexes with cationic lipids or polymers to form lipoplexes or polyplexes respectively. These nonviral vectors protect the plasmid from degradation and facilitate its entrance into the cell,¹³ however, these complexes have varied stability and processing conditions must often address aggregation. Viral vectors are the most efficient, yet they may have a short half-life and have the potential to lose efficacy during processing. Viral vectors generally pose greater safety concerns than nonviral vectors. For example, integration of the viral genes into the chromosome may disrupt gene function and lead to cancer. Additionally, there is a risk of the emergence of replication-competent viruses. Irrespective

of the vector used, the host immune response must be considered, as the inflammatory response may impact regeneration, whereas clearance of infected cells will abrogate transgene expression.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

The delivery of viral and nonviral vectors from microporous scaffolds and hydrogels were investigated in a series of three manuscripts, which identified mechanisms of biomaterial based gene transfer *in vivo* and developed technologies for enhancing delivery.^{14–16} The extent and duration of transgene expression *in vivo* and the identity of cells expressing the transgene were characterized for naked plasmid and lentivirus delivery from microporous scaffolds. Additionally, the delivery efficiency of nonviral vectors within hydrogels was investigated for hydrogels that had a varied affinity for binding the vectors. These studies identified critical design features that will guide the development of scaffolds for regenerative medicine applications.

Sustained release strategies have been hypothesized to maintain elevated plasmid concentrations locally and enhance gene transfer. The relationship between plasmid release kinetics and the extent and duration of transgene expression were investigated. Scaffolds were fabricated from microspheres composed of the copolymers of lactide and glycolide (PLG) modified with cationic polymer polydopamine (PD), with PD enhancing incorporation and slowing release.¹⁴ Layered scaffolds were fabricated, in which the plasmid is entrapped within a central layer of polymer, and a porous outer layer of scaffold surrounds the central polymer, allowing for cell infiltration. The central layer of the scaffold can then be manipulated to regulate DNA release (*e.g.*, addition of cationic polymer). *In vivo* implantation of scaffolds resulted in no significant changes in the level and duration of transgene expression between PD and unmodified scaffolds. Control scaffolds with dried plasmid on the exterior, which exhibited a rapid release, and scaffolds with extended leaching, to reduce initial plasmid quantities released, had similar levels and duration of expression. In contrast, changing the plasmid design, from a cytomegalovirus to an ubiquitin C promoter, substantially increased the duration of expression. These studies indicate that the initial dose released and vector design affect the extent and duration of transgene expression, which may be sustained over several weeks.

Localized and efficient gene transfer can be promoted by exploiting the interaction between

the vector and biomaterial. Regulation of the vector–material interaction was investigated by capitalizing on the binding between lentivirus and phosphatidylserine (PS), a component of the plasma membrane.¹⁶ PS was incorporated into microspheres composed of the copolymers of lactide and glycolide (PLG) using an emulsion process (Fig. 1A). Lentivirus, but not adenovirus, associated with PS-PLG microspheres, and binding was specific to PS relative to PLG alone. Immobilized lentivirus produced large numbers of transduced cells, and increased transgene expression relative to virus alone. Microspheres formed into porous tissue engineering scaffolds (Fig. 1B) exhibited increased retention of lentivirus and resulted in long-term and localized expression *in vivo* (Fig. 1C). Lentivirus delivery into a spinal cord model produced maximal expression at the implant and a gradient of expression rostrally and caudally. This specific binding of lentiviral vectors to biomaterial scaffolds may provide a versatile tool for numerous applications in regenerative medicine or within model systems that investigate tissue development. Note that lentiviruses are stably integrating vectors, though variants are being developed that do not integrate or in which the integration site can be controlled. Stable transfection may not be appropriate for situations where only transient expression of the protein is needed (*e.g.*, wound resolution). However, control mechanisms are being developed, such as the incorporation of additional genes that will effectively halt expression of the therapeutic gene once a specific drug is administered to the patient.³

Hydrogels capable of localized gene delivery are being developed to provide a support for tissue growth^{4,17} and as a versatile method to induce the expression of inductive proteins; however, the duration, level, and localization of expression is often insufficient for regeneration.¹ A hydrogel is a matrix of hydrophilic polymer that contains over 90% water, exhibits physical properties that resemble native tissue, and can be readily manipulated upon crosslinking conditions.¹⁸ Natural hydrogels (*e.g.*, collagen) have an innate ability to support cell adhesion; however, synthetic hydrogels based on poly(ethylene glycol) (PEG) must be modified to support cell migration. This functionality is achieved by incorporating peptide sequences during crosslinking that allow for cell adhesion to and degradation of the hydrogel.^{10,19} Cell migration then occurs by proteolytic degradation of the peptide crosslinkers by cell-secreted enzymes (Fig. 2). Initial studies demonstrated that transgene expression correlated with cell migration through the

Figure 1. *In vivo* cell transduction on lentivirus-loaded PS-PLG scaffolds. **(A)** PS was encapsulated within PLG microspheres using an emulsion process. **(B)** Microporous scaffolds were formed from these microspheres, which supported the binding of lentivirus. Cells seeded on the scaffold *in vitro* were transduced (*i.e.*, expressed the transgene), as evidenced by the staining (dark regions) throughout the scaffold. **(C)** *In vivo* implantation resulted in long-term transgene expression, which was determined by delivering the gene encoding for chemiluminescent enzyme luciferase. Luciferase is commonly used as a reporter gene as the light emission can be quantified by bioluminescence imaging. PS, phosphatidylserine; PLG, poly(lactide-*co*-glycolide).

hydrogel;⁴ however, degradation of the matrix by migrating cells resulted in the loss of substantial quantities of DNA. We subsequently investigated the modification of hydrogels with affinity peptides to enhance vector retention and increase transfection within the matrix. PEG hydrogels were modified with lysine-based repeats (K4, K8), which retained $\sim 25\%$ more vector than control peptides.¹⁵ Transfection increased 5- to 15-fold with K4 and K8, respectively, over control hydrogels. K8- and K4-modified hydrogels bound similar quantities of vector, yet the vector dissociation rate was reduced for K8, suggesting excessive binding limited transfection. Thus, hydrogels modified with affinity peptides enhanced vector retention and increased gene delivery, and these hydrogels may provide a versatile scaffold for numerous regenerative medicine applications.

INNOVATION

Three systems that exemplify the potential and challenges of delivering plasmid, nonviral complexes, or viral vectors are presented and summarized in Table 1. The initial studies identified that the release rate is decoupled from the expression profile.¹⁴ The initial release from the scaffold significantly influences transgene expression *in vivo* at the implantation site, and a rapid release leads to expression primarily adjacent to the scaffold. To promote gene expression within the material, strategies that enhance vector retention within the scaffold were investigated. PS is a plasma membrane component capable of binding lentivirus and provides a small molecule that can be presented on the scaffold surface to mediate specific interactions with the virus.¹⁶ Alternatively, peptides were incorporated into

Figure 2. Hydrogels with affinity peptides to enhance vector retention and increase transfection within the matrix. Hydrogels are formed by crosslinking four-arm poly(ethylene glycol) (PEG) that contain either adhesion peptides or affinity peptide, with a cell-degradable crosslinker. DNA lipoplexes are encapsulated within the hydrogels upon gelation and retained by the affinity peptide. Cell migration into the hydrogel is supported by interactions with the adhesion peptides and enzymatic degradation of the crosslinks. As cells contact the lipoplexes, they become transfected and express the target gene.

hydrogels to hinder vector release.¹⁵ The modification of hydrogels with peptides represents a versatile strategy that can be readily incorporated into a range of hydrogels to delay release through reversible association with the vector. The mechanistic insights into gene transfer combined with the development of novel technologies are expected to provide the enabling tools to temporally

and spatially modulate gene transfer at any *in vivo* site.

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

Inducing transgene expression by vector delivery from biomaterial scaffolds represents only the

Table 1. Comparison of discussed modalities for controlled gene delivery

	Modality		
	PLG scaffolds modified with cationic polymers	PLG scaffolds modified with phosphatidylserine	PEG scaffold modified with affinity peptides
Biomaterial	Microporous scaffold	Microporous scaffold	Hydrogel
Vector	Naked plasmid	Lentivirus	Plasmid complexed with lipids (lipoplex)
Modification	Incorporation of polydopamine (PD)	Incorporation of phosphatidylserine (PS)	Incorporation of lysine-based repeats
Results	PD enhanced plasmid incorporation and decreased release rate. No significant change in the level and duration of transgene expression with PD. Control scaffolds with dried plasmid on the exterior had similar levels and duration of expression.	PS provides specific binding of lentivirus to the biomaterial. Extent and duration of transgene expression increased in PS modified scaffold relative to unmodified scaffold.	Lysine-based repeats led to increased vector retention. Transfection increased 5–15-fold over unmodified hydrogel.
Conclusions	Initial dose of plasmid release is an important factor in the extent and duration of transgene expression.	PS can induce specific binding of lentiviral vectors to biomaterial scaffolds, which increases efficacy of gene transfer.	Hydrogels modified with affinity peptides enhance vector retention and increase gene delivery.

initial step toward wound healing or tissue regeneration. The extent of expression is critical, as insufficient expression is ineffective and excessive expression may lead to the formation of disorganized tissues. Similarly, the duration of expression must match the needs for tissue formation, as short-term expression may be ineffective at directing tissue growth and extended expression may inhibit the subsequent steps necessary for regeneration. The location of transgene expression can influence whether regeneration occurs around the implanted material or throughout the implant. The cell type expressing the transgene, such as endothelial cells, fibroblasts, or macrophages, can also influence the subsequent cellular processes. Thus, although the combination of tissue engineering and gene delivery provides a platform technology, the design parameters for the material and vector that promote precise control over the expression profile are only emerging. The characteristics of the expression profile will be critical for inducing the complex cascade of processes that leads to the restoration of tissue function.

FUTURE DEVELOPMENT OF INTEREST

Systems for efficient localized gene transfer have been applied as a means to control the microenvironment and promote wound healing or tissue regeneration. Strategies are emerging with the potential to spatially regulate gene transfer on the scale of tens to hundreds of microns,²⁰ and promoters are being developed to control expression at defined times or within specific cell types. A complementary aspect to creating a permissive microenvironment is to enhance the number of progenitor cells. The gene delivery systems described herein could potentially be employed with *in situ* cell programming. Further developments with transdifferentiation or with induced pluripotent stem cells may identify the factors that could be delivered locally to safely create tissue-specific progenitor cells *in vivo* with greater potential for promoting regenera-

TAKE-HOME MESSAGES

Basic science advances

- Biomaterial-based gene delivery can be categorized into two basic mechanisms: encapsulation and immobilization. Encapsulation involves entrapment of the vector within the material. Immobilization requires interaction between the vector and material. Both approaches function to maintain elevated concentrations locally, which can enhance the delivery efficiency. Additionally, the association of the vector with the material may stabilize the vector and thereby increase its activity.
- The initial release of vectors following implantation significantly impacted the extent of transgene expression. For implantation, sustained release did not enhance transgene expression, and further studies are needed to identify the dependence on release rate and expression profile. Long-term transgene expression can be obtained from this initial release of vectors, and the choice of promoter may be employed to control the duration of expression.
- Enhancing interactions between the vectors and the biomaterial can increase the extent of transgene expression and lead to enhanced expression within the injury or wound. Hydrogels modified with affinity peptides retained lipoplexes, whereas microporous scaffolds modified with PS selectively bound lentivirus.
- The material design must be considered within the context of the cellular processes associated with wound healing and also gene transfer. For example, the degradation rate of the hydrogel influences the rate of cell migration, which subsequently impacts the extent and duration of gene transfer.

Clinical science advance

These studies demonstrate that biomaterials used in the clinic, such as hydrogels, can be readily modified with phospholipids, polymers, or peptides to provide controlled localized delivery of genes encoding for therapeutic proteins.

Relevance to clinical care

Biomaterials have been employed as wound dressings, and their modification to deliver genes encoding for tissue inductive factors provide a promising platform for regenerating complex tissues such as skin, blood vessels, and nerves.

tion than the endogenous progenitors recruited to an injury.

ACKNOWLEDGMENTS AND FUNDING SOURCES

Support for this work was provided by the National Institutes of Health (RO1EB005678, RO1EB009910, and PL1EB008542)

AUTHOR DISCLOSURE AND GHOSTWRITING

The authors have no conflicting financial interests. Ghostwriters were not used to write this article.

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