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Engineering Biomaterial Systems to Enhance Viral Vector Gene Delivery

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Integrating viral gene delivery with engineered biomaterials is a promising strategy to overcome a number of challenges associated with virus-mediated gene delivery, including inefficient delivery to specific cell types, limited tropism, spread of vectors to distant sites, and immune responses. Viral vectors can be combined with biomaterials either through encapsulation within the material or immobilization onto a material surface. Subsequent biomaterial-based delivery can increase the vector's residence time within the target site, thereby potentially providing localized delivery, enhancing transduction, and extending the duration of gene expression. Alternatively, physical or chemical modification of viral vectors with biomaterials can be employed to modulate the tropism of viruses or reduce inflammatory and immune responses, both of which may benefit transduction. This review describes strategies to promote viral gene delivery technologies using biomaterials, potentially providing opportunities for numerous applications of gene therapy to inherited or acquired disorders, infectious disease, and regenerative medicine.

Received 9 March 2011; accepted 10 May 2011; published online 31 May 2011. doi:10.1038/mt.2011.111

INTRODUCTION

Gene therapy has shown increasing promise in clinical trials for disorders including Parkinson's disease,[1,2] X-linked adrenoleukodystrophy,[3] hemophilia B,[4] and Leber's congenital amaurosis.[5–7] Basic research and technological developments (*e.g.*, sequencing of the human genome) are aiding the identification of new genetic targets involved in human disease. Additionally, the discovery of RNA interference has provided new molecular options to treat such illnesses.[8,9] In parallel, progress in the development of gene delivery systems must continue to build upon the recent successes in the field and further improve the efficiency and safety of gene carriers.[10]

The higher gene transfer efficiencies of viral relative to nonviral vehicles have supported their use in the majority of clinical applications. Viral vectors—such as ones based on murine retrovirus, lentivirus, adenovirus, and adeno-associated virus (AAV)—are created by stripping the virus of its own genetic cargo and subsequently packaging the genes of interest into the viral capsid. The resulting vectors are typically administered by direct injection, which can be accompanied by either local or systemic spread[11] that can increase immune responses against the vector or its gene product, as well as risk side effects arising from gene expression in off-target regions. Furthermore, viral vectors may suffer from low transduction efficiencies for some therapeutically relevant cell types, due to low viral binding to the cell surface or subsequent gene transfer steps.[12] Strategies to address these concerns may enhance the translation of viral gene delivery to the clinic.

In this review, we describe the potential for combining viral gene delivery with engineered biomaterials to overcome some limitations related to nonlocalized gene delivery, insufficient transduction, and long-term transgene expression. In particular, biomaterials can increase the residence time within the target site through releasing vectors in a sustained manner, which can potentially enhance the delivery efficiency and extend the duration of gene expression. Furthermore, manipulating the properties of biomaterials—such as their size, architecture, and molecular composition—can modulate numerous cellular processes associated with gene delivery. Moreover, biomaterials can also shield viral vectors from components of the host immune response via encapsulation or immobilization, ultimately protecting them from degradation and potentially reducing inflammatory and immune responses. Finally, bioactive materials can be utilized to modulate the tropism of viruses, thereby more effectively specifying the cell population that will express the transgene. Taken together, these biomaterial strategies provide opportunities to more effectively deliver vectors for treating numerous disorders and for regenerative medicine (**Figures 1** and **2**).

STRATEGIES FOR LOCALIZED AND ENHANCED GENE DELIVERY

Biomaterials have been employed clinically for localized and controlled release of numerous small molecule drugs (*e.g.*, sirolimus) and proteins (*e.g.*, growth hormone),[13,14] and these systems may also be applied to the delivery of viral vectors. In particular, vectors have been interfaced with materials in two ways:

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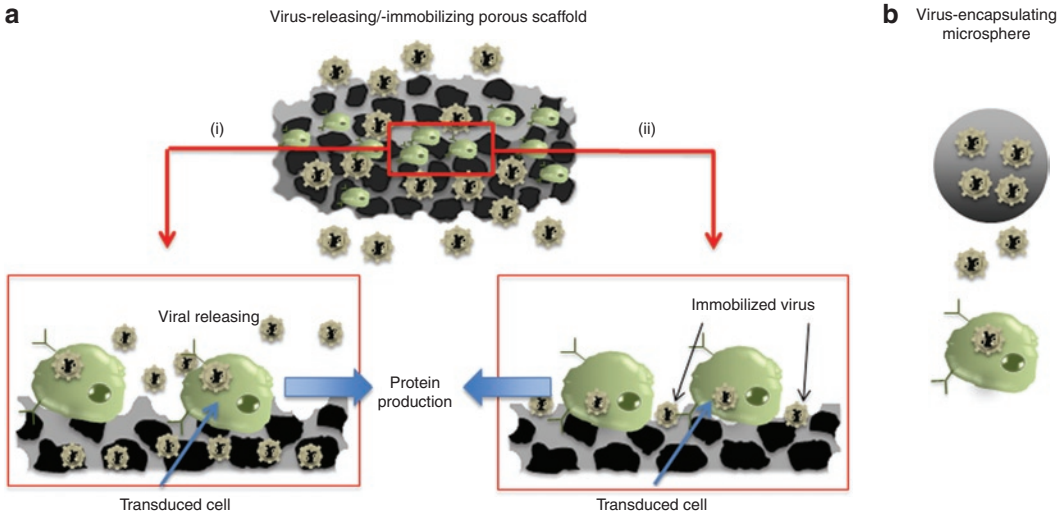

Figure 1 Schematic illustrating (**a**) porous scaffolds with virus that is (i) encapsulated or (ii) surface immobilized for delivery. (**b**) Microspheres with encapsulated virus for localized release.

encapsulation of the vector within a material (*e.g.*, microspheres, scaffolds) and immobilization of the vector to a material surface, a process termed reverse transfection, solid-phase delivery, or substrate-mediated delivery.[15,16] The material may enhance delivery in multiple ways (**Figure 1**). Material-vector nanoparticles may be directly internalized by cells, thereby providing a mechanism for cellular entry. Alternatively, the vector may be released in a sustained manner, which thus maintains a locally elevated concentration. Finally, immobilization to the material surface can colocalize cells with the vector and thereby overcome mass transport limitations.[17]

In addition to enhancing delivery, these strategies aim to localize gene expression to the microenvironment surrounding the material and minimize systemic spread of viral vectors *in vivo*. Direct injection of vector in solution can lead to undesired, widespread distribution to other tissues.[11,18,19] As one example, in an osteogenic gene delivery study, widespread transgene expression caused by direct injection has led to deleterious effects, such as heterotopic ossification or fusion of adjacent tissues (*e.g.*, cartilaginous and ligamentous tissues), resulting in joint dysfunction.[20] Additionally, even local injection of viral vectors into an organ can lead to widespread biodistribution including the liver and lymph nodes,[21] and it is well known that vector delivery to the liver can be associated with antigen-specific T-cell responses against the vector.[22] This section describes a range of material formulations and their application to and mechanisms of gene delivery with a focus on the integration of unmodified vectors with materials, and subsequent sections discuss strategies to directly modify the virus for controlled delivery.

Encapsulation within biomaterials

Biomaterials encapsulation is typically employed to protect a drug from the environment and thereby stabilize it against degradation. If the challenge of stabilizing the drug during the encapsulation process itself can be overcome, the resulting material presents numerous means to control the subsequent delivery process. For example, factors can be encapsulated into materials of different

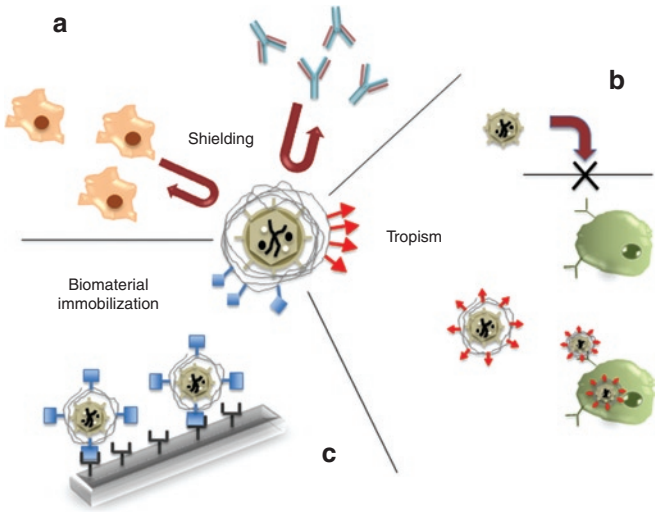

Figure 2 Schematic illustrating biomaterial modification of viruses for: (**a**) shielding against immune responses, (**b**) altering cellular tropism, and (**c**) functionalization for biomaterial immobilization.

compositions designed for the specific applications, and the materials can be formulated into structures of varying sizes, ranging from nanometer to micrometer, in order to control release properties. Furthermore, the material can be readily varied from two-dimensional substrates that are placed onto a surface to three-dimensional structures that fill a defined space *in vivo*.

Nano- and microparticles. Vector-encapsulated particles can be injected for noninvasive delivery within a tissue or can be added topically to an injury site to provide localized release. To date, microsphere particles have been formed with diameters on the order of 1–6 μm. Spheres with diameters exceeding a few microns are in general not readily internalized; however, they can adhere to cell surfaces or be retained within the tissue, and the ensuing localized virion release may increase contact between cells and the vector to enhance transduction. In addition, particles with

diameters on the order of a few μm can be phagocytosed by macrophages,[23] and smaller particles can be internalized by pathways such as clathrin-mediated endocytosis, caveolae, or macropinocytosis.[24,25] For example, nanoparticles with entrapped nonviral vectors can be directly internalized,[26] though this approach has not yet been extended to viral vectors. Such entrapment may present opportunities to overcome rate limiting steps for some viruses, as the surface properties of particles can be designed to mediate cellular interactions on behalf of the virus, with the potential to influence cell tropism. However, achieving high-viral internalization efficiency following encapsulation may be challenging, as will nanoparticle encapsulation of viral vectors—which have diameters ranging from 20 to several 100 nm.[27]

A number of biomaterial platforms have been harnessed for such applications. For example, synthetic, biodegradable polymers, such as poly (lactide-co-glycolide), have been extensively used for drug delivery formulations and have more recently been applied analogously for the delivery of viral vectors. Adenoviral vectors have been loaded using a double emulsion process,[28–33] with the particle size controlled through the extent of mixing and the emulsifying conditions. The encapsulated vector is then released through a combination of polymer degradation and subsequent diffusion from the particle, and degradation can be controlled by tuning the polymer formulation to modulate its hydrolysis rate. Particles loaded with viral vectors have been administered through various routes (*e.g.*, subcutaneously,[31] intracranially,[32] intramuscularly,[33] and intraperitoneally[34]). Administration of particles encapsulating viral vectors has yielded effective and localized gene expression while preventing systemic vector spread as compared to the free vector,[24] and shielding vectors within the particles has substantially reduced immune responses against the vector, ultimately extending the duration of transgene expression[26] or enhancing transduction efficiency[34] compared to nonencapsulated free virus.

Although virus-loaded particles have been developed using the double emulsion process, the organic–aqueous interface, shear stresses, and potentially low pH environment present during the emulsification can denature or inactivate the viral vector. Virus stability, defined as the maintenance of infectivity, has been addressed through the inclusion of stabilizers in the primary emulsion, such as bovine serum albumin/glycerol,[32,33] poly-L-lysine,[30] and polyethylene glycol (PEG).[29] These components may protect the adenoviral capsid under harsh conditions,[29] thereby enhancing the physical stability of the vector and increasing transduction. Finally, a challenge of the emulsion process is the relatively low efficiency of virus loading into the final particles (<25%), which can limit the potential utility of this approach.

Microporous scaffolds. Microporous scaffolds provide a structural support that upon implantation *in vivo* can maintain a space for tissue growth, as the porosity supports cell infiltration throughout the scaffold. The scaffolds may have a predefined architecture to organize tissue growth, or may be designed to "space fill" and solidify upon injection.[35] The pore sizes of these scaffolds are typically on the order of tens to hundreds of microns to facilitate rapid cell infiltration, and the release of vectors from the scaffold may transduce both cells that have infiltrated the material and cells within the surrounding tissue.[36]

One simple means to fabricate vector-releasing scaffolds is by fusing vector-loaded microspheres into a porous structure,[37] but they can also be generated by other means. For example, vectors based on as lentivirus or murine retrovirus—virions whose envelopes pose challenges for particle stability—have been loaded onto preformed microporous scaffolds by simple absorption into the pores, which avoids the challenges associated with polymer encapsulation.[37,38] This strategy thus maintains the vector activity; however, a rapid release or burst of the vector can occur for pores sizes that are substantially larger than the vector. Adenoviral vectors have also been encapsulated within the core of fibers by coaxial electrospinning, a versatile fabrication process to form micro- or nano-fibers by applying electrostatic charges on polymers under a high-voltage gradient.[39] Electrospun fibers formulated highly porous structures, where adenoviral vectors encapsulated within the core of fibers were released in a porogen-assisted manner.[39] Controlled release of adenoviral vectors from the resulting electrospun fibrous scaffolds prolonged the duration of transgene expression of cells seeded within the fibers over a month and reduced macrophage activation compared to freely dispersed viruses.[39] However, the challenges of maintaining vector activity described for microsphere fabrication are also relevant to electrospinning.

Hydrogels. Viruses have also been delivered from hydrogels, materials with physical properties similar to many soft tissues that is composed mostly of water (>90% by weight). Hydrogels can be created from hydrophilic polymers that are crosslinked or self-assembled to form a network, and they are employed in numerous applications in drug delivery and regenerative medicine.[40,41] Alternatively, hydrogels can be formed from natural materials, such as collagen and fibrin, and can thus present intrinsic signals that support a number of cellular processes such as adhesion and migration.[42–44] Like microporous scaffolds, hydrogel materials can be engineered to fill a defined three-dimensional space within a tissue, and they also offer the advantage that they are generally formed under mild conditions that do not diminish virus activity.[34,45–52] Additionally, viral vectors can be mixed with liquid formulations and then injected to undergo gelation *in situ*, a minimally invasive approach.[48] For example, collagen matrices containing adenoviral vectors encoding platelet-derived growth factor B, which were premixed with the material before gelation, retained the viral vectors for extended time periods in a rabbit dermal ear wound model and enhanced healing through localized platelet-derived growth factor B expression.[53,54] Other examples of hydrogel formulations used with delivery of adenovirus or retrovirus include collagen,[45,55] fibrin,[45,51] alginate,[34,38,49] chitosan,[50] silk-elastin-like polymer,[46,47,56] and recombinant polymers.[48,52]

The high water content of the hydrogel, as well as swelling that occurs with some gels, can lead to rapid release of the vector, and effective transgene expression requires tuning material properties to modulate vector release rate. Hydrogel design parameters for manipulating viral delivery to target either the surrounding cells or cells infiltrating the hydrogel include the mesh size (*i.e.*, average distance between polymer crosslinks within the 3D network) and degradation rate. The hydrogel mesh size—determined by the identity of the polymer, its molecular weight, concentration, and

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extent of crosslinking—can range from as low as 40 Å to as large as 10 μm.[57–59] Mesh sizes considerably larger than the virus diameter are not necessarily useful, as they lead to unhindered vector diffusion from the gel that approaches the rapid delivery rate of a simple bolus injection. However, for mesh sizes smaller than the vector, hydrogel degradation must occur for virion release.[60] Matrix degradation often accompanies cell infiltration and proteolytic degradation of gel crosslinkers, and factors that enhance cell infiltration have increased the extent of transgene expression and yielded more homogeneous gene expression throughout the hydrogel.[60] One example of the role of gel properties on viral release on is adenoviral gene delivery from silk-elastin-like polymers.[46] Vector release occurred over extended time periods, and induced persistent gene expression for at least 15 days in tumor sites, whereas gene expression mediated by bolus virus injection decreased within 11 days.[52]

Substrate-mediated viral gene delivery

In addition to polymer encapsulation, vector immobilization to the surfaces of biomaterials can initially retain and progressively release the virus for cellular internalization, an approach that has been termed reverse transfection, solid-phase delivery, or substrate-mediated delivery.[15] In contrast to encapsulation, the materials can be synthesized and manipulated first, and the vector added in the final steps, thereby avoiding exposing the virus to harsh processing steps. Like biomaterial incorporation, the subsequent administration of vector-presenting substrates to cells or tissues can place the cells and vector in close proximity during delivery and may thereby function to overcome mass transfer limitations to enhance the delivery efficiency.[16,61]

Nonspecific binding. The most straightforward means to immobilize vectors is via nonspecific binding, including electrostatic, van der Waals, and hydrophobic interactions.[15] Such viral vector adsorption onto preformed hydrogel structures and nano- or microparticles have been shown to enhance gene delivery.[37,53,54,62–75] Importantly, the extent of immobilization depends on the surface properties of biomaterials. For example, self-assembled monolayers that presented cationic groups ($-NH_2$) yielded higher level of retroviral vector mediated gene expression compared with surfaces presenting methyl ($-CH_3$) or carboxylic acids (–COOH), as the cationic surfaces promoted both virus adsorption and cellular attachment.[65]

Viral vectors may also interact with extracellular matrix proteins, which can readily be coated onto material surfaces. For example, immobilization of retrovirus onto a surface coated with fibronectin resulted in two to fourfold higher transduction efficiencies compared with bolus delivery.[67] In addition, material surface chemistry is important for extracellular matrix protein adsorption and for gene delivery. In one study, each combination of an extracellular matrix protein (*e.g.*, pronectin, collagen, and fibronectin) and surface chemistry (*i.e.*, NH_2, CH_3, and COOH) resulted in different adsorption and gene delivery of retroviral vectors, indicating that both electrostatic interactions and surface hydrophobicity can be central variables in controlling gene delivery from substrates upon virus adsorption.[65] In addition to retrovirus, other vectors have been adsorbed to biomaterials. For example, immobilization of lentivirus and adenovirus onto collagen, fibronectin, and poly(lactic-co-glycolic acid) scaffolds, via freezing and lyophilization of virus and scaffold, resulted in enhanced transduction efficiencies relative to bolus delivery, even with low quantities of virus and localized transgene expression *in vivo*.[37]

Specific binding. More recently, strategies have been developed for specific binding between the biomaterial and the vector, based on the interactions of functional groups on viral capsid and biomaterial surfaces. In principle, this approach provides the opportunity to tune vector affinity for the surface, thereby controlling release and gene delivery. For example, antiviral antibodies have been employed for vector immobilization to biomaterials.[19,76–79] In one study, such antibody tethering of adenovirus onto a vascular stent prevented systemic spread and resulted in-site-specific delivery to pig coronary arteries, whereas stents without tethering antibodies instead led to transduction at distal sites.[79]

We have recently modified AAV via insertion of six histidine residues (*i.e.*, hexahistidine) into a physically exposed loop of the AAV2 and AAV8 (*i.e.*, amino acid position 587),[80] and the resulting tagged virus could be specifically immobilized onto a surface presenting nickel ions chelated by biotin-nitrilotriacetic acid moieties.[61] The degree of immobilization could be controlled by varying both the histidine content on the viral capsid and the quantity of biotin-nitrilotriacetic acid on the material surface, and the resulting surfaces provided localized transduction. In earlier work with adenovirus, avidin-modified surfaces were used to tether biotinylated adenovirus and subsequently employed to infect target cells (*e.g.*, canine osteosarcoma or rat glioma cells) adhered on the surfaces.[62,81] This tethering limited virus diffusion and promoted gene delivery only to cells that come into contact with the substrate.

An alternative to modify the vector is to capitalize on its natural binding specificities. Phosphatidylserine, a component of the plasma membrane that has been linked with the association of the vesicular stomatitis virus glycoprotein to cell surfaces, was investigated as a means for immobilization of vesicular stomatitis virus glycoprotein-pseudotyped lentiviral vectors to biomaterials fabricated from the synthetic polymers lactide and glycolide (PLG).[82] Phosphatidylserine is a hydrophobic compound that can readily be incorporated into PLG microspheres, which are subsequently utilized as building blocks for fabricating three-dimensional scaffolds of an appropriate geometry.[82] Implantation of the resulting vector-loaded scaffolds either subcutaneously or into the spinal cord resulted in expression that persisted for at least 4 weeks at levels significantly increased relative to unmodified PLG scaffolds.

STRATEGIES FOR AVOIDING HUMORAL IMMUNE RESPONSES

A number of broadly utilized vectors are based on human viruses, such as adenoviruses and AAVs, and natural exposure to the parent viruses has led to pre-existing immunity against the recombinant vectors within much or even the majority of the human population. Both cellular and humoral immunity have been implicated as major problems in clinical trials.[22] A number of strategies

are under development to address cellular immunity,[83,84] and biomaterials may be harnessed to protect vectors from neutralizing antibodies, as well as potentially mitigate cellular immune responses against the vector that can preclude the potential for repeat administration (**Figure 2**).[85–88] Vector encapsulations within a material or attachment of materials onto the vector surface are two potential strategies to address these goals.

Physical encapsulation within biomaterials

Vector incorporation into biomaterials, a strategy discussed above for controlled vector release, has the potential to mitigate both pre-existing humoral immunity as well as immune responses against the injected vector. For example, adenovirus encapsulation within alginate microspheres has enabled the vector to evade pre-existing immunity, ultimately enhancing transduction efficiency.[34] Specifically, vector delivery from alginate microspheres yielded slightly reduced or equivalent levels of transgene expression in various organs in mice (*e.g.*, spleen, liver, lung, kidney, and lymph node) upon intranasal or intraperitoneal administration, even in the presence of adenovirus-specific neutralizing antibodies. In contrast, bolus injection led to significantly reduced transgene expression in immunized animals primarily due to immune responses against the vector. Another study explored the potential for biomaterial encapsulation to reduce antivector immune responses and found that adenovirus delivery from PLG microspheres resulted in 45-fold lower anti-adenovirus antibodies titers after delivery compared to direct injection of adenovirus.[32] The capacity for polymer-mediated delivery to shield viruses from neutralizing antibodies or downstream immune responses may enable higher transduction efficiencies, even for lower levels of injected vector, as well as enhance the potential for vector readministration. Future work may explore the generality of such results to other vector and routes of administration.

Chemical modification with biomaterials

In addition to protecting vector by encapsulation within a bulk material, direct chemical modification of viral capsids with biomaterials can aid viral gene delivery. For example, grafting synthetic polymers onto the virion surface can reduce innate immune responses and enable evasion of antivirus neutralizing antibodies, thereby increasing delivery efficiency and allowing repeated vector administration.[89] Such grafting is conducted through the chemical reaction of functional groups within the synthetic polymers to the side chains of several amino acids presented on the viral surface, such as lysines and cysteines. Polymeric materials utilized to date include PEG,[90–99] poly-*N*-(2-hydroxypropyl) methacrylamide (poly-HPMA),[100,101] polysaccharides,[102] and other bioreducible polymers.[103,104]

PEG—a nonimmunogenic and nontoxic material well known for its capacity to resist protein interactions and adsorption—has been widely used for covalent modification of proteins to evade immune responses or to extend circulatory half-life in blood.[105–108] Such PEGylation can reduce protein uptake by Kupffer cells,[109] likely through steric hindrance and masking of surface charges. Likewise, PEGylation has been utilized to protect viral vectors from neutralizing antibodies,[90–94,96,98,110] enable vector retargeting,[97] and enhance vector stability and transduction.[95] In one study, adenovirus with PEG covalently attached to surface lysine residues mediated equivalent transgene expression in adenovirus-immunized vs. naive animals, while nontreated adenovirus suffered an ~47% reduction in the former.[90,93] Interestingly, coadministration of methylprednisolone with PEGylated vector markedly suppressed chemokine expression in liver, neutrophil infiltration, and interleukin-6 plasma levels, as compared with unPEGylated vectors with or without methylprednisolone, or PEGylated vectors without methylprednisolone.[90] In another study, PEGylation reduced adaptive T-cell responses against adenoviral proteins and provided prolonged gene expression, as well as enabled readministration of the viral vectors without inducing significant immune responses.[110] Furthermore, the shielding of adenovirus with PEG significantly reduced nonspecific vector uptake by macrophages and Kupffer cells, without compromising transgene expression in most tissues, upon intravenous injection.[94]

PEGylated AAV vectors were also modestly protected from antibody neutralization. Conjugation with high molecular weight PEG reduced antibody neutralization, though exceeding the critical stoichiometry significantly altered the AAV particle properties (*i.e.*, size, shape) and decreased infectivity for both high- and low-molecular weight PEG, presumably due to steric hindrance of crucial domains for AAV infection by the PEG. However, moderate PEGylation was able to preserve infectivity while reducing neutralization.[90] Additionally, the mode of PEGylation apparently influences viral infectivity. AAV PEGylated with succinimidyl succinate was susceptible to neutralizing antibodies, whereas AAV modified with tresyl chloride more effectively protected the virus from neutralizing antibodies both *in vitro* and *in vivo*, promoting transduction levels for extended time periods.[111] Hydrolysis of succinimidyl succinate chains may expose antigen-binding sites, consequently resulting in reduced transduction upon repeated administration of viral vectors.

Cationic polymers have also been employed to modify viral vectors, either alone or in conjunction with PEG. Electrostatic complexation of an arginine-grafted, bioreducible polymer (ABP) with adenovirus decreased macrophage interleukin-6 production relative to nonmodified adenovirus after adenoviral infection *in vitro*, suggesting that the ABP can reduce vector immunogenicity.[112] This modification also improved transduction efficiencies in human cancer cell lines (*e.g.*, A549, MCF7) and coxsackie-adenovirus receptor (CAR)-negative murine cell lines. Such enhanced transduction with cationic polymers has also been observed for adenoviral vector infection of human bladder cell lines (*e.g.*, TCCSUP).[104] In an analogous strategy, noninfectious retrovirus-like particles lacking a viral envelope protein have been complexed with cationic polymers, such as poly-L-lysine or polyethylenimine, and the resulting combination of cationic polymers with non-infections virions created vectors able to mediate gene delivery into nondividing cells, as well as significantly enhanced the stability of the viral vectors.[113] Similarly, construction of hybrid vectors using murine leukemia virus-like particles with polyethylenimine enhanced delivery efficiencies and maintained vector stability even under harsh conditions (*e.g.*, freezing/thawing, ultracentrifuging).[114] In addition to cationic polymers, vectors such as adenovirus have been incorporated into "artificial" envelopes composed pH-sensitive lipid bilayers, such as dioleoyl phosphatidyl

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ethanolamine:cholesteryl hemisuccinate.[115] The resulting hybrid vector enhanced virion escape from the endosomal pathways after endocytosis and yielded substantial gene expression *in vitro* and *in vivo* compared to naked adenovirus. Similarly, adenoviral vector incorporation into self-assembled lipid bilayers, such as 1, 2-dioleoyloxypropyl *N*,*N*,*N*-trimethylammonium chloride/cholesterol (DOTAP/Chol) or dimyristoyl phosphatidylcholine/cholesterol (DMPC/Chol), resulted in enhanced penetration into a three-dimensional tumor spheroid, but delayed gene expression, compared to naked adenovirus.[116]

STRATEGIES FOR ALTERING CELL TROPISM

The majority of studies above grafted polymer onto a virion's surface with the goal of protecting the vector while maintaining its tropism; however, "over-shielding" a virus to override its natural specificity offers the potential to add additional functionalities to retarget the virus (**Figure 2**). For example, the interactions of adenoviral knob with CAR were blocked by conjugating a bifunctional PEG onto the viral capsid, and coupling an E-selectin-specific antibody to the other terminus enabled interaction with endothelial cells.[117] The resulting retargeted adenoviral vectors reduced transgene expression in CAR-positive cells and increased transduction of activated endothelial cells *in vitro* as well as *in vivo*. Additionally, attaching the E-selectin antibody to PEGylated virus enabled selective delivery within inflamed skin of mice, inducing local gene expression in the epithelium. In another study, adenoviral vectors conjugated with folate-PEG (fol-PEG) exhibited specificity for a folate receptor overexpressing cell line (KB cells), yet low affinity to a folate receptor deficient cell line (A549), while maintaining the capacity to evade innate immune responses.[97]

HPMA[100,101,118] has been used as an alternative to PEG. HPMA has multiple sites for reaction with the vector surface, and a fraction of the sites on HPMA remain unreacted, thereby providing sites for additional chemical modifications such as cell targeting ligands.[89] In one study with adenovirus, HPMA binding did not significantly alter the virion dimensions (104.8 ± 0.9 nm (unmodified virus) vs. 127.7 ± 1.5 nm (HPMAylated virus)).[100] Adenoviral vectors modified with poly-HPMA were additionally grafted with basic fibroblast growth factor-2 or vascular endothelial growth factor,[100,101] resulting in both improved resistance to antibody neutralization and targeting to the respective fibroblast growth factor-2 or vascular endothelial growth factor receptor receptor-bearing cell lines in mixed cell populations. Also, compared to parent adenovirus, vector decorated with fibroblast growth factor-2-HPMA had lower transduction of cells expressing high levels of CAR (*e.g.*, IGROV, A549), yet mediated higher gene expression in CAR-negative cell lines (*e.g.*, AB22, MC26). Interestingly, the presence of fibroblast growth factor extended blood circulation levels of the modified virus upon intravenous administration, consequently reducing transgene expression in numerous organs compared with the one by unmodified virus (*e.g.*, a 10,000-fold decreased transgene expression in liver). Analogously, intravenous injection of HPMAylated adenovirus presenting a laminin-derived peptide (*i.e.*, SIKVAV) to tumor-bearing mice resulted in extended circulation *in vivo* and reduced toxicity compared to nonmodified virus, and the modified virus also exhibited tumor tropism.[118]

In addition to PEG and HPMA, novel biocompatible polymers are being developed to enhance transduction and alter cell tropism. A naturally occurring and biodegradable polymer, polysaccharide mannan, was employed to chemically modify viral vectors,[102] similar to its prior use in synthetic vectors.[119] Modification of adenovirus with mannan altered vector tropism *in vivo*, decreasing transduction of CAR-positive muscle cells after intramuscular delivery and liver after systemic delivery.[102]

EMERGING OPPORTUNITIES: SPATIALLY PATTERNED GENE DELIVERY

The functionality of many natural tissues results from complex organization of cells into structures. The formation of these complex architectures arises, in part, from spatial patterns in gene expression. For example, the localized secretion of a diffusible factor from a signaling center can create concentration gradients that direct cell migration and influence the organization of cells into functional structures.[120–122] Likewise, extracellular signals can direct patterned expression of intracellular inductive factors to spatially regulate cell differentiation within a developing tissue.[123] By analogy, regenerative strategies for damaged tissue may need to recreate such architectures to restore function. Protein releasing systems have been employed to create concentration gradients that can orient tissue growth;[124,125] however, the delivery of gene therapy vectors from biomaterials offers the potential to spatially control expression on length scales of ten to hundreds of microns.[120,126] Gene delivery can also mediate the expression of intracellular protein and RNA products, which expands options relative to extracellular protein delivery.

Patterned gene expression can be achieved using the principles of biomaterial-based delivery presented above, with the inclusion of additional technologies to spatially regulate the distribution of vectors. Biomaterial surfaces can be patterned using techniques such as photolithography, microfluidics, and direct writing techniques,[127] and biomaterials based on self-assembly may provide additional mechanisms for spatially controlling the architecture. For example, microfluidic approaches have been employed to spatially regulate the deposition of nonviral vectors, for both *in vitro*[120,126] and *in vivo* delivery.[128] The *in vitro* systems demonstrated patterned expression on the length scales of 100 μm, which created concentration gradients that could pattern neuron survival[129] and directed neurite outgrowth.[120] However, the efficiency of the nonviral vectors has been a limitation, which viral vectors have the potential to overcome. For example, adenoviral vectors have been patterned using stamping technology.[74] The functionality of patterned delivery has been illustrated with the regeneration across the bone-soft tissue interface. Gradients of immobilized retrovirus, achieved via deposition of controlled poly(L-lysine) densities, resulted in spatial patterns of transcription factor expression, osteoblastic differentiation, and mineralized matrix deposition.[130] Such continuously graded vector presentation has the potential to significantly enhance the integration and biological performance of tissue substitutes.

In conclusion, the opportunities for gene therapy to treat a number of disorders continue to expand, yet efficient, safe, and controlled delivery remains a limitation. Biomaterials provide a modular and versatile tool to address some barriers associated

with viral gene delivery, namely inefficient delivery to specific cell types, limited tropism, spread of vectors to distant sites, and immune responses. The interaction of the vector and the material may be controlled to maintain vector activity, avoid recognition by the immune system, and provide locally controlled release. Alternatively, the vector may be modified with biomaterials to modulate interactions with the host immune system or target cells. These strategies may provide opportunities for numerous applications of gene therapy to inherited or acquired disorders, infectious disease, and regenerative medicine.

ACKNOWLEDGMENTS

We acknowledge support from the National Research Foundation (NRF) grant funded by the Korea government (MEST) through the Active Polymer Center for Pattern Integration (No. R11-2007-050-00000-0) and the Seoul R&BD Program (10816). We also acknowledge funding from NIH (R01HL081527, RO1EB005678).

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