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Hydrogels for Lentiviral Gene Delivery

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Abstract

Introduction—Gene delivery from hydrogel biomaterials provides a fundamental tool for a variety of clinical applications including regenerative medicine, gene therapy for inherited disorders and drug delivery. The high water content and mild gelation conditions of hydrogels support their use for gene delivery by preserving activity of lentiviral vectors and acting to shield vectors from any host immune response.

Areas Covered—Strategies to control lentiviral entrapment within and retention/release from hydrogels are reviewed. We discuss the ability of hydrogel design parameters to control the transgene expression profile and the capacity of hydrogels to protect vectors from (and even modulate) the host immune response.

Expert Opinion—Delivery of genetic vectors from scaffolds provides a unique opportunity to capitalize on the potential synergy between the biomaterial design for cell processes and gene delivery. Hydrogel properties can be tuned to directly control the events that determine the tissue response to controlled gene delivery, which include the extent of cell infiltration, preservation of vector activity and vector retention. While some design parameters have been identified, numerous opportunities for investigation are available in order to develop a complete model relating the biomaterial properties and host response to gene delivery.

Keywords

gene therapy; hydrogels; lentivirus; tissue engineering

1. Introduction

Delivery of genetic vectors represents a promising approach to treat a wide range of diseases and disorders. For example, gene therapies for Parkinson's disease, hemophilia B, muscular dystrophy, and 1-antitrypsin deficiency are in phase I and phase II clinical trials [1–4]. In the preclinical settings, gene delivery has shown promise for regenerative medicine applications where induced expression of a transgene has been employed to promote the formation of tissues, such as bone [5,6], spinal cord [7–12] or the eye [13]. Gene delivery can provide either short-term or long-term expression of the transgene at specific sites, which can have either local or systemic effects. Notably, gene therapy allows for changes to the gene sequence or delivery of multiple genes without redesigning the delivery system. From a research perspective, this flexibility makes biomaterial-mediated gene delivery a versatile tool with which to identify the appropriate factor or combination of factors that yield the most therapeutic benefit. Efficient delivery systems has proved challenging for many

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applications, with virus activity and dose and the host immune response contributing to limited gene expression and threatening patient safety. The majority of strategies to improve gene delivery have focused on vector optimization [14]. Alternatively, biomaterial platforms provide an opportunity to improve gene transfer by enhancing vector stability and shielding the immune system, while promoting and/or controlling cell-vector interactions in order to modulate the location and duration of transgene expression.

Viral vectors have demonstrated better clinical potential because of their ability to more efficiently transfer therapeutic genes and achieve long-term transgene expression *in vivo* compared to their non-vial counterparts [15–19]. Non-viral approaches include direct delivery of naked plasmids or oligonucleotides, and the complexation of these nucleic acids with cationic polymers or lipids. While complexation can enhance delivery efficiency, maintenance of vector stability of the complexes and establishment of long-term transgene expression represent significant challenges [17–19]. Viral vectors are derived from viral pathogens, in which the harmful sequences have been removed and therapeutic sequences have been inserted [14,19,20]. Several viral vectors are currently being tested in clinical trials for various therapies, including retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses [1–4,14,21–26]. Improved safety and efficacy of lentiviral vectors in recent years has greatly enhanced their feasibility for clinical use over other viral vectors [14, 19]. Lentiviral vectors infect both dividing and non-dividing cell populations, integrate the delivered gene into host chromosomes to enable long-term expression, and are relatively easy to produce [14,16,18,19]. For an extensive review of recent advances and translational potential of lentiviral vectors, please refer to Sakuma, et al. 2012 [14]. Their efficiency and considerable clinical potential has motivated their delivery from biomaterials; however, many reports are descriptive with regards to biomaterial design. We have supplemented our discussion of biomaterial design with reports that employed alternative vectors, with the objective of highlighting design parameters that should be considered for lentiviral vectors.

Compared to other biomaterial delivery systems (e.g., microporous scaffolds based on polylactide-co-glycolide (PLG) or ceramic materials), hydrogels provide a hydrated, tissue-like environment and typically mild, aqueous fabrication conditions that enable encapsulation of active vectors. While this review focuses on hydrogel-based delivery systems, other categories of materials have been reviewed for their potential to deliver viral [19] and non-viral [15,16] vectors. Hydrogels are formed by the crosslinking or self-assembly of hydrophilic polymers, which can be formed from naturally occurring (e.g., fibrin, chitosan and hyaluronan) or synthetic (e.g., polyethylene glycol (PEG) and polyvinyl alcohol) materials. Furthermore, hydrogels can be customized for many applications. For example, they can be designed to be injectable or environmentally responsive, to encourage infiltration of specific cell types and to acquire various geometries. Control over delivery of genetic vectors can be achieved by altering physical properties of the hydrogel carrier, such as pore size and degradation kinetics. Importantly, transgene expression can be designed to enhance or synergize with the intrinsic bioactivity of the scaffold and thereby create an environment that promotes tissue formation for regenerative medicine (Figure 1). For instance, the biomaterial provides a support for cell adhesion and an architecture that can serve to organize cells while transgene expression can target cellular processes (e.g., proliferation, differentiation) that complement these structural functions. Interactions between gene therapy vectors and biomaterial scaffolds can be tuned to modulate the release rate of vector, target specific internalization pathways, and potentially enhance intracellular trafficking [16,19,27]. As biomaterial delivery of lentiviral vectors is an emerging technology, most publications have been descriptive (i.e., demonstrating feasibility) or have investigated design of lentiviral vectors to modulate the host response. The following sections describe the established design parameters for gene delivery from hydrogels, and will focus on the emerging literature describing delivery of lentiviral vectors (Table 1).

2. Lentiviral Vectors for Gene Therapy

Viral vectors available for gene therapy can be categorized as integrating or non-integrating. Integrating vectors such as adeno-associated virus (AAV) and retrovirus (including lentivirus) have the ability to insert their viral genome into the chromosomal DNA of host cells. Nonintegrating vectors such as adenovirus and herpes simplex virus type 1 (HSV-1) deliver their genomes into the nucleus of the host cell, but remain episomal. Because integrating vectors get inserted into the host genome, their expression can be long-term and potentially lifelong, whereas non-integrating vectors tend to have short-term expression [19, 28], though there are examples of expression that persists for years (e.g., AAV) [29]. The duration of expression is critical to the choice of vector, as applications such as wound healing may require transient expression, whereas hemophilia, sickle cell anemia, and other genetic disorders require life-long genetic modification.

Lentiviruses are a subset of retrovirus that have gained popularity due to their ability to infect both nondividing and dividing cells, broad tropism, integration into the host genome which enables long-term availability of the encoded therapeutic protein and relative ease of production and the availability of large libraries of constructs [14,19]. Retroviruses are enveloped viruses containing single-stranded RNA that replicate by reverse transcription of the viral RNA into linear double-stranded DNA and subsequent integration into the genome of the host cell [30]. However, for use in gene therapy applications, retroviruses must be engineered to be replication defective otherwise they will produce and release virions leading to pathogenic effects. For example, lentiviral vectors were developed based on the human immunodeficiency virus type 1 (HIV-1), but have been engineered to enhance patient safety [14,20]. Specifically, the genes required for virus replication, packaging, and export from an infected cell have been removed from the viral genome. Thus transient transfection systems employing packaging cell lines *in vitro* are employed to produce replication defective viruses [20,31].

In brief, the packaging cells are transfected with a vector expression plasmid that encodes for the transgene along with packaging constructs, which are plasmids that supply the packaging cells with the genes needed to produce and export the viral particles (e.g., reverse transcriptase, envelope proteins). To further increase patient safety, packaging constructs have been optimized to decrease risk of homologous recombination, which could lead to a replication competent virus. An attractive feature of this production scheme is the envelope proteins can be changed by switching out the plasmid that encodes for these proteins without altering the other packaging constructs, a process called pseudotyping. Since the envelope proteins dictate cell association and internalization that influences the target cell types, changing the envelope plasmid may allow for the targeting of specific cell types (tropism) [32]. While investigation into new viral envelopes is ongoing, most lentiviral vectors are pseudotyped with the vesicular stomatitis virus glycoprotein (VSV-G) due to its broad tropism [20,33].

3. Encapsulation of Lentiviral Particles within Hydrogels

The retention of vectors within hydrogels can facilitate gene transfer to cells infiltrating the scaffold, as opposed to released vector that may target cells adjacent to the scaffold (Figure 1). The method of scaffold fabrication and the material properties influence the extent to which vectors are retained. Vector release from hydrogels typically occurs by diffusion, with the rate of release influenced by scaffold design parameters such as porosity, tortuosity, hydrophobicity or hydrophilicity, rate and mechanism of degradation and interactions between the vector and the material. In addition to the degree of virus retention, the ability of cells to infiltrate hydrogels is a key determinant of transgene expression. Natural

materials can be advantageous because of biocompatibility and their inherent biological interactions with cells that effect cell attachment, migration and differentiation. Alternatively, synthetic polymers offer the potential to create hydrogels with more precise control over the physical properties (e.g., mechanics, degradation) that directly affect both tissue formation and gene delivery. Hydrogel platforms for gene therapy have the potential for much improvement over other strategies and, relative to bolus injection, can enhance the extent and duration of transgene expression at an implantation site [34]. The overall efficiency of delivery is the sum of effects of the hydrogel on host response (e.g., cell infiltration) and the vector activity/accessibility (e.g., release rate), which combine to determine the overall efficiency of gene delivery. The entrapment of vectors within hydrogels is typically employed to provide a sustained release that will maintain elevated concentrations of the vectors locally to increase the opportunity for cellular internalization.

Both natural and synthetic hydrogels have been used to entrap gene therapy vectors as these hydrogels form under mild conditions that do not significantly impact vector activity (Table 1) [34–40]. The initial factor impacting retention versus release from the hydrogels is the mesh size. Hydrogels with a mesh size larger than the vector diameter (50–100 nm) typically exhibit a rapid release governed by diffusion, while those with a mesh size less than the vector diameter exhibit release rates that are highly dependent on the degradation rate of the hydrogel. The mesh size of both natural a synthetic hydrogels can be varied over a wide range (<50 nm to over 1500 nm) [41–43] and macropores (>10 μm) can be added to further increase mass transfer and cell infiltration [40]. Notably, imperfections in the hydrogel networks may enable diffusion of lentiviral vectors even in hydrogels with a theoretical mesh size smaller than the vector radius [44,45]. Environmentally responsive hydrogels (e.g., temperature or pH sensitive hydrogels), in which pore size changes in response to stimuli, have been used to achieve even greater dynamic control over vector release [46].

A second factor influencing release versus retention are interactions between vectors and hydrogels, which can prevent vector diffusion away from the hydrogel thereby increasing the local vector concentration that facilitates more effective gene transfer [38,39,45,47]. Additionally, the association of a vector with a biomaterial substrate that supports cell adhesion can co-localize virus and cells to enhance delivery. Either vectors or the hydrogel backbone can be modified prior to encapsulation to mediate non-specific or specific interactions. Hydrogels made from cationized gelatin, PEG modified with cationic peptides and positively charged chitosan have all been used to increase the non-specific retention of gene therapy vectors [10,38,46,48,49]. Alternatively, the specific interactions between avidin and biotin [50] or antibodies and vectors [51] have been exploited to provide vector-material interactions to promote vector retention. More recently, the lentiviral capsid proteins have been engineered to covalently bind fibrin during polymerization, which can release the vector as the hydrogel degrades [45]. Various strategies found in the literature to enhance the retention of lentiviral vectors encapsulated in hydrogels are summarized in Table 1. Importantly, the strength of interactions between scaffolds and vectors must be optimized so that vectors are adequately retained, but not so strong as to prevent vector dissociation, which is required for the vector to interact with the nearby cells and be internalized [38]. Similarly, the diffusion rate must be balanced to maintain spatial localization of gene transfer (Figure 1). If diffusion is too rapid, lentivirus may travel to a different area of tissue in which transgene expression is not desired or where target cells are inaccessible [35,45]. The addition of interconnected macropores into the hydrogels can further increase the probability that infiltrating cells will internalize vectors and thereby improve transgene expression (Figure 2) [40]. In addition, cells encapsulated within 3D hydrogels in the presence of adenoviral vectors expressed significantly higher levels of delivered transgene than those transduced in traditional cultures on polystyrene [44,47]. These results, which would likely translate to lentiviral transduction, suggest that the 3D

geometry directly enhances transduction by ensuring a higher degree of co-localization entrapped cells.

3.1 Natural Hydrogels

Hydrogels based on natural polymers readily support cell adhesion and migration and can be used to entrap lentiviral vectors. In fibrin hydrogels, low to medium fibrin concentrations, which partially determines mesh size, have been reported to allow for maximal levels of infection by encapsulated lentivirus *in vivo* and *in vitro* [34,35,39,52]. At higher fibrin concentrations, both vector diffusion and cell infiltration were relatively inefficient. Compared to collagen and alginate hydrogels, fibrin supports the highest levels of transgene expression for at least one month after intraperitoneal implantation (Figure 3) [34]. Fibrin contains multiple sites for cell adhesion and rapid cell infiltration was observed *in vivo*. Although this study reported that collagen supported similar levels of cell infiltration as fibrin *in vivo*, transgene expression was significantly reduced and instead resembled that of non-cell adhesive alginate [34]. This result is likely due to a faster degradation of fibrin, compared to collagen, in this implantation site. Taken together, these studies demonstrate that vector-material interactions may contribute to gene transfer beyond those that determine cell infiltration. These interactions may increase lentiviral stability or directly facilitate lentiviral infection [10,34,37,39].

3.2 Synthetic Hydrogels

Although these studies demonstrate the potential utility of natural hydrogels for localized gene delivery, the mode and dynamics of cell infiltration and hydrogel degradation can be more tightly controlled in hydrogels based on synthetic materials, such as PEG. For example, PEG hydrogels can be crosslinked via protease-sensitive peptides [43]. As cells migrate through the hydrogels, these crosslinks are locally degraded to allow for simultaneous cell infiltration and vector release. The use of synthetic hydrogels also allows for the addition of specific and protease-susceptible peptides that can be chosen to preferentially encourage the infection of specific cell types [36]. To enable robust cell infiltration, interconnected macropores can be incorporated into synthetic hydrogels using various methods [40,53–57]. For example, macroporous PEG hydrogels have been fabricated by embedding a secondary material (e.g., gelatin microspheres) that serves as a sacrificial template after hydrogel formation (Figure 2) [40]. Modulating the macropore size and density has the potenital to control cell infiltration (macroporous) independently of hydrogel degradation and vector release. Hydrogels can be designed to further encourage infiltration and transduction of specific cell types by incorporating short, bioactive peptides derived from the extracellular matrix (e.g., RGD) into scaffolds. In synthetic hydrogels, precise control over the density of adhesive sites can be used to modulate the strength of cell-scaffold interactions and the type of extracellular receptors that mediate these interactions. Along with the density of adhesion sites, the degradation rate of the matrix modulates cell migration through the material, which influences integration with host tissue. Integration of biomaterials tailored to present specific cues (e.g., chemical, mechanical, spatial) and gene therapy vectors represent a promising strategy to promote tissue formation and regeneration. In summary, the hydrogel provides a physical space containing a provisional matrix that promotes cell infiltration, transduction, and trophic factor expression that lead to tissue induction.

4. Hydrogels to Increase Lentivirus Stability

Many hydrogels can be formed under conditions that do not compromise activity; however, the stability of viral vectors remains a critical design parameter as hydrogels loaded with viral vectors may not used in applications that result in immediate cellular internalization of

vectors. For example, hydrogels used for regenerative medicine may require days to weeks for cells to fully infiltrate the scaffold and thereby encounter the encapsulated vector. Hydrogel systems have been developed that are capable of providing sustained release of small molecules and proteins over the time scale of days to weeks. Sustained release of vectors from materials is capable of producing increasing levels of gene transfer with time [19,58]. In contrast, sustained release may have no impact at some implantation sites. For delivery from scaffolds to the mouse peritoneal fat pad [34], expression was determined by the amount of vector release shortly after implantation, with no observed effect of release rate. Lentiviral vectors have a reported half-life of approximately 24 hours, and strategies may need to be developed that maintain the virus activity for several days [17,34,37].

Vector entrapment within hydrogels has the potential to increase lentivirus stability through protection from extracellular degradative enzymes and camouflage from the immune system [39,59–61]. Gene transfer with lentiviral vectors entrapped within collagen or fibrin hydrogels has been increased by the addition of hydroxyapatite (HA) nanoparticles [37,39]. For collagen hydrogels, the hydroxyapatite association of the vector increased the half-life to more than 30 hours [37]. More recently, the incorporation of HA nanoparticles within fibrin hydrogels significantly enhanced the duration of transgene expression *in vivo* (at least two additional weeks), though the mechanism appeared to be related to cell infiltration and hydrogel stability rather than vector stability (Figure 4) [39]. Taken together, hydrogels can be combined with strategies to preserve lentiviral activity in order to enhance gene transfer.

5. Immune Response and Lentiviral Vectors

The host response to the lentiviral vectors can significantly influence gene transfer and many researchers have focused on both understanding and attenuating the immune response to lentiviral vectors. Within hours of systemic injection, lentiviral vectors can trigger an inflammatory response characterized by a transient cytokine surges (e.g., interleukin-6 or tumor necrosis factor-α) and a type I interferon response that limits transgene expression [62,63]. This effect has been observed for multiple virus pseudotypes, and *in vitro* challenge of antigen-presenting cells suggested that plasmacytoid dendritic cells initiated the response. The mechanism of this response is hypothesized to involve activation of toll-like receptor 7 (TLR7) and/or TLR9, which recognize single-stranded RNA and unmethylated CpG, respectively. In addition to toll-like receptors, the complement system, a family of proteins that bind pathogens and aide in their clearance from the body, is well documented to target VSV-G envelope proteins, which leads to vector inactivation [64]. These studies indicate that stable transgene expression depends on the ability of the delivery system to protect the vector from the innate immune system and provides an opportunity to design and employ instructive hydrogels capable of blocking inflammation while delivering their viral vector payload to target cells.

The innate immune system may also facilitate an adaptive immune response to the vector, the transgene product or transduced cells. Some patients may have pre-existing antibodies to the envelope proteins used to pseudotype lentiviral vectors interfere with viral transduction and threaten patient safety, requiring patient screening prior to administration [14,28]. However, exposure to the lentiviral vector will cause the immune system to produce vector-specific antibodies that can neutralize the vector following re-administration, which may limit the therapeutic benefit. In addition, antibodies and antigen-specific T cells may develop recognition of the transgene product or viral vector components, leading to destruction of transduced cells that produce the therapeutic protein.

5.1. Hydrogels that Block Immune Recognition

Hydrogels can be utilized to protect vectors from neutralizing immune complexes and mitigate immune responses due to inflammatory cytokine release by immune cells. Vector encapsulation within hydrogels and attachment of hydrogels onto the vector surface are two potential strategies to address these goals. Hydrogels can be designed with a pore size to limit antibody and complement protein diffusion. Thus viral vectors are protected from the immune system simply by being encapsulated. Alternatively, individual viral particles can be masked from the immune system by modifying the envelope proteins that are recognized by host pattern recognition receptors (e.g., TLR7). For example, PEG can be covalently attached to a VSV-G psuedotyped lentiviral vector, a method known as PEGylation. PEGylation has been shown to extend the circulation half-life of the active vector in mice by a factor of 5 and reduced the rate of vector inactivation in the serum by a factor of 1000 without affecting the number of virus particles present in the circulation [65]. Furthermore, increased vector circulation time of the vector led to significantly enhanced transduction efficiency in the bone marrow and spleen. In sum, PEGylation protects lentivirial vectors from inactivation by serum compliment proteins and, as a result, improves the transduction efficiency of VSV-G pseudotyped lentiviral vectors *in vivo* [65].

5.2. Hydrogels for Local Immunosuppression

Hydrogels can be engineered to locally suppress inflammation and immunity within the matrix. Peptide sequences that bind and inactivate inflammatory cytokines or their receptors can be integrated into the hydrogel matrix in the same manner as adhesive peptides and degradable linkages (see **Synthetic Hydrogels** section) [66,67]. These strategies can result in decreased immune cell migration and activation within the hydrogel. Alternatively, ligands or antibodies that induce T cell apoptosis through Fas signaling can be incorporated into the hydrogel providing local immunosuppression [68].

Engineering hydrogels to locally suppress inflammation and immunity may be a promising strategy to promote long-term gene expression. This hypothesis is supported by long-term transgene expression in the liver following systemic vector delivery to mice deficient in the receptor for IFNα and IFNβ, key anti-viral cytokines [63]. Thus, immunomodulatory hydrogels could facilitate stable gene transfer to both immune and non-immune cells by suppressing activation of the immune system to the lentivirus or transduced cells. Alternatively, hydrogels may eventually be engineered to induce tolerance to both the vector and the transgene, thereby allowing for multiple administrations of the vector and long-term expression.

6. Conclusions

Hydrogels offer the opportunity to improve current gene delivery technologies for a range of applications in gene therapy and regenerative medicine. In contrast to bolus injection of naked lentiviral vectors, encapsulation of vectors within hydrogels or direct chemical modification of vectors with hydrogel materials can increase residence times *in vivo* and prevent vector inactivation by the immune system. Furthermore, these systems can maintain localized, high vector concentrations that overcome mass transport limitations to gene transfer. In addition, hydrogels can be engineered to present peptide motifs that support cell adhesion and vector retention. This strategy results in increased co-localization of vectors and target cells, thereby promoting gene transfer.

Hydrogel biomaterials have enormous potential for a range of applications in gene therapy and regenerative medicine. Hydrogels enhance the potential of lentiviral therapies by retaining vectors at local tissue sites, shielding vectors from the innate immune response and

increasing transduction efficiency. For regenerative medicine and tissue engineering applications, hydrogels can be tuned to present cell adhesive, mechanical and biodegradation properties that mimic the tissue of interest *in vivo*. For detailed reviews of gene delivery from biomaterial scaffolds for tissue engineering applications, see De Laporte and Shea 2007 [69] and Gower and Shea 2012 [27]. Incorporation of lentiviral vectors further enhances the regenerative capacity of hydrogels by adding a mode by which to locally deliver transgenes that regulate direct expression of choice soluble factors and perturb intracellular signaling pathways that determine cell phenotype.

7. Expert Opinion

Hydrogel biomaterials offer the opportunity to control the level, duration, and cellular targets of transgene expression, which are major barriers for gene therapy. Encapsulation of vectors within hydrogels or modification of vectors with hydrogels can shield the vector from clearance or inactivation by the immune system. Furthermore, hydrogels can be modified with biomimetic features to encourage adhesion and migration of specific cell types, thereby increasing transduction of this cell population. The design of hydrogel-based gene delivery systems for regenerative medicine should balance the need to minimize the immune response and limit vector clearance while promoting cell infiltration. Hydrogels with encapsulated vectors that are designed to limit immune cell activation may have limited gene transfer, which could result from the low cell recruitment due to minimal cell-matrix interactions typical of hydrogels that limit immune cell activation.

For regenerative medicine, the material can be designed to promote cellular processes associated with tissue formation, and vector delivery can further increase its bioactivity. Efficient gene transfer reflects a balance between vector retention versus release within the hydrogel, the rate of cell infiltration, and the stability of the vector within the system. Numerous opportunities remain for further refining these design parameters, developing technologies to maximize transduction, and providing spatial and temporal control over gene expression that can mimic the complex patterns of gene expression present during tissue development. Hydrogel biomaterials provide a unique set of tools that can be designed to synergize the requirements for gene transfer with those for promoting tissue formation.

List of Abbreviations

3D	three-dimensional
AAV	adeno-associated virus
HA	hydroxyapatite
HSV-1	herpes simplex virus-type 1
IFNαβ	interferon-αβ
PEG	poly(ethylene glycol)
RGD	Arginine-glycine-aspartic acid peptide
RNAi	interference RNA
TLR	toll-like receptor
VSV-G	vesicular stomatitis virus glycoproteins

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Highlights

- Lentiviral vectors are attractive for their relative ease of production, availability of extensive transgene libraries, and ability to infect both dividing and non-dividing cells.
- For tissue engineering applications, the hydrogel provides a physical space that presents specific cues to promote cell infiltration, and access to entrapped lentiviral vectors encoding for inductive factors that influence tissue formation.
- Delivery of lentiviral vectors from hydrogels can enable sustained, localized expression within the implantation site.
- Hydrogels engineered to mediate non-specific and specific chemical interactions with lentiviral vectors can be designed to either retain vectors within the hydrogel, or release vectors from the hydrogel, which influences the distribution of transduced cells.
- In combination with vector retention, the extent and location of transgene expression is also influenced by the rate of cell infiltration into the hydrogel.
- Gene delivery is also influenced by the ability of the hydrogel to stabilize vector activity and shield the vector from the host immune response.

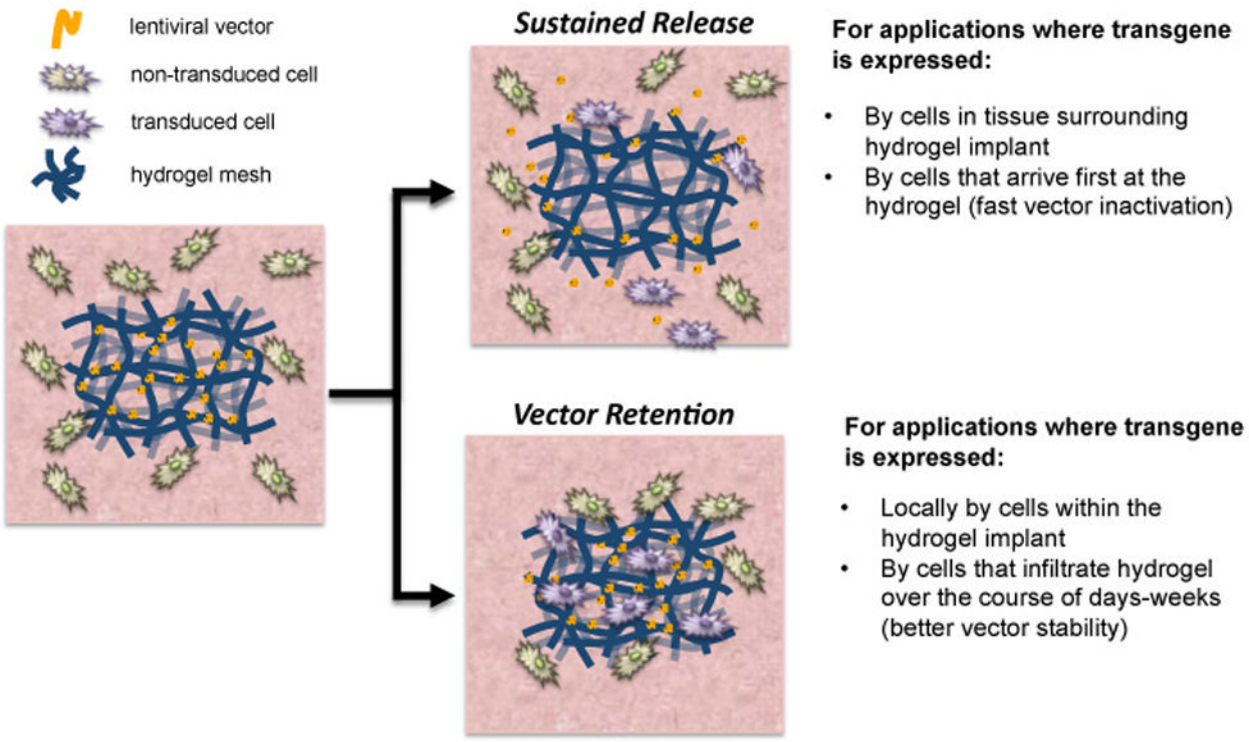

Figure 1.

Gene delivery strategies using hydrogels. The hydrogel design parameters for delivery of gene therapy vectors can be modulated to achieve different transgene expression profiles. Hydrogels can be designed to enable sustained release of vectors to target the cells surrounding the hydrogel. Alternatively, hydrogels can be designed to retain vectors within the scaffold to target infiltrating cells and better preserve vector activity.

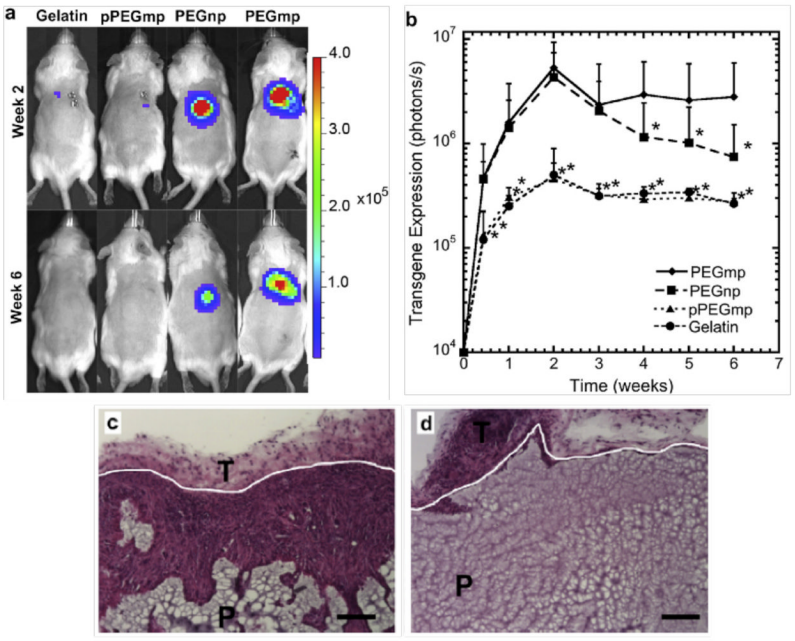

Figure 2.

In vivo transgene expression from lentivirus loaded hydrogels with and without macroporous structure. (a) Representative bioluminescence images following subcutaneous implantation of hydrogels. Three virus loading configurations into the PEG hydrogels are shown: (i) non-macroporous PEG (no gelatin) (PEGnp) hydrated with virus solution following gelation, (ii) PEG encapsulating gelatin microspheres (PEGmp) hydrated with virus solution following gelation, and (iii) PEG encapsulating gelatin microspheres that have been loaded with virus prior to gelation (pPEGmp) by swelling gelatin microspheres with virus solution. A gelatin only control is also shown. (b) Transgene expression was measured as integrated photon flux (photons/s) using an IVIS bioluminescence imaging system (b). Asterisks represent statistical difference ($p < 0.05$) relative to the PEGmp condition at each time point based on a KruskaleWallis test. (c), (d) Hematoxylin and eosin staining showing cell infiltration into PEGmp (c) and PEGnp (d) hydrogels 6 wks after subcutaneous implantation. The labels P and T denote PEG and tissue surrounding the implant, respectively. White lines denote hydrogel-tissue interface. Scale bars = 100 μm. Reprinted from Biomaterials, 33, Shepard JA, Virani FR, Goodman AG, Gossett TD, Shin S, Shea LD, Hydrogel macroporosity and the prolongation of transgene expression and the enhancement of angiogenesis, 7412–7421, 2012, with permission from Elsevier.

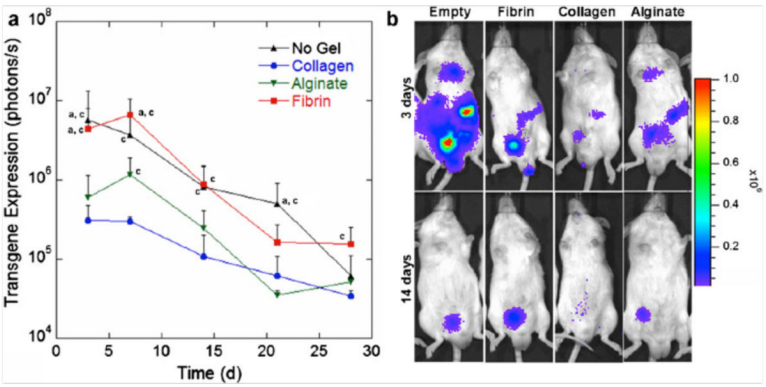

Figure 3.

In vivo transgene expression from hydrogel-filled polylactide-glycolide scaffolds as a function of time. (a) Hydrogel (collagen, fibrin, or alginate) precursors were mixed with a lentivirus encoding for the reporter gene luciferase, loaded into the pores of a PLG scaffold, and implanted in the fat pad of mice. Transgene expression was then measured using an *in vivo* bioluminesence imaging. Letters indicate statistically significant differences ($p < 0.05$) compared to (a) alginate or (c) collagen analyzed by a Kruskal–Wallis test (n= 3). (b) Representative bioluminescence images from scaffolds at 3 and 14 days post-implantation. Reprinted with permission from Springer Science+Business Media: Drug Del Transl Res, Hydrogels to modulate lentivirus delivery in vivo from microporous tissue engineering scaffolds, 1, 2011, 91–101, Aviles MO, Shea LD, Figure 4.

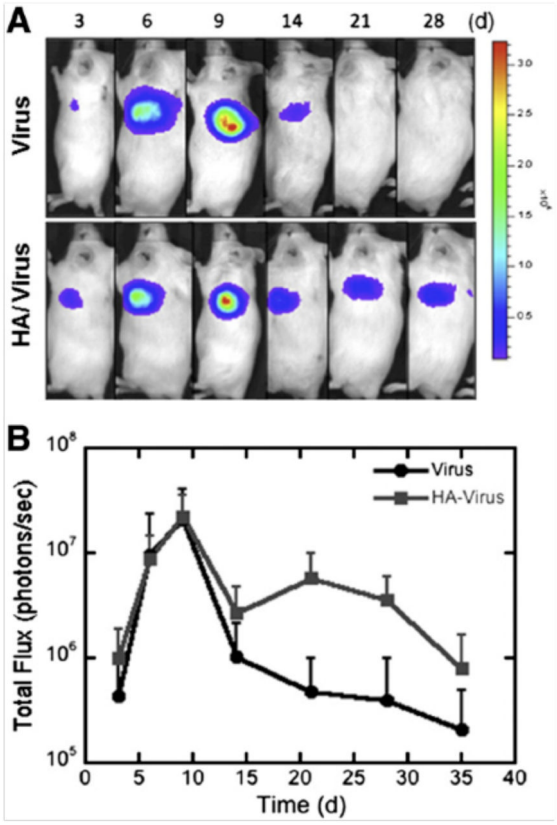

Figure 4.

In vivo transgene expression within fibrin hydrogels. Fibrin hydrogels containing either virus alone or HA/virus complexes were transplanted subcutaneously and expression profile was monitored for 28 days. (a) Represenative images of *in vivo* luciferase expression using bioluminescence imaging. (b) Quantification of luminescence as a function of time (n=6). Values at day 21 are statsitically different. Reprinted from J Control Rel, 157, Kidd ME, Shin S, Shea LD, Fibrin hydrogels for lentiviral gene delivery in vitro and in vivo, 80–85, 2012, with permission from Elsevier.

Table 1

Hydrogels for Lentiviral Delivery

Hydrogel Material	Strategy for Enhanced Transduction	Application	Reference
Collagen	Hydroxyapatite nanoparticles (non-specific vector retention)	Subcutaneous implant	[37]
Collagen	Encapsulation only	In vitro cell culture	[48]
Fibrin	Encapsulation only	In vitro cell microarrays	[35,70]
Fibrin	Hydroxyapatite nanoparticles (non-specific vector retention)	Subcutaneous implant	[39]
Fibrin	Fusion peptide/VSV-G envelope (conjugation to vector)	In vitro cell microarrays	[45]
Chitosan/β-Glycerol Phosphate blend	Positive charge on chitosan (non-specific vector retention)	In vitro cell culture	[46]
PEG	Encapsulation only	3D stem cell culture	[44]
PEG/Gelatin	Macropores (increased cell infiltration)	Subcutaneous gene transfer	[40]