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Hydrogels to modulate lentivirus delivery in vivo from microporous tissue engineering scaffolds

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Abstract

Numerous strategies to induce tissue regeneration employ scaffolds to create space and present biological cues that promote development. In this report, microporous scaffolds that provide structural support were filled with hydrogels to regulate cell adhesion and migration and were investigated as delivery vehicles for gene therapy vectors in vivo. Porous scaffolds were filled with either lentivirus-entrapped collagen or fibrin hydrogels, both of which support cell adhesion yet have varied rates for degradation and cell infiltration. Empty scaffolds and alginate hydrogels were employed as controls, with the latter not supporting cell infiltration. Hydrogel-filled scaffolds retained the lentivirus more effectively than empty scaffolds, and transgene expression was observed for all scaffold conditions. Empty and fibrin-filled scaffolds had maximal transgene expression in vivo, followed by collagen and alginate, with similar levels. Transduced macrophages and dendritic cells were initially present at the scaffold boundary and adjacent tissue and within the scaffold at later time points for all but the alginate condition. At days 3 and 7, expression was also imaged throughout the spleen and thymus, which may result from cell migration from the implant. These studies demonstrate that hydrogels can modulate gene delivery from scaffolds used in cell transplantation and regenerative medicine.

Keywords

Biomaterial scaffolds; Hydrogels; Collagen; Fibrin; Alginate; Lentivirus

Introduction

Tissue engineering scaffolds are being designed to control the local microenvironment in order to promote regeneration of tissues lost to disease or injury. These scaffolds must provide a structural support that creates and maintains a space for tissue growth, and must also function as a support for cell adhesion and migration to promote engraftment within the

host. Porous scaffolds can be fabricated into 3D structures, with the pores providing the path for cells to infiltrate into the scaffold (Hollister 2005). Hydrogels have been used to fill the pores of the structure and has been employed with cell transplantation to provide the biological cues for the transplanted cells (Nicodemus and Bryant 2008). Natural materials such as collagen, fibrin, and Matrigel have commonly been employed as they form gels under mild conditions and support or promote cell adhesion, migration, and proliferation (Drury and Mooney 2003; Malafaya et al. 2007). These natural materials also influence the endogenous host cells, through the intrinsic biological cues and the physical properties of the material (e.g., degradation rate and pore size). The combination of the properties of microporous scaffolds and hydrogels can be employed to create an environment with suitable mechanical properties while also providing the biological cues to support and promote cell adhesion and migration.

The scaffolds can also serve as a vehicle for the localized delivery of tissue inductive factors that can promote regeneration. Delivery of gene therapy vectors that encode for the inductive factors is attractive, as the vectors are versatile and can induce expression of one or more factors for extended time periods, which can potentially be controlled through the use of tissue-specific or inducible promoters. These vectors target the infiltrating cells, which subsequently function as localized bioreactors producing the factors. Porous scaffolds and hydrogels have been employed for the delivery of nonviral and viral vectors (De Laporte and Shea 2007; Pannier and Shea 2004; Putnam 2006). For porous scaffolds, transgene expression was a function of the plasmid dose, yet did not depend strongly on the release rate (Avilés et al. 2010). Additionally, while initial transgene expression was localized to the scaffold, long-term expression was observed adjacent to the implant (Salvay et al. 2010). Promoting tissue regeneration throughout the implant would benefit by obtaining transgene expression within the implant rather than in the adjacent tissue. Conversely, hydrogels have a different dependence on release and transgene expression. Hydrogels typically have pores whose size is on the same order of magnitude as the vector; thus, the hydrogel provides a barrier to diffusion and thus release (Shin and Shea 2010). Additionally, transgene expression for hydrogels is a function of the rate of cell migration (Shepard et al. 2010).

In this report, we investigated transgene expression by porous scaffolds filled with natural hydrogels that have encapsulated lentivirus. The porous scaffolds are composed of poly(lactide-co-glycolide) (PLG) formed by a gas foaming process, which provide mechanical support to the implantation site and have been widely used for cell transplantation and regenerative medicine. Collagen and fibrin hydrogels filled the pores of the scaffolds, both of which support cell adhesion yet have varying degradation and migration rates (Malafaya et al. 2007; Nair and Laurencin 2007). In addition to modulating cellular interactions, these hydrogels are hypothesized to limit the release of entrapped lentiviral vectors. In vitro vector release and cell infiltration in vivo were determined for the hydrogel/scaffold combinations. The location and level of gene expression was determined dynamically using a bioluminescence imaging system. Additionally, the distribution and identity of transduced cells was investigated. Hydrogel-filled scaffolds capable of sustaining gene delivery have a multitude of applications in gene therapy, cell transplantation, and tissue engineering applications.

Materials and methods

Lentivirus production

Lentivirus was produced in HEK-293 T cells grown in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA) at 37°C, and 5% CO₂. The lentiviral packaging vectors (pMDL-GagPol,

pRSV-Rev, and pIVS-VSV-G) were cotransfected along with plenti-cytomegalovirus enhanced green fluorescent protein (CMV-EGFP), or plenti-CMV-luciferase into 293 T cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). After 48 h of transfection, the supernatant was collected and filtered (0.45 μ m). Viruses were then concentrated using PEG-it (System Biosciences, Mountain, CA), with the precipitated lentiviruses suspended with PBS. The virus titer was determined by HIV-1 p24 Antigen ELISA Kit (ZeptoMetrix Co., Buffalo, NY).

Scaffold fabrication

PLG microspheres are used as the building block to make the scaffolds using a gas foaming technique. Microspheres were prepared as previously described (Jang et al. 2005). PLG (75% D, L-lactide/25% glycolide, one fourth i.v., 0.76 dL/g; Lakeshore Biomaterials, Birmingham, AL) was dissolved in dichloromethane to make a 6% (w/w) solution, which was then emulsified in 1% poly(vinyl alcohol) (PVA) to create microspheres. The microspheres were collected by centrifugation, washed four times with deionized water to remove residual PVA, and lyophilized overnight. Scaffolds were prepared using a previously described gas foaming/particulate leaching process (Mooney et al. 1996; Rives et al. 2009; Shea et al. 1999). Scaffold were constructed by mixing 2.5 mg of PLG microspheres with 80 mg of NaCl (250 μ m < day < 425 μ m) and then compressing the mixture in a 5-mm KBr die at 1,000 psi using a Carver press. The scaffolds are then equilibrated with high-pressure CO₂ gas (800 psi) for 16 h in a custom-made pressure vessel. Afterwards, the pressure was released over a period of 25 min, which serves to fuse adjacent microspheres creating a continuous polymer structure. To remove the salt, scaffolds were leached in water for 1 h while shaking. Scaffolds were then disinfected in 70% ethanol and stored in sterile water (not more than 24 h).

Hydrogel incorporation into scaffolds

Hydrogels were formed within the pores of the microporous PLG scaffolds to regulate gene delivery and impact cell interactions in vivo. The hydrogels collagen, fibrin, and alginate were used to fill the pores of the PLG scaffolds. To add the gel inside the porous scaffolds, the scaffolds were blot dried and the hydrogel precursor was added within 5 min. Virus loaded scaffolds were made by mixing the lentivirus suspension with the hydrogel prior to filling the pores. For collagen-filled scaffolds, acid-soluble rat tail collagen type I (BD Biosciences, San Jose, CA), was diluted with PBS and lentivirus to 0.15 mg/ml, neutralized with sodium hydroxide, and added to the scaffolds. The scaffolds were then incubated at 37°C for 30 min. For alginate-filled scaffolds, alginate (FMC BioPolymer, Philadelphia, PA) was diluted with PBS and lentivirus to 1 mg/ml and pipetted into the scaffolds and incubated in an excess solution of 50-mM calcium chloride for 10 min. Fibrin-filled scaffolds were made by diluting fibrinogen (Tisseel™, Baxter Healthcare, BioScience Division, Westlake Village, CA) with PBS and lentivirus to 25 mg/ml. The fibrinogen was pipetted into the scaffolds pores, and the scaffold incubated in a 50 U/ml thrombin solution for 15 min at 37°C. Lentivirus was added to empty scaffolds by directly pipetting the lentivirus into the scaffolds. Scaffolds were used immediately upon gelation or the addition of the lentivirus.

A Sirius red stain was employed to determine the hydrogel coverage within the scaffold. Sirius red staining is commonly used for collagen quantification by birefringence (Junqueira et al. 1979), yet the dye acts by electrostatic interaction with basic groups in amino acids, which results in nonspecific staining of other proteins (Nielsen et al. 1998; De Laporte et al. 2009; Salvay et al. 2008). Scaffolds were incubated in a solution of Sirius red (1% in 1.3% picric acid, Sigma-Aldrich, St. Louis, MO) for 1 h, and then incubated in acidified water (5-ml acetic acid (Sigma-Aldrich, St. Louis, MO) in 1-L water,) for 1 h. Hydrogel-filled scaffolds were incubated in DMEM with 10% FBS at 37°C and stained after 1 and 14 days.

In order to determine if the gels were stable at 14 days, hydrogel-filled scaffolds were compared with stained scaffolds in which the hydrogel was degraded using collagenase (1 mg/ml, Sigma-Aldrich, St. Louis, MO) or alginate lyase (10 IU/ml, Sigma-Aldrich, St. Louis, MO).

Lentiviral release from hydrogel-filled scaffolds

Hydrogel-filled scaffolds were incubated in media in order to determine the release rate of the lentiviral vector from the scaffolds. Hydrogel-filled scaffolds were prepared as described with 3.5×10^8 lentivirus particles per scaffolds. The scaffolds were incubated in DMEM media with 10% FBS at 37°C. After incubation for 24 and 48 hours, scaffolds were moved to fresh media, and the supernatant stored at -80°C until analyzed. Virus concentration in the supernatant was determined by a HIV-1 p24 Antigen ELISA Kit (ZeptoMetrix Co., Buffalo, NY).

In vivo transgene expression

In vivo transgene expression was monitored over time to determine the extent and the level of transgene expression. Animal studies were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and protocols were approved by the IACUC at Northwestern University. Hydrogel-filled and empty scaffolds loaded with a luciferase-encoding lentivirus (1.7×10^9 lentivirus particles) were implanted into the right intraperitoneal fat pad of CD1 male mice (Charles Rives, Wilmington, MA; 24–26 g), as previously described (Blomeier et al. 2006). In vivo luciferase expression was monitored using an IVIS imaging system (Xenogen Corp., Alameda, CA). For imaging, the animals were injected IP with D-luciferin (150-mg/kg body weight, 20 mg/ml in PBS; Molecular Therapeutics Inc., Ann Arbor, MI) using 28 G insulin syringes. The animals were placed in a light-tight chamber and bioluminescence images were acquired (every 2 min for a total of 20 min) until the peak light emission was confirmed. Gray scale and bioluminescence images were superimposed using the Living Image software (Xenogen Corp., Alameda, CA). A constant size region of interest was drawn over the scaffold implantation site and at another site on top of the animal as a background. The signal intensity was reported as an integrated light flux (photons/s) subtracting background, which was determined by IGOR software (WaveMetrics, Portland, OR). To determine the specific organs that had expression, mice were sacrificed after injecting D-luciferin, and the thymus, heart, the lymph nodes (inguinal, brachial, and axillary), liver, kidneys, fat pad, testicles, and lungs were retrieved and imaged as before.

Histological analysis and immunohistochemistry

Histological analysis was performed to determine the cellular distribution and identity of transduced cells. Scaffolds loaded with a lentivirus encoding for EGFP (1.7×10^9 lentivirus particles) were retrieved 7 and 21 days post-implantation and frozen in an isopentane bath cooled over dry ice to -50°C. Tissue samples were embedded in Tissue-Tek O.C.T. compound (Sakura Finetek, Torrance, CA), and sections were cut at 14-μm thickness using a cryostat. Prior to staining, sections were fixed with 4% paraformaldehyde for 10 min and washed in PBS. The extent of cellular infiltration into scaffolds was visualized by hematoxylin and eosin (H&E) staining of tissue sections retrieved at 7 and 21 days post-implantation. The distribution of transduced cells was determined by performing immunohistochemistry on tissue section retrieved at 7 and 21 days post-implantation using an antibody directed against green fluorescent protein (polyclonal rabbit anti-EGFP (1:500 dilution; Invitrogen, Carlsbad, CA)) with an anti-rabbit horseradish peroxidase secondary antibody (Vector Labs, Burlingame, CA). Hematoxylin was used as a counter stain.

The identity of transduced cells was identified by a double-label immunofluorescence. Sections from the scaffolds were stained with a polyclonal rabbit anti-EGFP (1:500 dilution; Invitrogen, Carlsbad, CA) as a primary antibody and Alexa Fluor 488 goat anti-rabbit (1:500 dilution, green; Invitrogen, Carlsbad, CA) as a secondary antibody. A second cell-specific staining was also performed on these sections with (1) rat anti-mouse F4/80 (1:100 dilution; AbD Serotec, Raleigh, NC); and (2) hamster anti-mouse CD11c (1:100 dilution; Novus Biological, Littleton, CO). Alexa Fluor 546 goat anti-rat (red; Invitrogen, Carlsbad, CA) and Texas Red anti-hamster (red; Novus Biological, Littleton, CO) were used as secondary antibodies, respectively. A Hoechst stain (10 mg/ml, 1:2,000 dilution; Invitrogen, Carlsbad, CA) was coincidentally performed to identify cell nuclei.

Statistical analysis

For multiple pairs' comparison, an analysis of variance (ANOVA) with post hoc Tukey's test was performed with a p level of 0.05. For comparison of the levels of transgene expression, a Kruskal–Wallis test was performed with a p level of 0.05. Error bars represent standard errors in all figures. Experiments were performed in triplicate.

Results

Hydrogel-filled scaffolds

Initial studies investigated the process for loading hydrogels into the scaffold in order to achieve complete filling of the pores. The scaffolds were formed by foaming PLG microspheres with salt as a porogen, and were 98% porous with pores that ranged between 250 and 425 μm . Solutions of collagen, fibrin, or alginate were pipetted onto the PLG scaffolds until the microporous structure was saturated, with subsequent gelation in situ. Sirius red staining indicated that all three hydrogels completely filled the porous structure (Fig. 1a–g). Additionally, the hydrogels were stable within the scaffolds for at least 14 days in vitro as indicated by the Sirius red stain at 14 days compared to degraded gels. (Fig. 1h, i).

The rate of cell infiltration into the hydrogel-filled and empty scaffolds were subsequently investigated by implantation into the peritoneal fat pad of mice. Empty scaffolds retrieved 7 days post-implantation had a dense cell population to a depth of approximately 0.75 mm from the scaffold edge, with a lower cell density observed throughout the scaffold interior (Fig. 2a, b). Fibrin and collagen-filled scaffolds demonstrated similar partial occupancy of the scaffold at 7 days, with the depth of dense cells approximately 0.5 and 0.6 mm, respectively (Fig. 2c, d). Alginate-filled scaffolds had no cells infiltrating or within the interior of the scaffold, as expected. (Fig. 2e, i). No obvious differences in the foreign body response were noted between conditions based on H&E staining. At 21 days post-implantation, the empty scaffold and those filled with collagen and fibrin were densely occupied by cells throughout the entire scaffold (Fig. 2j–h). For alginate-filled scaffolds, cells were not observed within the scaffold interior, consistent with earlier time points where alginate did not support cell infiltration (Fig. 2i).

Lentiviral release from hydrogel-filled scaffolds

We subsequently investigated the release of lentivirus from the hydrogel-filled scaffolds, which impacts the availability of the vector to infiltrating cells. Lentivirus was either directly pipetted into an empty scaffold, or the lentivirus vector was entrapped within the hydrogel matrix filling the PLG scaffold pores. Empty scaffolds released 68% of the vector within 24 h, and had released 98% by 48 h (Fig. 3). This rapid release was expected since the lentivirus does not interact strongly with PLG (Shin et al. 2010a; Shin et al. 2010b). For the hydrogel-filled scaffolds at 24 h, release was 42.9%, 11.1%, and 7.4% for collagen,

alginate, and fibrin, respectively (Fig. 3). By 48 h, release was 59.3%, 32.4%, and 16.3%, respectively. The hydrogel-filled scaffolds had a substantially decreased release relative to the empty scaffolds. The hydrogels are not expected to bind the lentiviral vectors (Shin and Shea 2010), and the increased retention likely resulted from the relatively small pores of the gels that provide a barrier to release. The observed release correlates with the pore sizes of the gels. Collagen, which has pores on the order of a few microns (Yang et al. 2010), had the fastest release while fibrin and alginate, which have sub-micron pores (Chan and Neufeld 2009; Karp et al. 2004), had the slowest release.

In vivo transgene expression

Scaffolds loaded with a lentiviral vector were implanted to investigate the extent and duration of transgene expression. Expression at the implantation site was observed for 28 days post-implantation, with the greatest expression obtained for the empty and fibrin-filled scaffolds (Fig. 4a). Collagen and alginate-filled scaffolds had expression that was decreased by an order of magnitude relative to the maximal expression levels. Overall, maximal transgene expression occurred within 3 to 7 days post-implantation for each condition, with expression declining steadily, consistent with previous reports and potentially resulting from gene silencing (Avilés et al. 2010; Rives et al. 2009; Zhang et al. 2009).

Maximal expression was observed at the implant site throughout the study duration; however, at 3 and 7 days post-transplantation, bioluminescence imaging indicated expression at off-target sites (Fig. 4b). The presence of the hydrogel minimized the diffuse luminescence around the scaffold. Specific expression was observed at two sites other than the implant for all conditions (Fig. 5a). To identify these sites, multiple organs were excised for subsequent imaging. The two primary sites for expression distant from the scaffold were the spleen and the thymus, both of which had luminescence throughout the organ (Fig. 5b). Expression at the implanted fat pad accounted for 73% of the total photons observed by imaging whereas the spleen and thymus accounted for 8.2% and 7.2%, respectively (Fig. 5c). Luminescence was also observed in the liver, lungs, testicles, kidneys, and the nonimplanted fat pad, though the expression levels were significantly lower and the luminescence was punctate rather than distributed throughout the organ (Fig. 5b). The liver, lungs, left fat pad, testicles, and kidneys each accounted for less than 3% of total expression. No expression was observed within the axillary, brachial, and inguinal lymph nodes, or the heart. After 14 days, expression was observed at the implant site only (Fig. 4b).

Distribution and identity of transduced cells

The distribution and identity of transduced cells was subsequently investigated at the implant site. At 7 days post-implantation, GFP-positive cells (brown) were localized at the outer areas of the scaffolds and the tissue adjacent to the scaffolds (Fig. 6a–d). GFP-positive cells were seen within the outer boundary of the scaffolds, yet the majority of the infected cells are localized in the tissue adjacent to the scaffolds. By 21 days, GFP-positive cells were observed both within the scaffolds and in the adjacent tissue for empty, collagen-, and fibrin-filled scaffolds (Fig. 6e–g). For the alginate-filled scaffolds, transduced cells were observed only in the tissue adjacent to the scaffold (Fig. 6h).

The identity of the transduced cells was investigated by double staining with antibodies to macrophages (F4/80) and dendritic cells (CD11c). At day 7, GF-positive stained cells (green) were typically observed at the outer regions of the scaffold (Figs. 7a and 6a), as before. Antibody staining for macrophages (Fig. 7a, red) and dendritic cells (Fig. 7b, red) co-localized with GFP staining. Similarly, GFP-positive cells (green) were found at the spleen, and co-localized with both, antibody staining for macrophages (Fig. 7c, red) and dendritic cells (Fig. 7d, red).

Discussion

We report on gene delivery from PLG microporous scaffolds, whose pores are filled with hydrogels, and demonstrate that the gel properties influence transgene expression *in vivo*. Biomaterial scaffolds serve a fundamental role in regeneration by providing structural support that creates and maintains a space for tissue growth. These scaffolds also function as a support for cell adhesion and migration, which facilitates cell infiltration and engraftment within the host. The extent of transgene expression by gene delivery from porous scaffolds has been reported to be primarily a function of the vector dose, particularly the dose released shortly after implantation (Avilés et al. 2010). Additionally, transgene expression is initially observed within the scaffold; however, after extended times, transgene expression within the scaffold may decline, yet can potentially increase in the surrounding tissue (Salvay et al. 2010). Filling the pores with hydrogels was investigated as a means to modify vector release and enhance vector retention within the scaffold. The hydrogel also provides an internal matrix for cell adhesion, migration and proliferation, with physical properties similar to that of many soft tissues. The combination of hydrogels and microporous scaffolds has previously been employed for cell transplantation, for applications such as in cartilage regeneration (Karp et al. 2004; Ameer et al. 2002; Gong et al. 2007) or enhancement of angiogenesis.

Lentiviral delivery from hydrogel-filled scaffolds supported gene delivery *in vivo*, with the extent of transgene expression dependent upon the hydrogel properties. Expression was highest for fibrin-filled and empty scaffolds, with decreased levels for both alginate and collagen. The empty scaffolds provide the most rapid rate of cell infiltration and also the most rapid release of the vectors, both of which may contribute to maximal transgene expression (Avilés et al. 2010; Shepard et al. 2010). Fibrin and collagen-filled scaffolds had similar rates of cell infiltration, yet surprisingly fibrin produced a greater level of transgene expression. Additionally, the levels of transgene expression did not correlate with the *in vitro* release profile, as fibrin had the slowest release *in vitro* and collagen had the fastest. Both cell infiltration and vector release are influenced by the degradation of the matrix, and our *in vitro* release studies may not effectively mimic the *in vivo* situation. Fibrin hydrogels, which degrade by the actions of plasmin, provide a rapid rate of degradation (Fisher et al. 2007; Petter-Puchner et al. 2010) that may contribute to gene transfer *in vivo*. Previous reports have indicated that fibrinolysis is necessary for expression from lentivirus-loaded fibrin gels (Raut et al. 2010), and fibrin degradation may be occurring more rapidly *in vivo* than in our *in vitro* studies. In contrast, alginate hydrogels, which do not degrade and have only minimal cell infiltration, produced transgene expression levels similar to those observed with collagen.

Loading hydrogels into the scaffold pores was hypothesized to increase transgene expression within the scaffold by retaining the vectors for transduction of infiltrating cells. The enhanced retention of viral vectors within hydrogels has previously been reported to enhance the extent of transgene expression (Shin and Shea 2010). Transduced cells were identified by immunostaining for EGFP and were observed within the pores of the scaffold. At 7 days, most of the staining occurred in the periphery of the scaffolds, and by day 21, only sporadic staining was observed within the scaffolds. Some of the transduced cells were identified as macrophages (F4/80+) and dendritic cells (CD11c+). Transduced cells present in the scaffold likely results from a fraction that were transduced outside the scaffold and subsequently infiltrated, and a fraction that were transduced within the scaffold. While the hydrogel did not increase expression within the scaffolds, expression was more localized within the implant with hydrogels filling the pores relative to empty scaffolds. Increasing the number transduced after cell infiltration will require retaining the vector within the scaffold while maintaining the activity of the vector. By the time the cells completely infiltrate the

scaffold (after day 7), the quantity of vector retained likely decreases substantially due to degradation of the hydrogel. Additionally, the lentivirus has a half-life of approximately 24 h at 37°C (Shin and Shea 2010), and by day 7 the activity would have decayed to approximately 1% of its original value. Thus, strategies must continually be developed that can effectively retain the vector while also maintaining its activity.

Interestingly, bioluminescence imaging identified transgene expression at off-target sites during the initial 7 days after implantation. This off-target expression likely results from a combination of vector release from the scaffold, as well as the movement of locally transduced cells. Expression in the thymus and spleen was observed throughout the organ; whereas expression in the other tissues was sporadic. Within the spleen and thymus, transduced cells identified as macrophages and dendritic cells, consistent with the cell types transduced at the scaffold. Macrophages and dendritic cells are recruited to the scaffold following implantation as a component of the foreign body response (Babensee 2008; Babensee et al. 1998), and these cells can subsequently distribute to other tissues in the body (Ali et al. 2009). Transgene expression throughout the thymus and the spleen may result from the movement of these cell types after they are locally transduced at the scaffold (Alvarez et al. 2008). The transport of vector from the scaffold to the spleen and thymus, while possible, would also be expected to significantly transduce cells in the liver, lungs and bone marrow, which was not observed (Tsui et al. 2002). This transduction of immune cells could potentially be of use in applications aimed at immuno-modulation, such as vaccination (Ali et al. 2009; Hubbell et al. 2009; Reddy et al. 2006). Punctate expression was observed in the adjacent testicles, opposite fat pad, liver, kidney, and lungs. This expression pattern is consistent with the transport of the released vector to these tissues. Off-target transduction is a concern for these efficient vectors, and may be addressed through the development of improved strategies to retain the vector locally (i.e., binding to scaffold (Shin and Shea 2010; Shin et al. 2010b; Stachelek et al. 2004)).

Conclusions

We have developed hydrogel-filled scaffolds capable of delivering lentivirus, using the natural hydrogels collagen, alginate, and fibrin to fill the pores of microporous PLG scaffolds. The hydrogel-filled scaffolds induced transgene expression in vivo, with the level of expression correlated to the gel properties. Expression was sustained for 4 weeks post-implantation, with empty scaffolds and fibrin-filled scaffolds having maximal expression compared with alginate- and collagen-filled scaffolds. We identified infected macrophages and dendritic cells in the scaffold, as well as the spleen and thymus, with the latter locations occurring in part through localized transduction of immune cells that subsequently migrate from the implant. Overall, these studies demonstrate the ability to combine the properties of hydrogel and polymeric scaffolds, and that it is possible to use the properties of the hydrogels in order direct gene delivery, which has numerous applications in tissue engineering, cell transplantation, and gene therapy.

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Fig. 1. Hydrogel-filled scaffolds. Hydrogel-filled scaffolds were stained using Sirius red. Images are (a) top view of an empty scaffold, (b–d) top and (e–g) cross-sectional views of scaffolds filled with (b, e) fibrin, (c, f) collagen, and (d, g) alginate 1 day after adding the gel. Representative top view of a (h) hydrogel-filled scaffold after 14 days indicates that the gel was stable compared with a (i) scaffold in which the hydrogel was degraded. *Scale bar* is 1 mm

Fig. 2.

Cell migration within hydrogel-filled scaffolds. H&E stains of hydrogel-filled scaffolds implanted in the fat pad of mice retrieved 7 and 21 days post-implantation. (a) Cross-sectional view of an empty scaffold retrieved 7 days post-implantation. The image was taken at $\times 100$ and stitched together using the software PtGui. Scale bar is 200 μm . (b–e) Sections from the tissue-scaffold boundary retrieved at 7 days, (f–i) the center of the scaffolds retrieved at 7 days, and (j–m) the center of the scaffolds retrieved at 21 days. The scaffolds were either empty (b, f, j), or loaded with a fibrin gel (c, g, k), a collagen gel (d, h, l), or an alginate gel (e, i, m). Images were taken at $\times 200$. Scale bar is 100 μm . Images are representative of three animals per condition

Fig. 3.

Lentivirus release from hydrogel-filled scaffolds. Scaffolds were incubated in DMEM media, and the supernatant was analyzed to determine lentivirus release using a p24 ELISA at 24 and 48 h after gelation. *Error bars* represent standard error ($n=3$). *Letters* indicate statistically significant differences ($p<0.05$) compared to (a) alginate, (c) collagen, or (f) fibrin at the same time point analyzed by a one-factor ANOVA post hoc Tukey's test

Fig. 4.

In vivo transgene expression from hydrogel-filled scaffolds as a function of time. **(a)** Hydrogel (collagen, fibrin, and 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 measured using an IVIS bioluminescence system. *Error bars* represent standard error ($n=3$). *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

Fig. 5. Localization of in vivo transgene expression. (a) Bioluminescence image of empty scaffolds at 3 days showed that expression was localized to other sites besides the implantation site (right fat pad). (b) Bioluminescence imaging at 3 days of mice organs showed robust expression at the implantation area, the thymus and the spleen. *Scale bar* is 2 mm. (c) Percentage of total transgene expression at each organ, quantified as the percentage of total photons. *Error bars* represent standard error ($n=3$). *Asterisk* indicates that expression at the implant was statistically higher ($p<0.05$) than the other tissues, analyzed by a one-factor ANOVA post hoc Tukey test ($n=3$)

Fig. 6. Distribution of transduced cells. (a, e) Empty scaffolds and scaffolds loaded with the hydrogels (b, f) fibrin, (c, g) collagen, and (d, h) alginate were retrieved at (a–d) 7 and (e–h) 21 days post-implantation, and analyzed by GFP antibody staining with a hematoxylin counter stain. Images at 7 days are taken at the tissue-scaffold boundary and at 21 days at the center of the scaffold. *Scale bar* is 200 μm . GFP-positive staining is *dark brown*

Fig. 7. Identity of transduced cells. Scaffolds and the spleen from the empty scaffold group were retrieved 7 days post-implantation and analyzed by immunohistochemistry, dual stained against GFP antibodies and the cell markers (**a, c**) F4/80 for macrophages and (**b, d**) CD11c for dendritic cells. Figures show an overlay of the GFP antibody staining (*green*) and the cell marker staining (*red*) for scaffolds (**a, b**) and the spleen (**c, d**), with co-localized cells in *yellow*. The cells marked by the *white squares* were magnified in the *inset*, showing co-localization of GFP (*green*), cell marker (*red*), and cell nuclei (*blue*). Scale bar is 100 μ m