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Fibrin-mediated lentivirus gene transfer: implications for lentivirus microarrays

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Abstract

We employed fibrin hydrogel as bioactive matrix for lentivirus mediated gene transfer. Fibrin-mediated gene transfer was highly efficient and exhibited strong dependence on fibrinogen concentration. Efficient gene transfer was achieved with fibrinogen concentration between 3.75 – 7.5 mg/mL. Lower fibrinogen concentrations resulted in diffusion of virus out of the gel while higher concentrations led to ineffective fibrin degradation by target cells. Addition of fibrinolytic inhibitors decreased gene transfer in a dose-dependent manner suggesting that fibrin degradation by target cells may be necessary for successful gene delivery. Under these conditions transduction may be limited only to cells interacting with the matrix thereby providing a method for spatially localized gene delivery. Indeed, when lentivirus-containing fibrin microgels were spotted in an array format gene transfer was confined to virus-containing fibrin spots with minimal cross-contamination between neighboring sites. Collectively, our data suggest that fibrin may provide an effective matrix for spatially-localized gene delivery with potential applications in high-throughput lentiviral microarrays and in regenerative medicine.

Keywords

Fibrin hydrogels; fibrinolysis; matrix-mediated transduction; cell-controlled gene delivery; lentiviral microarrays

INTRODUCTION

In recent years biomaterial-mediated gene delivery has emerged as a promising alternative to conventional gene transfer technologies. Natural and polymeric biomaterials have been employed to embed or immobilize pDNA complexed with lipids (lipoplexes) or cationic polymers (polyplexes) to improve gene transfer efficiency [1]. Increased gene delivery maybe due to a number of mechanisms such as increased local vector concentration [2–3], improved vector stability [4] or reduced cell cytotoxicity [5]. Additionally, biomaterials can be designed to provide spatial and temporal control of gene delivery, thereby improving transgene expression while minimizing gene transfer to non-target tissues [6–9]

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Natural and polymeric biomaterials have also been employed to facilitate gene transfer of recombinant viruses. For instance, adenovirus delivery using collagen matrix resulted in prolonged PDGF-BB expression at the wound site, enhanced wound healing and minimized the inflammatory response [10]. Also, a collagen/gelatin mixture promoted cell migration into the matrix, resulting in localized adenoviral delivery only to the matrix infiltrating cells [11]. Fibrin scaffolds were reported to preserve adenovirus activity [12] and provided an effective platform for localized and sustained adenovirus release to challenging target sites such as the esophageal epithelium [13]. Similarly, fibrin-mediated adenovirus delivery to the wound site [14] resulted in enhanced expression of KGF [15] or eNOS [16] and faster wound reepithelization. Finally, implantation of hydroxyapatite disks coated with BMP2-encoding rAAV resulted in significant bone formation in a rat animal model [17].

Cell-based arrays provide a platform for a wide spectrum of high throughput studies with a wide range of applications such as discovering new gene functions [18], comparing gene expression profiles between undifferentiated vs. differentiated cells [19], identifying substrates for cell adhesion and proliferation [20], examining cell interactions with various ligands [21–24] or drug screening [25–26]. Despite promising results, most microarrays are based on reverse transfection, which suffers from low gene transfer efficiency and short term transgene expression. To overcome these shortcomings, VSV-G pseudotyped lentivirus has been used to overexpress or to knockdown genes for high throughput analysis of gene functions [27–28]. However, the small amount of viral particles that can be immobilized within the small area of each spot may still limit gene transfer efficiency, especially when using difficult to transduce primary cells or stem cells.

Recently our laboratory demonstrated that fibrin-entrapped DNA lipoplexes can serve as a platform for enhanced and confined gene delivery in a microarray format [6]. Based on these findings we hypothesized that fibrin may also be used as a bioactive matrix for lentivirus delivery and set out to determine the optimal parameters for gene transfer. We evaluated the effects of fibrinogen concentration on lentivirus diffusion from the gel pores and on fibrin-mediated transduction efficiency. We also examined the importance of fibrin degradation for successful gene transfer, as well as the presence of polycations (polybrene) in this process. Finally, we demonstrated the use of fibrin hydrogels to achieve spatially-confined lentivirus-mediated gene delivery in the context of a cell-transduction microarray.

MATERIALS AND METHODS

Lentivirus Preparation

The lentiviral vectors pCS_hGHpA_ZsG_phPGK and pCS_pA_DsRed2_CMV (for details see supplementary materials and methods) were used to produce ZsGreen- or DsRed2-encoding lentiviruses, respectively as described elsewhere [29]. Virus was concentrated by centrifugation for 2 hr at 50,000g and 4°C (Avanti™ centrifuge J-25I; Beckman Coulter, Palo Alto, California). The pellet was resuspended in 200 µl fresh culture medium to create 100X virus stocks and stored at –80°C.

Transduction Methods

Plasminogen-depleted human fibrinogen (Enzyme Research Laboratories, South Blend, IN) was diluted in Phosphate Buffered Saline (PBS) to the desired concentration. The diluted fibrinogen (150 µl) was added to the wells of a tissue culture treated 24-well plate (Greiner Bio-One, Monroe, NC). Fibrinogen was deposited on the surface by evaporation for 2 hr in a laminar flow hood before addition of PBS (200 µl) containing thrombin (2.5 U/ml) and virus in the range of 10–50 fold dilution of the concentrated stock. In some experiments this solution also contained aprotinin (1 µg/ml to 100 µg/ml; Sigma, St Louis, MO) or ε-aminocaproic acid

(εACA), 0–20 mg/mL Calbiochem, La Jolla, CA). The plate was incubated at room temperature for 45 min to allow gel polymerization before addition of 500 µL cells on top of the gel at the indicated densities. At the same time cells were seeded in tissue cultured treated 24-well plate under similar conditions and transduced with the traditional transduction protocol (TTP) as described previously [30]. Briefly one day post-cell seeding, 500 µl virus was diluted to the desired virus strength in fresh medium. The diluted virus in the presence or absence of polybrene (PB; Sigma) was added over these cells overnight. Afterwards, the samples were replenished with 500 µl of fresh medium every 2 days until the gels were degraded, at which time the samples were processed for flow cytometry.

Flow Cytometry

The cells were resuspended in PBS and analyzed for transduction efficiency and fluorescence intensity using flow cytometer (FACSCalibur; Becton Dickinson, San Jose, California) as described previously [6,30–31].

Lentivirus arrays

A virus-thrombin solution was prepared as described in the “Transduction Methods” section and 0.25–1.0 µl of this solution was spotted on tissue culture treated surface at RT by manual pipetting. On complete drying, fibrinogen solution at the indicated concentration was carefully added over the thrombin spots while keeping the plate on ice. The plate was left at 4°C for 15 to 20 min followed by incubation at 37°C for 45 min to allow for gel polymerization. Subsequently, cells were seeded at near confluent densities and fluorescent cells were visualized by microscopy at approximately one week post-seeding.

Fluorescence microscopy

The cells were imaged at 5X magnification using a Zeiss Axio Observer (Carl Zeiss MicroImaging, Thornwood, NY) inverted microscope equipped with a moving stage, 37°C incubator and an ORCA-ER CCD digital camera (Hamamatsu, Bridgewater, NJ). Photographs of the transduced cell images were merged using the AxioVision software (Carl Zeiss) and analyzed using ImageJ (version 1.42; National Institutes of Health, Bethesda, USA).

Statistical Analysis

The results were analyzed using ANOVA for multiple comparisons followed by the Fisher's t-test for pairwise comparisons (significance $p < 0.05$). The sample size was $n = 3-4$ and each experiment was repeated three times unless indicated otherwise.

RESULTS

Efficient transduction with lentivirus embedded in fibrin hydrogels depends on FBG concentration

We examined whether fibrin can support efficient gene delivery to target cells using lentiviral vectors. To this end, we embedded lentivirus encoding for ZsGreen in fibrin hydrogels that were formed with various concentrations of fibrinogen (FBG) ranging from 0.75 – 15 mg/mL. Afterwards, 293T cells were seeded on top of the gel at two different densities (5×10^4 or 2.5×10^5 cells per cm^2) to initiate gene transfer. Cells transduced with the traditional transduction protocol (TTP) i.e. by adding virus on the cell monolayer on tissue culture plastic served as controls. The % transduced cells (transduction efficiency, TE) and mean green fluorescence intensity (GFI, an indication of transgene expression) were measured by flow cytometry after the hydrogels were degraded by target cells.

We found that at low cell densities (5×10^4 cells per cm^2) fibrin gels prepared with 0.75 mg/ml FBG yielded similar transduction efficiency ($89.6 \pm 0.8\%$ vs. $89.3 \pm 0.6\%$, $p = 0.5$ ZsGreen+ cells, Fig. 1A) but higher transgene expression (858 ± 27 vs. 776 ± 17 , $p = 0.002$ fluorescence intensity units (FIU)) than TTP (Fig. 1B). Interestingly, both the transduction efficiency and transgene expression decreased with increasing FBG concentration (Fig. 1A, B).

On the other hand, when target cells were transduced at high cell densities (2.5×10^5 cells per cm^2) both transduction efficiency and fluorescence intensity were independent of FBG concentration in the range of 0.75 – 7.5 mg/ml. At the highest FBG concentration (15 mg/ml), the transduction efficiency decreased slightly to ~85% (Fig. 1A) and transgene expression decreased by 15% (from 715 ± 59 FIU at 0.75 mg/ml to 613 ± 24 FIU at 15 mg/ml FBG, $p = 0.02$) (Fig. 1B). Interestingly, the TTP protocol yielded the lowest transgene expression level, suggesting that fibrin hydrogel might enhance the number of gene copies per cell under these conditions.

Although the trend was similar, the dependence on FBG concentration was more prominent for NIH-3T3 fibroblasts. Specifically, at low cell density (2.5×10^4 cells per cm^2) the percent ZsGreen+ cells dropped dramatically from $82.4 \pm 1.7\%$ for 0.75 mg/ml FBG to $30.3 \pm 9.2\%$ for 15 mg/ml FBG ($p = 3.1 \times 10^{-5}$, Fig. 2A). Transgene expression level also showed a considerable decrease from 479 ± 36 to 212 ± 21 FIU ($p = 1.4 \times 10^{-5}$, Fig. 2B). Similar results were obtained with high target cell density (1×10^5 cells per cm^2) as well (Fig. 2A, B).

Interestingly, in this case fibrin mediated gene transfer (0.75 mg/ml FBG) was more efficient than TTP ($64 \pm 5\%$ vs. $52 \pm 1\%$ ZsGreen+ cells, $p = 0.003$) but transgene expression was similar (408 ± 22 vs. 404 ± 7 FIU, $p = 0.76$).

High FBG concentrations prevent virus diffusion from fibrin hydrogels

FBG concentration has been shown to affect the structure of fibrin gels with low concentrations resulting in formation of fewer fiber bundles and larger pore size gels [32]. These results prompted us to examine the effect of FBG concentration on the diffusion of lentiviral particles through the fibrin gel pores. To this end, lentivirus was embedded in fibrin gels containing various FBG concentrations (1.5 – 30 mg/ml). Then the gels were overlaid with PBS and at the indicated times, the supernatant was collected and used to transduce 293T cells. After the last time point all gels were degraded with plasmin to measure the activity of fibrin-associated lentivirus. We observed a considerable amount of lentivirus loss from gels formed with 1.5 mg/ml FBG with maximum virus activity measured at one hour after gelation (Fig. 3A). For gels prepared with 3 mg/mL FBG concentration, only a small fraction of virus was lost throughout the time period tested. No virus activity was detected in the supernatant throughout the experiment when fibrin hydrogels were prepared with greater than 7.5 mg/ml FBG. In agreement, plasmin-degraded gels exhibited the reverse trend with the lowest or highest virus activity observed at 1.5 mg/mL or 7.5 mg/mL FBG, respectively (Fig. 3B). Together, these data indicated that the amount of lentivirus activity entrapped within the fibrin gel is a function of FBG concentration.

Fibrinolysis is necessary for gene transfer

Our results to this point indicated that gene transfer decreased with increasing FBG concentration despite improved lentivirus retention. One possible explanation maybe that the increased density of fibrin gels leads to slower hydrogel degradation. Since lentivirus has a finite half-life in culture, the virus may lose bioactivity before hydrogel degradation and cellular uptake, thereby compromising transduction efficiency. To address this hypothesis we added different concentrations of fibrinolytic inhibitors - aprotinin or ϵ -aminocaproic acid - into gels prepared with 1.5 mg/ml or 3.75 mg/ml FBG and evaluated gene transfer efficiency using either 293T or NIH-3T3 cells.

In gels containing 3.75 mg/mL FBG aprotinin reduced gene transfer in a dose-dependent way for both cell types. Compared to control (no aprotinin), 100 μ g/mL aprotinin decreased gene transfer to 293T cells by ~47% (from $37.5 \pm 2.0\%$ to $19.9 \pm 2.7\%$, $p = 5 \times 10^{-5}$) (Fig. 4A) and to NIH-3T3 cells by 59% (from $62.5 \pm 1.0\%$ to $26 \pm 8\%$, $p = 1.2 \times 10^{-4}$) (Fig. 4B). Similarly, addition of 100 μ g/mL aprotinin decreased transgene expression by 23% (from 398 ± 15 to 307 ± 14 FIU, $p = 1.2 \times 10^{-4}$) in 293T cells (Fig. 4C) and 47% (from 189 ± 13 to 101 ± 10 FIU, $p = 4 \times 10^{-5}$) in NIH-3T3 cells (Fig. 4D). On the other hand, for hydrogels prepared with 1.5 mg/mL FBG aprotinin did not prevent gel degradation and consequently had no effect on gene transfer in the range of concentrations tested.

Next we employed another fibrinolytic inhibitor - ϵ -aminocaproic acid (eACA) – to confirm our findings. Regardless of cell type, eACA decreased gene transfer in a dose-dependent way and to a greater extent than aprotinin. Compared to controls without fibrinolytic inhibitor, addition of 20 mg/mL eACA reduced the fraction of GFP+ 293T cells by 29% (from $64 \pm 3\%$ to $45.5 \pm 8.2\%$, $p = 0.008$) or 87% (from $58.4 \pm 3.3\%$ to $7.5 \pm 3.8\%$, $p = 9.1 \times 10^{-7}$) for hydrogels formed with 1.5 or 3.75 mg/mL FBG, respectively (Fig. 5A). Similar results were observed for NIH-3T3 cells. In particular, the fraction of GFP+ NIH-3T3 cells decreased by 52% (from $76 \pm 4\%$ to $37 \pm 5\%$, $p = 3 \times 10^{-5}$) or 58% ($69 \pm 4\%$ to $29 \pm 7\%$, $p = 8.7 \times 10^{-5}$) for gels containing 1.5 or 3.75 mg/mL FBG, respectively (Fig. 5B). As expected neither aprotinin nor eACA decreased gene transfer with TTP (data not shown), suggesting that neither compound had adverse effects on cell physiology.

Interestingly, eACA did not influence transgene expression to the same extent as the fraction of transduced cells. For NIH-3T3 cells, transgene expression was not altered by addition of eACA. On the other hand, in the case of 293T cells, addition of 20 mg/mL eACA decreased transgene expression by ~28% and ~55% for gels made with 1.5 and 3.75 mg/mL FBG, respectively. Taken together these results clearly indicated that fibrin gel degradation by target cells is required for efficient lentiviral transduction.

Polybrene enhances fibrin-mediated lentiviral transduction

Traditional retroviral transduction requires addition of cationic polymers such as polybrene to neutralize the electrostatic repulsion between viral particles and target cells [33–35]. On the other hand, polybrene decreases retrovirus binding to fibronectin, thereby decreasing retroviral gene transfer on fibronectin-coated surfaces [30,36]. These results promoted us to examine the effect of polybrene on fibrin-mediated retroviral gene transfer. To this end, lentivirus-containing fibrin gels were prepared with or without 8 μ g/mL polybrene and target cells were overlaid to initiate gene transfer.

Regardless of FBG concentration, polybrene increased both transduction efficiency and transgene expression of NIH-3T3 cells by 2- to 4-fold either at low (2.5×10^4 cells per cm^2) or high (1×10^5 cells per cm^2) target cell densities (Fig. 6). As expected, polybrene improved traditional gene transfer on tissue culture treated surfaces, especially at low target cell density (~10-fold increase). Interestingly, while the efficiency of traditional transduction decreased at high target cell density (lower multiplicity of infection, moi), fibrin-mediated transduction increased. Transgene expression followed the same trend, with higher fluorescence intensity values observed at high target cell density.

Spatially localized gene transfer on fibrin-based lentivirus arrays

As we showed above, lentivirus can be successfully immobilized within fibrin gels, which must be degraded by target cells for successful gene transfer. Based on these results, we hypothesized that fibrin hydrogels can be used to achieve spatially localized lentiviral gene transfer in an array format. To obtain proof-of-concept, we created a 25-spot array of virus-

containing fibrin microgels (Fig. 7A). As expected, the spot size decreased as the volume of fibrin decreased with the smallest diameter (~ 1 mm) attained at 0.25 μ l (Fig. 7B).

Next, fibrin gels (0.25 μ l) containing ZsGreen or DsRed-encoding lentivirus were positioned in alternating columns and target 293T cells were seeded at high cell density (2.5×10^5 cells per cm^2) throughout the whole surface area to initiate gene transfer. Although target cells covered almost all the area inside and outside the array, transduction was constrained mainly onto the virus-containing fibrin spots (Fig. 7C). Specifically, the fluorescence intensity measured outside of the spots was $8.8 \pm 1.3\%$ ($n=15$) of the total fluorescence intensity over the whole array surface. In addition, cross-contamination (i.e. the presence of DsRed+ cells in spots containing ZsGreen encoding virus and vice versa) was less than 0.01% (fewer than 5 cells among $\sim 5 \times 10^4$ cells seeded on the spots). These results demonstrate that fibrin can be used to achieve spatially controlled lentivirus gene transfer, thereby enabling engineering of cell transduction microarrays for high-throughput gene transfer experiments.

DISCUSSION

Fibrin hydrogels have been used extensively in tissue engineering and regenerative medicine for a variety of applications ranging from wound healing [37–39] to engineering blood vessels [40–43] and neovascularization [44]. In addition to cell delivery, fibrin has been used for protein and gene delivery [6,44–49]. Here we demonstrate that fibrin can be used for delivery of recombinant lentivirus in vitro in an efficient and spatially-controlled manner.

Our results indicated that fibrin-based lentivirus transduction depended strongly on fibrinogen concentration. Gene transfer efficiency decreased with increasing FBG concentration because denser hydrogels were more difficult for target cells to degrade and uptake the entrapped virus before losing its biological activity. Indeed, inhibition of fibrin degradation by fibrinolytic inhibitors acting through distinct mechanisms, namely aprotinin or ϵ -aminocaproic acid, decreased gene transfer in a dose-dependent manner. Aprotinin prohibits fibrin degradation by forming an enzyme-inhibitor complex with plasmin, while ϵ -aminocaproic acid binds reversibly to plasminogen, preventing its activation and transformation to plasmin [50]. In addition, plasminogen - the precursor of plasmin –enhanced transduction efficiency (supplementary material, Fig. S1). Taken together, our data suggested fibrin matrix degradation by target cells is necessary for lentivirus transduction.

In some cases, for example upon treatment with eACA, changes in transduction efficiency were not reflected in transgene expression (Fig. 5). This result is likely due to the small number of integrated transgene copies in NIH-3T3 as opposed to 293T cells - most likely one provirus per cell, as reflected in the low levels of fluorescence intensity. The presence of eACA results in decreased fraction of transduced cells but the ones that remain transduced show the same level of fluorescence intensity because they carry the minimum number of transgene copies (any less and they would be non-transduced). On the other hand, more easily transducible cells, such as 293T cells may contain multiple integrated copies of the transgene. Therefore, increasing eACA decreases the number of transgene copies per cell as manifested in decreased fluorescence intensity values as well as the fraction of transduced cells.

Notably, fibrin-mediated transduction efficiency and transgene expression increased with increasing target cell density, while the opposite is usually true in TTP. This is an intriguing observation that can be explained by considering the rate-limiting steps in each mode of gene transfer. It has been shown that TTP is limited by slow diffusion and decay of viral particles, thus limiting the number of active virus reaching the target cells [51–52]. On the other hand, in fibrin-mediated gene delivery target cells must degrade the gels in order to encounter the entrapped viral particles. It is also possible that hydrogel degradation allows previously

entrapped viral particles to diffuse more freely and thus experience more virus-cell encounters. Either way this mode of delivery is limited by rate of matrix degradation, suggesting that the higher transduction efficiency at high cell density may be the result of faster fibrinolysis. Alternatively, adherence to fibrin gels may affect cell cycle kinetics or other aspects of cellular physiology ultimately affecting lentivirus gene transfer in ways that are not currently understood.

In addition to degradation, FBG concentration determines the porosity of fibrin hydrogels thereby affecting diffusion of viral particles from the matrix. Low FBG concentration yields gels with fewer fiber bundles and more porous fibrin network [32], facilitating diffusion of viral particles out of the matrix. Under these conditions, transduction of target cells may occur by lentiviral particles that have escaped in the medium similar to traditional transduction protocol, alleviating the need for fibrin degradation. Indeed, neither aprotinin nor plasminogen had any effect on gene transfer when FBG concentration was 1.5 mg/ml or less (Fig. 4 and Fig. S1). For the same reason, ϵ ACA did not result in consistent decrease of gene transfer with gels containing 1.5 mg/ml FBG (Fig. 5). Collectively, our results suggested that an optimum FBG concentration in the range of 3.75–7.5 mg/ml minimizes virus diffusion without preventing fibrinolysis thereby yielding high gene transfer efficiency to target cells.

Our data also showed that polybrene increased fibrin-mediated transduction efficiency and transgene expression. In TTP, electrostatic repulsion dominates over thermal forces, preventing the virus from coming in close proximity to the target cell and bind to its receptor. In this case, polybrene is effective in promoting gene delivery by enhancing virus adsorption to the target cell surface [35]. The strong dependence of fibrin-mediated gene transfer on polybrene suggests that electrostatic repulsion may be important in this setting as well, necessitating the presence of polycations to enhance the success rate of virus-cell encounters. Our study cannot exclude the possibility that polybrene may act by a different mechanism when combined with fibrin gels, however evidence in this direction is currently lacking.

A potential limitation of fibrin-mediated lentivirus delivery is that this approach may not be applicable towards cells that lack the fibri(nogen) receptor, $\alpha v \beta 3$. In fact A431 cells that do not express $\alpha v \beta 3$ displayed impaired binding to fibrin [53], resulting in a low level of gene transfer. In this case, adsorption of fibronectin on the gel surface enhanced gene transfer (Fig. S2), possibly by promoting cell adhesion and survival. Therefore, addition of ECM molecules on the surface of fibrin gels can be used to generalize this approach for cell types that are not compatible with the fibrin matrix.

Since fibrin hydrogels can effectively entrap lentivirus and mediate high levels of gene transfer, we hypothesized that fibrin may be a promising substrate for spatially controlled gene delivery. For example, implantation of lentivirus-containing fibrin gels in vivo may allow gene modification to tissue cells that are capable of migrating into the matrix thereby minimizing non-specific gene delivery to surrounding tissues or to the systemic circulation. Alternatively, fibrin hydrogels can be used to create lentivirus microarrays for high throughput gene transfer studies. In contrast to the current surface immobilization techniques for making cell transduction arrays [27–28], the 3-dimensional fibrin matrix can incorporate more lentivirus per unit area, thereby increasing the probability of transduction and consequently the signal-to-noise ratio for each spot. In conjunction with nano-printing technology for automated surface deposition, fibrin-based transduction can become an effective platform for generating transduction microarrays for high-throughput gene transfer studies to primary cells and stem cells.

CONCLUSIONS

The present study added to our understanding of matrix-mediated gene transfer and identified conditions of optimal gene delivery in a cell-controlled manner. Fibrin hydrogels provided a bioactive matrix for lentivirus presentation in a way that depended on fibrin gel degradation by the target cells. Appropriate fibrinogen concentration prevented lentivirus diffusion through the pores of the hydrogel thereby necessitating fibrin degradation for successful gene transfer. Most important, this mode of cell-controlled gene delivery enabled lentivirus transduction in a spatially-controlled manner with potential applications in engineering lentiviral microarrays to facilitate high-throughput gene transfer studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Gene transfer on fibrin gels is dependent on the fibrinogen concentration
 293T cells (5×10^4 and 2.5×10^5 cell/cm²) were transduced by embedding lentivirus in fibrin gels formed with the indicated fibrinogen concentrations. Cells transduced with traditional transduction protocol (TTP) served as control. (A) The fraction of transduced cells and (B) the mean fluorescence intensity were measured by flow cytometry after the entire gel was degraded. All values are the mean $\pm$ standard deviation of quadruplicate samples in a representative experiment (n=3). The asterisks (*) and pounds (#) indicate significant difference between the indicated samples and TTP at the respective cell density (p<0.05).

Figure 2. Gene transfer to NIH-3T3 cells is strongly dependent on fibrinogen concentration NIH-3T3 cells (2.5×10^4 and 1×10^5 cell/cm²) were transduced by embedding virus in fibrin gels formed from the indicated fibrinogen concentrations. Cells transduced with the traditional transduction protocol (TTP) served as control. (A) % of transduced cells and (B) the level of gene expression were determined by flow cytometry after the hydrogels were completely degraded. All values are the mean $\pm$ standard deviation of quadruplicate samples in a representative experiment (n=3). The asterisks (*) and pounds (#) indicate significant difference between the indicated samples and TTP at the respective cell density (p<0.05).

Figure 3. Lentivirus diffusion from hydrogels is dependent on fibrinogen concentration

Virus-containing fibrin gels were prepared with fibrinogen at the indicated concentrations and overlaid with PBS. At the indicated time points, the supernatant was collected and gels were overlaid with fresh PBS. **(A)** Viral activity in the supernatant was determined by transducing 293T cells (1×10^5 cell/cm²) using the traditional transduction protocol. **(B)** At the conclusion of the experiment, the remaining gels were completely hydrolyzed using plasmin (0.1–0.2 U/ml) and remaining viral activity was determined as in (A). All values are the mean $\pm$ standard deviation of triplicate samples in a representative experiment (n=3).

Figure 4. Aprotinin reduces fibrin-mediated gene transfer in a dose-dependent manner

Lentivirus-containing fibrin gels were prepared with 1.5 mg/ml or 3.75 mg/ml fibrinogen and aprotinin at the indicated concentrations. After polymerization, 293T (1×10^5 cell/cm²) or NIH-3T3 (2.5×10^4 cell/cm²) were added over the gels to initiate gene transfer. The fraction of transduced cells (**A, C**) and transgene expression (**B, D**) of 293T and NIH-3T3 cells were plotted as a function of aprotinin concentration. All values are the mean $\pm$ standard deviation of quadruplicate samples in a representative experiment (n=3). The asterisks (*) and pounds (#) indicate significant difference from the samples without aprotinin for gels prepared with 1.5 and 3.75 mg/ml fibrinogen, respectively (p<0.05).

Figure 5. eACA reduces fibrin-mediated gene transfer in a dose-dependent manner

Lentivirus-containing fibrin gels were prepared with 1.5 mg/ml or 3.75 mg/ml fibrinogen and eACA at the indicated concentrations. After polymerization, 293T (1×10^5 cell/cm²) or NIH-3T3 (2.5×10^4 cell/cm²) were added over the gels to initiate gene transfer. The fraction of transduced cells (**A, C**) and transgene expression (**B, D**) of 293T and NIH-3T3 cells were plotted as a function of eACA concentration. All values are the mean $\pm$ standard deviation of quadruplicate samples in a representative experiment (n=2). The asterisks (*) and pounds (#) indicate significant difference from the samples without eACA for gels prepared with 1.5 and 3.75 mg/ml FBG, respectively (p<0.05).

Figure 6. Polybrene enhances lentivirus gene transfer on fibrin hydrogels

Lentivirus-containing fibrin gels were prepared in the presence or absence of polybrene (8 μ g/ml). Following polymerization, NIH-3T3 cells at 2.5×10^4 cell/cm² or 1×10^5 cell/cm² were seeded on the hydrogels to initiate gene transfer. Cells transduced with the traditional transduction protocol (TTP) served as control. **(A)** Transduction efficiency and **(B)** transgene expression were determined by flow cytometry after gel degradation. All values are the mean $\pm$ standard deviation of quadruplicate samples in a representative experiment (n=3).

Figure 7C

Figure 7. Transduction arrays using fibrin-entrapped lentivirus

Lentivirus-containing fibrin arrays were created by first spotting virus-containing thrombin on the surface. Subsequently, fibrinogen was added on top of the thrombin spots to form hydrogels. (A) A representative 5×5 array is shown. The inset shows fibrin gel spot at higher magnification (scale bar = $200 \mu\text{m}$). (B) Fibrin spots were made with the indicated volumes of FBG (5 or 10 mg/ml). The spots were photographed and the area (A) of each spot was

determined using the Image J software. The spot diameter was calculated as $D=\sqrt{(4A/p)}$ and plotted as the mean $\pm$ standard deviation of quadruplicate samples (n=4). (C) A 5×5 array of fibrin spots containing alternating columns of ZsGreen- or DsRed-encoding lentivirus and 293T cells were added at 2.5×10^5 cells per cm^2 . Gene transfer on the array was visualized with a Zeiss Observer.Z1 fluorescence microscope and the images were merged using the AxioVision software.