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Biomaterial-mediated retroviral gene transfer using self-assembled monolayers

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Abstract

Biomaterial-mediated gene delivery has recently emerged as a promising alternative to conventional gene transfer technologies that focus on direct delivery of viral vectors or DNA-polymer/matrix complexes. However, biomaterial-based strategies have primarily targeted transient gene expression vehicles, including plasmid DNA and adenovirus particles. This study expands on this work by characterizing biomaterial properties conducive to the surface immobilization of retroviral particles and subsequent transduction of mammalian cells at the cell-material interface. Self-assembled monolayers (SAMs) of functionally-terminated alkanethiols on gold were used to establish biomaterial surfaces of defined chemical composition. Gene transfer was observed to be greater than 90% on NH_2-terminated surfaces, approximately 50% on COOH-functionalized surfaces, and undetectable on CH_3-terminated SAMs, similar to controls of tissue culture-treated polystyrene. Gene delivery via the NH_2-SAM was further characterized as a function of retrovirus coating time, virus concentration, and cell seeding density. Finally, SAM-mediated gene delivery was comparable to fibronectin- and poly-L-lysine-based methods for gene transfer. This work is significant to establishing safe and effective gene therapy strategies, developing efficient methods for gene delivery, and supporting recent progress in the field of biomaterial-mediated gene transfer.

Keywords: Fibroblast; Genetic engineering; Gene therapy; Gene transfer; Self-assembly; Alkanethiol

1. Introduction

Genetic engineering of mammalian cells is central to numerous strategies for disease treatment, tissue regeneration, and the study of protein function and cellular processes [1–3]. Conventional methods for gene delivery include both *in vitro* and *in vivo* gene transfer based on viral vectors or DNA-polymer/matrix complexes. *In vitro* gene transfer typically involves the direct transfection or transduction of cultured cells that are subsequently analyzed for transgene activity and/or implanted for a therapeutic purpose. *In vivo* gene therapy involves the direct application of the gene carrier to the injured or diseased tissue. However, these approaches are often limited by inefficient transgene delivery and poor specificity. Recent attempts to overcome these limitations have focused on biomaterial-mediated gene delivery, wherein the gene carrier is immobilized to, or encapsulated within, a biomaterial support [1–3]. The hybrid gene-activated biomaterial is then seeded with cells *in vitro* or implanted. By co-localizing the gene delivery vehicle and cell adhesion, this method enhances gene transfer and specifically targets cells at the biomaterial interface, thereby reducing the risks associated with direct injection of the gene carrier. Therefore, this approach provides several advantages over conventional gene delivery modalities, including reduced cytotoxicity and immunogenicity of freely diffusible gene carriers, limited ectopic transgene expression in

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neighboring tissues, improved stability of the gene carrier, and controlled levels of gene transfer and expression.

Strategies for the integration of biomaterials and gene delivery have targeted both viral and plasmid DNA-based gene carriers. Fang et al. originally developed this method in the form of a gene-activated matrix (GAM) in which plasmid DNA was loaded onto collagen sponges prior to implantation into segmental bone defects [4]. Plasmid DNA was later incorporated into poly(lactide-*co*-glycolide) (PLG) scaffolds with tunable degradation properties, allowing for sustained release of DNA from the scaffold for up to 1 month [5]. Subsequently, numerous studies have evaluated strategies for controlled release of plasmid DNA from polymeric scaffolds or immobilization of DNA at biomaterial surfaces [1]. Methods for plasmid release have primarily focused on DNA incorporation into biodegradable polymers, including collagen [4,6], hyaluronan [7,8], PLG [5,9], poly(ethylene glycol) [10], and ethyl vinyl-co-acetate [11]. Immobilization approaches typically utilize the interaction of DNA with cationic agents, including poly-L-lysine [12], polyethylenimine [13–15], chitosan [16], or dendrimers such as polyamidoamine [17]. More recently, efforts have focused on incorporating the high gene transfer efficiencies of viral vectors into biomaterial-based gene delivery. For example, adenovirus particles have been suspended in hydrogel microspheres of fibrin [18], collagen [18], alginate [19], and poly(lactic-*co*-glycolic acid (PLGA) [20,21] and implanted for controlled delivery *in vivo*. Additionally, Schwarz and colleagues have developed a technique for freeze-drying recombinant adeno-associated virus particles onto implants to enhance tissue repair [22].

Although many strategies exist for biomaterial-mediated gene therapy with plasmid DNA or adenoviral particles, both of these methods generate only transient transgene expression. There are many examples of biomedical applications that require prolonged or sustained expression of the transgene. Therefore, an unfulfilled need exists for methods that immobilize or release gene carriers that permanently modify the cellular DNA, such as retroviruses and lentiviruses. In contrast to adeno- and adeno-associated viruses, these particles consist of an unstable lipid bilayer and RNA genome that present unique challenges for incorporation into biomaterials. Retrovirus immobilization to human plasma fibronectin or recombinant fibronectin fragments has been widely utilized to enhance gene delivery to several cell types [23–25]. However, fibronectin is an intrinsically bioreactive molecule that directs confounding effects on cell differentiation and proliferation [26–28]. Cationic and anionic polymers have been used to form large virus-polymer complexes and increase the rate of virus sedimentation onto cultured cells [29–31]. Building on these results, other studies have analyzed the effects of cationic surfaces or molecules to regulate the immobilization and delivery of negatively-charged retrovirus particles [32–34]. The present study expands on this work by evaluating the effects of surface chemistry on biomaterial-mediated gene delivery.

Self-assembled monolayers (SAMs) represent a robust, controlled, and stable biomaterial for analyzing the effects of surface chemistry on biological phenomena [35]. SAMs have been used extensively to study and control protein adsorption and cell adhesion and function *in vitro* and *in vivo*. More recently, plasmid DNA has been immobilized onto SAMs for biomaterial-directed gene delivery [36–38]. In the present work, we demonstrate SAM chemistry-dependent effects on retrovirus immobilization and gene delivery.

2. Materials and methods

2.1. Cell culture

NIH3T3 murine fibroblasts (CRL-1658, American Type Culture Collection, Manassas, VA) were cultured in DMEM, 10% fetal bovine serum, 100 U/ml penicillin G sodium, and 100 μg/ml streptomycin sulfate in a humidified 5% CO_2 atmosphere at 37 °C. Cell culture media and antibiotics were obtained from Invitrogen (Carlsbad, CA), fetal bovine serum was purchased from Hyclone (Logan, UT), and all other cell culture supplements and reagents were acquired from Sigma (St. Louis, MO).

2.2. Self-assembled monolayers

Alkanethiols 1-dodecanethiol (HS-$(CH_2)_{11}$-CH_3) and 11-mercaptoundecanoic acid (HS-$(CH_2)_{10}$-COOH) were purchased from Aldrich Chemical (Milwaukee, WI) and used as received. The amine-terminated alkanethiol 12-amino-1-mercaptododecane (HS-$(CH_2)_{12}$-NH_2) was synthesized and characterized by our group [39]. SAMs of their respective alkanethiols are referred to hereafter as CH_3, COOH, and NH_2. Gold-coated substrates (35 mm tissue culture-treated polystyrene dishes) were prepared by sequential deposition of optically transparent films of 75 Å Ti and 150 Å Au using an electron beam evaporator (Thermionics, Hayward, CA) at a deposition rate of 2 Å/s and a chamber base pressure of approximately 2×10^{-6} Torr.

For SAM assembly, freshly prepared Au-coated substrates were immersed overnight in alkanethiol solutions (1.0 mM in absolute ethanol). SAMs were validated by contact angle goniometry and X-ray photoelectron spectroscopy [39]. Following assembly, SAMs were rinsed with 95% ethanol and equilibrated in PBS for 10 min before addition of viral supernatants or matrix molecules. To study the effects of extracellular matrix molecules on retrovirus immobilization, SAMs were incubated with Pronectin (Sigma), bovine dermal type I collagen (Cohesion, Palo Alto, CA), human plasma fibronectin, or poly-L-lysine (MW: 70–150 kDa) at 20 μg/ml in PBS for 1 h. Matrix-coated SAMs were washed twice in PBS prior to exposure to viral supernatant and cell seeding.

2.3. Retrovirus production

Retroviral supernatant was produced with the pTJ66 vector from a stable ΦNX amphotropic producer cell line. The pTJ66 retroviral vector uses the promoter activity of the 5′ long terminal repeat (LTR) followed by an internal ribosomal entry site (IRES) to express a zeocin resistance-enhanced green fluorescent protein fusion protein (Zeo(r):eGFP), allowing for noninvasive analysis of transduction efficiency [40]. Plasmid DNA was purified from transformed *E. coli* using Megaprep kits from Qiagen (Valencia, CA). Helper-virus free ΦNX amphotropic producer cells were transiently transfected with plasmid DNA as previously described [41]. Four days following transfection, ΦNX cells were cultured in growth media (DMEM, 10% fetal bovine serum, 100 U/ml penicillin G sodium, 100 μg/ml streptomycin sulfate) supplemented with 200 μg/ml zeocin. After 2 weeks of antibiotic selection, individual zeocin-resistant colonies were isolated and characterized for production of pTJ66 retrovirus.