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Surface polyethylene glycol enhances substrate-mediated gene delivery by nonspecifically immobilized complexes

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Abstract

Substrate-mediated gene delivery describes the immobilization of gene therapy vectors to a biomaterial, which enhances gene transfer by exposing adhered cells to elevated DNA concentrations within the local microenvironment. Surface chemistry has been shown to affect transfection by nonspecifically immobilized complexes using self-assembled monolayers (SAMs) of alkanethiols on gold. In this report, SAMs were again used to provide a controlled surface to investigate whether the presence of oligo(ethylene glycol) (EG) groups in a SAM could affect complex morphology and enhance transfection. EG groups were included at percentages that did not affect cell adhesion. Nonspecific complex immobilization to SAMs containing combinations of EG- and carboxylic acid-terminated alkanethiols resulted in substantially greater transfection than surfaces containing no EG groups or SAMs composed of EG groups combined with other functional groups. Enhancement in transfection levels could not be attributed to complex binding densities or release profiles. Atomic force microscopy imaging of immobilized complexes revealed that EG groups within SAMs affected complex size and appearance and could indicate the ability of these surfaces to preserve complex morphology upon binding. The ability to control the morphology of the immobilized complexes and influence transfection levels through surface chemistry could be translated to scaffolds for gene delivery in tissue engineering and diagnostic applications.

Keywords: Gene delivery; Reverse transfection; Atomic force microscopy (AFM); Self-assembled monolayers

1. Introduction

Developing systems capable of controlled and efficient gene transfer is a fundamental goal of biotechnology, with applications including functional genomics, gene therapy and tissue engineering. The primary challenge in applying gene delivery to these applications is inefficient delivery, with extracellular and intracellular barriers both limiting the efficiency. In non-viral approaches, plasmid is complexed with cationic lipids or polymers to facilitate transfection in vitro and in vivo [1–3]. Complexation can enhance interactions between positively charged DNA complexes and the negatively charged cellular membrane, in addition to providing stability against degradation [4] and facilitating intracellular trafficking.

Controlled release systems for DNA delivery have the potential to overcome extracellular barriers that limit gene transfer and enhance gene delivery relative to more traditional delivery methods [5]. These systems include delivery through polymeric release, in which the DNA is released from a polymer scaffold, or substrate-mediated delivery, in which DNA is retained at the surface of a substrate. Substrate-mediated delivery, also termed solid phase delivery or reverse transfection, involves the immobilization of DNA, complexed with non-viral vectors, to a biomaterial or substrate that supports cell adhesion [6]. Cells cultured on the substrate are exposed to elevated DNA concentra-

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tions within the local microenvironment, which enhances transfection.

DNA complexes can be immobilized on the substrate through specific or nonspecific interactions for delivery from the surface. Specific interactions can be introduced through complementary functional groups on the vector and surface, such as antigen–antibody or biotin–avidin [7–9]. Poly(L-lysine) (PLL) and polyethylenimine (PEI), modified with biotin residues, were complexed with DNA and bound to a neutravidin substrate [7,8], resulting in 100-fold increased transgene expression from the immobilized complexes relative to bolus delivery of complexes [7]. Plasmid DNA or DNA complexed with cationic polymers or lipids can also interact with substrates through nonspecific mechanisms [10–18]. Polyplexes and lipoplexes nonspecifically immobilized to substrates enhanced the extent of transgene expression in both cell lines and primary human-derived cells, along with an increased cellular viability [13]. This enhancement was dependent on the properties of both the complex (e.g. complexation agent, nitrogen to phosphate (N/P) ratio) and the substrate.

Surface chemistry has been shown to affect substrate-mediated delivery of nonspecifically immobilized complexes, impacting both the initial binding and also subsequent transfection. Self-assembled monolayers (SAMs) of alkanethiols on gold were used to provide a flexible system for regulating surface chemistry [19–21] to examine complex–substrate interactions. DNA, complexed with cationic lipids, was immobilized through nonspecific mechanisms to SAMs presenting combinations of hydrophilic and charged (COO^-), hydrophilic and uncharged (OH), and hydrophobic terminal functional groups (CH_3) [15]. Surface hydrophilicity and ionization were found to mediate both DNA complex immobilization and transfection, but had no effect on complex release. The greatest amounts of binding and transfection were observed on surfaces presenting charged, hydrophilic groups, suggesting that electrostatic interactions allow for reversible interactions between the substrate and complexes and result in efficient gene delivery [22]. Hydrophobic substrates bound similar quantities of DNA as the hydrophilic surfaces, yet transfection was significantly reduced, suggesting the conformation of the DNA complexes may be altered upon binding to hydrophobic surfaces and result in low transfection levels [13,15].

However, as in traditional gene delivery approaches, further improvements are still needed for substrate-mediated gene delivery to address issues that limit gene transfer, including complex size stability, complex aggregation and strong interactions between the surface and complexes [9,13]. Polyethylene glycol (PEG), which has the monomeric repeat unit [–CH_2–CH_2–O]–, is widely used in drug and gene delivery and has been incorporated into DNA complexes of several cationic polymers, including polymethacrylate [23], PEI [24–32], PLL [33–35] and poly(amidoamine)s [36]. PEG reduces the surface charge of the complexes [25,26,31,34], which in turn reduces cytotoxicity [25,27,31]. The shielding effect of PEG also reduces the interaction between the complex and blood components (plasma proteins and erythrocytes) [25], and can prolong circulation of the complexes in the blood stream [25,32]. Furthermore, polyethylene glycosylation (PEGylation) can prevent salt-induced aggregation through steric stabilization [25,26,29–31,33,34]. Additionally, PEG is often used as a spacer for targeting ligands since the shielding effect of PEG is able to decrease nonspecific interactions with negatively charged cellular membranes, which results in reduction of nonspecific cellular uptake [37]. While some PEGylation strategies have had no effect on transfection efficiency in vitro [25,31,33] or in vivo [25], or even enhanced transfection [27,34], others have reported that PEGylation resulted in poor transfection [28,29,32], presumably due to interference with complexation [36]. These effects due to PEGylation have been associated with the extent of PEGylation, which may shield the surface charge [24,26], thus reducing cell binding and transfection, or alternatively, induce membrane leakage, resulting in enhanced cytoplasmic release [33,34].

In this report, SAMs of alkanethiols on gold were used to investigate substrate-mediated transfection by nonspecifically immobilized complexes on surfaces containing varying densities of PEG functional groups. We hypothesize that, rather than attach PEG to the complexes directly, its presence in a SAM could enhance substrate-mediated transfection by conveying the desired properties of PEG on gene delivery (reduced complex aggregation, and complex size stability), and promote interactions between the complexes and cell membrane. SAMs presenting oligo(ethylene glycol) (EG) groups [38,39] have previously been used to resist protein adsorption according to the length of the EG chain and its percent composition within the monolayer [39,40], and are used here to modulate DNA complex adsorption for substrate-mediated gene delivery. In our studies, EG-terminated alkanethiols were incorporated into SAMs at concentrations that do not limit cell adhesion and combinations of EG- and COO^--terminated alkanethiols were examined for their ability to bind and release complexes and to subsequently support transfection. Complex morphology, a factor in gene delivery, was examined by atomic force microscopy (AFM) on these surfaces. The correlation between surface chemistry and morphology of immobilized complexes must be a design consideration for translating substrate-mediated gene delivery to biotechnology applications.

2. Material and methods

2.1. Gold slide preparation and monolayer self-assembly

Gold-coated glass slides were prepared using e-beam evaporation (Edwards Electron Beam Evaporator, Wilmington, MA), and consisted of a 5 nm titanium adhesion layer and 50 nm of gold. A diamond-tipped glass cutter was used to cut the gold-coated slides into smaller pieces that fit into standard 48-well tissue culture plates. Gold

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