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Multilayer mediated forward and patterned siRNA transfection using linear-PEI at extended N/P ratios

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Abstract

Gene delivery from a substrate depends, in part, on the vector–nucleic acid complex that is bound to the surface and the cell adhesive properties of the surface. Here, we present a method to deliver patterns of small interfering RNA (siRNA) that capitalize on a forward transfection method (transfection by introducing siRNA transfection reagent complexes onto plated cells); herein denoted as multilayer mediated forward transfection (MFT). This method separates the substrate-mediated delivery from the cell adhesive properties of the surface. pH responsive layer-by-layer (LbL) assembled multilayers were used as the delivery platform and microcontact printing technique (μCP) was used to pattern nanoparticles of transfection reagent–siRNA complexes onto degradable multilayers. Efficient MFT depend on optimal formulation of the nanoparticles. 25 kDa linear polyethylenimine (LPEI) was optimized as the siRNA transfection reagent for normal forward transfection (NFT) of the nanoparticles. A broad range of LPEI–siRNA nitrogen/phosphate (N/P) ratios (ranging from 5 to 90) was evaluated for the relative amounts of siRNA incorporated into the nanoparticles, nanoparticle size and NFT efficiencies. All the siRNA was incorporated into the nanoparticles at N/P ratio near 90. Increasing the amount of siRNA incorporated into the nanoparticles, with increasing N/P ratio correlated with a linear blue shift in the ultraviolet/visible (UV/vis) absorbance spectrum of the LPEI–siRNA nanoparticles. NFT efficiency greater than 80% was achieved with minimal cytotoxicity at N/P ratio of 30 and siRNA concentration of 200 nM. Similarly, MFT efficiency $\geqslant$60% was achieved for LPEI–siRNA nanoparticles at N/P ratios greater than 30.

Keywords: siRNA transfection; Patterned transfection; Layer-by-layer (LbL) assembly; LPEI–siRNA nanoparticles; N/P ratio

1. Introduction

RNA interference (RNAi) is a sequence-specific post-transcriptional gene silencing process triggered through small interfering RNAs (siRNAs) [1,2] which serves as a powerful therapeutic tool [3,4] in gene therapy. An important aspect of gene therapy for regenerative medicine and organized tissue formation is to manipulate the location of transfected cells, requiring the generation of gene expression patterns in spatially controlled environments [5,6]. Patterned delivery of DNA has been demonstrated with cells seeded onto modified surfaces, where vector–DNA complexes were immobilized onto chemically modified surfaces, including self-assembled monolayers (SAMs), using different patterning techniques [6–8]. Delivery of patterned siRNA from a substrate to adherent cells for high-throughput functional genetic analysis has been demonstrated with reverse transfection-based RNAi microarrays [9,10]. Reverse transfection plates the cells at the time of transfection [10], whereas forward transfection plates the cells to allow them to first attach and grow, prior to transfection. Reverse transfection-based approaches for gene delivery or RNAi microarrays requires that the cells must be able

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to adhere to the surface containing the expression vector, or the substrates must be chemically modified to immobilize the non-adherent cell lines [11]. Gene delivery from a substrate depends, in part, on the vector–nucleic acid complex that is bound to the surface [6]. Various parameters such as surface charge, hydrophobicity/hydrophilicity [8], rigidity of the cell adhesion substrates [12] all contribute to the molecular interactions between the vector and the polymer on the surface. Any chemical modification of the substrates that may enhance cell adhesion could adversely affect the release of the vector–nucleic acid complexes from the surface and thus interfere with cellular internalization of the polymer–nucleic acid complexes [6], and efficient gene delivery. The present study describes a method for forward transfection of siRNA, yielding micron-sized patterns of transfected mammalian cells. With this method, the cells are cultured separately from a degradable LbL assembled multilayer arrayed with the siRNA, thereby separating the two issues, the complex release *from*, and the cell adhesion *on* the substrate.

The layer-by-layer (LbL) assembly method introduced by Decher and co-workers [13,14] for multilayer thin film formation is an attractive approach for controlled release of biomolecules from surfaces [15,16]. LbL thin films provide flexibility in terms of their choice of substrate and constituent components, surface patterning techniques, fabrication conditions, and tunable structural properties [16]. Other advantages include their ease of preparation and cost-effectiveness. Different patterning techniques can be employed to conjugate biomolecules, such as nucleic acids, to multilayer structures. Soft-lithographic microcontact printing (μCP) [17,18] is one such technique, which has emerged as a platform of choice for biochips and drug delivery applications [19]. Various "inks", including, proteins, DNA, RNA, and polyelectrolytes have been used in μCP to pattern surfaces without the need for dust-free environments and harsh chemical treatments [20].

A previous method of *in vitro* localized transfection of cultured cells from multilayer thin films did not involve patterned delivery of DNA from these films [21]. Nonetheless, LbL thin film application of reverse transfection of DNA to form cell microarrays have been previously demonstrated [22], but the method is not easily applied to siRNA due to the enhanced susceptibility of siRNAs to degradation as compared to DNAs [3,23]. Approaches to embed the polymer and nucleic acid alternatively to form LbL films [24] or embed the polymer–nucleic acid complexes within the multilayers [25] have not involved patterned delivery. Thus, spatially controlled delivery of siRNA based on thin film chemistries has yet to be realized.

Here, we describe the application of a LbL assembled degradable multilayer film for patterned delivery of siRNA using a forward transfection approach. The transfection process involved the following steps (Fig. 1). (1) pH controlled, biocompatible, and degradable multilayers were fabricated using LbL assembly under acidic conditions [26]. (2) Nanoparticles of vector–siRNA complexes pre-

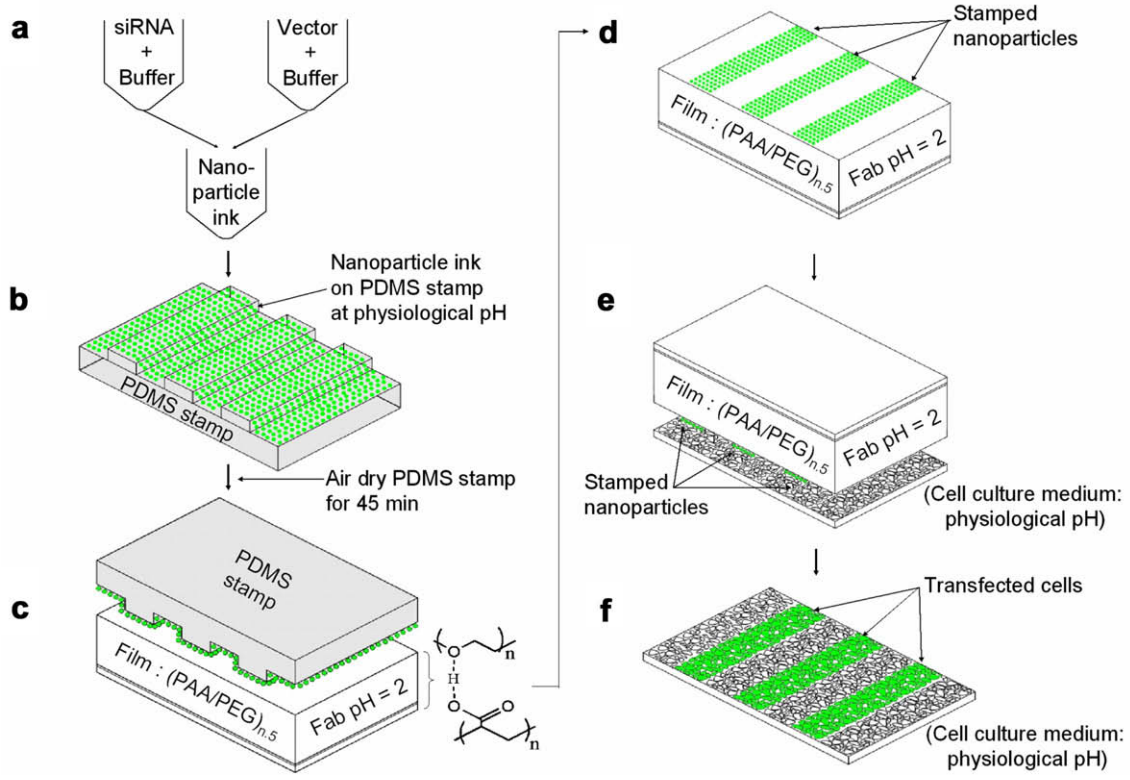

Fig. 1. Diagram illustrating the multilayer mediated forward transfection (MFT) of cationic vector complexed siRNA for patterned delivery.

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