Layer-by-Layer Delivery of siRNA

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INTRODUCTION

Layer-by-layer (LBL) assembly of polyelectrolyte thin films has been used for a wide range of applications due to its simplicity and robustness. The construction of LBL films for the release of many types of therapeutics has been investigated previously, including small molecule drugs, growth factors, and DNA, but there have not been any studies to date to show the successful delivery of siRNA from these assemblies. This work is focused on the delivery of siRNA from LBL assemblies for local applications.

EXPERIMENTAL

Materials. All siRNA was received as a part of the funding of this research from Sanofi-Aventis. Linear poly(ethyleneimine) (LPEI, MW = 25 kDa) and dextran sulfate (DS, MW = 500 kDa) were obtained from Polysciences. chitosan (LMWC, MW = 15 kDa) was obtained from Sigma-Aldrich. Polymer 2 (Poly2, MW = 10 kDa) was synthesized as previously described. Phosphate buffered saline solution (PBS, 10x) and Lipofectamine 2000™ were obtained from Invitrogen.

Film Assembly. All films were assembled on silicon wafers (Silicon Quest International) with 10 baselayers of (LPEI/DS). Assembly of all films was carried out in 0.1 M sodium acetate solution at pH 5.0. Polymer solutions used were made at a 2 mg/mL concentration and siRNA solutions used were at a 20 µg/mL concentration. Film thickness was measured using both ellipsometry (XLS-100 Spectroscopic Ellipsometer J.A. Woollam Co., Inc) and profilometry (Dektak 150 Profilometer).

Degradation Studies and Release Characterization. Film degradation was carried out in PBS at pH 7.4 at 37°C. Release of siRNA from films was quantified with both UV-Vis absorption, using a Cary 6000i UV-Vis-NIR Spectrophotometer, and Quant-iT™ PicoGreen dsDNA Assay.

Characterization of in Vitro Knockdown. The functionality of the siRNA released from the LBL films was assessed through FACS analysis of GFP knockdown in NIH-3T3s constitutively expressing GFP (purchased from Cell Biolabs, San Diego, CA). Flow cytometry analysis was performed using a BD FACSCalibur flow cytometer.

RESULTS

As siRNA has a strong negative charge associated with its phosphate backbone, initial work focused on the determination of an appropriate polycation pairing for optimum siRNA incorporation within the LBL film assembly. Many polycations were investigated for this purpose including multiple molecular weights of LPEI, branched PEI, Poly(allylamine hydrochloride), chitosan, poly(L-lysine), and protamine sulfate (data not shown). Of the polycations investigated LPEI (MW 25 kDa) and Chitosan (MW 15 kDa) were found to have the most favorable interaction with siRNA.

Film Release Studies. Studies were done over a period of 10 days, during which films were kept hydrated in PBS and release was measured daily. Film thickness varied greatly between the different architectures, though this thickness did not correlate with siRNA incorporation within the film. The release characterization of the films featured above was determined by taking daily measurements of the eluent from the films. These films had very different release characteristics, varying from immediate bolus release to sustained release profiles. Figure 1 shows the release profile for the $(Chitosan/siRNA)_{50}$ film. This film showed a bolus release of approximately 25% of its loaded siRNA immediately upon exposure to the release media. It also had prolonged release over a period of 1 week that was nearly uniform over that period.

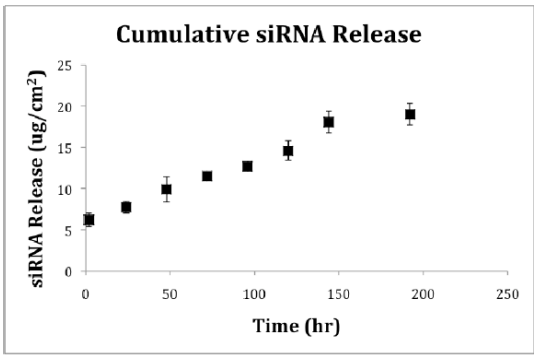

Figure 1. Cumulative release of siRNA from the $(chitosan/siRNA)_{50}$ bilayer film on degradable baselayers.

Functionality assessment of siRNA released from the films showed insignificant knockdown of GFP expression. However, when film eluent was combined with Lipofectamine 2000™ knockdown was significantly improved, suggesting that the siRNA released from the films into PBS was functional but unable to transfect cells effectively (data not shown).

DISCUSSION

This work has shown that through a broad-range approach to LBL film assembly a number of potential siRNA delivering films can be created. The films as tested were not able to generate significant knockdown of GFP in NIH-3T3s, but with the use of a commercially available transfection vector, the siRNA released was shown to be function and was able to create significant knockdown.

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