Layer-by-layer films containing plasmid DNA and reducible TAT polypeptide

Jenifer Blacklock[a,b], Guangzhao Mao[c], David Oupicky[a].
[a]Department of Pharmaceutical Sciences, [b]Department of Biomedical Engineering, [c]Department of Chemical Engineering and Materials Science, Wayne State University, Detroit, MI 48202, USA

Statement of Purpose: DNA-containing thin films hold much promise for localized gene delivery with applications in the field of implantable materials, biomedical devices, and tissue engineering. Degradable cationic polymers can be bioengineered to sustain the release of DNA for controlled periods of time while functioning in an efficient and timely manner. By incorporating disulfide bonds in the structure of the polycations, degradation of the thin films can be controlled by reducing conditions. The existing materials offer only a limited control over the release of DNA and generally lack the functional sophistication required to control both the spatial distribution and temporal release of DNA.

Methods: Plasmid DNA containing green fluorescent protein reporter gene (gWIZ™ GFP, 5757 bp) and gWiz™ High-Expression SEAP (gWiz™-SEAP) containing secreted alkaline phosphatase reporter gene were purchased from Aldevron (Fargo, ND, 3233 15th St S). The TAT-based polypeptide (PTAT), containing reducible disulfide bonds in the backbone, was synthesized by oxidative polycondensation of the CGRKKRRQRRRGC peptide as previously described [1]. Rat aortic smooth muscle cells (SMC) (CRL-2018) and embryonic mouse fibroblasts (NIH-3T3, CRL-1658) were purchased from ATCC (Manassas, MA, P.O. Box 1549) and used for transfection studies. Multilayers of [PEI/(PSS/PAH)$_8$] where initially deposited onto si-wafers or glass substrates by a layer-by-layer (LbL) assembly via a dip-coating technique. Following the initial layers, multilayers of DNA and polycation were deposited containing either a reducible cationic polypeptide (PTAT) (PTAT/DNA)$_n$ or a non-reducible cationic control (PLL) (PLL/DNA)$_n$. The film assembly and disassembly were monitored by contact angle goniometry, ellipsometry, and atomic force microscopy (AFM). Transfection studies were conducted implementing gfpDNA with PTAT and SEAP DNA with PTAT on glass substrates. Fluorescence microscopy, optical microscopy and luminescence were used to track cell transfection and migration upon cell attachment.

Results/Discussion: AFM and ellipsometric studies were performed in situ to evaluate the assembly and degradation of (PTAT/DNA)$_n$ thin films. (PTAT/DNA)$_n$ thickness was found to increase exponentially with the number of layers. The exponential growth is due to the rigidity of DNA as well as the increased presence of particulate complexes found after (PTAT/DNA)$_{8.5}$ and the mobility of PTAT molecules within the multilayers. Other film features consistent with the exponential film growth mechanism include surface roughness increase with increasing number of deposited layers ((PTAT/DNA)$_{1\text{-}8.5}$; 0.75-7.4 nm). AFM roughness studies showed that DNA layers have a higher roughness when compared to the PTAT layers (~2 nm). When compared to the control films containing PLL, the overall thickness of the films were larger for PTAT/DNA films (44 nm) than PLL/DNA films (40 nm). The hydrophobicity, evaluated by the contact angle measurement, showed a periodic variation during the LbL deposition. It was shown that PTAT-containing films disassemble readily in the reducing environment as well as in the presence of proteolytic enzymes. In contrast, control LbL films containing PLL were stable in the reducing conditions and exhibited slow degradation in the presence of proteases. This proves that reduction is an effective trigger for DNA release from the LbL films by reducing PTAT into TAT peptide residues with reduced affinity to the DNA. Upon cell attachment, transfection studies were performed for SMC and fibroblast by analyzing the green fluorescent protein and SEAP expressions. It was shown through fluorescence microscopy that both cell lines contained green cells after 5 days, proving that transfection occured. The luminometer provided quantitative results that SEAP expressions where apparent with both cell lines after 2 days and continued for 26 days. It was also shown that RLU expressions were significantly higher for (PTAT/DNA)$_{8.5}$ films than (PLL/DNA)$_{8.5}$ films (~120,000 RLU's; ~180,000 RLU's respectfully over 26 days).

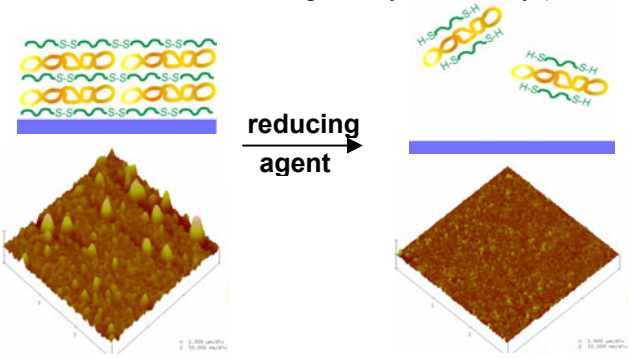

Figure 1. Assembly and disassembly of reducible PTAT/DNA films.

Conclusions: In summary the advantages of the flexibility, versatility, and tenability of the reducible LbL films in implants and tissue engineering, could offer additional benefits in controlled gene delivery since the film disassembly can be potentially triggered locally by the reducing microenvironment of cellular plasma membrane, increasing special and temporal gene delivery efficiency in tissue engineering applications.

References:

1. Soundara Manickam D. J Control Release. 2005; 102:293–306.