Engineering Nanolayers for Localized Delivery of siRNA

by

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Technical Summary

RNA interference (RNAi) is a promising technology for therapeutic application. The RNAi pathway involves sequence-specific gene silencing directed by RNA fragments of 21-23 nucleotides long known as short interfering RNA (siRNA). The great potential for siRNA to modulate gene expression has prompted research in treatment for diseases including inflammatory disorders, viral infections, and a host of cancers. Yet siRNA therapy is not without its challenges. Delivery barriers such as nuclease degradation, rapid clearance, cell membrane rejection, and lysosomal degradation must be overcome for effective siRNA therapy.

Local delivery of siRNA presents advantages including reducing off-target effects, increased efficacy at target site, and reduction in load requirements compared to systemic siRNA administration. Layer-by-layer (LbL) self-assembly technology is a promising method of nanolayer surface coating fabrication for the localized and controlled delivery of therapeutics. One area of particular interest for controlled localized siRNA delivery is the treatment of soft tissue wounds. Wound healing is a complex, multi-staged process wherein dysregulation in whichever healing phase may cause severe complications for patients.

Here we present the engineering of LbL thin films for localized delivery of siRNA. We design LbL films for release of multiple siRNAs. By tuning film architecture and incorporating barrier layers to prevent interlayer diffusion, we achieve sequential release of siRNA at physiological timescales relevant to a healing wound. To improve knockdown efficacy of released siRNA complexes, we investigate the assembly of a bilayer composed of siRNA and the polycation poly(β-amino ester) (PBAE). Through a fractional factorial design, we elucidate the effects of LbL assembly parameters on the resultant film's loading, composition, and *in vitro* efficacy. From these findings, we determine optimized assembly parameters for gene silencing.

Finally, we develop a mouse model for evaluating *in vivo* efficacy of LbL films assembled on sutures. Findings from a pilot study with our optimized films and recommendations for future studies are reported. This thesis work expounds the utility of LbL technology in assembling films for effective controlled localized siRNA delivery.

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