Characterizing the Interactions Between Gene Delivery Polyplexes and Bio-Inspired Polymeric Surface Coatings for Localized Gene Therapy

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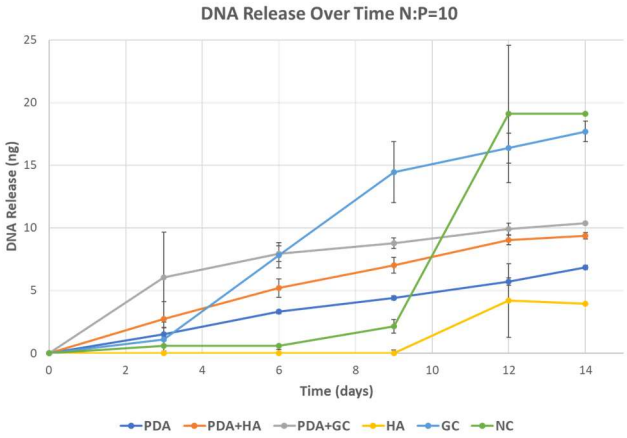
Statement of Purpose: Gene delivery has shown much promise as a strategy for the treatment of a variety of diseases, with several successful gene therapies approved for clinical use [1]. However, the typical approach involving systemic administration of gene delivery vectors faces several limitations, including endothelial barriers, poor targeting efficiency, systemic clearance and immune responses [2]. Localized delivery of gene delivery vectors from biomaterial surfaces or hydrogels can overcome many of these difficulties and increase transfection efficiencies by maintaining higher vector concentrations at the target site and controlling vector release kinetics [3]. This project aims to characterize the absorption and release interactions between a model polymeric gene delivery vector, polyethyleneimine (PEI), and surface coatings composed of bio-inspired and naturally derived polymers with the long-term goal of developing a surface coating that enables localized gene therapy for tissue engineering and regenerative medicine applications.

Methods: DNA-PEI polyplexes were formed via complexation of branched PEI (M_n = 20k) and plasmid DNA encoding GFP at several N:P ratios (0, 10, 20) in various media (PBS, DMEM). Resulting polyplex sizes and zeta potentials were characterized via Dynamic Light Scattering and ZetaPlus analysis (Brookhaven Instruments, USA). Surface coatings were formed at concentrations of 2 mg/mL and 5 mg/mL within tissue culture plates from the following polymers and polymer combinations: polydopamine (pDA); hyaluronic acid (HA); glycol chitosan (GLY-CHI); pDA + HA; and pDA + GLY-CHI. Uncoated wells (NC) were used as a control. Coatings were incubated with DNA-PEI polyplexes at various concentrations and N:P ratios. The DNA loading efficiencies on these coatings and the DNA release kinetics from these coatings over a period of 14 days in PBS at 37ºC were analyzed via the PicoGreen assay (Invitrogen, USA). Transfection experiments with NIH3T3 and HEK293 cell lines are currently being performed on these same coatings and will be analyzed for cytotoxicity via Live/Dead staining (Invitrogen, USA) and for transfection efficiency through the use of a fluorescence plate reader and fluorescence microscopy (Cytation 5, Biotek, USA) for GFP expression.

Results: Preliminary results suggest that DNA-PEI polyplexes formed in PBS at N:P ratios of 10 and 20 had significantly higher surface charges (~14 to 17 mV) and slightly lower sizes (~177 to 181 nm) than those formed in DMEM (~ -0.65 to 5.5 mV; ~ 207 to 211 nm); likely due to the presence of additional ionic species in DMEM. DNA absorption onto coatings and controls was found to be significantly higher for DNA complexed with PEI (N:P = 10 or 20) than for naked plasmid DNA (N:P = 0). DNA loading efficiencies were observed to be highest on coatings containing pDA (pDA; pDA + HA; pDA + GLY-CHI) and lowest on GLY-CHI coatings (≅ 18-22%). Although all coatings and N:P ratios showed relatively low amounts of DNA release in PBS, significantly more DNA is released over time in the case of coating-absorbed naked plasmid DNA (N:P = 0) than when DNA is complexed with PEI (N:P = 10 or 20). Preliminary results indicate that coatings composed of GLY-CHI exhibit the fastest and highest amount of DNA release, while coatings composed of HA show the slowest and least amount of DNA release (see **Figure 1**).



(Figure 1: Representative DNA release data from different coatings over 14 days at N:P = 10. Mean ± STD, n=3)

Conclusions: Coatings composed of GLY-CHI showed the lowest DNA loading efficiency and the fastest DNA release. This is likely due to charge repulsion between positively charged DNA-PEI polyplexes and cationic GLY-CHI. Similarly, anionic HA exhibited the slowest and lowest DNA release in PBS. coatings showed the highest DNA loading efficiencies. Experiments are currently underway to see how these results translate to substrate-mediated cellular transfection efficiencies. This work represents an important first step in the development of surface coatings that enable localized gene delivery for tissue engineering and regenerative medicine applications

References: [1] (Lapteva, L. Mol Ther Methds Clin Dev. 2020;19:387-389.)
[2] (Duan, D. Curr Opin in Viro. 2017;21:16-25.)
[3] (Youngblood, RL. Molec Ther. 2018;26:2087-2106.)