In Vivo Gene Delivery and Multimodal Imaging of Multifunctional Degradable Nanoparticles after Delivery to Lung

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Statement of Purpose: DNA plasmid delivery offers the promise of treating a number of diseases including acute lung injury and other pulmonary pathologies. However, a multitude of challenges must be overcome in order for these technologies to reach the clinic, including finding carriers that can bind and release DNA, deliver DNA to specific tissues and cells, facilitate cell uptake, minimize toxic responses, and clear from the body after delivery. Degradable nanoparticles offer multifunctional properties that can potentially help solve many of these problems. In this study, degradable cationic shell-cross-linked knedel-like nanoparticles (cSCKs) were used to deliver plasmid DNA for *in vivo* transfection in a mouse model.

Methods: Polylactic acid (PLA) cSCKs were synthesized through reported methods. Nanocomplex formation between cSCKs and luciferase expression plasmid DNA (pDNA) was characterized using dynamic light scattering (DLS), electron microscopy, atomic force microscopy (AFM), and gel electrophoresis on an agarose gel. Cell uptake and trafficking of fluorescently-labeled nanocomplexes was characterized *in vitro* using flow cytometry and optical imaging. pDNA transfection efficiency was characterized *in vitro* using RAW 264.7 cells and *in vivo* using a mouse model by quantifying luciferase-generated bioluminescent signal. Toxicity of nanocomplexes was also monitored both *in vitro* and *in vivo*. Further, cSCKs were radiolabeled with ^{124}I to perform quantitative *in vivo* biodistribution and characterize excretion kinetics through urine and feces. Finally, cSCK biodistributions were imaged with both positron emission tomographic (PET) imaging and Cherenkov radiation imaging at multiple time points following intratracheal administration into the lungs.

Results: Dynamic nanocomplexes were formed between degradable PLA cSCKs and pDNA, with full DNA binding occurring at an amine:phosphate ratio of 2:1. Cell uptake of the complexes occurred within 90 minutes, reaching the early endosome within 4 hours. *In vitro*, transfection efficiency of the degradable PLA cSCK was greater than a nondegradable counterpart, which was attributed to the more efficient release of DNA from the cSCKs over time. Successful transfection using the degradable cSCK occurred *in vivo*, with less toxicity compared to a polyethylenimine (PEI) control. Efficient delivery of nanocomplexes to the lung via intratracheal injection (> 50% ID/organ after 1 hour) was observed in the biodistribution studies, and significant clearance occurred over two weeks (8.7% ID/organ remained 14 days after injection). Evidence of blood and intestinal clearance was found based on activity in the urine and feces, which was highest in the 3 days after delivery. Finally, cSCKs were monitored with both PET and Cherenkov imaging (Figure 1). Validation of clearance was indicated by activity in the stomach and kidney, correlating with the quantitative biodistribution data. Further, cSCK clearance from the lung was observed with image analysis in both modalities. PET standardized uptake values (SUVs) decreased from 5.4 to 1.7 over 14 days, while normalized Cherenkov radiance values decreased from 5.4 X 10^5 to 2.4 X 10^5 p/s/cm^2/sr over the same time period. These two imaging modalities were linearly correlated ($r^2 > 0.99$).

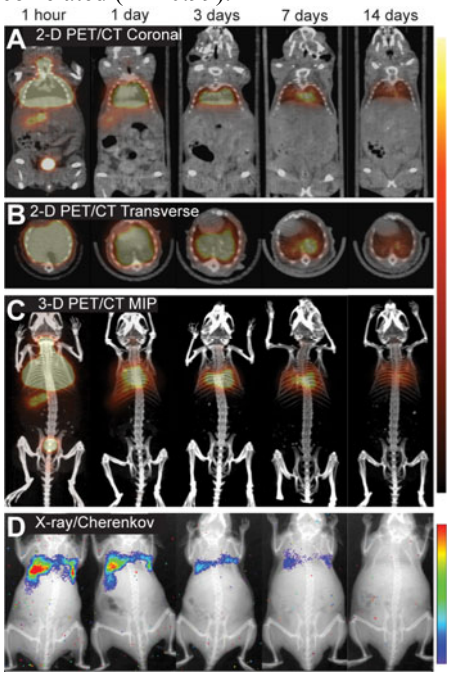

Figure 1. PET (A-C) and Cherenkov (D) imaging after administration of cSCK:pDNA nanocomplexes.

Conclusions: This study characterized the delivery of pDNA to the lung with degradable cSCKs for future gene therapy applications. Efficient transfection was observed *in vitro* and *in vivo*, causing significantly less toxicity than PEI carriers, a promising result in their translatability. The cSCKs were labeled with PET-active radionuclides and were tracked *in vivo*, which provided biodistribution, lung clearance quantification, excretion kinetics, and anatomical visualization. PET and Cherenkov imaging were both performed and provided quantitative visualization of clearance of the nanocomplexes from the lungs following delivery, correlating with the biodistribution data. Cherenkov imaging can potentially be used for quantitative imaging in small animal models to provide high throughput protocols, while PET imaging provides direct clinical relevance, giving this dual-modal imaging system complementary advantages on the path of translation between *in vitro* experiments, small animal imaging, and human clinical trials.

References: Samarajeewa et al. J. Am. Chem. Soc. 134(2): 1235-1242.