Porous Silicon Nanoparticle Delivery of Peptide Nucleic Acid Anti-MicroRNA Therapeutics

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Statement of Purpose: Inhibiting a single biomolecule to alter expression of an entire pathogenic gene network represents a powerful new therapeutic paradigm. MicroRNA (miRNA) are promising targets in such an approach because they act as master regulators of gene expression by preventing translation of thousands of messengerRNA (mRNA) into protein.[1] Short, synthetic oligonucleotides known as peptide nucleic acids (PNA) have demonstrated therapeutic potential as potent inhibitors of disease-associated miRNA. The main barrier to the clinical translation of PNA anti-miRNA therapeutics is their poor cellular uptake and rapid renal clearance (<5min) upon intravenous injection into the body.[2] We have previously shown that PNA cellular uptake can be increased by loading PNA into highly porous, biodegradable carriers known as porous silicon nanoparticles (PSNPs).[3] In this study, we investigate whether or not PSNPs improve the anti-miRNA activity of PNA in human liver cancer cells.

Methods: Multilayer PSi films were fabricated as previously reported,[3] then ultrasonically fractured to form PSNPs. These nanoparticles were size-sorted by ultracentrifugation and oxidized in H_2O_2 at 155°C for 4 hr. The resultant PSNP mean size (236 nm) and size distribution were determined by Nanoparticle Tracking Analysis[4] and transmission electron microscopy (TEM). A well-characterized anti-miRNA PNA, anti-miR122 (NH_2-ACA AAC ACC ATT GTC ACA CTC CA-cys-COOH), was used as the model therapeutic in this study. PNA was packaged in PSNPs by physical adsorption. Loading efficiency was quantified by high pressure liquid chromatography (HPLC), and validated by scanning transmission electron microscopy-energy-dispersive X-ray spectrometry (STEM-EDS). Cytotoxicity and therapeutic anti-miRNA activity were then evaluated in a Huh7-psiCHECK-miR122 cell line.[5]

Results:

PNA loading was determined to be 2 pmole/µg PSNP by HPLC. The presence of PNA within PSNP pores was confirmed by STEM-EDS analysis (Figure 1), which reveals localization of nitrogen-rich PNA (purple) within the PSNP matrix (red). These signals do not perfectly overlap (white circles), confirming that the signal from nitrogen is not an artifact of imaging.

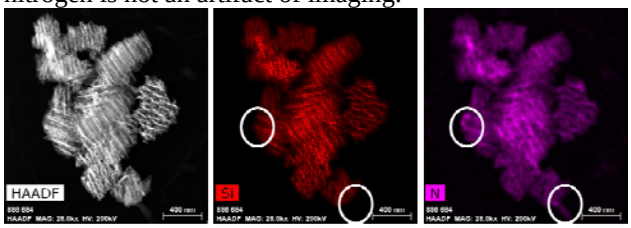

Figure 1. Elemental map of PNA (indicated in purple by nitrogen map) loaded PSNPs (indicated in red by the silicon map).

PNA-PSNP therapeutic activity was interrogated in Huh7 cells that stably express *Luciferase* as a marker for miRNA activity (Figure 2). Therapeutic inhibition of endogenous miR-122 results in increased luciferase expression. Encapsulation of PNA into PSNPs significantly enhanced anti-miRNA activity, as demonstrated by a 83% increase in *luciferase* activity when compared to no treatment. Furthermore, PNA-loaded PSNPs inhibited miR122 in a dose dependent manner, and the level of miRNA inhibition achieved by a 4µM PNA-PSNP dose was comparable to that of a 2µM dose of a "gold standard" anti-miRNA molecule (AMO) delivered with a cationic commercial transfection reagent. Importantly, PNA-PSNPs exhibited significantly less cytotoxicity than the AMO which was too toxic to be delivered at a 4 µM dose.

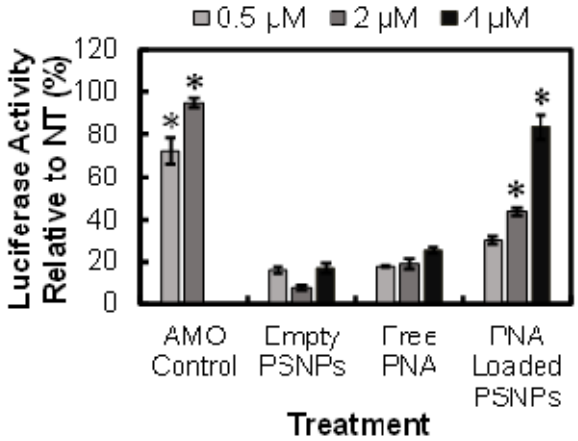

Figure 2. PSNPs increase the activity of a PNA anti-miR therapeutic 48 hours after treatment, when compared to free, unencapsulated PNA. ($p<0.05$). AMO control is a "gold standard" oligonucleotide delivered with a cationic commercial transfection reagent (*Note that the 4 µM AMO control dose has been omitted due to toxicity).

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Conclusions: In this study, we have shown that porous silicon nanoparticles can be used to increase PNA anti-miRNA activity in a dose dependent manner, without decreasing cell viability. As such, these particles may aid in the clinical translation of this promising class of therapeutics.

References:

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