Co-delivery of chemo drug and siRNA using layer-by-layer nanoparticles for triple negative breast cancer treatment

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Statement of Purpose Our recent studies have proposed that layer-by-layer (LbL) assembly of polyelectrolytes on nanoparticles provides a promising drug delivery system (Poon Z. Nano Lett. 2011;11:2096-2103; Poon Z. ACS Nano. 2011;5:4284-4292). These studies have investigated the impact of different film architectures on the nanoparticle surface, elucidating key control variables necessary to generate a serum-stable particle as well as the effect of terminal layers on the pharmacokinetics of the nanocarriers. We are now focusing on the design of multilayered nanoparticles that "shed" their layers step by step to release different cargoes at different times, as a platform for combination drug therapies for systemic delivery in cancer applications. The aim of the current work is to incorporate siRNA into nanoparticles through layer-by-layer assembly as means of demonstrating the versatility of the LbL nanoparticle platform as delivery systems. This work will be towards a combinatorial release design for more efficacious therapeutic treatment of cancer.

Methods: A library of polycations was firstly examined for optimal loading of siRNA/polycation LbL films on the nanoparticles, these including poly-L-lysine, poly-L-argine, poly-L-histidine, chitosan, polyethylenimine and poly (beta-amino ester). The size and zeta potential of the nanoparticles were assessed by dynamic light scattering. An approximately 2 nm increase in nanoparticle radius was observed after siRNA layering. The nanoparticles were well stabilized by the multiple siRNA/polycation bilayer, generating consistent and thin films on the nanoparticles. GFP-targeting siRNA were used to test the intracellular gene knockdown of the nanoparticles. Cell uptake and intracellular trafficking of the siRNA-LbL nanoparticles is assessed by flow cytometry and microscopy. Furthermore, a xenograft model of triple negative breast cancer using luciferase-expressing MDA-MB-468 cells was established to assess the systemic delivery of RNAi *in vivo*.

Results: We demonstrated that siRNA molecules could be efficiently incorporated to colloidal templates through LbL assembly. Initially, carboxyl modified latex nanoparticles have been selected as the cores for LbL assembly of siRNA, as these nanoparticles are a convenient alternative of drug-loaded PLGA nanoparticles. We investigated the size, zeta potential, stability and siRNA loading of polycation/siRNA assembly on the nanoparticles. This allowed us to find the optimal polycation candidate for the LbL-siRNA assembly.

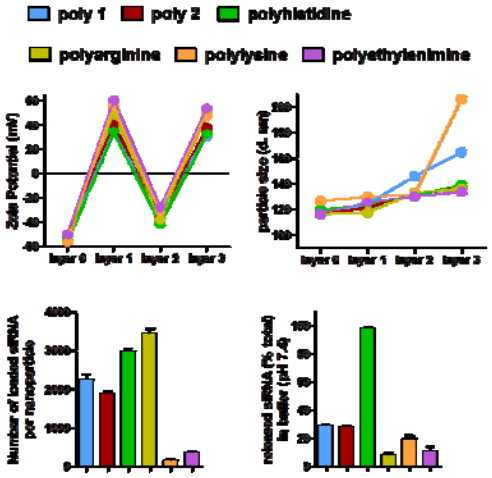

Figure 1 *ex vivo* characterization of LbL siRNA assembly on nanoparticles. Size, zeta potential, siRNA loading and stability in buffer were examined to identify the optimal polycation candidate.

The loading of LbL siRNA onto chemo drug loaded PLGA nanoparticles and liposomes are currently under investigation. Preliminary data suggests that similar loading properties were obtained on these nanoparticles, while a stagger release of the siRNA and chemo drug were observed. A co-delivery of doxorubicin with p-gp siRNA or Bcl-2 siRNA are currently tested both *in vitro* and *in vivo* for improved efficacy.

Conclusions: Using the LbL approach, siRNA molecules were incorporated with biodegradable biopolymers for improved loading, stability and biocompatibility. siRNA can be co-delivery with nanoparticle loaded with chemo drugs, e.g. doxorubicin or cisplatin for increased efficacy in cancer treatment.

References:

Poon, Z., Lee, J.B., Morton, S.W. & Hammond, P.T. Controlling in vivo stability and biodistribution in electrostatically assembled nanoparticles for systemic delivery. Nano Lett 11, 2096-2103 (2011).

Poon, Z., Chang, D., Zhao, X. & Hammond, P.T. Layer-by-layer nanoparticles with a pH-sheddable layer for in vivo targeting of tumor hypoxia. ACS Nano 5, 4284-4292 (2011).