Layer-by-layer assembled oligodeoxynucleotide sphere for multi-drug delivery

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Statement of Purpose: Nucleic acid has gained attention as potential drug in therapeutics[1]. Most efforts to date have utilized cationic polymers to form polyplex with nucleic acid drugs[2,3]. Unlike plasmid DNA, short oligodeoxynucleotides or siRNA has very low charge density, limiting the formation of condensed polyplex for great intracellular uptake and high drug efficacy. To achieve efficient drug delivery, a novel method for preparation of oligodeoxynucleotide (ODN) based carrier was developed. As carrier and drug, highly concentrated ODN formed sponge-like spherical structure by enzymatic elongation without any condensing agent. The size of this spherical particle dramatically decreased to favorable size for cellular uptake by a layer-by-layer assembly of poly-L-lysine (PLL), DNA strands, and polyethyleninmine (PEI), without losing the amount of ODN. This LbL-coated spherical sponge contained extremely high amount of ODN. In addition, LbL assembled spherical sponge showed significant improvement of stability in *in vivo* environment and gene knowdown.

Methods: To synthesize of ODN drug spheres, Circular DNA templates with sense ODN sequence were incubated with Φ29 DNA polymerase at 30 °C for overnight. The resultants were then sonicated for 10min to remove aggregates. After several washing steps by centrifugation with Milli-Q water, the sphere was mixed with PLL. The resulting PLL layered ODN (ODN/PLL) sphere was then assembled again with short DNA strands as anionic polymer. For the most outer layer, PEI was self-assembled onto the ODN/PLL/DNA sphere.

Results: To condense the ODN drug sphere for more highly localized concentration, PLL was used as cationic polymer. Due to the high negative charge density of the sponge, PLL was readily assembled onto it. The size of ODN drug sponge dramatically decreases from approximately 1800nm to 200nm. At low concentration of PLL (0.01mg/ml), ODN sponge formed aggregates, showing more than 50μm of effective diameter from dynamic light scattering (DLS) results. ODN sponge was significantly condensed with 0.25mg/ml PLL solution, resulting in 200nm of ODN nanosponge and did not show any significant change with higher PLL concentration (1mg/ml). By first layering process, we achieved the favorable size for cellular uptake and high local concentration of ODN for efficient drug efficacy. Interestingly, the sponge-like structure begun to be covered with PLL at 0.05mg/ml PLL solution. The

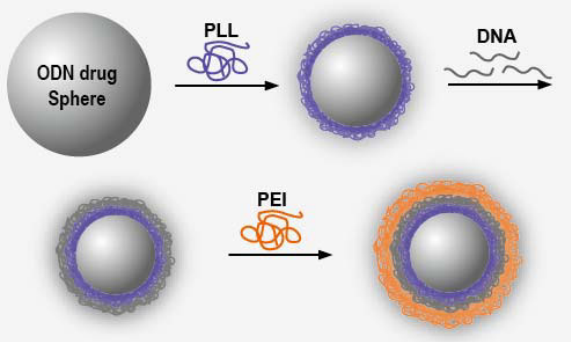

Figure 1. Schematic drawing of LbL assembly on ODN drug sphere

particle was totally layered by PLL at 0.25mg/ml PLL. Zeta potential also verified the self-assembly of PLL onto ODN sponge by showing the change of the value from -37mV to 32mV. The results also suggest that the first layer of ODN sponge was saturated at 0.25mg/ml of PLL solution because any significant increase of zeta potential was not observed with higher concentration of PLL. To build up more layers on the sponge, DNA strand was used as an anionic polymer because it is highly negative charged biodegradable polymer and can easily load chemotherapy drug. Self-assembly of DNA strands onto ODN/PLL particle were achieved by electrostatic interaction, resulting in the change of zeta potential value to -23mV. PEI which is great endosomal escape agent was applied for outer layer. Positive zeta potential value of ODN/PLL/DNA/PEI verified the assembly of PEI onto ODN/PLL/DNA particle. The size of ODN/PLL/DNA/PEI particle revealed about 200nm in diameter which is appropriate for cellular uptake.

Conclusions: We have demonstrated a new class of vehicle for nucleic acid drug delivery using DNA as a drug and carrier. The synthesis of layer-by-layer self-assembled ODN particle provides an advance for significant condensation of nucleic acid drug with high local concentration. Therefore, this approach would have great potential for therapeutic nucleic acid drug applications.

References:

1. Hida K. Adv Drug Del Rev. 2007;59: 1562–1578
2. Mok H. Nat Mater. 2010;9:272-278
3. Hammond P. Nat Mater. 2010; 9:292-293