Polysialic Acid-N-Trimethyl Chitosan Nanoparticles for Oligonucleotide Delivery

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Statement of Purpose: Nucleic acids that interfere with gene expression, particularly aptamers, antisense oligonucleotides (ODNs), and siRNA, have been proposed as promising therapeutics for a number of diseases. However, applications have been limited by a lack of stability in physiological environments and a low propensity for intracellular penetration. Nano-sized carrier systems have been suggested as a means of improving the effectivenss of nucleic-acid based treatments. Our lab has developed N-trimethyl chitosan (TMC)-polysialic acid (PSA) nanoparticles to serve as a drug delivery system in the treatment of rheumatoid arthritis.[1] In this study, we demonstrate that ODNs can be incorporated within TMC-PSA nanoparticles and that the resultant ODN-loaded TMC-PSA nanoparticles facilitate intracellular uptake.

Methods: A 20-base pair, Cy5-labeled oligonucleotide with a terminal G-T base pair was used to demonstrate our ability to encapsulate ODNs into the TMC-PSA nanoparticles. Prior to encapsulation, a calibration curve showing fluorescence intensity as a function of oligonucleotide concentration (0-500 nM) was generated to assess the loading efficiency and capacity. For encapsulation, 10 µg of ODN and 6.4 mg of TMC were dissolved in 0.3% aqueous acetic acid. Separately, 3.2 mg of PSA and 1.0 mg of sodium TPP were dissolved in 2 ml of DI water. The PSA solution was added drop-wise to the TMC solution, and the reaction mixtures was stirred at room temperature for 20 minutes (Fig. 1). A nanoparticle pellet was obtained by centrifugation at 3000 RPM for 15 minutes. A Malvern Zetasizer Nano was used to determine the size, polydispersity, and zeta potential of re-suspended nanoparticles. Controlled release of the ODNs from the nanoparticles into DI water at a temperature of 37°C was observed over a 72 hour time period. For observation of intracellular uptake, nanoparticles were prepared with fluorescein-tagged TMC. The ODN-loaded nanoparticles were administered to NIH3T3 cells on collagen-coated plates. At 90 minutes post-administration, the plates were washed with 1X PBS and imaged with a Nikon Eclipse Ti inverted microscope.

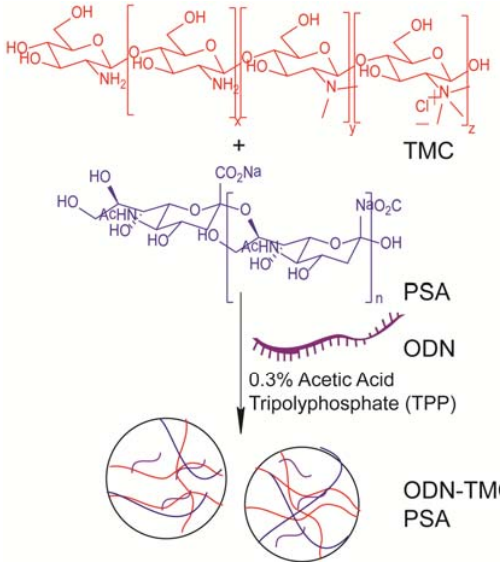

Figure 1. ODNs were encapsulated into TMC-PSA nanoparticles formed via ionic complexation.

Results: ODN-loaded TMC-PSA nanoparticles with a size and zeta potential of 215 nm and 10.5 mV, respectively, were successfully formed. The polydispersity was less than that of nanoparticles formed without ODNs, suggesting that the ODNs confer additional stability to the nanoparticles. The loading efficiency and loading capacity were determined to be approximately 100% and 10 µg ODN/mg TMS-PSA. Controlled release of the ODNs from the nanoparticles into DI water at a temperature of 37°C was observed over a period of 72 hours. The slow rate of release is consistent with significant interactions between the TMC and the ODNs. Microscopy confirmed that both the nanoparticles and ODNs were taken up by cells (Figure 2). As anticipated, this uptake was greater than that observed with ODNs alone (data not shown).

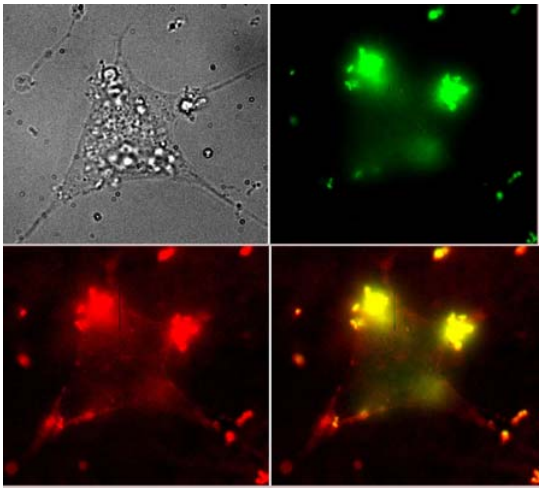

Figure 2. microscopy was used to demonstrate that cellular uptake of TMC-PSA nanoparticles (green) and the ODNs (red) occurred concurrently.

Conclusions: Stable ODN-loaded PSA-TMC nanoparticles were formed and were demonstrated to enhance intracellular penetration of the ODNs. Future studies will aim towards using ODN-loaded PSA-TMC nanoparticles to inhibit the NF-κB pathway in the pathogenesis of rheumatoid arthritis.

Reference:

1. Zhang N. NanoLife. 2012;2:EPub.

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