Histidine-Rich Glycol Chitosan Self-assembled with siRNA Decorated QDots as a Vehicle for SiRNA Delivery

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Statement of Purpose: RNA interference (RNAi) has proven to be a highly suitable mechanism to inhibit the expression of individual genes by the action of double-stranded RNA. Current therapeutics using siRNA, however, suffer from poor intracellular uptake due to the strong anionic charge of the RNA phosphodiester backbone, rapid degradation by nucleases along with limited blood stability.[1,2] We propose to engineer a delivery vehicle for siRNA to address the above limitations.

Methods: N-acetyl histidine conjugated to glycol chitosan (GC-His) and self-assembled with near infrared QDots decorated with siRNAs. Partial modification of glycol chitosan with N-acetyl histidine was done using the modified procedure of Park et al.[3] Streptavidin-coated QDs were mixed in a 1:1 and 1:5 molar ratios with biotinylated siRNAs (HDM2 targeting) in RNase free water. Verification of siRNA binding to QDs was done using zeta potential measurements. The siRNA-QDs conjugates were self-assembled with GC-His and characterized using dynamic light scattering. Uptake of siRNA was monitored using laser confocal microscopy.

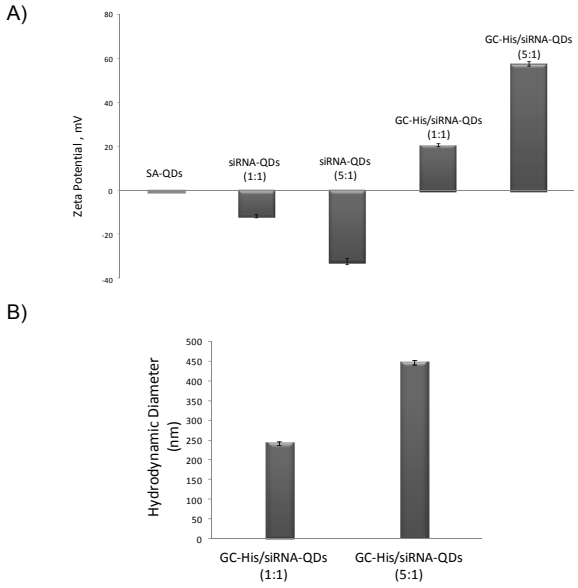

Figure 1: Characterization of nanoparticles using (A) zeta potential measurements and (B) dynamic light scattering.

Results: ^{1}HNMR confirmed the synthesis of GC-His through the characteristic peaks of the imidazole ring protons of histidine appearing around 6.0 and 8.0 ppm. Conjugated biotinylated siRNA to SA-QDs (siRNA-QDs) had a negative zeta potential value justifiying the presence of siRNAs on the surface of the QDs (Fig. 1). An inversion of zeta potential value verified the self-assembly of GC-His on the quantum dot surface (Fig. 1). Physical characterization by dynamic light scattering

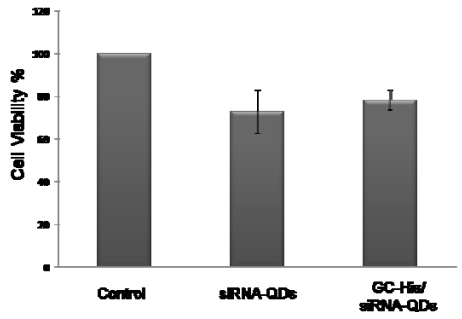

Figure 2: Viability of MCF-7 cells after treatment with various nanoparticle formulations using MTT assay.

revealed the average diameter of the nanocarrier with 1:1 siRNA-QDs to be 240 nm and 5:1 siRNA-QDs to be 440 nm. Cultured MCF7 breast cancer cells treated with these nanocarriers had minimal cytotoxcity. Preliminary results show the uptake efficiency of GC-His/siRNA-QDs nanoparticles in MCF7 breast cancer cells was nanoparticle size dependant and was considerably higher than the control siRNA-QDs as determined by immunofluorescence.

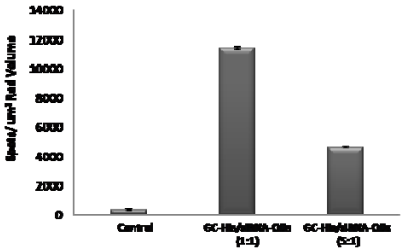

Figure 3. A comparison of various nanoparticle formulations uptake into MCF7 breast cancer cells using light microscopy.

Conclusions: Our results verify the self-assembly of siRNA-QDs inside GC-His nanocarrier and preliminary immunofluorescence study suggest that it has great potential for gene delivery.

References:

[1]Stein, C. A. *Trends in Biotechnology* **1996**, *14*, 147-149.
[2]Urban-Klein, B.; Werth, S.; Abuharbeid, S.; Czubayko, F.; Aigner, A. *Gene Ther* **2004**, *12*, 461-466.
[3]Park, J. S.; Han, T. H.; Lee, K. Y.; Han, S. S.; Hwang, J. J.; Moon, D. H.; Kim, S. Y.; Cho, Y. W. *Journal of Controlled Release* **2006**, *115*, 37-45.