Polycationic Hydrogel Nanoparticles for siRNA Delivery

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Statement of Purpose: RNAi-based therapeutics require effective drug delivery strategies. With tens of thousands of reports of siRNA delivery, there is no shortage of strategies; however, there are very few strategies that use simple and scalable carrier synthesis approaches that may improve ease of manufacturing. In addition, most reports emphasize systemic delivery approaches, and there are few examples of carriers designed for oral delivery. We present a robust synthesis approach using ARGET ATRP to create polycationic hydrogel nanoparticles to enable cellular uptake and endosomal escape for siRNA delivery. These nanoparticles are being developed in parallel with an oral delivery carrier to enable delivery to the intestines. Delivery of anti-TNF-α siRNA to macrophages in the intestines may reduce inflammation and thus treat inflammatory bowel disease. The hydrogel nanoparticles are synthesized in a one-pot, single-step reaction, unlike many crosslinked siRNA carriers that require an additional reaction for crosslinking, and the crosslinking is intended to provide enhanced stability in the gastrointestinal track.

Methods: The polycationic hydrogel nanoparticles were synthesized using an emulsion-based ARGET ATRP scheme in water adapted from a free radical photoemulsion polymerization developed by Fisher et al.[1] Cell viability was determined using an MTS assay. Membrane disruption was evaluated using hemolysis with sheep blood at pH 6.5 and 7.4 and using an LDH assay at pH 7.4. Dynamic light scattering was used to estimate size and surface charge. NBD-Cl was reacted with primary surface amines to create fluorescent NBD-NPs used for imaging of nanoparticle internalization. NBD-NPs were incubated for 2 h at 0.25 mg/ml with RAW 264.7 cells and then fixed and stained. Confocal microscopy images were acquired using a Zeiss LSM 710 confocal microscope.

Results: Polycationic hydrogel nanoparticles were synthesized and characterized for properties important for effective siRNA delivery. 30% knockdown in GAPDH expression was observed using 0.25 mg/ml polycationic hydrogel nanoparticles with 33 nM siRNA, compared to 55% knockdown using Lipofectamine™ 2000 (Invitrogen, Calsbad, CA) (Fig. 1a). Low cytotoxicity was observed in multiple cell lines, including RAW 264.7 murine macrophages (Fig. 1b). Negligible membrane disruptiveness was observed at extracellular pH 7.4 using hemolysis and an LDH assay, but nanoparticles were strongly membrane disruptive (~100%) at early endosomal pH 6.5. The polycationic nanoparticles have a positive surface charge for siRNA complexation, with zeta potentials of 45.4 and 13.2 mV in water and phosphate buffer at pH 7.4, respectively. The nanoparticles are monodisperse (pdi 0.208) with an average size of 106 nm in PBS 7.4 and swell as pH decreases.

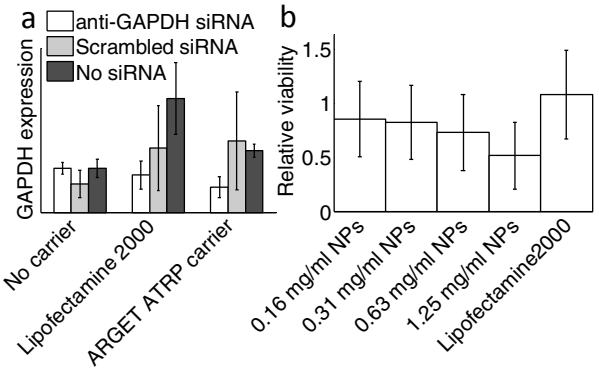

Figure 1 (a) siRNA induced knockdown of GAPDH expression after 48 h in PANC-1 cells with ARGET ATRP carrier. (b) Low cytotoxicity observed for the fluorescently labeled NBD-NPs with RAW 264.7 murine macrophages after 20h, comparable to that of Lipofectamine™ 2000.

Confocal microscopy images show green fluorescence located within the cell membrane (red), which is indicative of efficient NBD-NP internalization (Fig 2); green fluorescence was not observed in no-nanoparticle controls.

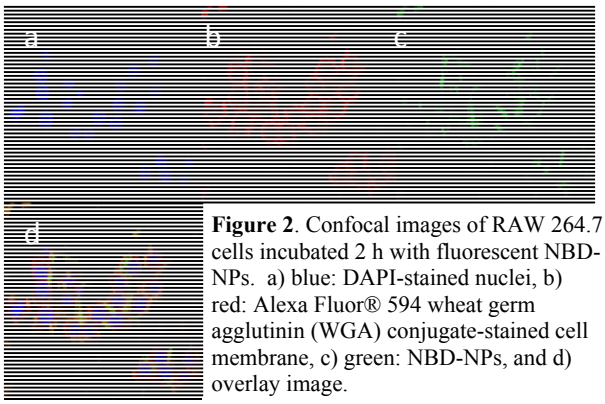

Figure 2. Confocal images of RAW 264.7 cells incubated 2 h with fluorescent NBD-NPs. a) blue: DAPI-stained nuclei, b) red: Alexa Fluor® 594 wheat germ agglutinin (WGA) conjugate-stained cell membrane, c) green: NBD-NPs, and d) overlay image.

Conclusions: The ARGET ATRP synthesis in water produced polycationic hydrogel nanoparticles that demonstrate valuable properties for drug delivery including pH-responsiveness and low cytotoxicity. Internalization is observed using confocal. The polycationic hydrogel nanoparticles induce knockdown of GAPDH expression when complexed with anti-GAPDH siRNA, demonstrating their utility as siRNA delivery agents.

References

1. Fisher O Pharma Res. 2009; 26:51-60.

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