Cationic Nanogels for Oral Targeted siRNA Delivery to Macrophages for Treatment of Inflammatory Bowel Diseases

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Statement of Purpose: RNA interference is an important treatment method that utilizes small interfering RNA (siRNA) to silence the production of specific strands of mRNA and thus reduce the production of that protein. It has been FDA approved for treatment of multiple diseases [1], and has promise as a treatment for inflammatory bowel diseases (IBDs) [2]. Current treatments for IBDs include immunosuppressant drugs and parenterally delivered biologics [3]. These treatments are delivered systemically and reduce immune system performance against normal pathogens and damage [4]. Further, parenteral delivery is not feasible for patients in poor areas of the world and requires meticulous care so as not to cause infection. Therefore, a *targeted* system that delivers siRNA *orally* to the site of inflammation in the intestines would be of immense benefit as it would reduce off-target immunosuppression and be more accessible to the global population. Several challenges associated with the oral delivery of siRNA include enzymatic degradation, extreme pH environments, targeting inflammatory cells, and the need to achieve intracellular delivery and endosomal escape while maintaining siRNA integrity [5]. We developed cationic nanogels to deliver siRNA to macrophages in the intestines after enteric delivery. Cationic nanogels were synthesized to be the appropriate size to undergo uptake by macrophages, and nontoxic with a pK_a to promote endosomal escape. The carriers can protect siRNA until after endosomal escape.

Methods: In this study cationic nanogels were synthesized using ARGET ATRP [6] with various cationic monomers: 2-(dimethylamino) ethyl methacrylate, 2-(diethylamino) ethyl methacrylate (DEAEMA) and 2-(diisoprpylamino) ethyl methacrylate. To adjust the pK_a to the pH of an endosome and to reduce toxicity, hydrophobic comonomers were added: tert-butyl methacrylate, cyclohexyl methacrylate, and hexyl methacrylate [7]. Additionally poly(ethylene glycol) methyl ether methacrylate was grafted to the nanogels to reduce aggregation and further reduce toxicity. Various formulations of nanogels were synthesized and characterized for swollen and collapsed size by dynamic light scattering, swelling ratio, pK_a by potentiometric titration, *in vitro* toxicity in multiple cell lines with MTS assay, and siRNA loading and release kinetics using QuantIT RNA detection assay. siRNA loading was performed for 1 hour (or 72 hours) at both charged (pH 5.5) and neutral (pH 7.5) conditions in buffer. Release was performed over 8 hours under sink conditions in buffers at both pH values.

Results: Increasing the hydrophobicity of the cationic monomer or increasing the content of hydrophobic comonomers in the particles increased the nanogel aggregation, reduced their volume swelling ratios, and reduced the pK_a. Therefore, it was determined that DEAEMA with a hydrophobic comonomer at 22 mole % should be used for desired pK_a and swelling behavior. Nanogels were nontoxic at concentrations of 50 nM in different cell models. Loading at pH 5.5 when nanogels were charged resulted in nearly 100% loading where loading at pH 7.5 resulted in less than 50% loading, as shown in Figure 1a. siRNA was released within ~8 hours, although much of the encapsulated siRNA had been released within 4 hours as shown in Figure 1b. However, phagocytosis of polymeric particles by macrophages has been shown to occur within minutes[8], so the quick release rate should be ideal for this application. To decrease the burst release, loading can be done under neutral pH conditions for longer time periods (72 hours). Release experiments were all done at pH 7.5, when nanogels are neutrally charged, because they have negligible release at pH 5.5 when charged.

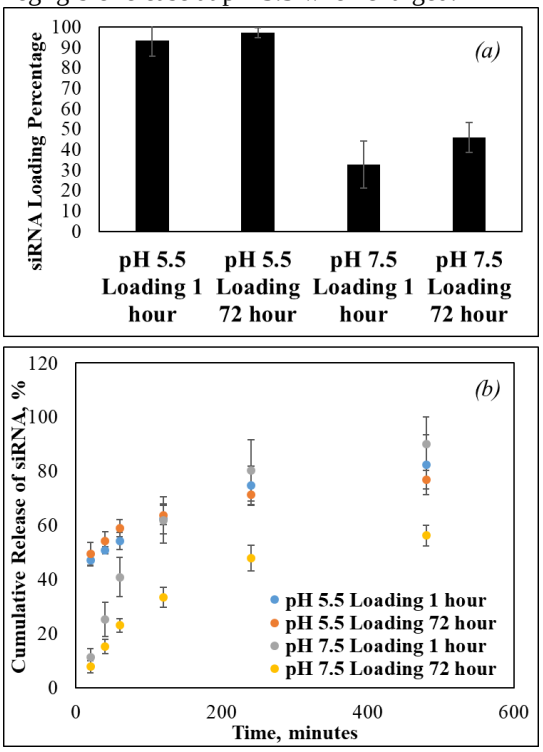

Figure 1. (a) Loading of siRNA into nanogels at different pH/time conditions; and (b) Release of siRNA from the nanogels at pH 7.5.

Conclusions: Cationic nanogels that are nontoxic with appropriate swelling, size, and pKa were synthesized. siRNA loading and release was shown under multiple pH conditions to optimize the system for future experiments.

References: **[1]** M. M. Zhang, R., et al, *Biochem. Pharmacol*., 2021; [2] Y. Zhang, et al, *Mol. Ther.*, 2006 [3]J. M. F. Chebli et al., *Med. Sci. Monit*., 2014, [4] R. R. Martins-Chaves, et al, *Braz. Oral Res*., 2020, [5] K. A. Whitehead, et al, *Nat. Rev. Drug Discov.* 2009, [6] D. C. Forbes, et al, ACS Nano, 2014, [7] D. S. Spencer, et al, *J. Control. Release,* 2021, [8] J. A. Champion, et al, *Pharm. Res*, 2008.