pH Responsive Nanoparticles-in-Microparticles System for Oral Delivery of siRNA

Laura E. Strong[1,4], Jennifer M. Knipe[2,4], and Nicholas A. Peppas[1,2,3,4]
Departments of [1]Biomedical Engineering, [2]Chemical Engineering, and [3]Pharmacy
[4]Institute of Biomaterials, Drug Delivery, and Regenerative Medicine
The University of Texas, Austin, TX

Statement of Purpose: A major challenge in the translation of siRNA therapy to the clinic is the efficient delivery of the molecule into target cells. Specifically, oral delivery of siRNA has yet to be achieved, though such a formulation would be especially conducive to treating diseases to the gastrointestinal (GI) tract, such as inflammatory bowl disease (IBD). IBD manifests from excessive intestinal inflammation, and delivery of siRNA targeting these inflammatory cytokines provides a novel treatment approach. Previously, polycationic nanoparticles composed of poly[2-(diethylamino) ethyl methacrylate-co- tert-butyl methacrylate] (DEAEM-co-tBMA) have been developed and validated for intracellular delivery of siRNA [1,2]. This work aims to create a platform for oral delivery of these siRNA-loaded nanoparticles using a material which would (1) protect the nanoparticles from the harsh acidic environment of the upper GI tract and (2) enzymatically degrade and release the nanoparticles once the platform has reached the diseased intestinal tissue. This platform consists of microparticles composed of poly(methacrylic acid-co-*N*-vinylpyrrolidone) [MAA-co-NVP] crosslinked with a trypsin-degradable peptide linker. The poly(MAA-co-NVP) backbone is designed to collapse around and protect encapsulated nanoparticles at the low pH levels seen in the stomach (pH 2-4). At pH levels of 6-7.5, as seen in the intestine, the poly(MAA-co-NVP) matrix swells, allowing for intestinal enzymes, such as trypsin, to diffuse in and degrade the matrix, resulting in delivery of the therapeutic nanoparticles to the intestine.

Methods: Poly(MAA-co-NVP) was synthesized via photoinitiated, free radical polymerization. A solution containing a 1:1 molar ratio of MAA:NVP was added to 1:1 H_2O:EtOH at a 1:3 (w/w) monomer:solvent ratio, followed by addition of Irgacure 184 ® to 1 wt% of the solution. This monomer solution was purged with N_2 for 20 min and polymerized via exposure to a UV point source for 30 min under stirring. The polymer product was purified by addition of 1 N HCl followed by centrifugation. Polycationic poly(DEAEM-co-tBMA) nanoparticles were synthesized using Activator ReGenerated by Electron Transfer- Atom Transfer Radical Polymerization (ARGET-ATRP) following previously described methods [1]. The polycationic nanoparticles were encapsulated in poly(MAA-co-NVP) hydrogels crosslinked with a trypsin-degradable peptide (GRRRGK). Covalent crosslinks between carboxylic acid groups on the polymer backbone and primary amine groups on the peptide were formed via reaction with 1-ethyl-2-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS). Hydrogel films were dried by lyophilization, and then crushed into a powder and sieved to obtain microparticles less than 30 μm.

Results: Resulting microparticles were characterized using confocal microscopy. The morphology of a typical poly(MAA-co-NVP) microparticle is seen in Figure [1A], with fluorescently-tagged poly (DEAEM-co-tBMA) nanoparticles distributed throughout the microparticle (Figure [1B]). Degradation of microparticles was assessed in PBS, PBS containing 0.3 mg/ml trypsin, as well as gastric and intestinal fluids harvested from Sprague Dawley juvenile male rats. Microparticle degradation was evaluated by measuring the relative turbidity of the samples every 5 min for 4 hr. Results (Figure [1C]) show minimal changes in samples incubated in PBS or gastric fluid, while samples in trypsin or intestinal fluid display a loss in turbidity over time, indicating microparticle degradation.

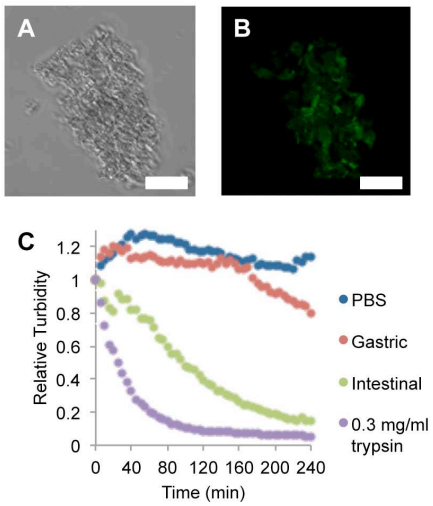

Figure 1. [A] Bright field image showing poly(MAA-co-NVP) microparticle morphology and [B] fluorescent image showing successful incorporation of NBD-Cl labeled poly(DEAEM-co-tBMA) nanoparticles. Scale bars = 30 μm. [C] Relative turbidity measurements indicate that the microparticle platform degrades in the presence of intestinal fluid or trypsin, but remains intact in presence of PBS or gastic fluid.

Conclusions: Enzymatically-degradable poly(MAA-co-NVP) microparticles were designed to be collapsed at low pH levels, thus protecting encapsulated siRNA-loaded nanoparticles throughout the upper GI tract. Once reaching the intestine, the microparticles swell and enzymatically degrade, releasing the therapeutic nanoparticles. Current and future efforts focus on characterizing the release of the poly(DEAEM-co-tBMA) nanoparticles from the microparticles, cellular uptake, and subsequent downregulation of inflammatory proteins associated with IBD.

References:

[1] Forbes D.C. ACS Nano 2014;8:2908-2917.
[2] Forbes D.C. Macromol Biosci 2014;14:1096-1105.