Evaluation of Surface Modified P(MAA-co-NVP) Hydrogels for Oral Protein Delivery
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Statement of Purpose: Complexation hydrogels composed of methacrylic acid (MAA) and N-vinyl pyrrolidone (NVP), are excellent candidates for the transmucosal delivery of protein therapeutics such as insulin and growth hormone. These carriers are ideal due to their ability to remain collapsed at low pH, such as that in the stomach, and swelling upon reaching more neutral pH, such as in the small intestine. Protein is encapsulated within the carrier, protected during its transit through the harsh conditions of the stomach, and released in the upper small intestine. P(MAA-co-NVP) has been optimized for oral delivery of insulin and shown success in in vitro models. The carrier must now be modified for the delivery of other protein therapeutics of different size and structure. This work focuses on the development and evaluation of surface-modified P(MAA-co-NVP) for the oral delivery of human growth hormone (hGH). This protein was selected for therapeutic relevance, used for treating conditions such as Prader-Willi syndrome and growth hormone deficiency, but also for its large size, ~ 4 times larger than previously delivered proteins. Surface modification will add either poly(ethylene glycol) or dextran tethers to the hydrogel carrier. These additions will increase the interaction of the carrier with the mucus lining of the upper small intestine and ensures that the larger protein therapeutic will have sufficient time to diffuse out of the carrier and be delivered across the intestinal wall.

Methods: Hydrogel synthesis. P(MAA-co-NVP) hydrogels are synthesized using UV-initiated bulk-free radical polymerization. MAA and NVP monomers are added in a 2:1 ratio to crosslinking agents of varying types and densities. PEG-functionalization. PEG functionalization was completed during synthesis by adding a monofunctional PEG monomer into the reaction mixture at a 2:1 MAA:NVP:PEG ratio. Dextran-functionalization. Dextran tethers have been successfully added in a post-synthesis scheme using carbodiimide-linking chemistry that joins primary amine-containing hydrogels to carboxymethylated dextran. Primary amines were added by including allylamine into the monomer solution prior to polymerization. Polymer characterization. Equilibrium and dynamic swelling studies were performed to determine the swelling profile of the hydrogel network at gastric and intestinal pH. SEM was also used to compare surface morphology of the hydrogel carriers. Confirmation of surface functionalization. Incorporation of PEG and dextran tethers was confirmed using Fourier Transform Infrared Spectroscopy (FTIR) and a phenol-sulfuric polysaccharide quantification assay, respectively. Protein loading and release. Both bovine serum albumin (BSA) and porcine growth hormone (pGH) were loaded into the P(MAA-co-NVP) hydrogel network by incubating the carriers in a protein-PBS solution at pH 7.4 for 6-24 hours. Particles were collected after reducing the pH to collapse the network and encapsulate the protein. Protein release was measured as a function of time after loaded particle introduction to acidic or neutral buffer. Protein quantification was completed using a microBCA assay. Mucoadhesion. Hydrogel discs were mounted onto an aluminum probe and allowed to swell to equilibrium. Using a texture analyzer, the hydrogel was introduced to excised porcine intestine for 5 minutes at 5g of force. The work of adhesion was then calculated. In vitro viability assays. Particle cytotoxicity was measured on Caco2 colon adenocarcinoma and HT29 goblet cells. Particles at varying concentrations were introduced to seeded cells for 2 hours. MTS assay was used to measure cell viability.

Results: P(MAA-co-NVP) hydrogels were successfully surface modified with crosslinking densities ranging from 0.75-1.25 mol% and both PEG- and dextran-incorporation. Swelling profiles of the synthesized formulations showed the desired behavior of remaining collapsed at low pH and swelling at neutral pH to open pores ~10x the dynamic radius of hGH. BSA loading efficiencies ranged from 40% for the highly crosslinked gels to 80% for the lesser crosslinked gels. BSA release at varying pH conditions is shown in Figure 1. PEG-containing hydrogels (+) released more protein as compared to their non-PEG-containing counterparts (-), with increased crosslinking density showing decreased protein release. Hydrogels were shown to have substantial interaction with the intestinal lining, with PEG-gels once again outperforming their non-PEG counterparts. No significant cytotoxicity was observed at particle concentrations less than 5 mg/mL.

Conclusions: In conclusion, we successfully synthesized surface modified P(MAA-co-NVP) hydrogels. It was shown that varying crosslinking type and density changed the swelling behavior and cytotoxicity. Furthermore, surface functionalization showed a marked effect on protein loading and release, as well as mucoadhesion. Surface-modified P(MAA-co-NVP) has been shown to be a viable candidate for oral delivery of a high molecular weight protein therapeutic.

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