In vitro Evaluation of Carbohydrate Decorated-Hydrogels for Oral Protein Delivery

Margaret A. Phillips and Nicholas A. Peppas
The University of Texas at Austin

Statement of Purpose: The development of an oral protein delivery system is a significant challenge in the field of biomaterials. The material requirements of an oral protein delivery system are two-fold: to protect the bioactivity of the protein drug through the digestive tract and to allow absorption of the active drug. The use of anionic complexation hydrogels of poly(methacrylic acid-grafted-ethylene glycol) (P(MAA-g-EG) have been developed in our laboratory for the oral delivery of insulin. These materials are advantageous for this application because hydrogen bonding between polymer chains in acidic conditions limits diffusion into and out of the gel which allows protection of the drug in the stomach. In more neutral conditions, diffusion is less limited due to the loss of hydrogen bonding which corresponds to release in the upper small intestine at the site of absorption. The goal of this work was to modify the surface of these P(MAA-g-EG) hydrogels with polysaccharides to improve interactions between the drug delivery system and the intestinal wall. In this work, we present the material synthesis, characterization, and in vitro evaluation of carbohydrate decorated-P(MAA-g-EG) hydrogels.

Methods: P(MAA-g-EG) thin films were prepared by a UV-initiated free radical solution polymerization according to published protocol (Woods et al). The resulting films were washed for 5-7 days, dried, and then crushed into microparticles. Nanoparticles were prepared in a similar fashion using a dispersion polymerization in which 800 µl of a solution of methacrylic acid (MAA), poly(ethylene glycol) methyl ether methacrylate (PEGMMA) with a molecular weight of 2000 Da, poly(ethylene glycol) dimethacrylate (PEGDMA), and Irgacure® 2959 were placed in 100 ml of Ultrapure water. The solution was purged with nitrogen for 20 minutes and polymerized for 30 minutes using a UV point source. The resulting nanogels were dialyzed for 5-7 days and lyophilized before use.

In order to couple the polysaccharides dextran, pullulan, and poly(galacturonic acid) to the surface of microgels and nanogels, a primary amine was added to the reducing end of the the polysaccharides (1-2 g) using hexane diamine (HMD) (0.5-1 ml of 60% HMD) by reductive amination in water (10-50 ml) with sodium cyanoborohydride (20-100 mg) for 24-72 hours. The aminated polysaccharides were dialyzed against water for 2 days and then lyophilized. Polysaccharides (100-500 mg) were coupled to hydrogels (100 mg to 1 g) via carbodiimide coupling using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (20 mg) and N-hydroxysuccinimide (NHS) (20 mg) overnight. Modified microparticles were washed in water to remove unreacted polysaccharides while nanogels were dialyzed. The modification of these gels was confirmed by FT-IR spectroscopy and staining with a fluorescently-labeled lectin.

To evaluate the in vitro performance of these hydrogels as oral protein drug delivery systems, dynamic and equilibrium swelling studies were performed according to published protocol to determine whether the addition of carbohydrate tethers disrupted the pH-dependent complexation behavior of these gels. In vitro loading and release studies using insulin as a model protein drug were also performed to determine the loading efficiency and release kinetics of insulin. The cytotoxicity of these materials was evaluated using an MTS assay using an intestinal epithelial model of Caco-2 cells.

Results: Results have shown that we have successfully synthesized P(MAA-g-EG) films modified with polysaccharides using FT-IR spectroscopy and fluorescence staining. These gels have shown that they maintain their pH-dependent swelling which is highly desirable for oral protein delivery. We have also shown that the swelling ratio can be adjusted by changing the monomer feed ratio of crosslinker (PEGDMA).

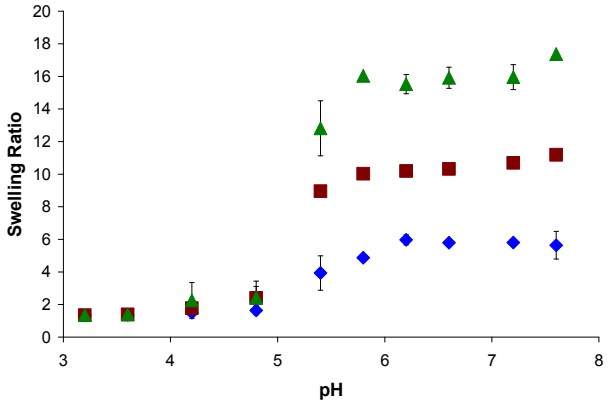

Figure 1: The swelling ratio of P(MAA-g-EG) films modified with dextran with crosslinking feed ratios of 5% (blue), 2% (red), 1% (green).

Additionally, we have shown that we can load these hydrogels with insulin resulting in comparable loading efficiencies to P(MAA-g-EG) controls. Preliminary cytoxicity studies have also indicated that the modified P(MAA-g-EG) gels do not have a statistically significant difference in cytotoxicity from controls.

Conclusions: In vitro studies have shown that carbohydrate decorated-P(MAA-g-EG) hydrogels are potential oral protein delivery systems because they demonstrated they exhibit pH-dependent complexation behavior, have suitable loading and release properties, and have an appropriate cytotoxicity.

References: Woods, KM., GM Stone, and NA Peppas. Biomacromolecules. 2008; 9:1293-1298.