pH-Responsive Polymeric Vehicles for the Oral Delivery of Hemophiliac Factor IX

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Statement of Purpose: Current treatment methods for hemophilia B, a hereditary bleeding disorder characterized by the deficiency of clotting protein, factor IX, rely on injections and infusions that cause pain and discomfort, leading to noncompliance and risk of subsequent bleeding.[1] Protein replacement therapy can prevent spontaneous bleeding and restore hemostasis, greatly improving the patient's lifespan and quality of life. However, such treatments are not readily available worldwide, and without treatment the median life expectancy is 11 years.[2] A non-invasive treatment based on an oral delivery system can both overcome such issues and increase global access to protein therapy. Anionic complexation hydrogels have been engineered to protect factor IX from the harsh environment of the GI tract and deliver it to the small intestine. We have successfully developed environmentally responsive polymeric systems based on poly(methacrylic acid)-grafted-poly(ethylene glycol) [P(MAA-g-EG)] as delivery vehicles for bovine serum albumin (BSA, 66 kDa) and human factor IX (hFIX, 57 kDa). We focused on optimizing P(MAA-g-EG)-based systems for oral delivery of human factor IX (hFIX) for hemophilia B treatment.

Methods: P(MAA-g-EG) hydrogels consist of a methacrylic acid (MAA) backbone with polyethylene glycol (PEG) tethers. Such hydrogels were synthesized at varying crosslinking densities (0.8-2.0 mol %) with either tetraethylene glycol dimethacrylate (TEGDMA) or poly(ethylene glycol) (MW=400) dimethacrylate (PEGDMA400) by bulk UV polymerization. Hydrogels were purified, dried, and crushed into <75 µm or <45 µm microparticles. We performed polymer characterization, including SEM for surface morphology and size distribution, potentiometric titration and FTIR for polymer composition, and swelling studies for pH-responsive behavior. Protein loading and release studies ensure that the microparticles are viable oral delivery vehicles. For loading, microparticles were placed in a protein solution at pH 7.4 and then collapsed to trap the protein in the polymer network. Release studies were performed at pH 2 (stomach) and pH 7.4 (small intestine). For optimization, we first tested BSA, a size and isoelectric point analog for hFIX. BSA was quantified by a microBCA assay, while hFIX was quantified by a hFIX-specific ELISA. Protein activity and structure were evaluated by activity assay, gel electrophoresis, FTIR, and thermal shift studies. For hFIX, a factor IX chromogenic assay was used to determine activity. Cytotoxicity was assessed in Caco-2 cells using an MTS assay to screen for potentially harmful carriers. Mucoadhesion leads to increased residence time in the small intestine, improving bioavailability. Microparticles were modified with mucoadhesive tethers, and bioadhesion was evaluated in Caco-2 cells by fluorescent intensity. Permeability of hFIX was determined by transport studies with in vitro intestinal epithelial models.

Results: P(MAA-g-EG) hydrogels contained 96.5-97.0 mol% MAA, as quantified by potentiometric titration. FTIR spectra of crosslinked P(MAA-g-EG) showed characteristics associated with MAA and PEG. SEM micrographs of crushed microparticles showed irregular morphology due to the crushing process, as well as a wide polydispersity of microparticle size attributed to sieving which only defines two of the three dimension. Loading and release studies showed that P(MAA-g-EG)-based systems are viable carriers for BSA and hFIX. We achieved a loading level of up to 60 µg hFIX per mg microparticle. Release of active hFIX was significant in the pH 7.4 solution (≤20% release) compared to the pH 2 solution (≤0.3%). Active hFIX was released at pH 7.2, while release at pH 2 indicated no detectable as determined by an activity assay. Exposure (2 h) to lower concentrations of all carriers showed no appreciable cytotoxicity (at least 0.8 relative absorbance) and cytotoxicity depends on concentration (Figure 1). Modifying carriers improved adhesion in vitro.

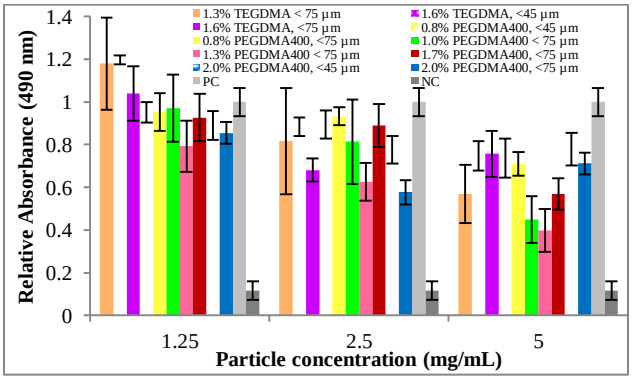

Figure 1. Cytocompatibility of P(MAA-g-EG) microparticles is concentration dependent.

Conclusions: Oral delivery of factor IX can be achieved by tailoring biomaterial-based microcarriers to improve protein release and increase bioavailability. Successful outcomes of this work will change hemophilia B treatment by offering a novel safe and needle-free protein replacement therapy with improved global accessibility.

References: (1) Berntorp E. Lancet. 2012;379:1447-1456. (2) Darby CS. Blood, 2007;110:815-825.

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