Growth Hormone Release from pH-Sensitive Complexation Hydrogels

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Statement of Purpose

The oral delivery of therapeutic proteins is a viable and advantageous alternative to injection as a means for drug administration. Success of oral delivery relies on the design of the carrier as the gastrointestinal tract presents major barriers to this route. Barriers to overcome include maintaining the activity of the protein in the harsh enzymatic environment of the stomach and releasing sufficient quantities of the drug in the small intestine so absorption may occur through the intestinal epithelial cell layer.

In this work, we have developed a hydrogel network of N-vinyl pyrrolidone (NVP) and methacrylic acid (MAA) for the oral delivery of proteins. MAA is used for its pH-sensitivity, and a high degree of complexation due to hydrogen bonding occurs between MAA and NVP. The motivation is to determine whether this biomaterial will allow for greater protection of the drug over previously designed oral delivery systems, ultimately resulting in an increased bioavailability.

Methods

Hydrogels were synthesized by UV-initiated, free radical polymerization of MAA and NVP in the presence of ethylene glycol dimethacrylate (crosslinking agent), 1-hydroxycyclohexyl phenyl ketone (photoinitiator), and water and ethanol (solvents). The polymerization was carried out for 30 minutes in a nitrogen environment with a UV intensity of 16-17 mW/cm^2. Following washing, the films were crushed into 90-150 μm microparticles.

Swelling studies were carried out using a DMGA/NaOH buffer (I=0.1M) between pH=3.2 and pH=7.6 to analyze the dynamic swelling properties of the formulations and to mimic *in vivo* pH changes between the stomach and small intestine.

Equilibrium partitioning was used for the loading of a 0.5 mg/mL stock solution of porcine somatotropin into microparticles. Release studies were carried out using a dissolution apparatus at 37°C and samples were taken at various time points over a 24-hour period. Protein concentrations for loading and release studies were measured using HPLC.

Cytocompatibility was measured by incubating microparticles with Caco-2 cells and testing for NADPH production using a cellular metabolic assay. Microparticle concentrations from 0.5-2.5 mg/mL were tested.

Results and Discussion

Swelling studies show that considerable swelling begins above the pKa of MAA (4.9) and that increasing the amount of NVP increases the swelling ratio at all pH values. This is due to the highly hydrophilic nature of NVP.

A hydrogel of MAA and poly(ethylene glycol) (PEG) was created in the same fashion as previously stated for system comparison. HPLC analysis shows that more hormone was loaded into the MAA-PEG system (71.86% ± 2.74) than the MAA-NVP system (67.29% ± 1.21). The addition of HCl to collapse the microparticles forces some hormone out of the network, resulting in lower efficiencies than those seen prior to collapse.

The release studies demonstrate that the concentration of hormone released from the MAA-NVP system (0.06 mg/mL) was higher than that from the MAA-PEG system (0.016 mg/mL) (Figure 1) after 1 hour. In addition to a higher concentration released, the slope of the MAA-NVP concentration release signifies a faster release of hormone over the MAA-PEG system.

Cytocompatibility studies show that MAA-NVP microparticles have minimal effect on the viability of living cells (Figure 2). At the highest concentration tested, 94% ± 6% of cells remained viable.

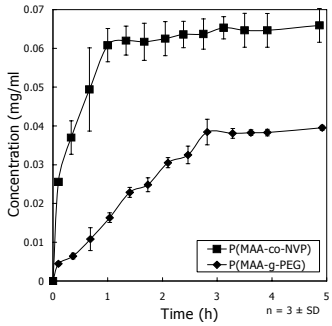

Figure 1: Growth hormone release concentrations for hydrogel systems

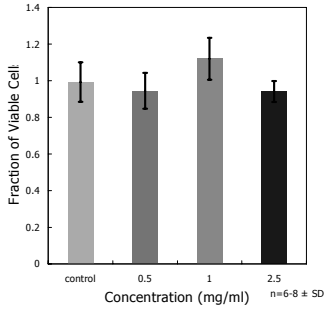

Figure 2: Cytocompatibility of P(MAA-co-NVP) with Caco-2 cells

Conclusions

Swelling studies confirm that this system can respond to a physiological pH change. The MAA-NVP system released a higher concentration of growth hormone and proved to be minimally toxic to viable cells. Further loading and release studies are needed for more formulations to have a comprehensive analysis of P(MAA-co-NVP) as a vehicle for oral delivery.

Acknowledgements

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