PROMPT: Polyanhydride-Releasing Oral Microparticle Technology as an Oral Vaccine Strategy

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Statement of Purpose: The Global Vaccine Action Plan (GVAP) has designated non-syringe delivery mechanisms to be a priority area of research for development of safe, cost-effective and easily distributable next-generation vaccines.[1] Oral delivery of protein antigens can improve upon traditional injection-based administration strategies by eliciting both mucosal and systemic immune protection in a noninvasive manner. Subunit vaccines (e.g. protein antigens) can improve vaccine safety. However, they are often poorly immunogenic, requiring adjuvants to achieve protection. In order to effectively induce immunity, an oral vaccine must overcome the challenges of maintaining antigen stability in the harsh conditions of the gastrointestinal tract, including acidic pH and proteolytic enzymes in the stomach, to reach the antigen-sampling cells of the small intestine. As a solution, we propose combining the unique properties of two promising biomaterial platforms into a solution for vaccination with protein antigens. First, the use of pH-responsive hydrogel enables safe transport of an antigenic payload through the stomach and delivery into the small intestine.[2] Second, polyanhydride nanoparticles (PNPs) with tunable polymer chemistry possess innate adjuvant properties that enable a modulated immune response and tailored antigen release kinetics, as well as activation of antigen presenting cells in a pathogen-mimicking manner. PNPs serve as both a delivery vehicle for protein antigens and adjuvant to direct immune response.[3] The proposed system is a Polyanhydride-Releasing Oral Microparticle Technology (**PROMPT**) in which microencapsulation of PNPs enable transport of a depot of nanoparticles to the small intestine for uptake by M cells of Peyer's Patches to prompt a mucosal immune response.

Methods: *Hydrogel Synthesis.* Two pH-responsive hydrogel networks were modified with degradable crosslinking strategies to achieve targeted depot release of PNPs in intestinal conditions. First, poly(methacrylic acid) backbone with grafted poly(ethylene glycol) tethers, denoted as P(MAA-g-EG), was synthesized by UV-initiated free radical polymerization of PEG mono-methylether monomethacrylate (PEGMMA), methacrylic acid (MAA), and dimethacryloyl hydroxylamine (DMHA) crosslinking agent.[2] DMHA is a biodegradable crosslinker, stable at pH<5 and hydrolytically cleavable in an alkaline environment, coinciding with particle swelling in the small intestine. Second, a copolymer of MAA and N-vinylpyrrolidone (NVP), denoted P(MAA-co-NVP), is crosslinked with a peptide via carbodiimide chemistry to achieve selective degradation by intestinal enzymes. PNPs of various copolymer compositions chemistries can be included during microgel synthesis to optimize vaccine formulation and antigen release kinetics. *PROMPT Characterization:* Degradation studies were performed to determine hydrogel behavior at gastric and intestinal conditions. First, gravimetric analysis was used to compare degradation of hydrogel discs in buffered conditions with differing DMHA crosslinking densities, measuring mass remaining as a function of time. Microgel degradation as a function of time, crosslinking density and environmental conditions was monitored by turbidity measurements for degradable system types. Nanoparticle inclusion and release was verified by incorporating PNPs containing quantum dots and visualizing with fluorescent light microscopy and imaging flow cytometry. *In Vitro Studies*: Cytotoxicity of degradation byproducts was evaluated using LDH Membrane Integrity Assay. Transport and internalization of PNP chemistries was compared in an intestinal co-culture and RAW264.7 macrophages, respectively.

Results: *Hydrolysis:* Mass loss of hydrogels with varying crosslinking density demonstrates hydrogel degradation is tunable by modifying DMHA crosslinking density, and is confirmed by turbidity measurement. P(MAA-g-EG) gels degrade more rapidly than P(MAA-co-NVP) counterparts. *Enzymolysis:* Peptide-crosslinked P(MAA-co-NVP) microgels selectively degrade in intestinal conditions (trypsin, intestinal fluid isolated from rats) within hours. Turbidity remains constant in gastric fluid. PNPs have been successfully incorporated into both systems, and are readily taken up by macrophages.

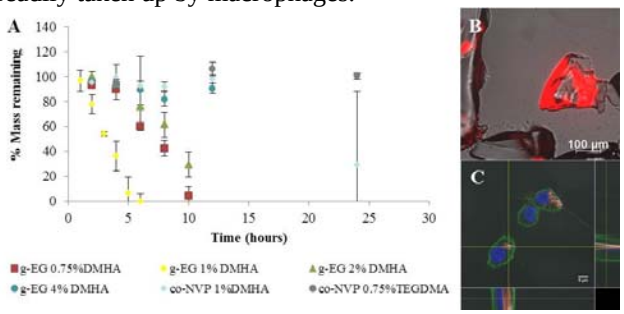

Figure 1. Hydrolysis rate is tunable by polymer composition and crosslinking density (A). PNPs are encapsulated into microgels (B) and endoctyosed by RAW macrophages (C).

Conclusions: In this study, pH-responsive hydrogels were adapted to incorporate two biodegradable crosslinking strategies and optimized for delivery of nanoparticles. Degradation studies indicate therapeutic release at physiologically relevant conditions, and *in vitro* assessment indicates no cytotoxicity and substantial internalization, indicating PROMPT has potential as an adaptable platform for oral vaccine administration.

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[1] WHO. *Global Vaccine Action Plan Strategic Objectives*. 2013. [2]Besheer A. et al. *J. Controlled Release*, 2006; 11: 73-80. [3]Ulery B. et al. *Sci Rep.* 2011, 1-9