BRIEF REPORT

Development of a pH-Responsive Particulate Drug Delivery Vehicle for Localized Biologic Therapy in Inflammatory Bowel Disease

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The treatment of inflammatory bowel disease (IBD\textsuperscript{†}) recently has been revolutionized by the introduction of protein-based biologic therapies. However, biologic therapy is complicated by the requirement for administration with a needle, systemic side effects, and high cost. Particulate drug delivery systems have been shown to deliver drugs locally to the intestinal mucosa via oral administration. However, these systems have been largely unexplored for the delivery of biologics due to harsh particle fabrication conditions and the tendency of many particulate formulations to dissolve in the acidic upper GI tract. We have, therefore, fabricated an inexpensive and non-toxic novel microparticle capable of encapsulating proteins. We establish that the particle retains its contents at acidic pH and releases them at neutral pH. We also demonstrate particulate encapsulation of interleukin-10 (IL-10), a protein relevant to the treatment of IBD, at an encapsulation efficiency of 14.3 percent. Such a vehicle is promising for its oral route of administration and potential to decrease side effects and increase potency of biologics.

Biologic therapies have greatly improved the treatment of inflammatory bowel disease (IBD), although their use can be limited by significant side effects, high cost, and the requirement of injection or infusion [1,2]. In addition, some promising therapies, such as interleukin-10 (IL-10), have proven ineffective due to inadequate local tissue concentrations when delivered systemically [3,4]. However, recent studies have demonstrated increased potency and decreased systemic toxicity of colitis therapies that are delivered locally by such carriers as drug-loaded micro- and nanoparticles [5,6].

Particulate drug delivery vehicles are especially well-suited to treating IBD because they selectively accumulate in areas

\textsuperscript{†}Abbreviations: IBD, inflammatory bowel disease; IL-10, interleukin-10; FDA, Food and Drug Administration; PBS, phosphate buffered saline; BSA-Rho, bovine serum albumin conjugated to rhodamine B; ELISA, enzyme linked immunosorbent assay; TNBS, trinitrobenzene sulfonic acid.

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of intestinal inflammation when delivered orally. This allows for the local delivery of therapeutics to areas of inflammation without the need to target ligands on the particle surface [7]. However, particulate delivery of biologics has remained largely unexplored, given the harsh physical conditions often involved in particle fabrication, and delivery to the upper GI tract has been limited by the tendency of particles to degrade in its low-pH environment.

Here, we designed and fabricated a novel particulate drug-delivery platform in order to encapsulate protein biologic therapies, protecting these drugs from the low pH of the upper GI tract and delivering them specifically to inflammatory lesions in the lower GI tract. Potential therapeutic use of this vehicle includes the oral administration and subsequent local delivery of both novel and existing Food and Drug Administration (FDA)-approved biologic therapies currently available only in systemically delivered, injectable forms. Local delivery of biologics via such a targeted platform could potentially increase the efficacy, decrease the side effects, and decrease the therapeutic dose of biologics used to treat IBD.

We designed a particle composed of an aqueous gelatin core surrounded by a coating of the commercially available pH-responsive polymer Eudragit FS30D (gift from Evonik Industries, Darmstadt, Germany) (Figure 1a). A protein therapeutic may be contained within the aqueous core and is easily incorporated during fabrication using a standard water/oil/water double emulsion technique that has been described previously [8]. Briefly, a solution of the encapsulant protein in 20 wt% gelatin was added drop wise to a solution of 0.5 mg/ml Eudragit FS30D in dichloromethane while vortexing. This water/oil emulsion was then added to a 5 percent aqueous solution of polyvinyl alcohol while vortexing, which was subsequently added to a large volume of phosphate buffered saline (PBS) acidified with hydrochloric acid to approximately pH 4. The solution was stirred for 1 hour to allow for dichloromethane evaporation, followed by washing of the particles with acidified PBS and storage at -80°C.

The particles were visualized by scanning electron microscopy, and size distribution was determined using ImageJ analysis software (National Institutes of Health). Visualization revealed round discreet particles with smooth surface morphology (Figure 1b). The particle size distribution is presented in Figure 1c.
Drug release as a function of pH was determined by encapsulating bovine serum albumin conjugated to rhodamine B (BSA-Rho) within the aqueous core. Particles were incubated on a rotary shaker at 37°C in citrate buffer for 2 hours at pH 2, 2 hours at pH 5, and finally 2 hours at pH 7, mimicking the pH environments encountered by particles delivered via the oral route. Particles were collected hourly via centrifugation and dissolved in dimethyl sulfoxide. BSA-Rho release was then determined via fluorescence spectroscopy (Figure 1d). After 2 and 4 hours, the particles were pelleted, washed, and re-suspended in the appropriate buffer. A robust pH response was observed with <10 percent BSA-Rho released under acidic conditions and a rapid burst release of >80 percent under neutral conditions, demonstrating the platform’s ability to deliver drugs to the lower GI tract via the oral route.

To establish the pertinence of our system to IBD, we chose to encapsulate IL-10 (BD Pharmingen, Franklin Lakes, N.J.), an anti-inflammatory cytokine that has shown promise as an IBD therapy when delivered locally [9,10]. Particles containing IL-10 were fabricated using the techniques described above, washed three times in acidified PBS to remove non-encapsulated IL-10, and subsequently dissolved in bicarbonate buffer of approximately pH 9 in order to release any IL-10 encapsulated within the particle. IL-10 in the supernatant was quantified via sandwich enzyme linked immunosorbent assay (ELISA) (BD Biosciences, Franklin Lakes, N.J.). The IL-10 encapsulation efficiency was determined to be 14.3 percent, demonstrating the ability to encapsulate proteins relevant to the treatment of IBD in our particulate system.

While detection via ELISA confirms the presence of IL-10, it does not necessarily guarantee its bioactivity. Further work, therefore, includes experiments to ensure the preservation of IL-10’s anti-inflammatory activity subsequent to release from the particle at neutral pH in vitro. Additional future studies include in vivo biodistribution studies and toxicity screens. Finally, the advantage of our novel platform must be determined by comparing it against freely administered IL-10 in an animal model of colitis, such as the acute trinitrobenzene sulfonic acid (TNBS) administration model or the chronic CD4CD45RB+ T-cell transfer model [11].

In conclusion, Eudragit-coated gelatin microparticles are easily fabricated from inexpensive non-toxic materials. Particle fabrication is a highly reproducible and flexible process allowing for the encapsulation of hydrophilic therapeutic agents within the aqueous core, including protein-based biologic drugs. The strong resistance of the particle to acidic degradation and its prompt dissolution in neutral environments demonstrate its feasibility for targeted colonic drug delivery via the oral route. Such a platform potentially could be used in the clinic to increase the efficacy, decrease the side effects, and ease the administration of currently available biologics. It also could be used to deliver promising new biologic therapies, such as IL-10, to the intestinal mucosa. Furthermore, our platform may accommodate the packaging and subsequent targeted delivery of several different biologics with synergistic mechanisms of action simultaneously within the same particle. This novel pH-responsive particulate drug delivery vehicle, therefore, represents a promising new modality for the treatment of IBD.

Acknowledgments: The authors thank Dr. Clara Abraham for valuable conversations and Dr. Ragy Ragheb for imaging assistance. This work was funded by an NIH CTSA T32 grant awarded through the Yale School of Medicine.

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