Controlled Local Delivery of Therapeutic Antibodies from Injectable Hydrogels

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Statement of Purpose: Therapeutic antibodies are currently used for the treatment of various diseases ranging from cancers to arthritis to immune and inflammatory disorders. As the popularity for using antibodies as therapeutic drugs continues to rise, and as large doses are typically used, the production capacity for these antibodies may not be able to keep up unless dosing regimens can be decreased through more effective delivery.[1] Furthermore, localized delivery is an important area of research to minimize possible systemic side effects. Injectable biopolymer systems are an attractive solution due to their minimally invasive potential for controlled release in a localized area. Alginate and chitosan are widely available naturally occurring biocompatible polysaccharides with unique properties. Alginate can be ionically crosslinked with calcium to form a hydrogel; however, this anionic hydrogel is not optimal for controlled antibody release. Introducing the polycation chitosan to this system will increase electrostatic interactions with antibodies, and compositional changes of the biopolymers could allow for control of antibody release rates.

Methods: In this study, hydrogels were formed from alginate (20/40, FMC Biopolymer) and chitosan (Protasan CL213, FMC) with calcium sulfate (Sigma Aldrich). Alginate release studies were conducted by encapsulating IgG, a model antibody, in 2%w/v alginate, and crosslinking with 105 mg/ml CaSO4. Hydrogels were formed at a thickness of 0.75 mm and a diameter of 11.1 mm. These disks were placed in 1 ml PBS and agitated at 37ºC. Samples were taken by changing the PBS and the IgG concentration was determined by a BCA Protein Assay (Pierce Biotechnology). Hydrogels of alginate and chitosan were formed at four ratios 100:0, 90:10, 70:30, and 50:50 alginate to chitosan. 2% wt alginate and 2% wt chitosan solutions were mixed in the appropriate volume ratios. Hydrogels with a 5 mg/ml initial IgG concentration were made into disks 2 mm in height and 12.7 mm in diameter. Disks were loaded into Transwell® membranes and placed into 1 mL of PBS solution and incubated at 37°C. PBS solution was replaced at various time intervals and IgG content was determined by a Micro BCA Protein Assay (Pierce Biotechnology).

Results: Alginate alone provides little diffusional resistance to control the delivery of IgG (Figure 1). From these hydrogels, 98% of IgG was released by the end of the first day. In contrast, Figure 2 shows the release profiles from several alginate-chitosan mixtures. The addition of chitosan to the alginate gels significantly decreased the release rate of IgG from the hydrogels. Compared to the burst release exhibited by alginate-only hydrogels in the first week, the 50:50 alginate-chitosan hydrogels exhibited an almost-linear release over the

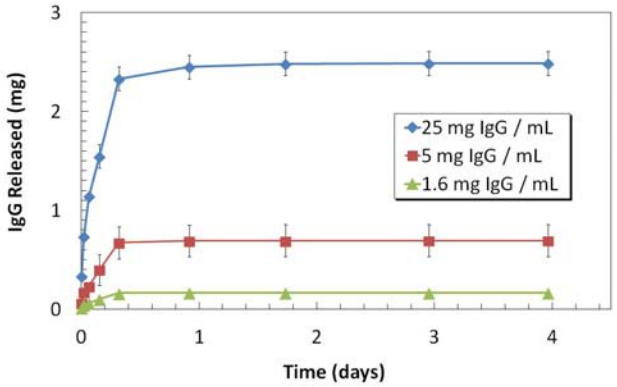

Figure 1. Total IgG released from alginate hydrogels over 4 days.

intermediate chitosan concentrations also delayed the release of the IgG to some extent. This decrease in IgG release rate in alginate-chitosan hydrogels is believed to be due primarily to increased electrostatic interactions between the overall negatively-charged IgG antibody and the positively-charged chitosan. The observed decrease in release rate could also be due to porosity changes to the material caused by ionic interactions between the alginate and chitosan. The drop in the release rate between the 70-30 and the 50-50 hydrogels is the largest observed in this experiment.

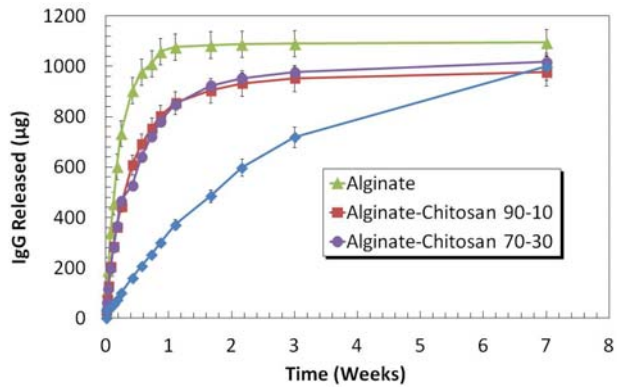

Figure 2. Total IgG released from alginate-chitosan hydrogels over 7 weeks.

Conclusions: Alginate hydrogels release antibody too quickly to provide controlled delivery on a useful time scale. The addition of chitosan to the alginate hydrogels provides substantial control over the rate of antibody release from the system and allows for controlled delivery over an extended time. We are also examining microsphere systems for additional control over the release profiles, and currently moving into cell studies with therapeutic antibodies for cancer.

References: 1) Grainger DW. Expert Opin Biol Ther. 2004;4:1029-1044.