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Controlled delivery of antibodies from injectable hydrogels

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ABSTRACT

Therapeutic antibodies are currently used for the treatment of various diseases, but large doses delivered systemically are typically required. Localized controlled delivery techniques would afford major benefits such as decreasing side effects and required doses. Injectable biopolymer systems are an attractive solution due to their minimally invasive potential for controlled release in a localized area. Here, alginate–chitosan hydrogels are demonstrated to provide controlled delivery of IgG model antibodies and also of Fab antibody fragments. Also, an alternate delivery system comprised of poly(lactic-co-glycolic acid) (PLGA) microspheres loaded with antibodies and encapsulated in alginate was shown to successfully provide another level of control over release. These biopolymer systems that offer controlled delivery for antibodies and antibody fragments will be promising for many applications in drug delivery and regenerative medicine.

1. Introduction

Therapeutic antibodies are clinically used for the treatment of a wide spectrum of diseases ranging from cancers and arthritis to immune and inflammatory disorders. Antibodies account for about 16% of the global biotechnology industry and 20% of the current drugs in clinical trials [1]. Antibodies are large proteins that bind specifically to a target protein or antigen. Antibody fragments are also being increasingly researched as therapeutics; they retain the targeting ability of the full-size antibodies but their smaller size provides better tissue penetration, lower immunogenicity, and often more economical production [2,3]. There are many instances when a localized delivery of an antibody therapeutic would be preferred over systemic delivery, as it would provide enhancement of the effect at the site of interest, and would decrease side effects, decrease dosing requirements and thereby also decrease associated cost. Also, with this rising popularity, production capacity may be seriously strained unless dosing can be decreased, or alternatives to therapeutic antibodies or their production methods are innovated [1]. Thus, it is critical to develop effective delivery strategies for antibody therapeutics.

Overall there has been relatively little work in the area of controlled antibody delivery. From the mid-1990s to early 2000s, one group published several papers describing the use of poly(ethylene-co-vinyl acetate) (EVAc) for the sustained release of antibodies [4–9]. In one of these, the injection of IgG antibody directly in the brain was examined, and the antibody levels dropped off considerably within the first few days; in contrast, antibody released from EVAc provided higher IgG levels within the brain for 28 days following implantation [4]. These studies demonstrate that a controlled release system for antibodies has the potential to be successful for providing sustained exposure of the area to the antibodies. However, these polymer implants examined were formed with harsh solvents, which have the potential to impact antibody effectiveness, and the polymer was not degradable, so the patient might require an additional surgery at the end of treatment to remove it. Another group has developed hyaluronic acid scaffolds with tethered nogo-66 receptor antibody to promote axonal growth after stroke or spinal cord injury [10–12]. An antiangiogenic antibody called bevacizumab (product name Avastin®, Genentech) which targets vascular endothelial growth factor (VEGF) is currently being examined for various clinical applications, including for the treatment of various cancers [13], for use in the eye for macular degeneration, and other conditions such as hypertension, thromboembolic events, intestinal perforation, and neutropenia [14]. One study examined the delivery of bevacizumab from blended glycol chitosan and oxidized alginate hydrogels for possible use in ocular drug delivery, but release was complete after 3 days [15]. To date, there have been no studies demonstrating the ability to provide controlled and sustained release over time of antibodies from degradable biopolymers.

The work presented here demonstrates several biopolymer systems (alginate hydrogels, alginate–chitosan hydrogels and PLGA microspheres embedded in alginate) that are capable of providing highly controlled, sustained, localized release of antibodies and antibody fragments. Injectable drug delivery biopolymer systems are attractive due to their minimally invasive potential for controlled drug release in a localized area. Alginate and chitosan are widely available, naturally occurring, biocompatible and biodegradable polysaccharides with unique properties. Alginate, an anionic polysaccharide comprised of β-

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D-mannuronate (M) and α-L-guluronate (G) subunits, is extracted from brown algae. It can be ionically crosslinked with calcium or other divalent cations to form a hydrogel. Chitosan is a cationic polysaccharide made from deacetylated chitin; it is acid soluble but can be modified to be water-soluble. These two charged polysaccharides can readily form ionic bonds with each other to form polyelectrolyte complexes [16]. Another widely used polymer in the controlled delivery field is poly(lactic-co-glycolic acid) (PLGA), which degrades by hydrolysis into the monomers lactic acid and glycolic acid. This hydrophobic polymer can be readily formed into microspheres; many properties of these microspheres can be adjusted to alter their degradation and release rate such as sphere diameter, ratio of lactic acid units to glycolic acid units, and production method [17–19].

Here, alginate and alginate–chitosan hydrogels are examined for their abilities to provide sustained release of IgG, a model antibody, and IgG Fab antibody fragments over time. The material properties of these hydrogels including permeability, diffusivity, degradation, and swelling were characterized. Also, a system consisting of IgG-laden PLGA microspheres embedded in alginate hydrogels was characterized for prolonged release of IgG over time. These various biopolymer systems providing controlled delivery of antibodies and antibody fragments over time hold much promise for future use in drug delivery and regenerative medicine applications.

2. Materials and methods

2.1. Materials

Protanal LF 20/40 alginate and Protasan UP CL 213 water-soluble chitosan were generous gifts from FMC BioPolymer (Philadelphia, PA). The alginate was purified by dialysis for 4 days, subjected to activated charcoal treatment, sterilized through a 0.22-μm filter, frozen and lyophilized until dry. Poly(lactic-co-glycolic acid) (PLGA) polymers with lactic acid to glycolic acid monomer ratios of 85:15, 75:25, and 50:50, all having inherent viscosities of 0.55–0.75, were obtained from LACTEL Absorbable Polymers (Birmingham, AL). Purified Immunoglobulin G (IgG) and IgG Fab antibody fragments derived from human plasma were purchased from Athens Research and Technology (Athens, GA). Polyethyleneimine (PEI) with an average Mw of 750,000 was obtained from Sigma (St. Louis, MO). Transwell® polyester membranes with 12 mm diameter and 0.4 μm pore size membranes were obtained from Corning Incorporated (Corning, NY). Phosphate buffered saline (PBS) with calcium and magnesium was obtained from HyClone (Logan, UT). Zeba Desalt Spin Columns were obtained from Pierce (Grand Island, NY). All other chemicals were obtained from Sigma.

2.2. IgG release from alginate–chitosan hydrogels

Hydrogels were formed using solutions of 2 wt.% alginate in PBS, 2 wt.% chitosan in PBS, a calcium sulfate slurry solution (105 mg/mL calcium sulfate in PBS), and 100 mg/mL IgG in PBS. For the alginate hydrogels, 2 mL of alginate solution and 100 μL of IgG solution (or PBS for controls) were loaded into one 3 mL syringe (Becton Dickinson), while 160 μL of the calcium slurry was added to a second 3 mL syringe. For the alginate–chitosan hydrogels, chitosan solutions were loaded into the second syringe with the 160 μL calcium sulfate slurry. The amount of alginate and chitosan solutions were determined based on the desired ratio of alginate to chitosan of the hydrogels, with 1.8 mL, 1.4 mL, and 1 mL of alginate solution mixed with 0.2 mL, 0.6 mL, and 1 mL of chitosan solution, respectively, for the 90:10, 70:30, and 50:50 alginate–chitosan hydrogels. The two syringes for each hydrogel type were then connected via a luer lock adaptor and mixed rapidly for 5 s. Then the contents of the syringes were placed between two glass plates separated by 2 mm spacers and allowed to set for 20 min. Then 3 disks, 1/2-inch in diameter, were punched out for each hydrogel type and placed into Transwell membranes. The Transwell membranes were placed into 12 well plates and the wells were filled with 1 mL of PBS prior to the plates being placed in a 37 °C humidified incubator. At pre-determined time points, release samples were taken by collecting the PBS from each well and replacing it with fresh PBS. The released IgG was measured via the microBCA protein assay (Pierce, Grand Island, NY) as per the manufacturer's instructions using IgG for the standard curve.

2.3. Antibody fab fragment release study

Alginate hydrogels were formed using solutions of 2 wt.% alginate in PBS, 2 wt.% alginate in PBS containing 4 mg/mL of Fab antibody fragment, 2 wt.% chitosan in PBS, and calcium sulfate slurry solution (105 mg/mL calcium sulfate in PBS). For the alginate hydrogels, 0.7 mL of alginate solution and 0.3 mL of alginate solution containing Fab antibody fragments (or more alginate solution for controls) was loaded into one 3 mL syringe, and 80 μL of the calcium slurry was added to another 3 mL syringe. For the alginate–chitosan hydrogels, the chitosan solution was loaded into the second syringe with the 80 μL calcium sulfate slurry. The amount of alginate or chitosan solution used depended on the desired ratio of alginate to chitosan of the final hydrogels. The contents were mixed as above, and then dispensed into three Transwell® supports in 250 μL aliquots via a 1 mL syringe (Thermo Fisher Scientific Pittsburgh, PA). The Transwell membranes were then placed into 12-well plates filled with 0.5 mL of PBS and put in a 37 °C incubator. At pre-determined time points, release samples were taken by collecting the PBS from each well and replacing it with fresh PBS. The released IgG was measured via the microBCA protein assay using Fab antibody fragments for the standard curve.

2.4. PLGA microsphere fabrication, characterization, and release

PLGA microspheres were made by the double emulsion evaporation extraction method similar to that described by Sah [20]. This process required the following solutions: 5% *w/v* PLGA in ethyl acetate, 100 mg/mL of IgG in PBS, 5% w/v poly(vinyl alcohol) (PVA, MW 9–10 kDa) in 7% ethyl acetate in water (secondary emulsion solution), and 0.3% w/v PVA in 7% ethyl acetate in water (extraction solution). Preparation of the primary emulsion required the addition of 100 μL of IgG solution to 1 mL of the PLGA solution, followed by 15 s of sonication at a frequency of 20 W in an ice bath. 1 mL of the secondary emulsion solution was then added and the mixture vortexed for 15 s. The secondary emulsion was then poured into 200 mL of the extraction solution and stirred continuously for 3 h. The extraction solution was then filtered, rinsed with DI water, and passed through a 70 μm cell strainer to remove larger clumps. The collected microspheres were then centrifuged for 10 min at 4696 g and then rinsed three times in water. They were flash frozen in liquid nitrogen for 5 min and lyophilized. Microspheres were formed at three lactic acid to glycolic acid ratios: 85:15, 75:25, and 50:50. Microspheres were formed with IgG encapsulated and also without (blank controls). Images of the microspheres were taken on a FEI Quanta 600i Environmental Scanning Electron Microscope with a voltage of 20 kV and 3500× magnification after gold sputter coating. The diameters of the microspheres were measured using ImageJ analysis of 3 scanning electron photomicrographs for each microsphere type. To form hydrogels with the microspheres and measure release, the microspheres were mixed with an alginate solution at a ratio of 20 mg microspheres/mL alginate, and then crosslinked with calcium sulfate to form hydrogels, and release measured over time as described in Section 2.2.

2.5. Swelling and degradation

Alginate–chitosan hydrogels were formed as described in Section 2.3 above, placed in 1.0 mL PBS, and then placed in a 37 °C humidified