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Controlled release of monoclonal antibodies from poly-L-lysine-coated alginate spheres within a scaffolded implant mitigates autoimmune responses to transplanted islets and limits systemic antibody toxicity

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ABSTRACT

Immunomodulatory monoclonal antibodies (IM-mAbs) are a cornerstone of modern immunotherapy; however, when administered systemically (*i.e.*, *via* injection), these agents can generate a variety of negative side effects. For many diseases, systemic delivery of IM-mAbs is the most effective mode of treatment, but in instances where the cellular target occupies a limited, well-defined space (*e.g.*, solid tumors or cellularized implants) local, controlled release of IM-mAbs might be desirable. Antibodies are highly sensitive to a variety of environmental conditions, which limit the kinds of polymers suitable for antibody retention and controlled release. The present study evaluates the release of antibodies from biocompatible, 2-mm diameter alginate spheres coated with poly-L-lysine and a thin outer layer of alginate (APA spheres). *In vitro*, rates of antibody release (including IM-mAbs) could be incrementally decreased and made linear by incrementally increasing the quantity of poly-L-lysine deposited on the alginate, with linear release lasting in one scenario for at least 46 days. To evaluate the bioactivity *in vivo* of IM-mAbs, APA spheres loaded with either anti-CD3ε or anti-CD95 mAb were incorporated into scaffolded islet implant (SI) test-beds and the SIs implanted into a mouse model of autoimmune (type 1) diabetes. Release of mAbs within the implanted SIs resulted in reduced autoimmune responses to both transplanted and native islets. Notably, mice implanted with APA spheres loaded with quantities of anti-CD95 mAb that would be lethal if given systemically showed immunomodulation with no toxic side effects. Collectively, our results indicate that APA spheres are a relatively simple means to evaluate the effects of local, controlled release of IM-mAbs in a way that preserves mAb function and limits systemic toxicity.

1. Introduction

Immunomodulatory monoclonal antibodies (IM-mAbs) are a cornerstone of modern immunotherapy, due to the high specificity of mAbs for their molecular targets and the diverse range of mAbs targeting different molecular/cellular components of the immune system. Approximately 50 mAbs are approved therapeutically [1] (most are IM-mAbs) and several hundred mAbs are investigational agents. Like other immunomodulatory compounds, IM-mAbs are usually administered systemically, which can generate negative side effects such as tissue/organ toxicity and enhanced susceptibility to infections and cancers. Some side effects can be severe enough to require treatment with additional drugs to control symptoms.

For many diseases, systemic delivery of IM-mAbs is the most effective mode of treatment; however, in instances where the cellular target occupies a well-defined space of limited volume, controlled release of IM-mAbs in the immediate vicinity of the target might be particularly beneficial. Such local delivery could provide concentrated, therapeutically-effective doses of mAb to the target while keeping systemic levels of mAb low, thereby mitigating side effects.

Pre-metastatic, solid tumors are perhaps the most obvious candidates for treatment with locally delivered mAbs; however, the approach might also be applied to some forms of cellular transplantation, such as strategies to treat type 1 diabetes (T1D) that utilize fixed-volume scaffolds loaded with donor islets or stem cell-derived islet equivalents. In the context of islet transplantation, we have developed *scaffolded islet implant (SI)* test-beds to evaluate the survival and function of transplanted islets in mesenteric or subcutaneous graft sites. The SIs, sized for mice, consist of a disk-shaped, open-pore polyvinyl alcohol (PVA) sponge scaffold containing one or more cavities filled with islets

Abbreviations: APA, alginate-poly-L-lysine-alginate; BGL, blood glucose level; HA, hyaluronan; IM-mAbs, immunomodulatory monoclonal antibodies; OVA, ovalbumin; PLL, poly-L-lysine; PVA, polyvinyl alcohol; SI, scaffolded islet implant; TCR, T-cell receptor; T1D, type 1 diabetes

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suspended in a collagen gel. SIs loaded with 400–500 syngeneic islets and implanted on the gut mesentery or under the skin of mice with chemically-induced diabetes become vascularized within a few weeks and mediate long-term reversal of diabetes [2, 3]. To address the major problems of: 1) lack of long-lasting immune tolerance to transplanted islets and 2) negative side effects of systemic immunosuppressive therapy (which is of lifelong duration in islet transplant patients), we are exploring the potential for time-limited, controlled release of IM-mAbs locally within the SI to protect transplanted islets from rejection.

Antibodies are highly sensitive to elevated temperatures, pH extremes, hydrophobic interfaces, and harsh chemical crosslinkers [4–7] – factors which limit polymers suitable for mAb retention and controlled release. Alginate gels are advantageous as they are chemically inert, non-toxic, non-immunogenic, form at physiological pH and temperature, are hydrophilic, and are crosslinked with non-toxic divalent cations (*e.g.*, Ca^{++}). Alginate has been used to deliver cationic bioactive proteins [8–10]; however, strong retention of mAbs by alginate alone is problematic, as many mAbs are not strongly cationic – isoelectric points of 12 FDA-approved intact mAbs averaged ~8.5 [11]. To more reliably control mAb release from alginate, we have experimented with poly-L-lysine (PLL)-coated alginate spheres.

Cationic polyamines such as poly[allylamine] and PLL have proven useful in a variety of strategies for controlled release. These molecules can interact with multivalent anions to form crosslinked polymer aggregates with a net positive charge that can direct the assembly of anionic silica nanoparticles into ordered, spherical microcapsules [12, 13]. PLL has also been incorporated into nanoparticles as: 1) a structural/stabilizing component, 2) a counterion to bind anionic cargoes, or 3) a factor to improve cellular uptake *via* engagement of cell membrane polyanionic phospholipids. Such microcapsular or nanoparticle constructs have been used for the delivery/controlled release of a variety of bioactive agents such as drugs (*e.g.*, curcumin [14–17] and paclitaxel [18]), DNA [19, 20], si/miRNA [21–23], hormones [20], and growth factors (*e.g.*, bone morphogenetic protein-2, vascular endothelial growth factor, tumor necrosis factor-α, and nerve growth factor [24–27]).

PLL can also bind electrostatically to the surface of micro- or macro (millimeter)-scale spherical alginate gels to form a thin, strong membrane [28, 29] that resists transit of chemical compounds. To improve biocompatibility [30], a thin alginate coating is applied over the PLL layer to create an *alginate-PLL-alginate (APA)* sphere. APA spheres loaded with therapeutic compounds of low molecular weight show short-term (48 h *in vitro* for thalidomide [31]) and longer-term (21 days *in vivo* for vancomycin [28]) release profiles. *In vivo* (in rabbits), APA spheres were resorbed slowly with only a mild host reaction [28]. To our knowledge, there are no published reports examining release of antibodies from APA spheres. The present study evaluates the characteristics of release of antibodies from macro-sized APA spheres *in vitro* and demonstrates that APA spheres loaded with IM-mAbs, incorporated in SIs, and implanted into mice can release biologically-active mAb that influences immune responses to transplanted islets.

2. Materials and methods

2.1. Scaffold fabrication

Disk-shaped SI scaffolds were made of 2-mm thick, medical-grade Merocel® CF90 PVA sponge (500 μm average pore size with no surfactant treatment; Medtronic, Minneapolis, MN). Scaffolds were 8 mm in diameter with one 3-mm diameter cavity to hold islets and three 2-mm diameter cavities to each hold one APA sphere. Disks and cavities were cut with seamless, sterile biopsy punches (Sklar Surgical Instruments, West Chester, PA) [2]. We have found that the PVA sponge material elicits no appreciable foreign body reaction and is well-tolerated by mice [2].

2.2. Preparation of APA spheres

All steps were performed at room temperature under sterile conditions. To prepare 2-mm diameter APA spheres containing antibodies, 4% aqueous stock solutions of Na alginate (Pronova™ UP-LVG from FMC Corp., Philadelphia, PA) was combined in a 1:1 volume ratio with antibody stock solution in phosphate-buffered saline (PBS). Aliquots of the mixture (10 μL or 15 μL) were micropipetted onto the end of a downward-projecting, sharply-pointed triangle made of hydrophobic Parafilm™ "M" (Pechiney Plastic Packaging, Chicago, IL) and allowed to fall 8 cm into a 60 mm plastic petri dish filled with 100 mM $CaCl_2$, which resulted in the formation of spheres. The spheres were incubated for 30 min to fully gel the alginate and then washed by gently swirling 3 × 1 min in normal saline supplemented with 25 mM HEPES and 2 mM $CaCl_2$, pH 7.5 ("SHC buffer") in the 60 mm petri dish. The washed spheres were then gently swirled on a rotating shaker for 30 min in the petri dish in saline containing various concentrations of PLL (15–30 kDa PLL HBr, Product 7890, Sigma-Aldrich). Subsequently, the spheres were washed 2 × 1 min in SHC buffer and then gently swirled for 5 min in saline-0.1% alginate. The completed APA spheres were stored in SHC buffer for < 1 h prior to use in experiments. Antibodies incorporated into the APA spheres were the following: Fluorescein isothiocyanate (FITC)-tagged polyclonal goat anti-mouse IgG (Product ab6785, Abcam, San Francisco, CA), FITC-tagged or non-tagged Armenian hamster anti-mouse CD3ε mAb clone 145-2C11 (Products 11–0031 and 16–0031, ThermoFisher, Waltham, MA), and FITC-tagged or non-tagged Armenian hamster anti-mouse CD95 mAb clone Jo2 (Products 554257 and 554254, BD Biosciences, San Diego, CA). For mAb experiments conducted *in vivo*, control APA spheres substituted PBS for the mAb solution. Initial tests of the barrier effects of APA coatings were performed on alginate spheres loaded with 16.8 μg of high molecular weight (1.5×10^6 Da) FITC-tagged hyaluronan (HMW-HA) (Lifecore Biomedical, Chaska, MN).

2.3. Measurement of HMW-HA and antibody release from APA spheres in vitro

To measure release of FITC-tagged antibodies or HMW-HA from APA spheres, wells of a 24-well tissue culture plate were filled with 1 mL of SHC buffer, a single APA sphere was placed in each well, and the plate was incubated at 37 °C and 100% humidity. Media were changed at intervals of 1 or 3 days for antibody-release experiments and 3–7 days for HMW-HA experiments. Levels of fluorescence in the collected media were determined by fluorescence spectrophotometry using an EnSpire™ multimode plate reader (PerkinElmer, Waltham, MA) and quantitated from fluorescence plots of serially-diluted standards.

2.4. Assembly of the SI with PVA scaffold, islets, and APA spheres

Isolation of islets from mice was performed as described previously [2]. The isolated islets were resuspended in a collagen solution containing one volume of rat tail native type I collagen stock in dilute acetic acid (Product 354236, Corning/Discovery Labware, Bedford, MA), 1/9 volume of 10-strength Medium 199 (Gibco/Life Technologies, Grand Island, NY) saturated with $NaHCO_3$, and sufficient Dulbecco's Modified Eagle Medium (DMEM; Gibco), normal mouse serum (NMS), and 1 N NaOH to yield a pH 7.4 solution containing 2.5 mg/mL collagen and 10% NMS. To assemble each SI, a PVA sponge scaffold was hydrated with sterile water, blotted on sterile gauze to remove excess water, and placed in a small, sterile, humidified chamber with a hydrophobic, nonstick surface. The APA spheres were placed in the 2-mm cavities of the sponge and a suspension of ~200 islets in the collagen solution was pipetted into the single 3-mm cavity. Subsequently, a small volume of the collagen solution was pipetted over the APA spheres to provide extra support. The assembled SI was then heated in the humidified chamber for 30 min in a 37 °C cell culture incubator to