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Prevention of foreign body reaction in a pre-clinical large animal model

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ABSTRACT

In this work, the foreign body reaction (FBR) to small subcutaneous implants was compared between small (rodent) and large (swine) animal species for the first time. Dexamethasone-releasing poly(lactic-co-glycolic acid) microspheres/polyvinyl alcohol hydrogel composite coatings were adapted to prevent FBR to small, subcutaneous implants in a large animal model (Goettingen minipigs). The implants consisted of small silicon chips (used to mimic small medical devices) that were coated with the composite formulations. The stages of the FBR were compared with previous studies in rats (that used the same-sized implants); the onset and severity of chronic inflammation (collagen deposition) was identified as a key difference between the two species. In the absence of inflammation control, fibrosis was observed from day 7 post-implantation in minipigs, whereas in rats this did not occur until day 14. This is significant as swine skin is the most commonly used model for pre-clinical testing of dermal formulations. It was determined that for long-term prevention of the FBR (longer than 24 h), a lag phase in dexamethasone release between days 1 and 10 did not affect the anti-FBR properties of the implant in rats. However, continuous release of dexamethasone, with no lag phase, was necessary to prevent inflammation in minipigs (effective dexamethasone dose was 100 μg delivered immediately after implantation and 10 μg/day delivered continuously thereafter). This study offers significant insight into the translation of anti-FBR strategies across species, and showcases the importance of tailoring the controlled release kinetics of the formulation to the host response.

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1. Introduction

Implantable biomaterials such as biosensors are recognized by the immune system as foreign; this leads to a cascade of events collectively known as the foreign body reaction (FBR) [1,2]. The FBR consists of two main phases: an acute phase, characterized by the infiltration of inflammatory cells, mainly neutrophils, and a chronic phase, characterized by the presence of active fibroblasts (cells that deposit collagen fibers around the implant) [1,3–5]. The collagen fibers will ultimately encapsulate the foreign body in a dense, fibrous collagen layer (fibrosis).

The FBR has been the focus of many research studies over the past decades. Most of the early work in this area was related to organ rejection prevention [6–8]. In recent years, the emergence of implantable medical devices has led to the FBR being investigated to extend device lifetime [9–12]. In the case of subcutaneously implanted devices, their size, shape, mechanical properties, type of biomaterial, implantation duration and even method of implantation can yield a different response. The FBR can be minimized by using materials with mechanical properties similar to those of the surrounding tissue [13–15], by incorporating hydrophilic coatings that prevent protein adsorption (biofouling) [16–20] and by using biocompatible materials that do not produce toxic or irritating by-products upon degradation [21–24]. However, these approaches only minimize the FBR but do not eliminate it altogether. The only method that has been shown to completely prevent the FBR is the use of local delivery of anti-inflammatory agents which prevent infiltration and further attraction of inflammatory cells [25–32]. Systemic administration of anti-inflammatory agents and immuno-suppressants is regularly used to prevent organ rejection [33–41] but it is not a desirable approach for medical devices and implantable biomaterials due to the high risk-to-benefit ratio.

Dexamethasone, a synthetic glucocorticoid, is the most commonly used anti-inflammatory agent to prevent FBR [26,27,29,31]. Due to its potency, only small amounts of dexamethasone are required; this is essential for small implants where space is limited. Implant coatings designed to suppress the FBR for implantable glucose biosensors have been previously reported and their efficacy has been tested in several rat models (normal, diabetic, and obese) for one and three-month implantation durations [9,11,28,42–46]. Dexamethasone was delivered throughout the implantation period by incorporation in poly(lactic-co-glycolic acid) (PLGA) microspheres that were embedded in a polyvinyl alcohol (PVA) hydrogel coating. While studying the efficacy of these coatings in small animals is necessary as proof-of-concept, it is accepted that studies in larger animals are required in order to extrapolate the results to design the first-in-human clinical trials. The Goettingen minipig,

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a breed of miniature swine, is a common animal model for dermal studies due to the similarities between human and swine skin [47–50]. The objective of the present work was to study the FBR to miniaturized implantable biomaterials in the Goettingen minipig, identify key parameters that will determine anti-FBR dosing regimens of dexamethasone, and apply the findings for long-term prevention of the FBR. To achieve this, PLGA microsphere/PVA hydrogel composites that release dexamethasone in various amounts and rates (as determined in previously published work from our group [51]) were prepared. The composites were used to coat silicon chips, mimicking an implantable biosensor, and were implanted in the subcutaneous tissue of Goettingen minipigs. The local tissue reaction to the implants was determined histologically at multiple time points after implantation.

2. Materials and methods

2.1. Materials

Dexamethasone was purchased from Cayman Chemical Company (Ann Arbor, MI). High-molecular weight poly(vinyl alcohol) (HMW-PVA, MW 30–70 kDa), was purchased from Polysciences, Inc. (Warrington, PA) and low-molecular weight PVA (LMW-PVA, 99% hydrolyzed, MW 133 kDa) was purchased from Sigma-Aldrich (St. Louis, MO). PLGA Resomer® RG503H (inherent viscosity 0.32–0.44 dl/g) was a gift from Boehringer-Ingelheim and PLGA DLG2A (inherent viscosity 0.15–0.25 dl/g), was a gift from SurModics Pharmaceuticals (Birmingham, AL). Methylene chloride and dimethyl sulfoxide (DMSO, ACS grade) were purchased from Fisher Scientific (Pittsburgh, PA).

2.2. PLGA microsphere preparation

Dexamethasone-loaded PLGA microspheres were prepared as previously described [9]. Briefly, 2 g of PLGA was dissolved in 8 ml methylene chloride (DCM). 200 mg of crystalline dexamethasone was added to the polymer solution and the mixture was homogenized at 10,000 rpm for 2.5 min using a T 25 digital ULTRA-TURRAX® homogenizer (IKA® Works, Inc.) to obtain a homogenous suspension. The suspension was subsequently transferred to 40 ml of 1% w/v LMW-PVA aqueous solution and homogenized for 1 min at 10,000 rpm to obtain a solid–oil–water emulsion. The emulsion was transferred to 500 ml of 0.1% w/v LMW-PVA aqueous solution and stirred at 600 rpm under vacuum for 3 h to remove the DCM. The hardened microspheres were purified via three centrifugation cycles at 3500 rpm for 2 min each, freeze dried and stored at 4 °C until further use. Blank microspheres were prepared in the same way without the addition of dexamethasone. PLGA polymers of two molecular weights were used in different preparations: 25,000 g/mol (50:50 Resomer 503H) and 12,000 g/mol (50:50 DLG 2A).

2.3. Preparation of implants

Implants were of cylindrical shape and consisted of a rectangular silicon chip core ($5 \times 0.5 \times 0.5$ mm) coated with PVA hydrogel embedded with PLGA microspheres (7–11 mm length, 1.5 mm diameter when hydrated). To coat the silicon chips, 75 or 150 mg of microspheres was suspended in 1 ml of 5% w/v HMW-PVA aqueous solution. The mixture was vortexed and placed in a sonicated bath for 10 s to achieve good microsphere distribution and break any aggregates. The PVA hydrogel was formed after physical crosslinking of the HMW-PVA via three freeze–thaw cycles. First, the suspension was subjected to one freeze–thaw cycle (2 h at −20 °C and 1 h at ambient temperature). After the first cycle, the partially thickened suspension was fed in a two-piece grooved mold (grooves of 1.5 mm in diameter). The silicon chips were sandwiched between the two mold pieces that were then subjected to two more freeze–thaw cycles to complete the PVA crosslinking and form a self-supporting hydrogel around the chips. Each mold was used to coat 30 silicon chips of approximately 2 mg weight. Please note that low-molecular weight PVA was used as a surfactant to improve emulsion stability during the microsphere preparation process, while high-molecular weight PVA was used to form a hydrogel. Hydrogel strips containing the silicon chips were air-dried and cut at 7, 9 or 11 mm length implants. The implants were placed in 16 gauge needles and stored at 4 °C until further use. Different implant formulations were labeled as shown in Table 1.

2.4. PLGA microsphere characterization

Particle size: An Accusizer 780 (Particle Sizing Systems) was used to measure the particle size of the PLGA microspheres. 3–5 mg of dried microspheres was suspended in 1 ml of 0.1% w/v LMW-PVA solution, bath-sonicated for 10 s and analyzed for volume-based average size.

Dexamethasone loading: 5 mg of dried microspheres or composites was dissolved in 1 ml DMSO and then diluted 10 times in phosphate buffered saline (PBS) pH 7.4. Dexamethasone concentration was determined via RP-HPLC (PerkinElmer, Inc.). Mobile phase: acetonitrile/water/phosphoric acid (30/70/0.5%, v/v/v); column: Zorbax® C18 (4.6 mm × 15 cm); detection wavelength: 240 nm; flow rate: 1 ml/min.

2.5. In vivo pharmacodynamics study

Seven young, female Goettingen minipigs were used as a large animal model to study the inhibition of the FBR to subcutaneous implants. Minipigs were studied in iterations of 2 or 3 animals. The number of animals that were utilized for each formulation is indicated in the figure legends. Each study lasted for 30 days. The implants that were tested are shown in Table 1. They were implanted at the back of the animals on days 0, 9, 16, 23, 27, and 29. All implants were spaced at least 5 cm apart to ensure no interference. The area right above the spinal cord was not implanted. The animals were sacrificed on day 30 and the implants with surrounding subcutaneous tissue were harvested and stored in 10% buffered formalin solution (Sigma-Aldrich Co. LLC.). This resulted in implants being excised on days 1, 3, 7, 14, 21, and 30 post-implantation. All time points were ±3 days to allow for unforeseeable delays. The extracted implants that prevented the FBR were analyzed for the remaining dexamethasone content. All animal studies were reviewed and approved by the University of Connecticut's Institutional Animal Care and Use Committee (IACUC) prior to the beginning of the experiments.

2.6. Histological evaluation

Fixed tissues were processed, embedded in paraffin and sectioned in 20 μm films at the University of Connecticut's Pathobiology Department. Tissue sections were stained with Hematoxylin & Eosin (H&E) and the presence and progress of the FBR were evaluated by observation under a light microscope. Normal connective tissue appears pink, adipose tissue white, deposited collagen fibers light pink, and inflammatory cells purple. Digital images representative of the tissue reaction around the implants are presented here.

Table 1
Implant composition and size.

Implant	Microspheres	PLGA per ml PVA solution (mg)	Length (mm)
Control	Blank	75	7
R	1M	75	7
R150	1M	150	7
R9	1M	75	9
R11	1M	75	11
R2W	2W	150	7

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