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PLGA microsphere/PVA hydrogel coatings suppress the foreign body reaction for 6 months

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ABSTRACT

The application of dexamethasone releasing poly (lactic-*co*-glycolic acid) (PLGA) microspheres embedded in a poly vinyl alcohol (PVA) hydrogel coatings have been successfully used in the suppression of the foreign body response (FBR) to implantable glucose sensors. In the current study, dexamethasone-loaded PLGA microspheres were prepared by blending two types of PLGA polymers (RG503H and DLG7E with MW of ca. 25 kDa and 113 kDa, respectively) to achieve long-term (6 months) inhibition of the FBR. The microsphere composition was optimized according to the in vitro drug release profiles. Microspheres with DLG7E/RG503H/dexamethasone = 70/13.3/16.7 wt% composition, when embedded in a PVA hydrogel, provided a continuous drug release for 6 months. By combining the aforementioned microspheres with microspheres composed solely of the DLG7E polymer within a similar PVA hydrogel realized an even longer (> 7 months) in vitro drug release. A heat map was constructed to depict the daily in vitro drug released and elucidate possible lag phases that could affect the pharmacodynamic response. These drug-loaded implant coatings were investigated in vivo (rat model) and showed inhibition of the foreign body response for 6 months. These results suggest that the minimum effective daily dose to counter chronic inflammation is ca. 0.1 μg per mg of coating surrounding a 0.5 × 0.5 × 5 mm silicon implant (dummy sensor). Accordingly, these drug-eluting composite coatings can ensure long-term inflammation control for miniaturized implantable devices.

1. Introduction

Diabetes mellitus is a chronic metabolic disease affecting ca. 10% of the U.S. population and is on a rapid growth trajectory [1]. Monitoring blood glucose levels is critical for diabetic patients to prevent hyper- and hypo- glycaemia, through allowing enhanced accuracy in medication dose control, as well as through adjustment of diet and life style [2]. Advancements in biosensing technologies provide new opportunities for diabetic patients to continuously monitor their blood glucose levels, which are far superior to intermitted finger pricking assessment [3]. These advancements hinge on the ability to suppress the foreign body response (FBR) that gradually impedes analyte (glucose) diffusion to the sensing element [4]. FBR is a series of immunological reactions marked by acute inflammation, chronic inflammation and the formation of a fibrous capsule [5] that necessitate frequent external sensor calibration using finger pricking test strips to account for sensor calibration drift [6].

Various coatings have been developed to overcome the FBR and extend sensor life-time. These include anti-biofouling coatings [7], porous coatings [8] [9], angiogenic agent releasing coatings [10–12], and anti-inflammatory drug eluting coating [13–17]. Dexamethasone, a potent anti-inflammatory drug, has shown significant promise in suppressing FBR for short periods of time [18]. This is based on its suppression of immune cell activation and promotion of anti-inflammatory cytokines [19,20]. Dexamethasone loaded PLGA microsphere/PVA hydrogel composite coatings have been developed to prevent the FBR through continuous release of the drug [14,17,21,22]. The depletion of dexamethasone can induce a delayed inflammatory reaction and therefore the efficacy of these coatings greatly depends on continuous drug release [17,18]. 3-month FBR inhibition was achieved by mixing two different populations of PLGA microspheres with complimentary drug release profiles [16]. Blending low and high molecular weight PLGA was further utilized to achieve a microsphere formulation with long-term drug release, and a 4.5-month efficacy was recently accomplished using this strategy [17]. Upon degradation of the low Mw component, its more hydrophilic PLGA fragments increase water absorption into the polymer matrix, as well as increase the acidity and thereby facilitate the autocatalytic degradation of the higher Mw PLGA

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component in these microspheres. This shortens the lag phase and results in a continuous release profile. In order to further extend drug release from such microspheres, it is necessary to fine tune the formulation and obtain accurate control of the lag phase, which otherwise may result in inflammation [23].

In the current study, dexamethasone loaded PLGA microspheres were prepared via blending two PLGA polymers, RG503H and DLG7E, and the ratio of the two polymers was optimized to achieve a long-term drug release profile of at least 6 months. RG503H is a low Mw PLGA resomer (ca. 25 KDa) that is end capped with a carboxylic group, making it relatively more hydrophilic. This polymer facilitates fast water absorption and the early matrix degradation onset of microspheres. DLG7E on the other hand is a higher Mw PLGA resomer (ca. 113 KDa) that is end capped with lauryl ester group, making it relatively more hydrophobic, thus providing a greater resistance against acid-catalyzed degradation. Here an optimized microsphere formulation composed of DLG7E/RG503H/dexamethasone = 70/13.3/16.7 wt % is described that was characterized in terms of its morphology and drug eluting profiles. This formulation was then investigated in three microsphere/PVA hydrogel composite coatings based on: 1) only the optimized microspheres; 2) a mixture of the optimized microspheres and microspheres prepared solely with the DLG7E polymer to achieve a longer release profile; and 3) a mixture of the optimized microspheres, the microspheres prepared solely with the DLG7E polymer (to achieve a longer release profile) and free dexamethasone powder (to enhance the acute inflammatory phase is controlled). In vitro drug release testing was performed on the coatings lasting for ca. 7 months and dexamethasone stability during the long-term study was monitored using HPLC. These composite coatings (with thickness of ca.150 μm) were applied to silicon implants (0.5 × 0.5 × 5 mm, dummy sensors) and implanted into the subcutaneous tissue of rats for evaluation of 6-month FBR suppression.

2. Material and methods

2.1. Materials

Dexamethasone was purchased from Cayman Chemical (Ann Arbor, MI), poly (vinyl alcohol) (PVA, Mw 30–70 KD, used as an emulsifier for microsphere preparation), sodium chloride (NaCl, ACS grade), sodium azide (NaN_3), sodium phosphate dibasic dihydrate ($Na_2HPO_4{\cdot}2H_2O$), sodium phosphate monobasic (NaH_2PO_4) and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO). PVA (99% hydrolyzed, Mw 133 KD, used for preparation of PVA hydrogel after freeze thaw cycles) was purchased from Polysciences, Inc. (Warrington, PA). PLGA RG503H with a low Mw (ca. 25 KDa) (RG503H, inherent viscosity 0.32–0.44 dl/g) and is a 50%50% resomer of lactic acid and glycolic acid with a carboxylic acid end group. RG503H was a gift from Boehringer-Ingelheim. PLGA 9010 DLG7E (DLG7E, inherent viscosity 0.6–0.8 dl/g) is a 90%10% copolymer of lactic and glycolic acid with its terminal carboxylic group end capped by lauryl ester. DLG7E was purchased from Lakeshore Biomaterials (Birmingham, AL). Methylene chloride (DCM), acetonitrile (ACN, HPLC grade), and tetrahydrofuran (THF, HPLC grade) were purchased from Fisher Scientific (Pittsburgh, PA). NanopureTM quality water (Barnstead, Dubuque, IA) was used for all studies.

2.2. Methods

2.2.1. Preparation and optimization of PLGA microspheres

Dexamethasone-loaded microsphere formulations were prepared using an oil-in-water (o/w) emulsion solvent extraction/evaporation technique as previously reported [23]. Briefly, both PLGA polymers at the specific ratio under investigation were dissolved in 2 ml of methylene chloride and dexamethasone was dispersed into this solution. Following a 20-min sonication period in a bath sonicator, the dispersion was further mixed using a T 25 digital ULTRA-TURRAX homogenizer (IKA Works, Inc., Wilmington, NC) at 10,000 rpm for 1 min. In order to form an emulsion, the organic phase was homogenized into a 10-ml PVA solution (1% (w/v), average Mw 30–70 KDa) at 10,000 rpm for 2.5 min. The emulsion was then transferred into a 125 ml aqueous PVA solution (0.1% (w/v), Mw 30–70 KDa) and stirred at 600 rpm under vacuum. After 2.5 h, hardened microspheres were transferred to 50 ml centrifuge tubes and collected via centrifugation at 1500 rpm for 2 min. The microspheres were then washed three times with deionized water (10 ml each time), recollected using the same centrifugation procedure and dried via freeze drying. The prepared microspheres were stored at 4 °C until further use. Blank microspheres were prepared using DLG7E polymer following the same procedure except that no dexamethasone was added. In order to achieve a formulation with long-term drug release of 6 months, microsphere composition optimization was conducted using a central composite design (3 factors and 5 levels) composed of 20 formulations as previously reported [23]. The design table and 20 formulations are listed in supplementary Table 1.

2.2.2. Characterization of optimized PLGA microspheres

2.2.2.1. Particle size and morphology. The particle size and size distribution was determined using an AccuSizer 780A autodiluter particle sizing system (Nicomp, Santa Barbara, CA). Approximately 5 mg of microspheres were dispersed in 1 ml of 0.1% (w/v) PVA solution (30–70 KDa) and 100 μl of the dispersion were injected into the system for particle size analysis. The morphology of the microspheres was determined using a scanning electron microscopy (a FEI Nova NanoSEM 450 unit). Samples were mounted on carbon taped aluminum stubs and sputter coated with gold for 1.5 min at 6 mA with a deposition rate of 6 nm/min before imaging.

2.2.2.2. Thermal analysis. A TA Q1000 differential scanning calorimeter (DSC) (TA, New Castle, DE) was used to determine the glass transition temperature (Tg) of the optimized microspheres (formulation CCD-18). Modulated DSC was performed with the cycle below: the samples were heated at a rate of 2 °C/min from 4 °C to 80 °C at a modulating oscillatory frequency of 1 °C per 50 s. The thermograms were analyzed using Universal Analysis software (TA Instruments) to determine the glass transition temperature. In addition to the microsphere formulation, thermal analysis was performed on the individual polymers, dexamethasone and the polymer/drug physical mixtures for contrast. The physical mixture was prepared by mixing solid polymer and drug at the same ratio as in the microspheres and grinding to achieve a homogeneous mixture.

2.2.2.3. Powder X-ray diffraction (PXRD). The crystallinity of the optimized microsphere formulation (CCD-18) was determined using PXRD. X-ray diffraction patterns were obtained using an X-ray diffractometer (Model D5005, Bruker AXS Inc., Madison, WI) with Cu-Kα radiation, with 40 kV accelerating voltage and 40 mA current. All the scans were performed with a scanning rate of 2°/min with steps of 0.02° from 5° to 40° at 2θ ranges. PXRD was also performed on the individual polymers, dexamethasone and the polymer/drug physical mixtures for contrast. The physical mixture was prepared using the method described in 2.2.2.2. above.

2.2.3. Preparation of PLGA microsphere/PVA hydrogel composites

2.2.3.1. Preparation of composite coated dummy sensors. Cylindrical implants were prepared using a two-piece grooved mold based method after three freeze-thaw cycles [22]. Four composite coatings were prepared (Table 1) with different microsphere and dexamethasone combinations to achieve various release profiles.

Briefly, the microspheres were suspended using 1 ml of 5% w/w PVA solution (133 KDa) and filled into 1-ml syringes. The PVA solution was pre-filtrated using 0.22-μm sterile filters to ensure sterility. The suspensions were sonicated in a bath sonicator for 10 s followed by one