International Journal of Pharmaceutics 384 (2010) 78–86

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

PLGA/PVA hydrogel composites for long-term inflammation control following s.c. implantation

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ARTICLE INFO

Article history:
Received 17 July 2009
Received in revised form
23 September 2009
Accepted 24 September 2009
Available online 2 October 2009

Keywords:
Biosensor
Continuous drug delivery
Dexamethasone
Foreign body reaction
Implants
Localized delivery
Microspheres

ABSTRACT

Dexamethasone loaded PLGA microsphere/PVA hydrogel composites were investigated as an outer drug-eluting coating for implantable devices to provide protection against the foreign body response. Two populations of microspheres were prepared: 25 kDa PLGA microspheres which had a typical triphasic release profile extending over 30–33 days; and 75 kDa PLGA microspheres which showed minimal release for the first 25 days and then increased to release over 80–85 days. Incorporation of the microspheres in the composites only slightly altered the release profile. Composites containing 25 kDa microspheres released dexamethasone over 30–35 days while composites containing combinations of 25 and 75 kDa microspheres in equal amounts released over 90–95 days. Pharmacodynamic studies showed that composites containing only 25 kDa microspheres provided protection against the inflammatory response for 1 month, however, a delayed tissue reaction developed after exhaustion of dexamethasone. This demonstrated that sustained release of the anti-inflammatory agent is required over the entire implant lifetime to control inflammation and prevent fibrosis. Composites fabricated using combinations of 25 kDa and 75 kDa microspheres controlled the tissue reaction for 90 days. This strategy of combining different microsphere populations in the same composite coating can be used to tune the release profiles for the desired extent and duration of release. Such composites offer an innovative solution to control the foreign body response at the tissue–device interface.

1. Introduction

During the past three decades, efforts have been focused on the development of implantable biosensors for the estimation of body metabolites and biochemical markers for the management of metabolic and other disorders, *e.g.* blood glucose monitoring for diabetes treatment and lactate analysis during cardiac surgery (Wilson et al., 1992). Developing a minimally invasive, implantable and continuous glucose biosensor is an area of intense scientific research because of its potential clinical and economical benefits. It has been demonstrated that intensive insulin therapy guided by frequent blood glucose monitoring effectively delays the onset and slows the progression of diabetic complications (DCCT, 1993; UKPDS, 1998). Continuous monitoring also provides the rate and direction of change in glucose levels which is important for early detection of hypo/hyperglycemic states (Gerritsen, 2000).

Even though advances have been made in analytic techniques, design and fabrication of glucose sensors, the estimation of glucose in physiological fluids can only be performed for short time periods at present (Shaw et al., 1991; Wilson et al., 1992; Daniloff, 1999; Kerner, 2001; Koschinsky and Heinemann, 2001; Wang, 2001; Liu and Ju, 2003; Hover et al., 2005). A progressive loss in sensitivity, accuracy and functionality of the sensor is observed *in vivo* (Moatti-Sirat et al., 1992; Wilson et al., 1992; Gilligan et al., 2004; Kissinger, 2005). The biological instability of the sensor is due to the hostile tissue environment encountered following device implantation (Anderson, 2001; Ratner and Bryant, 2004; van der Giessen et al., 1996), as a result of tissue trauma and associated biofouling (Wisniewski et al., 2001; Hickey et al., 2002b). Tissue trauma results from both injuries during implantation and the long-term presence of the implant. This triggers a cascade of inflammatory and wound healing responses typical of a foreign body reaction (FBR). FBR is characterized by an initial acute phase (minutes to days) and a chronic phase (days to years) with subsequent formation of a collagenous avascular fibrotic capsule around the implant (*i.e.* scarring) (Anderson, 2001; Mitchell and Cotran, 2002). Biofouling

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doi:10.1016/j.ijpharm.2009.09.046

involves adhesion of proteins and cells onto foreign materials and occurs immediately after implantation (Wisniewski et al., 2001). Inflammatory cells release various reactive oxygen species and secrete proteolytic enzymes resulting in decreased tissue pH. These changes may damage sensor components and impair sensor function (Gerritsen et al., 1999). Moreover, the avascular fibrous capsule and biofouling decrease the analyte transport to the sensor, compromising device sensitivity (Sharkawy et al., 1997; Wisniewski et al., 2001). Therefore, controlling inflammation and fibrous encapsulation at the implant site would appear to be critical to achieve a functional biosensor with extended lifetime.

Corticosteroids have been utilized both locally and systemically to prevent the immune response to implants, however, local delivery has shown superior results (Stone et al., 1989; Pepine et al., 1990; Strecker et al., 1998; Hickey et al., 2002b; Blanco et al., 2006). It is possible that therapeutic levels might not be achieved at the implant site after systemic administration (Strecker et al., 1998; Drachman and Simon, 2005). Moreover, long-term systemic use of corticoids produces a number of adverse and toxic effects and may complicate disease states, such as diabetes (Schimmer and Parker, 2001). Microsphere and nanoparticle based drug delivery systems, hydrogels, microparticles embedded in hydrogel matrices and implants containing drug-filled reservoirs have been investigated as a means to deliver anti-inflammatory drugs to the implant site (Hickey et al., 2002a,b; Stevens et al., 2002; Voskerician et al., 2003; Patil et al., 2004, 2007; Galeska et al., 2005; Bhardwaj et al., 2007). Earlier, we reported the development, characterization and *in vivo* evaluation of a composite for implantable devices, based on physically cross-linked (freeze–thaw cycling) poly(vinyl alcohol) (PVA) hydrogels containing dexamethasone loaded poly(lactic-co-glycolic acid) (PLGA) microspheres (Patil et al., 2004; Galeska et al., 2005). These composites have mechanical properties similar to the subcutaneous connective tissue (Galeska et al., 2005) and can be used as an external biocompatible drug-eluting coating for biosensors and other medical devices.

In a recent study, it was reported that controlling only the acute inflammatory phase using composites that release dexamethasone over 1 week did not provide long-term protection (Bhardwaj et al., 2007). A delayed inflammatory tissue response developed after exhaustion of drug from the composites (Bhardwaj et al., 2007). The current study was undertaken to further understand the temporal aspect of drug delivery and develop a composite capable of controlling the inflammatory response for a 3-month time period. Two strategies were explored in the present work to achieve this. First, composites containing PLGA microspheres providing 1 month release (Patil et al., 2004, 2007) were evaluated *in vivo* for 3 months to investigate whether immunosuppression can be maintained for 3 months. Second, composites were modified by incorporating PLGA microspheres capable of releasing dexamethasone over 3 months and these were evaluated *in vivo*. This work provides a strategy to control the inflammatory response to implantable sensors/devices for long-term.

2. Materials and methods

2.1. Materials

PVA (average molecular weight (MW) 30–70 kDa), humic acid and dexamethasone were purchased from Sigma (St. Louis, MO). PVA (99% hydrolyzed; MW 133 kDa) was purchased from Polysciences, Inc. (Warrington, PA). PLGA Resomer® RG503H 50:50 (MW 25 kDa) and PLGA Medisorb® 65:35 DLPF1 (MW 75 kDa) were gifts from Boehringer–Ingelheim, and Purdue Pharma, respectively. PLGA polymers Resomer® 503H (co-polymer ratio 50:50; MW 25 kDa) and Medisorb® DLPF1 (co-polymer ratio 65:35; MW 75 kDa) were selected based on their MWs (Zolnik et al., 2006). Henceforth, PLGA Resomer® 503H will be referred to as 25 kDa and Medisorb® DLPF1 as 75 kDa. Methylene chloride was purchased from Fisher Scientific (Pittsburgh, PA).

2.2. Preparation of PLGA microspheres

Dexamethasone loaded or blank PLGA microspheres (with 25 kDa and 75 kDa molecular weight PLGA) were prepared using a solvent evaporation method as described previously (Zolnik et al., 2005, 2006; Bhardwaj et al., 2007). Briefly, 2 g PLGA was dissolved in 8 ml methylene chloride. For dexamethasone loaded microspheres, 200 mg of dexamethasone was dispersed in this solution. This organic phase was emulsified in 40 ml of a 1% (w/w) PVA (average MW 30–70 kDa) solution and homogenized at 10,000 rpm for 1.5 min using a PowerGen 700D Homogenizer (Fisher Scientific, Pittsburgh, PA). The resultant emulsion was poured into 500 ml of a 0.1% (w/w) PVA (average MW 30–70 kDa) solution and stirred under vacuum to achieve rapid evaporation of methylene chloride. The hardened microspheres were washed three times with de-ionized water and collected by filtration (0.45 μm). The prepared microspheres were dried under vacuum and stored at 4 °C until further use.

2.3. In vitro release from microspheres

In vitro release studies were conducted as described previously (Zolnik et al., 2005, 2006; Bhardwaj et al., 2007). Briefly, *in vitro* release was analyzed at 37 °C using a modified USP apparatus 4 (Sotax *C7 piston pump*, Sotax, Horsham, PA) with flow-through cells (12 mm diameter) packed with glass beads (1 mm). Approximately, 40 mg of microspheres were dispersed in the flow-through cells and 250 ml of 0.1 M phosphate-buffered saline (PBS) was circulated through a fiberglass filter (0.45 μm) at a flow rate of 20 ml/min. One millilitre samples were withdrawn (and replenished) at each time point and analyzed by HPLC using acetonitrile/water/phosphoric acid (35:65:0.5, v/v/v) mobile phase with a Zorbax® Rx C_{18} column (4.6 mm × 15 cm) at flow rate of 1 ml/min. The values are reported as mean ± standard deviation ($n = 3$). The amount of drug in the microspheres was determined by dissolving about 15 mg of microspheres in 10 ml of acetonitrile, filtering (Millex-HV, 0.45 μm, Fisher Scientific, Pittsburgh, PA) and analyzing using the HPLC method, described above.

2.4. Preparation of composites

Microsphere/PVA composites were prepared as described previously (Patil et al., 2004, 2007; Bhardwaj et al., 2007). Briefly, PVA (MW 133 kDa) was dissolved in Nanopoure™ quality deionized water at 80 °C to obtain a 10% (w/w) solution and sterilized by autoclaving. A 4% (w/w) humic acids (HAs) solution was prepared in deionized water and sterilized by filtration. Equal volumes of PVA and HAs solutions were mixed to obtain 5% (w/w) PVA solution containing 2% (w/w) HAs. PLGA microspheres were dispersed in this solution to achieve a homogenous distribution. The dispersion was then filled into 18G needles and subjected to three freeze–thaw cycles. Each freeze–thaw cycle comprised 2 h of freezing at −20 °C followed by 1 h thawing at 24 °C. The prepared composites were stored at 4 °C until further use.

Three types of composites containing different types of PLGA microspheres were investigated: (1) the first type of composite contained 25 kDa microspheres only. For this, 100 mg of microspheres were dispersed per ml of PVA/HAs solution; (2) the second composite contained mixture of 25 kDa and 75 kDa microspheres. For this, 1 g of 75 kDa microspheres were pre-degraded by hydration in 10 ml of PBS for 15 days, filtered and vacuum dried. The