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

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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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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

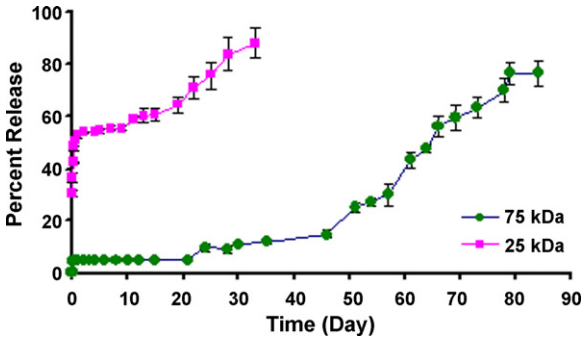

Fig. 1. Dexamethasone *in vitro* release from microspheres prepared using 25 kDa PLGA (Resomer® 503H) and 75 kDa PLGA microspheres (Alkermes DLPF1) in PBS 0.1 M, pH 7.4 at 37 °C using USP apparatus 4. Each value represents the mean ± SD ($n = 3$).

75 kDa microspheres were pre-degraded to avoid the lag phase of release (Fig. 1) and attain dexamethasone release before 25 kDa microspheres get depleted (please refer to Section 3.3.1 for details). One hundred milligrams of these pre-degraded and 100 mg each of 25 kDa and 75 kDa non-degraded microspheres were dispersed in PVA hydrogel to fabricate the composites (ratio 1:1:1) (this is referred as Formulation I hereafter); (3) the third type of composite contained 25 kDa and 75 kDa non-degraded microspheres. 100 mg of each were added to PVA hydrogel to fabricate the composites (ratio 1:1) (this is referred as Formulation II hereafter).

2.5. In vitro release from composites

In vitro release from dexamethasone loaded PLGA microsphere/PVA hydrogel composites were determined as described previously (Galeska et al., 2005). Briefly, composites were immersed in 250 ml Pyrex® glass bottles containing 200 ml of 0.1 M phosphate-buffered saline (PBS) (pH 7.4) and incubated at 37 °C under constant agitation (50 rpm). The release of dexamethasone was monitored by extracting one ml aliquots at each time point and replenishing with fresh PBS. Sink conditions were maintained. Dexamethasone was estimated using HPLC analysis as described in Section 2.3. Cumulative percent release at a given time point was calculated as: cumulative percent release = (amount released at sampling time/total amount released) × 100. The values are reported as mean ± standard deviation ($n = 3$).

2.6. In vivo pharmacodynamic study

All animal studies were conducted at the University of Connecticut in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines using an approved protocol. All procedures were performed as discussed previously (Patil et al., 2004, 2007; Bhardwaj et al., 2007). Composites were implanted into the dorsolateral subcutaneous (s.c.) tissue of male Sprague–Dawley rats using an 18G needle/plunger system. The same needles that were used to fabricate the composites were used for implantation. For implantation, rats were anesthetized using isoflurane (Forane; Baxter; Deerfield IL). The anesthesia was maintained during implantation with 2% (v/v) isoflurane in oxygen. The back of each animal was shaved and washed with betadine solution. Tissue inflammatory response was determined through serial sacrifice to investigate both the acute and the chronic stage anti-inflammatory effects ($n = 6$ at each time point). A histological evaluation of excised tissue samples from the site of implantation was performed after staining with hematoxylin and eosin (H&E) stain. Tissue samples were observed and digitally stored using an Olympus microscope (model A051, Olympus America, Melville, NY) and Bioquant software (BQ-TCWV3.00.6).

Two sequential pharmacodynamic studies were conducted in order to investigate the temporal aspect of drug delivery. For the first study, blank composites (control; no drug) and composites containing dexamethasone loaded 25 kDa microspheres were implanted in the back of the rats on either side of the midline. The study time points were days 30, 60 and 90. In the second study, Formulations I and II were implanted in the same animal (to minimize the number of rats used) and were implanted in the back of the rats on either side of the midline. The study time points were days 7, 14, 21, 28, 45, 60, 75 and 90.

3. Results and discussion

In this work, the feasibility of PLGA microspheres/PVA hydrogel based composite coatings to provide long-term protection against tissue response was investigated. As discussed above, controlling only the acute phase of the inflammatory reaction did not provide long-term protection (Bhardwaj et al., 2007). Therefore, it was first investigated whether controlling both the acute and chronic phase of the inflammatory reaction using composites containing 1 month dexamethasone releasing PLGA microspheres would provide long-term anti-inflammatory protection (over 3 months). Next, the coatings were modified to deliver dexamethasone for a 3-month period.

3.1. In vitro release from PLGA microspheres

The drug loading (7–8%) and particle size (average particle size ca. 20 μm) of both the 25 kDa and 75 kDa microspheres used in this work were similar to that reported earlier for different PLGA microspheres (Zolnik et al., 2006). Fig. 1 shows the *in vitro* release from the microspheres. The 25 kDa PLGA microspheres had a triphasic *in vitro* release profile of dexamethasone over 33 days, characterized by an initial burst release of approximately 50% (w/w) (24 h), a slow lag phase (day 1 to day 15) and then an apparent-zero-order second burst release phase (Fig. 1). The 75 kDa PLGA microspheres showed minimal release until 30 days with a small initial burst release (approximately 5%, w/w) followed by a lag phase (Fig. 1). Most of the drug was released from these microspheres between days 35 and 84. The *in vitro* release profiles from the 25 kDa and 75 kDa PLGA microspheres are consistent with the polymer properties as previously reported (Zolnik et al., 2006). Usually, high MW and higher lactide content of PLGA results in slower release and *vice versa* (Wu, 1995).

3.2. Composites containing 25 kDa PLGA microspheres

3.2.1. In vitro release

Fig. 2 shows release from PLGA microsphere/PVA hydrogel composite containing dexamethasone loaded 25 kDa PLGA microspheres. A triphasic release profile was observed with an initial burst release (ca. 33% in 24 h), followed by a lag phase till day 15 and then a second relatively rapid release phase. The release profile was qualitatively similar to that of 25 kDa microspheres alone (Fig. 1) with two differences: (1) a lower initial burst release and (2) a slightly faster "lag" phase release (days 1–15) were observed from the composites as compared to dexamethasone release from the microspheres. The decrease in the burst release might be due to incorporation of the microspheres into the PVA matrix which created another barrier for diffusion of dexamethasone into bulk media. Moreover, since burst release is due to release of drug that is close to microsphere surface, deposition of PVA onto the microsphere surface is likely to decrease burst release. The slightly faster release rate during the "lag" phase is probably a result of presence

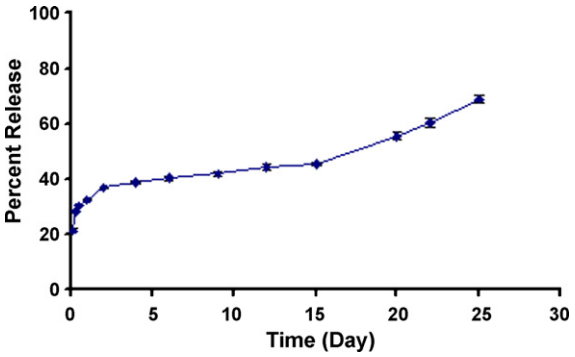

Fig. 2. Dexamethasone *in vitro* release from PLGA microsphere/PVA hydrogel composites containing 25 kDa PLGA (Resomer® 503H) microspheres in PBS 0.1 M, pH 7.4 at 37 °C. Each value represents the mean ± SD ($n = 3$).

of humic acid in the PVA matrix. As reported earlier (Galeska et al., 2005), humic acid increases PLGA degradation and dexamethasone release when incorporated in composites due to localized acidity as a result of its surface active properties. This *in vitro* release profile suggested that dexamethasone release over 25–30 days may be achieved *in vivo*. Accordingly, these composites were evaluated *in vivo* to investigate whether long-term protection from the foreign body response could be achieved.

3.2.2. In vivo pharmacodynamics

Fig. 4 shows the histopathology of the representative tissue sections taken from the vicinity of the control composite coatings containing blank 25 kDa microspheres without drug (Fig. 4A–C) and composite coatings containing dexamethasone eluting 25 kDa microspheres at days 30, 60 and 90 (Fig. 4D–F). The implant is denoted by an asterisk in all photomicrographs. At day 30, a small number of mixed inflammatory cells (predominantly neutrophils and macrophages; black arrow) were present in the tissue surrounding the dexamethasone eluting implants (Fig. 4D). Occasional interstitial infiltrates in the collagenous connective tissue were also observed in the normal s.c. tissue (Fig. 3). This is in agreement with our previous findings that these composites provide anti-inflammatory protection for 4 weeks (Galeska et al., 2005; Patil et al., 2007). On the other hand, in the control tissue samples the implant is surrounded by a granulomatous inflammation characterized by macrophages, multi-nucleated giant cells and a thick fibrous connective tissue (Fig. 4A). These features persisted at days 60 and 90 in control implant tissue samples (Fig. 4B and C). In addition, fragments of the PVA hydrogel matrix were observed and these were surrounded by numerous multi-nucleated giant cells on day 90 in the tissue surrounding the control implants (Fig. 4C). It appears that although the inflammatory cells were unable to digest the implant over time, the giant cells attempted to phagocytose fragments (Anderson, 2001). On day 60, a delayed inflammatory reaction was observed in the tissues surrounding the dexamethasone eluting composites. The inflammatory cells were predominantly macrophages and lymphocytes (black arrows). Abutting this inflammatory reaction is a thick band of fibrous connective tissue (dark green arrow) (Fig. 4E). However, no multi-nucleated giant cells were observed. By 90 days, a fully developed granulomatous reaction was observed in tissue samples surrounding the implants containing the 25 kDa dexamethasone microspheres (Fig. 4F). These results indicate that once dexamethasone release stopped and/or tissue levels fall to sub-therapeutic level, the foreign body inflammatory stimulus prevails. Therefore, a sustained release of dexamethasone is required to prevent tissue response for the lifetime of the implantable device.

3.3. Composites containing mixed population PLGA microspheres

3.3.1. In vitro release

In order to provide sustained drug levels for 3 months, 25 kDa and 75 kDa microspheres were combined in the PVA hydrogel composite as described in Section 2. Based on the *in vitro* release, it was hypothesized that by adding both 25 kDa and 75 kDa microspheres in the same implant composite, sustained dexamethasone levels may be achieved for a 3-month time period. During the first 30–35 days, the 25 kDa microspheres are expected to release dexamethasone and following that the 75 kDa microspheres are expected to take over. However, a close look at *in vitro* release profiles revealed that while the 25 kDa microspheres were exhausted of drug between day 30 and 35, release from the 75 kDa microspheres was very slow. It was anticipated that the dexamethasone levels might not be sufficient during this period and a delayed inflammatory reaction could develop. Therefore, a portion of the 75 kDa microspheres were pre-degraded in PBS for 15 days. These pre-degraded 75 kDa microspheres would release drug earlier than the non-degraded 75 kDa microspheres. When used in combination with non-degraded 75 kDa microspheres, pre-degraded 75 kDa microsphere may provide adequate drug release during day 30 and 35. Therefore, two different composite formulations were developed: (1) 25 kDa non-degraded + 75 kDa non-degraded + 75 kDa pre-degraded microspheres (Formulation I); and (2) 25 kDa non-degraded + 75 kDa non-degraded (Formulation II) in the PVA hydrogel matrix.

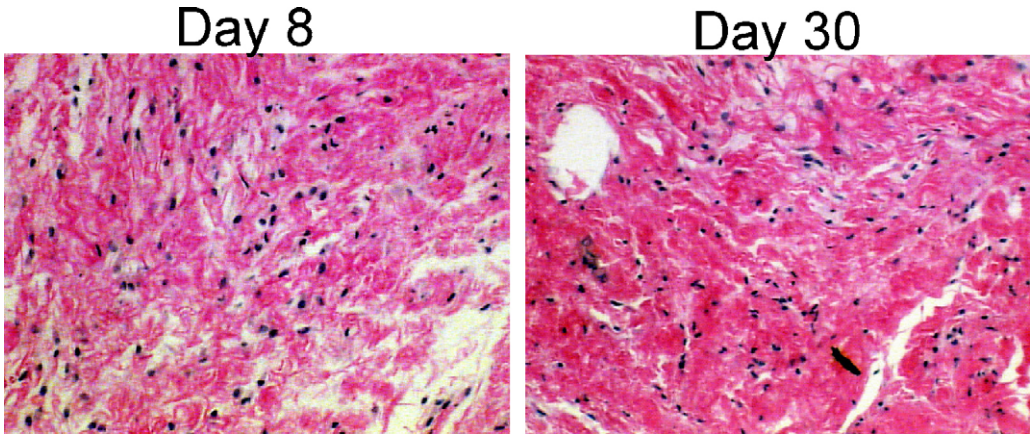

Fig. 3. Representative tissue sections from normal tissue of Sprague–Dawley rats. The tissues were taken from the same rats that were implanted with composites to remove any bias.

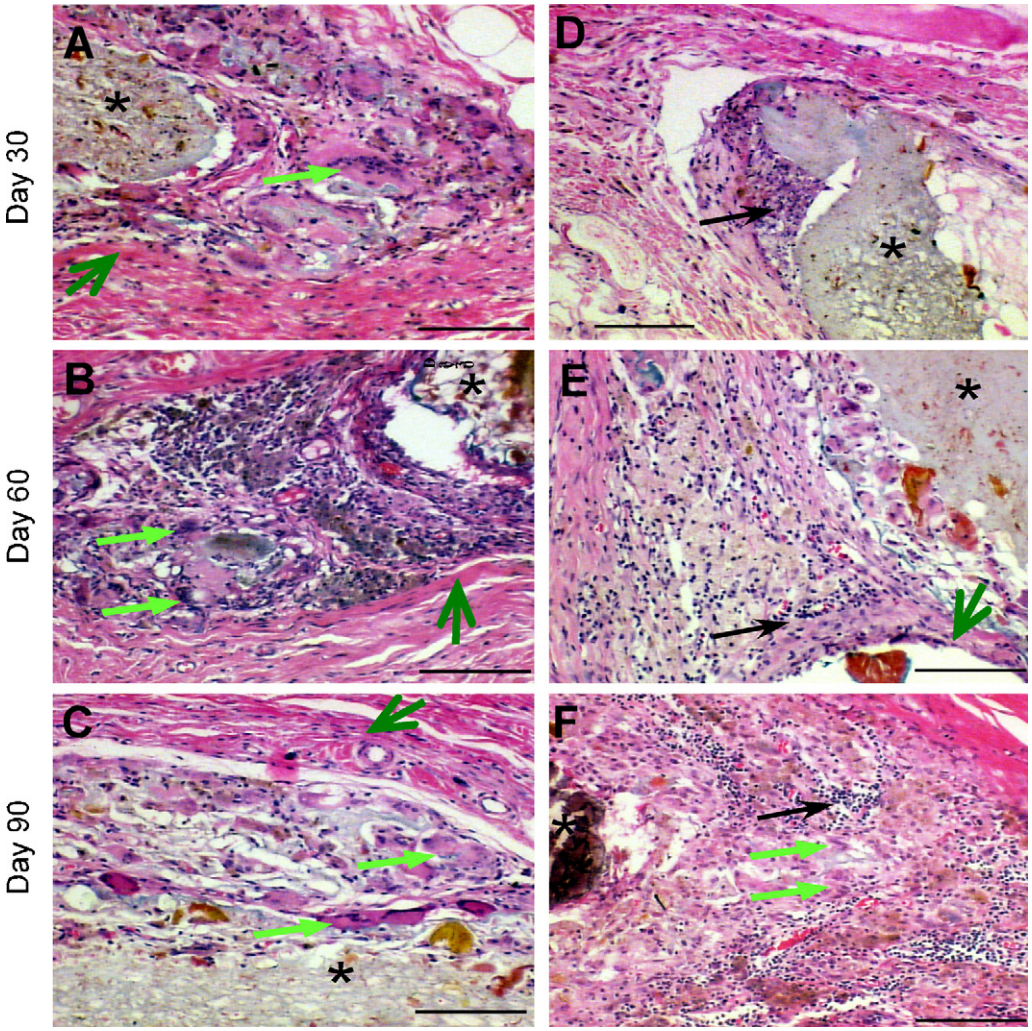

Fig. 4. Pharmacodynamic changes in representative tissue sections taken from the s.c. tissue of rats implanted with 25 kDa PLGA microsphere/PVA hydrogel composites: (A–C) without dexamethasone (control) and (D–F) with dexamethasone. *Symbols*: asterisk-implant, dark green arrow-fibrous tissue, light green arrow-multinucleated giant cells, black arrow-aggregates of neutrophils and macrophages. Bar: 100 μm.

Fig. 5 shows dexamethasone release from composites containing mixed populations of microspheres (Formulations I and II) and composites containing only 75 kDa microspheres. *In vitro* release from 75 kDa microspheres and composites containing them were qualitatively similar with a small burst (day 1), lag (lasting around day 25) followed by a second fast release phase. However, a slightly higher burst was observed for composites (ca. 5% for microspheres vs. ca. 8% for composites). As discussed above for the 25 kDa microspheres, this may be due to the presence of the surface active humic acids creating an acidic microenvironment at the surface of the microspheres. Cumulative percent releases of dexamethasone from Formulations I and II were similar (Fig. 5) and appeared to be a combination of the release profiles of the 25 kDa and 75 kDa microspheres. An initial burst release (ca. 15% in 24 h) was observed followed by a slow release phase similar to that obtained for the 25 kDa microspheres containing composites (Fig. 2) until day 12–13. A continuous release phase was observed from day 12 until day 90–95. It is worth mentioning here that although the normalized cumulative percent release profiles from both Formulations I and II appear similar, the total amount released was higher for Formulation I after day 12. This is due to the fact that at day 12, 75 kDa microspheres (Formulation I) degraded for 15 days should start releasing dexamethasone (since the second fast release from 75 kDa non-degraded microspheres was observed between days 26 and 29). However, this coincides with the second fast release phase from 25 kDa microspheres. Therefore, a higher contribution from 25 kDa microspheres in cumulative release masks the contribution of 75 kDa degraded microspheres in cumulative percent release calculations. Thus, the similarity between the cumulative percent release profiles is a result of normalized calculations. Incorporation

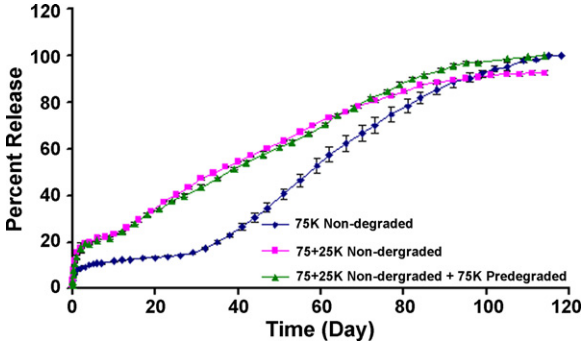

Fig. 5. Dexamethasone *in vitro* release from PLGA microsphere/PVA hydrogel composites containing 75 kDa PLGA microspheres and combinations of 25 kDa and 75 kDa PLGA microspheres (Formulation I and II; see text) in PBS 0.1 M, pH 7.4 at 37 °C. Each value represents the mean ± SD ($n = 3$).

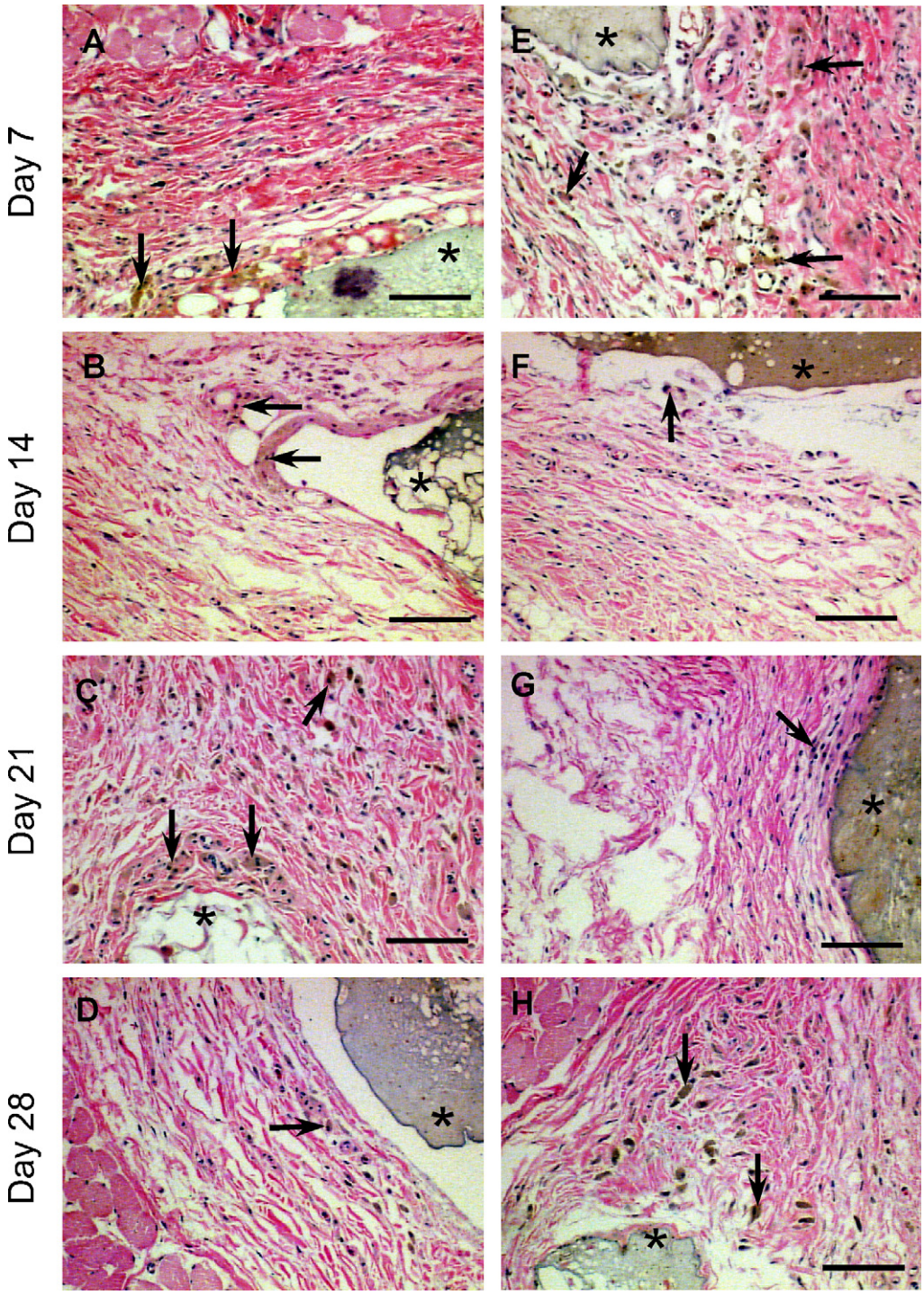

Fig. 6. Pharmacodynamic changes in representative tissue sections taken from the s.c. tissue of rats implanted with mixed PLGA microsphere/PVA hydrogel composites from days 7 to 28: (A–D) Formulation I and (E–H) Formulation II. *Symbols*: asterisk-implant, black arrow-hemosiderophages. Bar: 100 μm.

into the PVA/humic acid matrix did not significantly change the release profile of microspheres. These *in vitro* release data support our the hypothesis of combining 25 kDa and 75 kDa microspheres in the same composite to provide continuous sustained release of dexamethasone for the intended 90-day duration.

3.3.2. *In vivo pharmacodynamics*

The Formulation I and II composites were evaluated *in vivo* after implantation into rats. Tissue sections taken from the vicinity of the implanted composites are shown in Fig. 6 for days 7–28. No inflammatory reaction was observed for either Formulation I (Fig. 6A–D) or Formulation II (Fig. 6E–H). Only a few hemosiderophages (histiocytes that have phagocytosed red blood cells; indicated by black arrows) were observed in the vicinity of the implant. Hemosiderophages are present in the tissue resulting from blood vessel injury during implantation. Other than the hemosiderophages, the tissue surrounding the implant is similar to normal s.c. connective tissue (Fig. 3). These results demonstrated that both formulations were able to protect against tissue reaction for 4 weeks, which is consistent with previous results using PVA composites containing 25 kDa PLGA microspheres (Patil et al., 2004). This protection against inflammation was extended till day

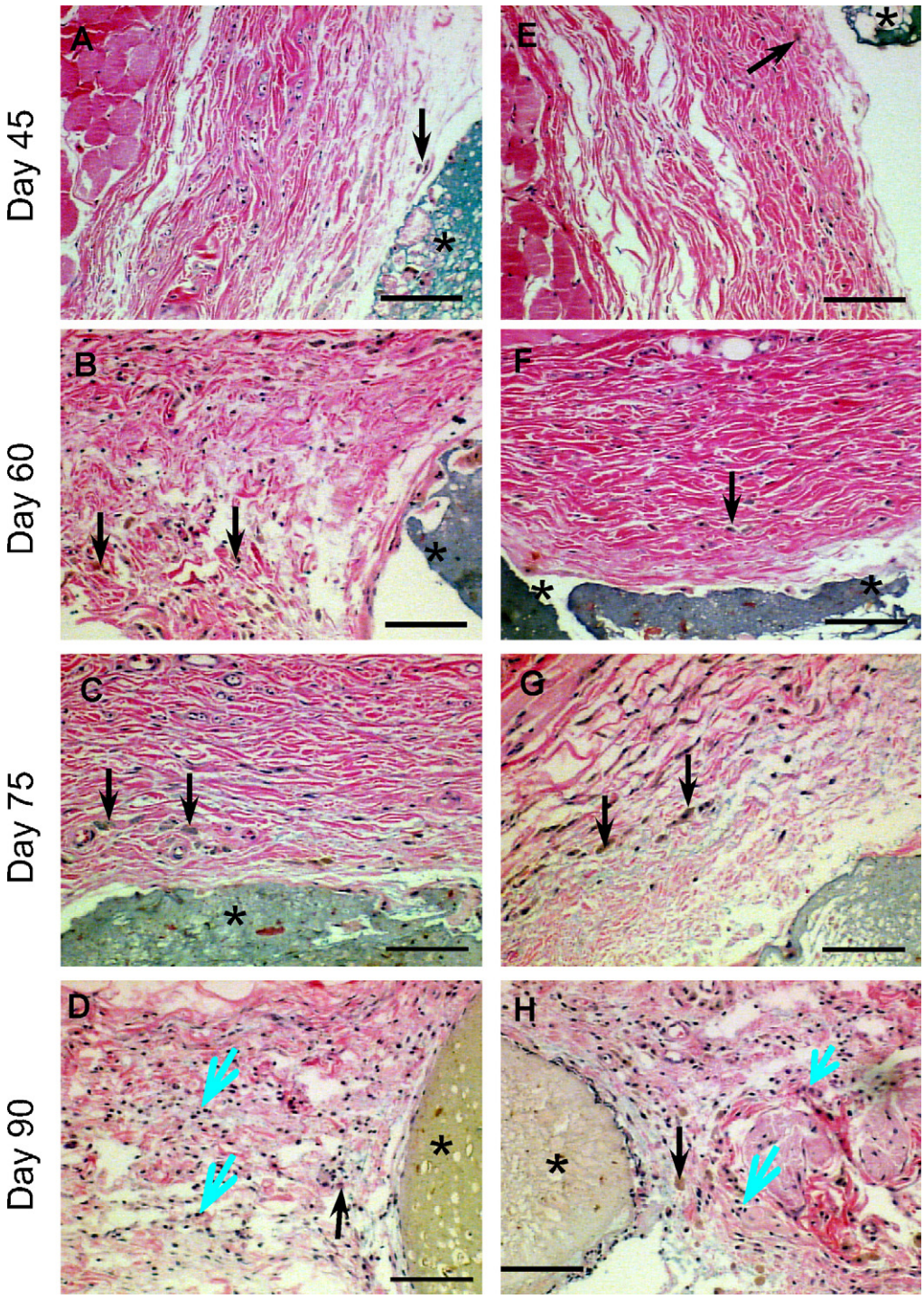

Fig. 7. Pharmacodynamic changes in representative tissue sections taken from the s.c. tissue of rats implanted with mixed PLGA microspheres/PVA hydrogel composites from days 45 to 90: (A–D) Formulation I and (E–H) Formulation II. *Symbols*: asterisk-implant, black arrow-hemosiderophages, light blue arrow-macrophages. Bar: 100 μm.

75 in both the formulations as no inflammatory cells were observed at days 45, 60 and 75 (Fig. 7A–C: Formulation I; E–G: Formulation II) with only a few hemosiderophages present in the vicinity of the implants. On day 90, a small number of macrophages (light blue arrows) were observed in tissue sections taken from both Formulations I and II (Fig. 7D and H). However, these features were observed in only 2 out of the 6 animals in the study group, the tissue sections from the other four animals were similar to the control tissue with no signs of inflammation. This might be due to animal to animal variation commonly encountered in biological studies.

This study was followed at frequent time points, initially every week for 4 weeks and then every 15 days to evaluate whether the drug release was sufficient to provide continuous anti-inflammatory protection. Both these composite coatings were able to control the tissue response for the entire 3-month period (Figs. 6 and 7). These results indicate that the combination formulations provided a sustained and continuous dexamethasone release *in vivo*. As mentioned earlier, cumulative amounts of dexamethasone released from Formulation I were higher than that from Formulation II due to due to presence of additional 75 kDa predegraded microspheres. However, the pharmacodynamic response

was similar for both formulations. Hence, it would appear that Formulation II can provide adequate amounts and addition of predegraded 75 kDa microsphere is not required. These results prove the versatility of this composite coating to provide long-term localized drug delivery and minimize tissue reaction.

The present work is a continuation of earlier efforts to understand the temporal aspects of drug delivery to prevent the foreign body response to implantable sensor/device and to develop a composite coating for implantable devices to provide long-term anti-inflammatory protection. Knowing that sustained release of drug(s) is required for intended lifetime of biosensor/device implantation, a suitable composite can be designed to provide the desired release profile using the strategy employed in this work. This can be achieved by utilizing microspheres prepared with different polymers and polymer grades such as PLGA (as shown in this work), poly lactide (PLA), *etc*. and/or with different delivery vehicles such as microspheres, nanoparticles, liposomes, *etc*. In addition, different drugs can be released simultaneously or in sequential manner depending upon the desired application. Therefore, the PLGA microspheres/PVA hydrogel based composite has potential for use as an outer drug-eluting coating for implantable biosensors or other implantable devices to enhance their functionality and prolong the lifetime.

4. Conclusions

Sustained release of the hydrophobic drug dexamethasone over 3 months was achieved using a PVA composite containing a combination of medium MW 25 kDa and high MW 75 kDa PLGA microspheres. Incorporation of only 25 kDa PLGA microspheres in PVA composite coatings did not provide long-term protection against inflammation and a delayed foreign body response developed after dexamethasone exhaustion from the composites. These results emphasized that a sustained delivery of the anti-inflammatory drug dexamethasone is required for the entire implant lifetime. The strategy of combining 25 kDa and 75 kDa microspheres in the same coating could successfully prevent the foreign body response for the desired 3-month time period. This approach can be utilized to design a coating for delivering tissue response modifying drugs for a required time period. These composite coatings will help in the development of a clinically useful implantable biosensor for metabolic monitoring.

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