Journal of Controlled Release 169 (2013) 341–347

Contents lists available at SciVerse ScienceDirect

Journal of Controlled Release

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

Polymeric "smart" coatings to prevent foreign body response to implantable biosensors

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ARTICLE INFO

Article history:
Received 13 October 2012
Accepted 25 December 2012
Available online 5 January 2013

Keywords:
PLGA/PVA "smart" composite coatings
Implantable biosensors
Diabetic rats

ABSTRACT

Application of implantable glucose biosensors for "real-time" monitoring is reliant on controlling the negative tissue reaction at the sensor tissue interphase. A novel polymer coating consisting of poly(lactic-co-glycolic) acid (PLGA) microsphere dispersed in poly(vinyl alcohol) (PVA) hydrogels was evaluated in combination with dummy sensors as a "smart" drug eluting biocompatible coating for implantable biosensors to prevent the foreign body response, and thus enhance sensor performance *in vivo*. The polymeric microspheres slowly release tissue-modifying drugs at the implantation sites to control the inflammation and fibrous encapsulation, while the hydrogel allows rapid analyte diffusion to the sensing elements. Dummy sensors with identical dimensions to that of the functional glucose sensors (0.5×0.5×5 mm) were coated with the PLGA/PVA composites using a mold fabrication process. Both normal and diabetic rats were used in the current study to investigate the effect of the diabetic state on tissue sensor interactions. It was evident that the PLGA/PVA hydrogel composite was able to form a uniform coating around the dummy sensor and stayed intact throughout the course of the study (one month). Tissue samples containing dummy sensors that were coated with dexamethasone free composites exhibited acute and chronic inflammation as well as fibrous encapsulation in both normal and diabetic rats. However, the diabetic rats exhibited decreased intensity and delayed onset of the foreign body response following implantation of drug free dummy sensors in comparison to those of normal rats. On the other hand, tissues containing dummy sensors that were coated with dexamethasone containing composites remained normal (*i.e.* similar to untreated tissues), with no inflammatory reaction or fibrous encapsulation occurring over the one-month period in both the normal and diabetic rats. The feasibility of utilizing PLGA microsphere/PVA hydrogel composites as coatings for implantable biosensors was demonstrated. This polymeric composite is an innovative approach to control the foreign body reaction at the tissue–device interface to prolong biosensor lifetime.

1. Introduction

Continuous glucose monitoring (CGM) is a technology that is able to provide continuous "real-time" monitoring of blood glucose levels and allows realization of the "artificial pancreas" concept as well as ensures optimal insulin dosing [1]. This would significantly enhance diabetic management and reduce the risk of complications associated with this chronic disease. Despite the significant progress made in the development of this technology in the past several decades, its application is hampered by the poor performance of the glucose biosensors post implantation, which is manifested as a rapid functionality loss and eventual sensor failure [1–3]. Considering that: 1) conventional glucose meters fail to provide an overall pattern of change in blood glucose levels with an individual's daily activity; and 2) the diabetes mellitus population continues to increase at an alarming rate, there is an urgency to develop a strategy to enhance the *in vivo* performance of the implantable biosensors to enable their application.

The rapid functionality loss of implantable sensors post implantation is mainly due to the foreign body reaction at the implant site [4,5]. Tissue trauma generated during implantation and the poor biocompatibility of sensor materials induce a series of events including biofouling, inflammation and fibrous encapsulation. Inflammatory cells release various reactive oxygen species that may damage the sensor components, and impair their functionality. In addition, fibrous encapsulation decreases glucose transport towards the sensing element thus compromising sensor sensitivity. Therefore, modifying the tissue reaction without compromising glucose diffusion towards the sensing elements at the implant site would appear to be critical to achieve a functional biosensor *in vivo*.

Many polymeric materials (*e.g.* pellethane™, polyallylamine, horseradish peroxidase derivatives, polyethylene glycol) have been investigated as coating materials to improve the biocompatibility of biosensors by

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http://dx.doi.org/10.1016/j.jconrel.2012.12.028

reducing biofouling and the foreign body response [6,7]. However, none of these materials has been able to fully eliminate the inflammatory response. Consequently, anti-inflammatory drugs have been used to control inflammation at the implantation sites [8–10]. Implantable biosensors are intended for long-term use and it has been reported that anti-inflammatory drugs are needed throughout the lifetime of the biosensors [9,11]. Local delivery is preferred since long-term systemic administration of anti-inflammatory drugs may cause serious side effects and may not result in effective therapeutic concentrations at the local site. Drugs cannot be directly loaded into the traditional polymeric coating materials, as their hydrophilic nature cannot provide long-term drug delivery. Long-term drug delivery can be obtained using hydrophobic polymers. However, these materials are not suitable for biosensor coating, since analytes cannot easily diffuse through. Therefore, a "smart" coating that allows rapid glucose diffusion towards the sensing element and yet elutes drugs in a controlled manner over the desired lifetime of sensors is needed.

To achieve this goal, new polymeric materials may be developed. However, development and characterization of new materials are time consuming and costly due to the need for a comprehensive understanding of the physicochemical properties as well as for exhaustive toxicology studies. In the particular case of drug eluting coatings for implantable biosensors, the regulatory burden of these drug device combination products is already high. Therefore, the use of existing polymers is desired. Our group has designed a "smart" coating comprised of poly (lactic-co-glycolic acid) (PLGA) microspheres and poly vinyl alcohol (PVA) hydrogels, which allowed rapid influx of analyte (glucose) through the hydrogel matrix and slow release of drug from the microspheres [12]. The general physicochemical properties of both materials and their safety profiles have been well documented. PLGA has been widely used in FDA approved products. In addition, there are some unique advantages afforded by this composite coating: 1) PVA hydrogels can be formed by physical crosslinking *via* freeze and thaw cycling, therefore, the risk of destabilizing the sensing element during chemical crosslinking is avoided; 2) the mechanical properties of PVA hydrogels are very similar to those of human soft tissues; 3) various desired drug release profiles can be achieved by altering the molecular weights and co-polymer ratios of PLGA polymers; and 4) different populations of PLGA microspheres containing different drugs can be used in combination. It has been demonstrated that local delivery of dexamethasone successfully suppressed inflammation and fibrous encapsulation at the implant site for extended periods without causing any systemic side effects. However, only the PLGA microsphere/PVA hydrogel "smart" coating was investigated in the previous work using normal healthy rats [9,11,13]. In order for this polymeric "smart" coating to be utilized for implantable biosensors, several critical questions still need to be addressed, such as: 1) whether the composite is able to form uniform coatings with adequate adhesion to the sensor which remain intact throughout the intended sensor lifetime; and 2) the impact of diabetes on the inflammation and vascularity of the surrounding tissues.

The current work involved *in vivo* studies to assess the performance of dexamethasone-releasing (one month) composites composed of PLGA microspheres and PVA hydrogels when used together with dummy sensors (oxidized silicon chips) that have the same dimensions ($0.5 \times 0.5 \times 5$ mm) as the glucose biosensors. The first part of the study examined the tissue reaction to the composite coated dummy sensors in normal healthy rats to determine: 1) whether the composite can form a uniform coating around the sensor and retain its integrity following implantation, and 2) whether the presence of the sensor (as a hard core) alters the dose of dexamethasone required to suppress the foreign body response. Three different dexamethasone doses were tested for optimization purposes. Following determination of the optimal dexamethasone dose in normal healthy rats, the impact of the diabetic condition on the extent of the foreign body reaction was investigated. Two different diabetic animal models (representative of type 1 and type 2 diabetes) were used to determine the effect of the type of diabetes on the tissue reaction.

2. Materials and methods

2.1. Materials

Dexamethasone, poly(vinyl alcohol) (PVA, MW 30–70 kDa), sodium chloride (ACS grade) and sodium azide were purchased from Sigma-Aldrich (St. Louis, MO). PVA (99% hydrolyzed, MW 133 kDa) was purchased from Polysciences, Inc. (Warrington, PA). PLGA Resomer® RG503H (inherent viscosity 0.32–0.44 dl/g) was a gift from Boehringer-Ingelheim. Methylene chloride, sodium mono-hydrogen phosphate (ACS grade), acetonitrile (ACN, HPLC grade), dimethyl sulfoxide (DMSO, ACS grade) and tetrahydrofuran (THF, HPLC grade) were purchased from Fisher Scientific (Pittsburgh, PA). Disodium hydrogen phosphate (ACS grade) was purchased from VWR International. NanopureTM quality water (Barnstead, Dubuque, IA) was used for all studies.

2.2. Methods

2.2.1. Preparation of PLGA microsphere/PVA hydrogel composite coated dummy sensors

2.2.1.1. Preparation of PVA solutions. PVA (99% hydrolyzed, MW 133 kDa) aqueous solution (5% w/v) was prepared by mixing polymer powder (1 g) with distilled water (20 ml). The mixture was heated to 80 °C for about 45 min, while slowly stirring. When all the polymers were dissolved and the mixture becomes clear, the solution was cooled back to room temperature and kept overnight to remove all the air bubbles and allow the dissolved polymer to reach equilibrium.

2.2.1.2. Preparation of PLGA microspheres. Dexamethasone loaded/blank PLGA microspheres were prepared using an oil-in-water emulsion solvent extraction/evaporation technique as described previously [14–16]. Briefly, 2 g of 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 Power Gen 700D Homogenizer (Fisher Scientific, Pittsburgh, Pennsylvania). 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 3 times with deionized water and collected *via* filtration (0.45 μm). The prepared microspheres were kept under vacuum overnight and later stored at 4 °C until further use.

2.2.1.3. Preparation of PLGA microsphere/PVA hydrogel composite coated dummy sensors. A mold fabrication process was used to coat the dummy sensors with the PLGA microsphere/PVA hydrogel composite. The dummy sensors were silicon chips that have the same dimension and composition as the actual biosensors. These chips were cut from a p-type Si (4 in. in diameter) using a dicer. Briefly, PVA (MW 133 kDa) was dissolved in deionized water at 80 °C to obtain a 5% (w/w) solution and sterilized by autoclaving. PLGA microspheres were dispersed in this solution to achieve a homogenous distribution. The grooved mold was filled with this PLGA microsphere containing dispersion and dummy sensors were placed into the mold. These were then 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.

Four groups of composite coatings containing different amounts of PLGA microspheres were investigated: (1) 75 mg of dexamethasone-loaded microspheres was dispersed per ml of PVA solution; (2) 100 mg of dexamethasone-loaded microspheres was dispersed per ml of PVA

solution; (3) 150 mg of dexamethasone-loaded microspheres was dispersed per ml of PVA solution and (4) 100 mg of blank microspheres was dispersed per ml of PVA solution. The fourth group was used as a control group.

2.2.2. Characterization of PLGA microsphere/PVA hydrogel composite coated dummy sensors

2.2.2.1. High performance liquid chromatography (HPLC). The concentration of dexamethasone was determined using a Perkin Elmer HPLC system (series 200) with a UV absorbance detector (Perkin Elmer, Shelton, CT) set at 240 nm. The mobile phase consisted of acetonitrile/water/phosphoric acid (30/69.5/0.5, v/v/v). A Perkin Elmer C18 (4.6 mm × 15 mm) analytical column was used with the flow rate set at 1.2 ml/min. The chromatographs were analyzed by PeakSimple™ Chromatography System (SRI instruments, Torrance, CA).

2.2.2.2. Particle size analysis. The mean particle diameters of the prepared microspheres were determined using an AccuSizer 780A autodiluter particle sizing system (Santa Barbara, CA). Approximately 25 mg of microspheres was dispersed in 1 ml of 0.1% (w/v) PVA solution. 200 μl of the dispersion was used for particle size analysis. All measurements were conducted in triplicate and the results are reported as the mean ± SD.

2.2.2.3. Drug loading tests of the composites. The amount of dexamethasone in each PLGA/PVA composite coated dummy sensor was determined by dissolving the coating in 1 ml DMSO. The dexamethasone concentration was determined using high performance liquid chromatography (HPLC) as described above. The drug loading was then calculated as: percentage drug loading = total amount of dexamethasone determined/weight of composite coating. All measurements were conducted in triplicate and the results are reported as the mean ± SD.

2.2.2.4. Thickness measurement of the composite coatings. The thickness of the composite coating was measured using a caliper and determined as: (thickness of composite coated dummy sensor − thickness of dummy sensor)/2. To check the uniformity of the composite coating, each sample was measured at three different sections: the middle point as well as the two ends of the dummy sensor.

2.2.2.5. Mechanical property of the composite coatings. The Young's modulus of the PLGA microsphere/PVA hydrogel composite coatings was obtained from uniaxial tensile testing using thermomechanical analysis (TMA). Rectangular samples (approximately 6.38-mm wide, 0.06-mm thick, and 20-mm long) were used for the tensile tests. The force was linearly ramped from 30 to 200 mN at a constant rate of 25 mN min^{-1} and the Young's modulus was ascertained from the resulting stress–strain curves.

2.2.2.6. In vitro release study. In vitro release tests of dexamethasone loaded PLGA microsphere/PVA hydrogel composites were determined as described previously [9,11]. Briefly, the 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 (100 rpm). Sink conditions were maintained. At pre-determined time points, 1 ml samples were taken and replaced with fresh PBS. The concentration of dexamethasone in each sample was determined using HPLC as described above. 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).

2.2.3. In vivo pharmacodynamic study using a normal rat model

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 (#A11-010). PLGA/PVA composite coated dummy sensors were prepared as described above and were implanted into the interscapular subcutaneous tissue of male Sprague-Dawley rats (weighing ~200 g) using a 16-gauge thin wall needle. Each rat received four implants, which were one drug free and three dexamethasone-containing composites. To implant the coated dummy sensors, rats were initially anesthetized using isoflurane (Forane; Baxter; Deerfield, Illinois). The anesthesia was maintained during the implantation with 2% isoflurane in oxygen. The back of each animal was shaved and washed with betadine solution. All of the procedures were conducted under aseptic conditions. Tissue inflammation response was determined through serial sacrifice to investigate both the acute (day 3) and the chronic (7, 14, 21 and 28 days) phase anti-inflammatory effects (n = 6 at each time point). A histologic evaluation of excised tissue samples from the site of implantation was performed after staining with hematoxylin and eosin (H&E) stain for inflammation. Tissue samples were fixed in 10% neutral buffered formalin processed through serial alcohol and toluene and embedded in paraffin. Tissue sections of 4 μm thickness were cut and stained with H&E. The dummy sensors (silicon implants) were removed prior to sectioning. The inflammatory cells stain basophilic (purple) and the connective tissue surrounding the implant stains eosinophilic (pink). Tissue samples were observed and digitally stored using an Olympus microscope (model A051, Olympus America, Melville, NY) and Bioquant software (BQ-TCWV3.00.6). The photomicrographs at lower magnification (100×) shows the intensity of the inflammatory reaction around the entire implant. The photomicrographs at the magnification of 200× were used to count the number of inflammatory cells in the vicinity of the implant (ten boxes (1 × 1 in.) randomly distributed in the vicinity of the implants and inflammatory cells were counted manually). The highest magnification photomicrographs 400× reveal the details of the different types of inflammatory cells involved (*i.e.* neutrophils, macrophages and giant cells).

2.2.4. In vivo pharmacodynamic studies using diabetic rat models

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 (#A09-030). Two different diabetic rat models were investigated in the studies, which were Zucker diabetic fatty rats representing type 2 diabetes mellitus and streptozotocin (STZ) induced diabetic rats representing type 1 diabetes mellitus. For STZ induced diabetic animals, each one received a single intraperitoneal (i.p.) injection of STZ (60 mg per kg body weight). The values of blood glucose concentrations of both types of diabetic animals were determined using a commerical glucose meter by pricking the tail veins. All the animals were weighed daily and their general condition was monitored throughout the course of the study.

For the pharmacodynamic studies, each rat received two composite coated dummy sensors: 1) composite containing blank microspheres and 2) composite containing dexamethasone-loaded microspheres. In order to determine the effect of the diabetic condition on the dexamethasone dose required to suppress the foreign body reaction, only the lowest dexamethasone dose determined using normal healthy rats was tested. Details of the pharmacodynamic studies were the same as described in Section 2.2.3.

3. Results and discussion

3.1. Characterization of PLGA microsphere/PVA hydrogel composite coated dummy sensors

The characteristics of the PLGA/PVA composite coated dummy sensors investigated in this work are given in Table 1. Dummy sensors were oxidized silicon chips with identical dimensions of 0.5 × 0.5 × 5 mm (the same dimensions as our glucose biosensors). The average drug loading and particle size of the PLGA microspheres used were 7.6 ± 0.24% (w/w) and 7.43 μm (number based mean diameter) ± 6.64 μm (SD),

Table 1
Microsphere loading, dexamethasone loading and thickness of PLGA microsphere/PVA hydrogel composite coatings.

Formulation	Microsphere loading (mg/ml PVA solution)	Dexamethasone loading per implant (%,w/w)	Coating thickness (dry) (μm)
1	75	3.43 ± 0.15	250 ± 83.66
2	100	3.95 ± 0.27	246 ± 57.91
3	150	5.91 ± 0.19	251 ± 53.96

respectively. Three microsphere concentrations (75, 100, and 150 mg/ml hydrogel) were used to prepare the composite coatings. Composite coatings containing 100 mg microspheres per ml of hydrogel (with no dummy sensor core) have previously been shown to be sufficient to prevent the inflammatory reaction at the implantation site over a one-month period [13]. The higher concentration (150 mg microsphere per ml hydrogel) was selected since the incorporation of the dummy sensor increases the rigidity of the implants, which may cause more mechanical resistance to the surrounding tissue leading to a higher level of negative tissue reaction. The lower concentration (75 mg microspheres per ml hydrogel) was chosen for optimization purposes. The average thicknesses of the composite coatings containing different amounts of microspheres (75, 100, 150 mg/ml hydrogel) were 250, 246, and 251 μm, respectively. However, there was no statistically significant difference in the thickness of the three coatings. Scanning electron microscope pictures revealed that the PLGA microspheres were solid spherical particles and they were fully covered by the physically cross-linked PVA hydrogels (Fig. 1). The Young's modulus of the PLGA/PVA composite coating is 0.39 ± 0.11 GPa.

3.2. In vitro release from PLGA microsphere/PVA hydrogel composite coatings

The dexamethasone release profiles from PLGA microspheres and PLGA microsphere/PVA hydrogel composites are shown in Fig. 2. Triphasic release profiles were observed in both cases with an initial burst release followed by a lag phase till day 10 and then a secondary apparent zero-order release phase. The release profile of the PLGA/PVA composites was qualitatively similar to that of PLGA microspheres alone with two differences: (1) a slightly delayed initial burst release and (2) a slightly faster secondary apparent zero-order release was observed from the composites. It is known that burst release is mainly due to the release of surface associated drug. Deposition of PVA onto the microsphere surface is likely to delay the burst release, since the PVA hydrogel acts as another barrier to diffusion of dexamethasone into the bulk media. However, once the PVA hydrogel is fully hydrated, its highly porous microstructure allows rapid diffusion of dexamethasone into bulk media. The slightly faster release rate during the secondary zero-order release phase is probably a result of the increased local acidity generated by the PLGA oligomers being retained in the PVA hydrogel. Despite these two differences, the presence of the PVA hydrogel did not change the mechanism of dexamethasone release. The drug release from the composites is a combination of diffusion and polymer erosion.

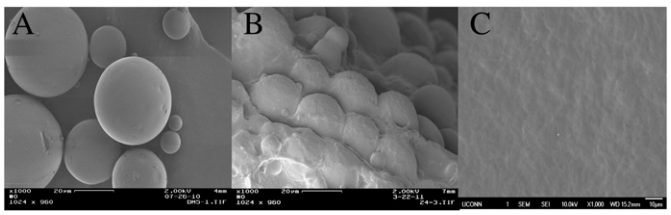

Fig. 1. Scanning electron microscope pictures of: (A) PLGA microspheres; (B) PLGA microsphere/PVA hydrogel composites; and (C) PVA hydrogel.

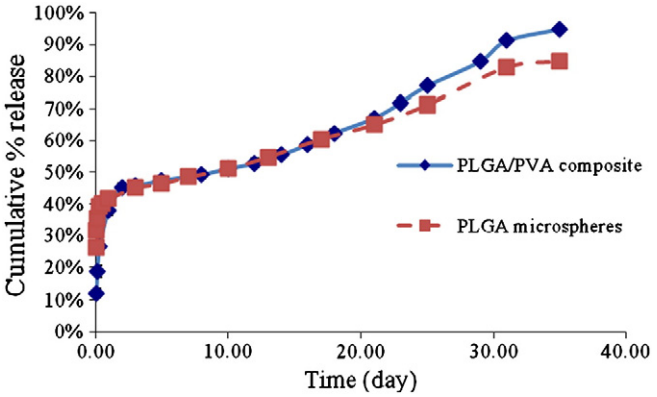

Fig. 2. *In vitro* release profiles of PLGA microspheres and PLGA microsphere/PVA hydrogel composite coatings (n = 3 ± SD) at 37 °C, phosphate buffer solution (pH = 7.4 ± 0.1).

3.3. In vivo pharmacodynamics using a normal rat model

Each rat received four composite coated dummy sensors, which were one control without dexamethasone and three with different amounts of dexamethasone as mentioned above. Fig. 3 shows tissue sections taken from the vicinity of the implants on the predetermined days covering both the acute and chronic inflammantory phases. Composites without dexamethasone resulted in an intense inflammatory response characterized by a large number of neutrophils (polymorphonuclear leukocytes, PMNs) surrounding the implant (Fig. 3A). On the other hand, the dexamethasone-loaded composites suppressed the acute inflammatory reaction (Fig. 3B) and these tissue sections were comparable to that of normal tissue (Fig. 3I). Only a few lymphocytes and plasma cells were

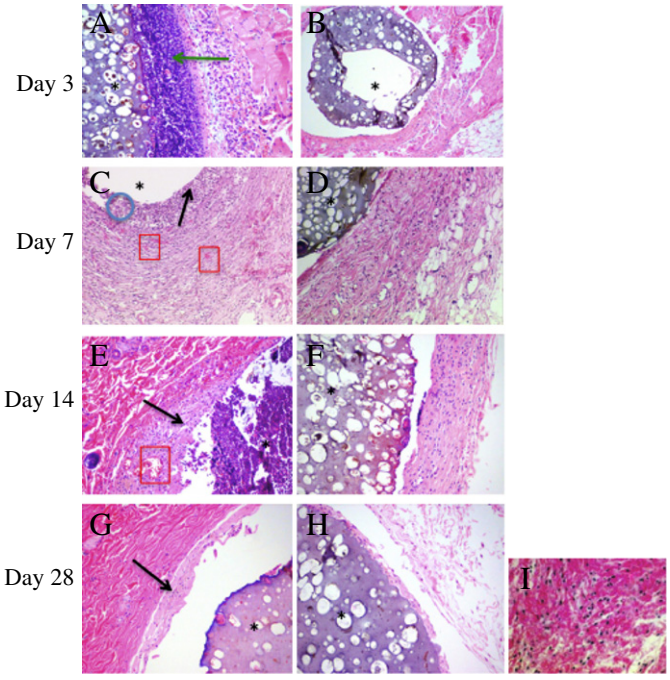

Fig. 3. Pharmacodynamic changes in representative tissue sections taken from the subcutaneous tissue of rats implanted with PLGA microsphere/PVA hydrogel composite coated dummy sensors on days 3, 7, 14, and 28: (A, C, E, and G) without dexamethasone; (B, D, F, and H) with dexamethasone; and (I) normal subcutaneous tissue. Hematoxylin & Eosin (H&E) stains inflammation mediating cells basophilic (purple) and s.c. connective tissue eosinophilic (pink). The asterisk indicates the position of the hydrogel part in the tissue. The green arrow points to neutrophils, the blue circle indicates macrophages, the red square indicates thin wall capillaries, and the black arrow points to fibrous encapsulation.

present in these tissue sections. By day 7, a chronic inflammatory tissue response was identified in the vicinity of the composites without dexamethasone by the presence of mononuclear cells, *i.e.* monocytes and lymphocytes (Fig. 3D). Neo-vascularization was also observed by this time point in the drug free composites, which is evident by the presence of thin-walled blood vessels in the surrounding fibrous tissue (Fig. 3D). However, no such response was visible in the tissue sections surrounding the dexamethasone containing composites at day 7. Fig. 3E, and F shows tissue section taken from the vicinity of the implants on day 14 after implantation. The drug-free composite coated dummy sensor exhibited a chronic inflammatory reaction by day 14 as characterized by fibroblasts, macrophages and multinucleated giant cells (30–50 μm in diameter) (Fig. 3E). Deposition of collagen in the area around the drug-free composite coated sensors started to occur and a thin band of fibrous connective tissue was evident around the dummy sensor. This is the usual reaction of the body to the continuous presence of any non-absorbable/degradable foreign material. In comparison, the tissue samples taken from the vicinity of the implants containing dexamethasone showed no signs of chronic inflammation (Fig. 3F) and resembled untreated tissue samples (Fig. 3I). Fig. 3G, and H shows tissue sections taken from the vicinity of the implants on day 28 post implantation. A foreign body reaction characterized by the presence of fibrous encapsulation was observed at the site of the dexamethasone free composite coated dummy sensor at day 28 (Fig. 3G). On the other hand, the foreign body reaction is successfully supressed by dexamethasone. The absence of: inflammatory cells, collagen deposition and neovascularization in the tissue sections containing dexamethasone-loaded composite coated dummy sensors is in agreement with previous reports that dexamethasone impairs the normal wound healing process. Skin wound healing is a dynamic process comprised of four continuous, overlapping and precisely ordered phases: hemostasis, inflammation, proliferation, and tissue resolution. This process demands cooperative efforts of many different cells including but not limited to neutrophils, macrophages, lymphocytes, keratinocytes, fibroblasts, and endothelial cells. Any interruptions, aberrancies, or prolongation in the process may lead to delayed or modified wound healing [17,18]. Dexamethasone inhibits recruitment of various inflammatory cells and gene expression of many key wound healing cytokines and growth factors [19]. In addition, dexamethasone also inhibits fibroblast proliferation and impairs the synthesis of several dermal extracellular matrix (ECM) proteins. The absence of neo-vascularization in the tissue surrounding the dexamethasone containing composite coated dummy sensors indicates suppression of the immunogenic reaction, which indicates that the body fails to recognize the dummy sensor as a foreign object.

One concern was whether the composite coating can stay intact throughout the course of study. The presence of the dummy sensor could present mechanical stress and in the case of the dexamethasone free composite coatings, the coatings might experience additional environmental stress induced by the inflammantory response. Fig. 4A and B are representative tissue sections containing the dummy sensor alone and the dummy sensor with the composite coating, respectively at day 30. The mechanical property of the composite coating as well as its adhesion to the dummy sensor was sufficient.

Comparing the histological results obtained in current study to the previous results using composite coatings without dummy sensors [9,13], the presence of the dummy sensor did not provoke a more severe negative tissue reaction including both the acute and chronic inflammatory phases even though larger needles (16 gauge compared to 18 gauge) were used for implantation since the overall size of the coated sensors was slightly larger. The hydrogel coating was able to increase the biocompatability of the dummy sensor and minimize any mechanical stress to surrounding tissues.

3.4. Diabetic rat models

Two diabetic rat models were used in this work, streptozotocin induced diabetic rats and Zucker fatty diabetic rates. Both of these are well documented models of experimental diabetes. Fig. 6 shows the blood glucose concentrations of Sprague Dawley rats following a single i.p. injection (60 mg/kg body weight) of STZ. Streptozotocin is a pancreatic cell toxin that induces rapid and irreversible necrosis of the pancreatic cells. The rats became hyperglycemic within two days post administration and the blood glucose level continued rising in the first two weeks till it reached a plateau at 541 ± 53.8 mg/dl. The animals remained diabetic throughout the course of the study. As shown in Fig. 5 (right), the blood glucose concentrations of ZDF rats (558 ± 45 mg/dl) were comparable to those of the STZ induced diabetic rats.

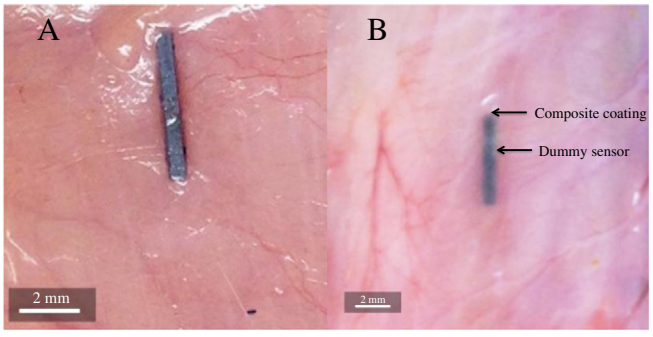

Fig. 4. Subcutaneous tissue containing dummy sensor without composite coating (left) and composite coated dummy sensor (right), scale bar = 2 mm.

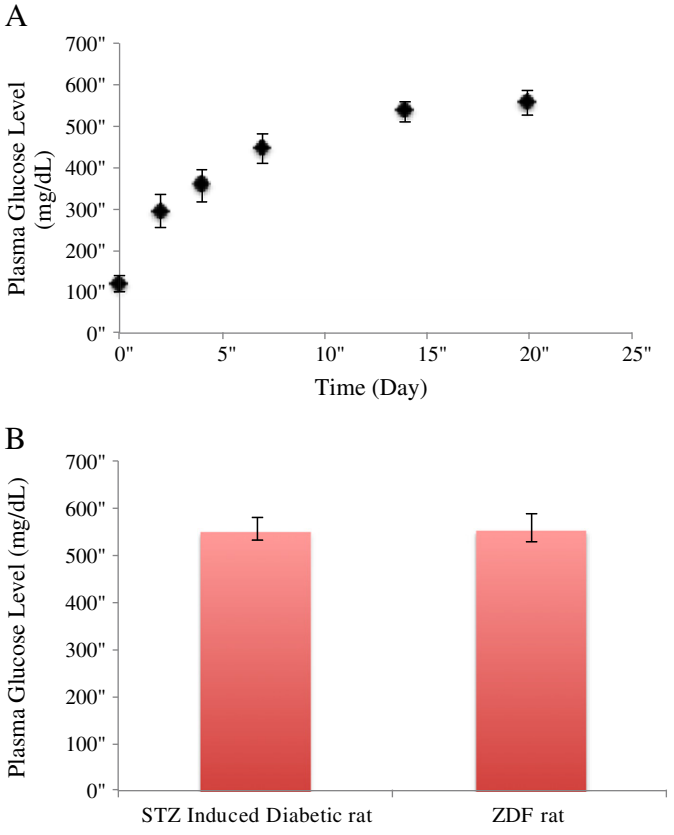

Fig. 5. A: Plasma glucose concentrations of Sprague Dawley rats following a single i.p. injection of streptozotocin (STZ) (60 mg/kg body weight). B: Comparison of the plasma glucose concentrations between STZ induced diabetic rats and ZDF rats.

3.5. In vivo pharmacodynamics using diabetic rat models

Each diabetic rat (both STZ induced diabetic rats and ZDF rats) received two composite coated dummy sensors: one was drug free (control group) and the other was loaded with dexamethasone. Only the lowest efficient dexamethasone dose (75 mg microspheres per ml PVA hydrogel) determined using normal healthy rats was tested to determine the effect of the diabetic condition on the dexamethasone dose required to suppress the foreign body response.

Figs. 6 and 7 show tissue sections taken from the vicinity of the implants on predetermined days covering both the acute and chronic inflammantory phases for STZ induced diabetic rats and ZDF rats, respectively. In both cases, the composite loaded with blank microspheres resulted in an acute inflammatory response characterized by the presence of neutrophils accumulated at the implantation site (Figs. 6A and 7A). On the other hand, this acute inflammatory phase was not observed when dexamethasone was loaded into the composite coatings (Figs. 6B and 7B). Following the acute inflammatory phase, a network of fibrous tissue together with lymphocytes, macrophages, and multinucleated giant cells gradually developed along the surface of the sensors without dexamethasone (Figs. 6C, E, 7C, and E). Tissues containing dummy sensors coated with dexamethasone-loaded composites showed no sign of chronic inflammation (Figs. 6D, F, 7D and F) and resembled the untreated tissues (Figs. 6G and 7G).

No difference in the level of the inflammatory reaction was observed in the control groups (no dexamethasone) of the STZ induced diabetic rats and the ZDF rats. The type of diabetes did not affect the foreign body response. However, the intensity of the acute inflammatory response in the diabetic rats was markedly decreased in comparison to the normal rats. As shown in Fig. 8, the average number of inflammatory cells at the implant sites in diabetic rats is much less than that of the normal healthy rats. In addition, the number of inflammatory cells in normal rats decreased with time (from day 3 on) as the acute inflammatory phase progressed to the chronic phase. In the case of diabetic rats, a delayed onset of the inflammatory response occurred. This observation is in agreement with Sheldon et al. who have reported that diabetes leads to metabolism change, which impairs the function of the tissue mast cells and interferes with the inflammantory response [20]. The tissue mast cells in rats play an important role in the development of hyperemia, vascular permeability, edema formation and wound healing, which are essential components of the normal inflammatory and reparative reaction. Following the initial injury caused during implantation, mast cells degranulate to release histamine that dilates the blood vessels around the implantation site allowing neutrophils and other plasma cells to migrate from the capillaries/vessels towards the implant sites. In diabetic rats, the degranulation of the mast cells is inhibited, and therefore, leads to a markedly reduced acute inflammatory phase and a delayed onset of chronic phase.

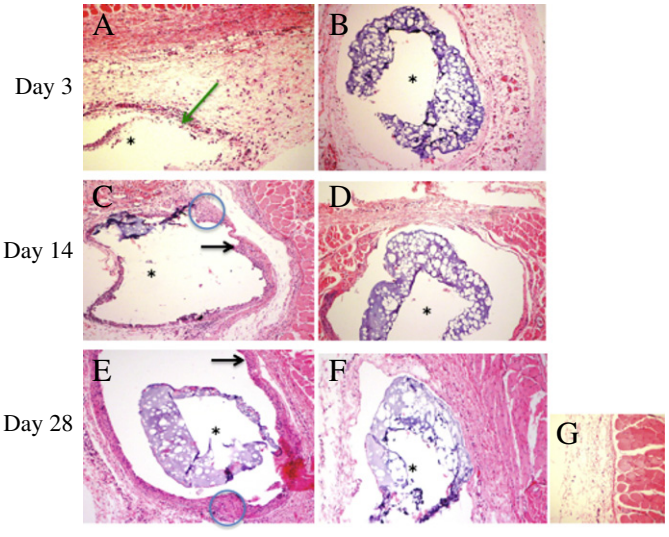

Fig. 6. Pharmacodynamic changes in representative tissue sections taken from the subcutaneous tissue of STZ induced diabetic rats implanted with PLGA microsphere/PVA hydrogel composite coated dummy sensors on days 3, 14, and 28: (A, C, and E) without dexamethasone; (B, D, and F) with dexamethasone; and (G) normal subcutaneous tissue. Hematoxylin & Eosin (H&E) stains inflammation mediating cells basophilic (purple) and s.c. connective tissue eosinophilic (pink). The asterisk indicates the position of the hydrogel part in the tissue. The green arrow points to neutrophils, the blue circle indicates macrophages, and the black arrow points to fibrous encapsulation.

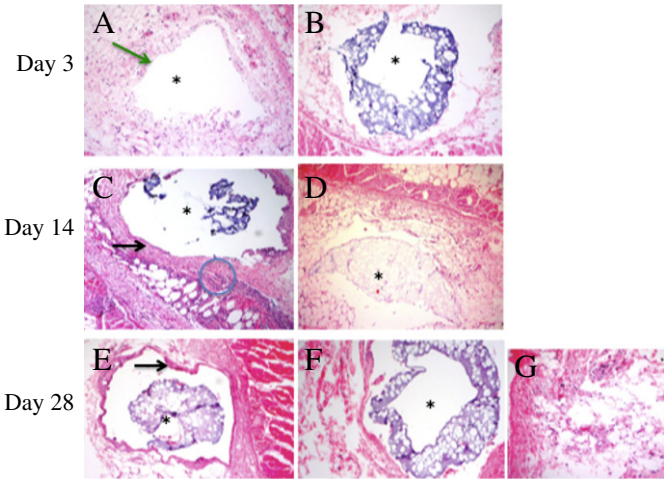

Fig. 7. Pharmacodynamic changes in representative tissue sections taken from the subcutaneous tissue of ZDF rats implanted with PLGA microsphere/PVA hydrogel composite coated dummy sensors on days 3, 14, and 28: (A, C, and E) without dexamethasone; (B, D, and F) with dexamethasone; and (G) normal subcutaneous tissue. Hematoxylin & Eosin (H&E) stains inflammation mediating cells basophilic (purple) and s.c. connective tissue eosinophilic (pink). The asterisk indicates the position of the hydrogel part in the tissue. The green arrow points to neutrophils, the blue circle indicates macrophages, and the black arrow points to fibrous encapsulation.

4. Conclusions

The current work provides a proof of concept for utilizing "smart" PLGA microsphere/PVA hydrogel composites as outer polymeric coatings for implantable biosensors to suppress the negative tissue reaction at the implantation sites, and thus facilitate the *in vivo* application of the biosensors. No significant change in the dexamethasone release profile was

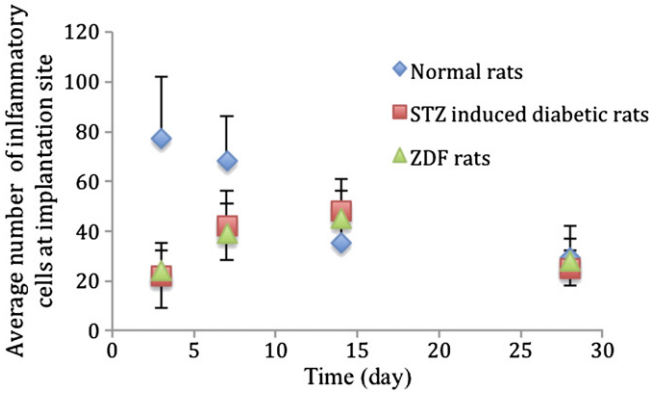

Fig. 8. Effect of diabetic condition on the inflammatory-mediating cell population in the vicinity of the implantation site. Each data point represents an average of 10 randomly chosen unit regions ± standard deviation from each photomicrographs.

observed when the PLGA microspheres were embedded inside the PVA hydrogel. This indicates that PVA hydrogel is a good "dispersing" matrix for microspheres or other similar drug delivery systems to enhance their biocompatibility and thus decrease local tissue irritation, without altering the release kinetics. The drug-free composite coated dummy sensors resulted in a foreign body response at the implantation sites in both normal and diabetic rats. The difference observed in extent and onset of the negative tissue reaction between diabetic rats and normal rats indicates that the diabetic condition (metabolic disorder) alters the immune response. This observation may be related to dysfunction of key inflammatory mediators (*i.e.* mast cells and fibroblasts) and growth factors (*i.e.* VEGF). The comparison of the negative tissue reactions between type 1 diabetic rats and type 2 diabetic rats shows that the diabetic type does not affect the tissue immune response to foreign objects in rats. This information will be helpful for the future development of implantable sensors targeted at diabetic populations. Dummy sensors coated with dexamethasone-loaded composites did not result in any negative tissue reactions at the implant sites over the study period of one-month in both normal and diabetic rats. This composite coating holds promise to enhance lifetime and performance of implantable biosensors.

Acknowledgments

Financial support for this study was obtained from the US Army Medical Research Grant (W81XWH-07-10688), and NIH grant (1-R21-HL090458-01).

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