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Dexamethasone/PLGA microspheres for continuous delivery of an anti-inflammatory drug for implantable medical devices

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Abstract

The purpose of this research was to develop polylactic-*co*-glycolic acid (PLGA) microspheres for continuous delivery of dexamethasone for over a 1-month period, in an effort to suppress the acute and chronic inflammatory reactions to implants such as biosensors, which interfere with their functionality. The microspheres were prepared using an oil-in-water emulsion technique. The oil phase was composed of 9 : 1 dichloromethane to methanol with dissolved PLGA and dexamethasone. Some microspheres were predegraded for 1 or 2 weeks. Ten percent of polyethylene glycol was added to the oil phase in alternative formulations to delay drug release. The in vitro release studies were performed in a constant temperature (37°C) warm room, in phosphate-buffered saline at sink conditions. Drug loading and release rates were determined by HPLC-UV analysis.

The standard microsphere systems did not provide the desired release profile since, following an initial burst release, a delay of 2 weeks occurred prior to continuous drug release. Predegraded microspheres started to release dexamethasone immediately but the rate of release decreased after only 2 weeks. A mixed standard and predegraded microsphere system was used to avoid this delay and to provide continuous release of dexamethasone for 1 month.

Keywords: Microspheres; Dexamethasone; Continuous release; PLGA; Implant; Biosensor; Anti-inflammatory

1. Introduction

A critical problem for implantable devices, such as glucose sensors, is the inflammatory response of the body to tissue injury on implantation and to the materials of the device. The inflammatory response consists of several phases: acute, chronic, and fibrotic encapsulation [1,2]. The acute inflammatory reaction occurs within 24–48 h and when the triggering condition continues past the end of this response without any suppressive agent, a chronic inflammatory reaction occurs. This reaction continues for 1–2 weeks, after which either the fibrotic tissue is deposited or normal tissue regrowth occurs. These tissue reactions affect the normal function of numerous implants such as biosensors, pacemakers and bioartificial organs. For example, the accumulation of inflammatory cells and the presence of a fibrous capsule around a glucose sensor reduce the transport of glucose from the capillaries to the sensor [3–5]. Thus, in order for a biosensor to function accurately in vivo, the inflammatory response must be suppressed for the effective life of the implant. Suppression of the inflammatory response will not only minimize tissue injury, but will also allow normal tissue regrowth and limit fibrotic encapsulation that would cut off the blood supply to the sensor thus ending its functionality [1,2].

Glucocorticoids are used to prevent or suppress inflammation in response to multiple inciting events, including radiant, mechanical, chemical, infectious, and immunological stimuli [6]. They inhibit the production of factors that are critical in generating the inflammatory response, including vasoactive and chemoattractive factors, and lipolytic and proteolytic enzymes. In addition, they decrease the extravasion of leukocytes to the injury site [7]. Dexamethasone, with its high potency and effectiveness on multiple organ systems was chosen for this research. However, dexamethasone can

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have serious systemic side effects. Consequently, the controlled, continuous local delivery of dexamethasone at the implantation site via a microsphere system was considered as a means to avoid these side effects and achieve the goal of suppressing the local inflammatory response.

Microspheres are used as controlled drug delivery systems for a variety of applications including chemotherapy, cardiovascular disease, hormone therapy, therapeutic protein delivery and vaccine development [8–10]. Some of the advantages of microspheres as controlled drug delivery devices are: a decrease in single dosage size, a continuous drug release, decrease in systemic side effects, reduced possibility of dose dumping, reduced frequency of administration, and, therefore, increased patient compliance [8]. Poly(lactic acid), poly(glycolic acid) and their copolymers are well-known biodegradable, biocompatible aliphatic polymers, and are preferred for microsphere preparation, in part because of their slow and reproducible degradation rates. The biodegradation of PLGA ranges from 2 to 5 months depending on the copolymer ratio [11].[1] PLGA has been used in several FDA approved parenteral drug applications that are currently on the market (e.g. Lupron Depot and Zoladex) [12,13].

Sustained release of drugs from biodegradable matrices is accepted to occur by three mechanisms: diffusion through the polymer continuum, liberation from the matrix via polymer degradation, or a combination of drug diffusion and polymer degradation [14]. PLGA microspheres often do not release the entrapped drug for 7–14 days. This delay reflects the time during which the hydrolytic processes begin the degradation and consequent pore formation and fragmentation of the microspheres thus enhancing drug egress. The release kinetics and length of this delay are governed by microsphere particle size and surface morphology, polymer physicochemistry (e.g. molecular weight, copolymer ratio and crystallinity), as well as the physicochemical properties of the drug [14,15]. Detailed models of the degradation/erosion of the microspheres have been previously developed [16–21]. The goal of our study was to develop a microsphere system that can be used for continuous delivery of an anti-inflammatory drug for implantable medical devices, using a simple single emulsion system that had shown promising preliminary in vivo results in our laboratory.

In this paper, a new dexamethasone/PLGA microsphere system is reported which eliminates the delay in release by using a mixture of standard and predegraded microspheres to provide immediate and continuous release of dexamethasone for over 1 month. This dexamethasone/PLGA microsphere system has been designed to provide localized dexamethasone delivery at an implant site to suppress the inflammatory response.

2. Materials and methods

2.1. Materials

Poly(lactide-co-glycolide) 50 : 50 (PLGA; ave. mol. wt. = 40,000–70,000), polyvinyl alcohol (PVA; ave. mol. wt. = 30,000–70,000), and dexamethasone were purchased from Sigma Chemicals (St. Louis, MO). Methylene chloride, methanol, acetonitrile, and isopropanol were obtained from Fisher Scientific (Suwanee, GA) in high performance liquid chromatography (HPLC) grade. Distilled deionized water from a Millipore Milli-Q water filtration system was used in the formulation of the microspheres and preparation of all solutions. Saline, 0.9% (w/v) sodium chloride (USP), was used in the in vivo studies.

2.2. Microsphere formulation

PLGA microspheres loaded with dexamethasone were prepared by an oil-in-water (o/w) emulsion/solvent evaporation technique. The oil phase consisted of 20 mg of dexamethasone added to 5 ml of a mixture of 9 : 1 dichloromethane to methanol in which was dissolved 100 mg of PLGA (2% w/v). The dexamethasone and PLGA were used as received from Sigma. This oil phase was added to 100 ml of 0.2% (w/v) PVA solution, which was stirred at 1250 rpm for 30 min to achieve emulsification and the desired droplet size range. The resulting emulsion was stirred on a magnetic stir plate for $\sim$16 h to allow complete solvent evaporation. Predegradation of the microspheres was achieved by stirring for 1 or 2 weeks in the PVA solution. Alternative formulations were made with the addition of polyethylene glycol (PEG) of 8000 (or 3350) molecular weight to the (2% w/v) oil phase. Ten percent of the PLGA dissolved in the mixture of 9 : 1 dichloromethane to methanol was replaced by PEG.

The resulting microspheres were collected from the PVA solution by centrifugation at 8000 rpm (6500g). The microspheres were washed twice with distilled, deionized water, and lyophilized to dry, remove any trace of solvents, and extend the storage life.

2.3. Microsphere particle size analysis

The mean particle size and size distributions of the microspheres were measured using a Model 770 Accusizer (Particle Sizing Systems, CA). This system detects particle size based on the light blockage

[1] Website of polymer properties and technical information for Birmingham Polymers: http:www.birminghampolymers.com/tech.html.

principle, from 0.5–500 μm in diameter. A small volume of suspended microspheres, usually 0.5 or 1 ml was added to 50 ml of continuously stirred deionized distilled water. Each measurement reported was the mean of three samples per batch of microspheres for at least three different batches. Mean values and standard deviations were reported.

2.4. Microsphere image analysis

Each microsphere system was examined at 200×, 400×, and 600× magnification using a Nikon microscope with a digital camera attached. The morphology of the microspheres was examined as well as the presence of any non-encapsulated dexamethasone. Samples of predegraded and non-predegraded microspheres were placed on top of carbon tape, and covered with 20 nm of gold using a sputter coater following a standard procedure for scanning electron microscopy (SEM) studies. These samples were examined at 150–3000× magnification in an JEOL JSM-6320F SEM.

2.5. Microsphere encapsulation analysis

A few milligrams of microspheres, washed to eliminate free drug, were dissolved via sonication in 10–25 ml of acetonitrile. Drug concentration was analyzed using a Varian HPLC consisting of a model 345 dual wavelength UV detector, a Prostar 210 solvent delivery system with an inline filter and degasser and Dynamax HPLC Method Manager analytical software. Analysis was performed at 246 nm using a Water's 290 mm reversed phase μBondapak C-18 column with a mobile phase of 40% 2 mM acetate buffer (pH 4.8) and 60% acetonitrile flowing at 1 ml/min [22].

2.6. In vitro release of dexamethasone from microspheres

The in vitro release study was performed in phosphate buffered saline (PBS) with 0.01% (w/v) sodium azide under sink conditions. The in vitro release studies were performed on a stir plate in a constant temperature (37°C) warm room. Samples of 5–10 mg microspheres were added to 100 ml of PBS in sealed amber jars. At set time intervals, 2 ml samples were taken for analysis by syringe through a sterile 0.22 μm filter. The microspheres pulled into the syringe filter were returned by "back washing" 2 ml of replacement PBS through the syringe filter into the jars. Analyses of the release samples were performed using HPLC, as described above, with a mobile phase of 50 : 50 2 mM acetate buffer (pH 4.8) to acetonitrile flowing at 1 ml/min [22]. Three batches of microspheres were investigated and the means and standard deviations reported.

2.7. Dexamethasone degradation study

Since our initial in vitro release results suggested a progressive degradation of dexamethasone with time, the degradation of dexamethasone was studied in PBS and at 37°C to confirm this observation. For this study, three 25–30 ml aliquots of a dexamethasone calibration standard in PBS with and without sodium azide were placed in amber vials in the same constant temperature warm room (37°C) as used for the in vitro release studies. The aliquots without azide were kept in sealed containers and opened aseptically, as necessary, in a laminar flow hood. Every couple of days, 0.5 ml was removed from each aliquot by syringe. These samples were analyzed using the HPLC method described above for the in vitro release studies. All data points for each of the three samples at each time point were included in the graph to determine the degradation rate.

2.8. Mixed predegraded microsphere system

A mixed system of predegraded microspheres was developed to provide continuous release of dexamethasone starting immediately after implantation to up to 4 weeks. This microsphere system was an equal mixture (1 : 1 : 1) of microspheres which had been predegraded for 1 week, 2 weeks, and not at all. The microspheres were washed twice in isopropanol for a few minutes and collected by filtration (sterile 0.2 μm filters). After the isopropanol had completely evaporated, the microspheres were washed again with water and collected by filtration to eliminate any excess free drug. In vitro release of dexamethasone by this mixed microspheres system was evaluated for over 4 weeks in PBS at 37°C. Dexamethasone concentration in the release medium (PBS) was measured (using the HPLC) at various time points and the means and standard deviations (for $n = 3$) reported.

3. Statistical analysis

Three in vitro release studies were performed under the same conditions for each microsphere system. The means and standard deviations were calculated at each time interval. The means were graphed for each release profile with the standard deviations included as the error bars. Linear regression was performed on the dexamethasone concentration loss as a function of time and as a function of the area of the peak for the suspected degradation product. There were three samples for each time interval. All the data were used in the linear regressions. The coefficients of determination (r^2) for the regressions are given with the linear regression function on the two graphs for the degradation of dexamethasone.

4. Results and discussion

In this research, a microsphere system was developed using a simple o/w (single) emulsion process and a PLGA of median range molecular weight. Varied polymer physicochemistry and microsphere characteristics could have been used to change the release kinetics. However, for this study, our goal was to develop dexamethasone/PLGA microspheres, using a simple formulation process with a medium range molecular weight as a starting point, which could be used in vivo to show the effectiveness of dexamethasone released from microspheres to reduce inflammation to medical implants. In future research, we plan to extend the microsphere development into other molecular weights (M_w) of PLGA such as lower molecular weight. Lower M_w PLGA has been shown to have a faster release profile in other microsphere systems [23–28].

Although other researchers have used different geometries of PLGA drug delivery devices to control the release profile, we need to keep the microsphere geometry for ease of incorporation with the implant and injectibility [27,29]. Coatings or layers of different polymers have also been added to the outside of the PLGA by other groups to control the release kinetics [29–32]. Salts and surfactants have been used by other researchers to create pores in the PLGA and change the release profile to a zero order release [30,33–37]. However, the PLGA systems in these cases were formulated using casting, pressing, double emulsion or other more complicated processes. For our application, the complexity of the formulation process should be minimized, as each additional step adds additional risk of contamination. (For example, the additional risk of incomplete solvent evaporation in multiple emulsion techniques.) The use of additives or additional chemicals will provide new possible sources of tissue response/toxicity.

4.1. Characterization of the microspheres

The microspheres had a regular spherical morphology as shown in Fig. 1. The predegraded microspheres had surface irregularities. The microspheres had a Gaussian distribution of particle sizes ranging from 1 to 50 μm in diameter. Approximately 100,000 particles were counted for each sample. The average diameter size was 11 ± 1 μm for the standard microspheres and 12 ± 2 μm for predegraded microspheres. Predegrading microspheres caused no significant change in particle size.

An excess of dexamethasone was used in formulating the microspheres in order to maximize the amount encapsulated. Theoretically 16% encapsulation was possible, but not realistically expected. The encapsulation percentage for the microspheres was low, $\cong 4\%$ once any free dexamethasone was removed. The encapsulation percentage of the predegraded microspheres was further lowered by approximately the amount of the burst release. The PEG formulation of the microspheres had $\cong 3\%$ encapsulation.

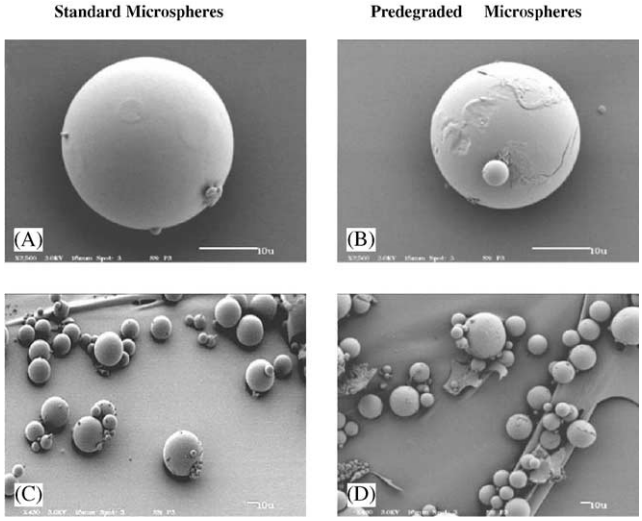

Fig. 1. SEM images of standard and predegraded microspheres. The standard microspheres are shown on the left (A and C) and the predegraded microspheres are shown on the right (B and D). Both types of microspheres have a regular spherical morphology. The surfaces of the two types differ, as can be seen at high magnification (A and C). The surface of the standard microspheres (A) was smooth, while the surface of the predegraded microspheres (B) had surface irregularities. The irregularities in the surface of the microspheres are caused by the degradation of the PLGA. Ranging from dimples reflecting a channel into the PLGA, to entire regions with a rough irregular degraded surface. In the low magnification images (C and D) the surface topography is not visible, but a representative distribution of the microsphere sizes for both standard and predegraded microspheres are shown.

4.2. Dexamethasone degradation

Our initial release studies showed a loss of dexamethasone after correction for dilution due to replacement of samples with fresh PBS solution (data not shown). A secondary HPLC peak developed and increased in size with increase in the elapsed time for the in vitro release samples. This secondary peak was a dexamethasone degradation product, indicating that degradation was the probable cause of the reduction in dexamethasone concentration.

The degradation of dexamethasone may be due to microbiological action or environmental factors such as temperature, exposure to PBS (water), or pH. The pH of the PBS in the release studies, was checked after 2 months had elapsed and was still within the range of 7.0 to 7.4. Therefore, the volume of PBS necessary to maintain sink conditions and its buffering capacity was sufficient to diminish any effects of the PLGA degradation on bulk solution pH. However, this did not

eliminate the possibility that lowered pH inside the microspheres may contribute to dexamethasone degradation. Previous in vitro studies have shown that PLGA degradation causes a decrease in pH in the interior of the microspheres [38,39]. Therefore, to eliminate the possibility of degradation by low pH, degradation studies were performed on dexamethasone (without PLGA) in PBS solution at pH 7.4. A decrease in dexamethasone concentration was still observed despite the constant pH of 7.4. This ruled out pH as being the cause of degradation of dexamethasone in the microsphere release studies. Dexamethasone standards prepared in PBS with and without sodium azide were used to comparatively study drug degradation by microbiological action at elevated temperatures. Aliquots of these dexamethasone standards were placed in the same constant temperature (37°C) warm room as used for the in vitro release studies. The dexamethasone concentrations in all cases decreased steadily as shown in Fig. 2, with and without sodium azide. Therefore, degradation was not due to microbiological action.

A plot of the loss of concentration of dexamethasone versus the area of the new secondary peak (standardized for the injection volume) resulted in a linear relationship (see Fig. 3), inferring that the loss of dexamethasone in the in vitro release studies was a consequence of the production of this degradation product. The above function was subsequently used to correct the in vitro release study results to account for dexamethasone degradation. Previous studies from our laboratory have shown the importance of drug stability on the analysis of release data from controlled release microspheres, and a model was developed to account for drug degradation [40].

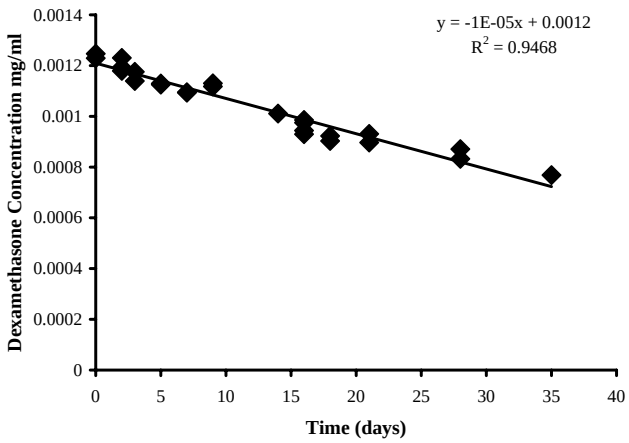

Fig. 2. Dexamethasone degradation with time in PBS (pH 7–7.4) containing sodium azide at 37°C. The dexamethasone concentration (mg/ml) in dexamethasone standard solutions, as determined by the HPLC, is plotted as a function of the elapsed time the solutions have spent in a 37°C warm room. The concentration of dexamethasone decreases linearly with time.

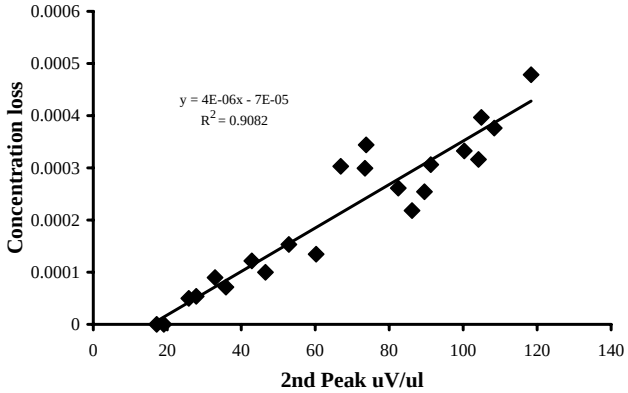

Fig. 3. Dexamethasone degradation function in PBS (pH 7–7.4) containing sodium azide at 37°C. As the loss in dexamethasone concentration (mg/ml) in the standard solutions increased with the elapsed time the solutions have spent in a 37°C warm room, a secondary HPLC peak increased. The loss in concentration for the dexamethasone standard samples taken at different elapsed time intervals was plotted as a function of the secondary peak area. The graph of the samples showed a linear slope. Linear regression of this data provided a function to correlate the secondary peak to the amount of dexamethasone degraded. This function as well as the correlation constant is provided in the graph above.

4.2.1. Dexamethasone release studies

Three in vitro release studies were performed under the same conditions for each microsphere system. The standard deviation increased for the later readings due to the cumulative error of small quantities of microspheres lost during sample collection even though filtered samples were back flushed into the release media.

Fig. 4 shows that the standard microspheres (without predegradation) had an initial burst release followed by a delay and then continued release of dexamethasone. The initial burst release was due to microsphere surface associated drug. The delay reflected the time necessary for PLGA hydrolysis to erode sufficient PLGA to allow dissolution and release of entrapped drug [41]. In biomedical applications (such as biosensor implants), this delay would prevent availability of drug for inflammation suppression during the crucial first 2 weeks post-implantation. The second period of drug release continued over the 1-month study period. Both periods of release, 1 day to 2 weeks and 2 weeks to 1 month, appeared to have linear or zero order release rates. Release rates follow the rate equation given below [42].

$$C = C_0(e^{-kt}), \quad (1)$$

C is the concentration of the released drug, C_0 the release rate coefficient or the slope of the release plot, k the release constant and t the time.

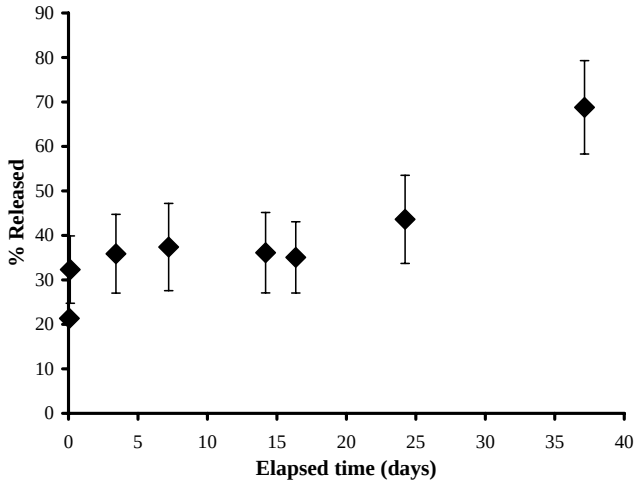

Fig. 4. Cumulative dexamethasone released from standard microspheres during the in vitro release studies in PBS (pH 7–7.4) containing sodium azide at 37°C ($n = 3$). The total amount of dexamethasone released into the PBS as a percentage of the total amount of dexamethasone encapsulated into the microsphere system was plotted as a function of the elapsed time from the beginning of the release studies. The initial burst of dexamethasone released is due to the dexamethasone associated with the surface of the microspheres. The plateau, which follows, reflects the delay needed for PLGA hydrolysis to erode the microsphere sufficiently to allow drug egress. The release of dexamethasone continues at a linear rate after this delay of approximately 2 weeks.

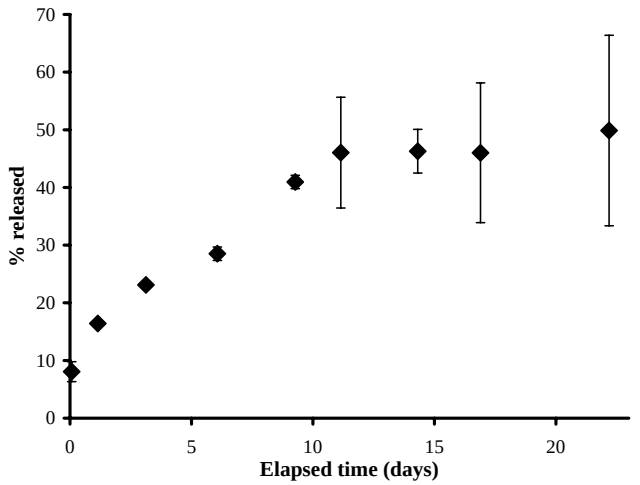

Fig. 5. Cumulative dexamethasone released from predegraded microspheres during the in vitro release studies in PBS (pH 7–7.4) containing sodium azide at 37°C ($n = 3$). The total amount of dexamethasone released into the PBS as a percentage of the total amount of dexamethasone encapsulated into the microsphere system was plotted as a function of the elapsed time from the beginning of the release studies. The initial burst of dexamethasone released is due to the dexamethasone associated with the surface of the microspheres. The release of dexamethasone from the predegraded microspheres begins immediately and continues at a linear rate for approximately 2 weeks before decreasing. There is no delay for PLGA hydrolysis to erode the microsphere sufficiently to allow drug egress. The decrease in the release rate is due to the depletion of dexamethasone from the microspheres.

The predegraded microspheres released dexamethasone continuously with no delay (Fig. 5). The initial burst release of dexamethasone was due to the dissolution of dexamethasone in the outer surface of the microspheres. The release rate appeared to have a linear or zero order release rate from days 1 to 12. The release rate decreased as the microspheres became depleted of drug.

The predegraded and standard microspheres were then combined to provide continuous release over 1 month (Fig. 6). One-third of the combined batches was predegraded for 2 weeks, another third for only 1 week and one-third was standard microspheres. All batches were washed with isopropanol to eliminate any free drug crystals.

The release profile of this mixed predegraded microsphere system began with an initial burst release and continued with an approximately zero order rate for 1 month (Fig. 6). The initial burst release was due to diffusion of the dexamethasone on or near the surface of the microspheres.

PEG was added during microsphere preparation to determine if it could extend the microsphere release. The addition of 10% w/w PEG to the PLGA in the oil phase extended the delay in the dexamethasone release from 11 to 21 days compared to standard microspheres (Fig. 7). Mixing these microspheres with the predegraded and standard microspheres could be used to continue zero order release beyond the 1-month period.

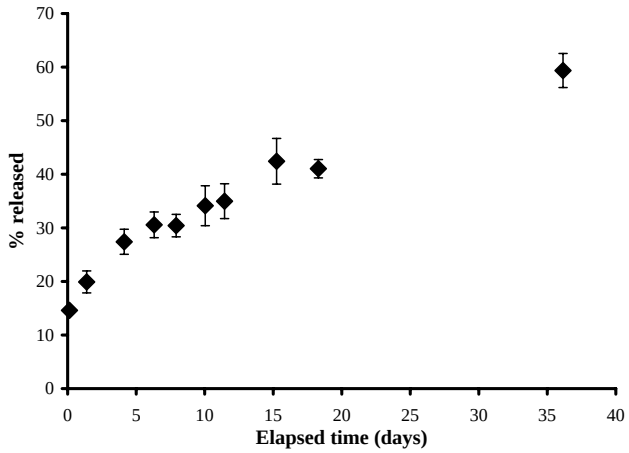

Fig. 6. Cumulative dexamethasone released from mixed predegraded microsphere system during the in vitro release studies in PBS (pH 7–7.4) containing sodium azide at 37°C ($n = 3$). The total amount of dexamethasone released into the PBS as a percentage of the total amount of dexamethasone encapsulated into the microsphere system was plotted as a function of the elapsed time from the beginning of the release studies. The initial burst of dexamethasone released is due to the dexamethasone associated with the surface of the microspheres. The release of dexamethasone from the predegraded microspheres begins immediately and continues at a linear rate over 1 month.

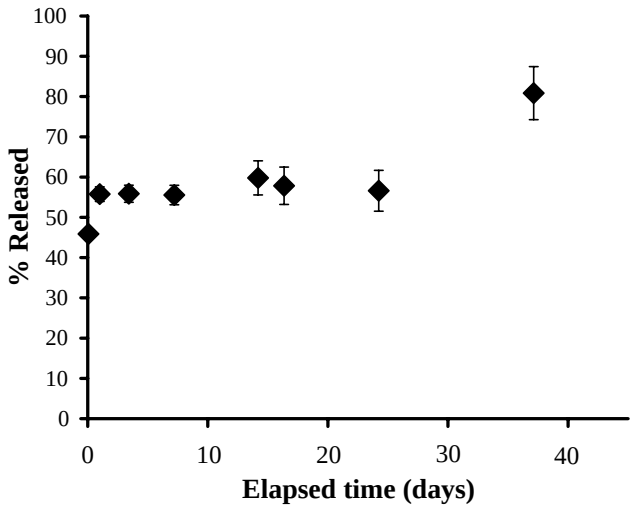

Fig. 7. Cumulative dexamethasone released from PLGA microspheres with 10% (w/w) PEG added, during the in vitro release studies in PBS (pH 7–7.4) containing sodium azide at 37°C ($n = 3$). The total amount of dexamethasone released into the PBS as a percentage of the total amount of dexamethasone encapsulated into the microsphere system was plotted as a function of the elapsed time from the beginning of the release studies. The initial burst of dexamethasone released is due to the dexamethasone associated with the surface of the microspheres. The plateau, which follows, reflects the delay needed for PLGA hydrolysis to erode the microsphere sufficiently to allow drug egress. The release of dexamethasone continues at a linear rate after this delay of approximately 4 weeks.

5. Conclusions

A PLGA microsphere system was developed that continuously releases the anti-inflammatory drug dexamethasone over a 1-month period in vitro. The standard PLGA microspheres formulation did not provide release of dexamethasone during the crucial period of 2 days to 2 weeks in the in vitro release studies. Although the predegraded microspheres did provide release of dexamethasone during the first 2 weeks, they did not sustain this release rate over the 1-month study period. A mixed system of predegraded and standard dexamethasone PLGA microspheres was able to provide continuous delivery of dexamethasone for 1 month. The addition of PEG to the microsphere system delayed the start of the linear release period and thus adding these microspheres to the mixed predegraded and standard microsphere system may extend the continuous release beyond 1 month. We expect that continuous release of dexamethasone by this mixed microsphere system will be able to control inflammation around implants and that as a result the function and longevity of these implants in vivo will be enhanced.

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