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Polyelectrolyte-coated alginate microspheres as drug delivery carriers for dexamethasone release

R. D. Jayant¹, M. J. McShane², and R. Srivastava¹

¹School of Biosciences and Bioengineering, IIT Bombay, Powai, Mumbai, India

²Biomedical Engineering Department, Texas A& M University, College Station, TX, USA

Abstract

Alginate microspheres loaded with dexamethasone were prepared by the droplet generator technique. Important parameters affecting drug release, including initial drug content, the type of polyelectrolyte coating, and a combination of different ratios of coated and uncoated microspheres were investigated to achieve in vitro dexamethasone delivery with approximately zero order release kinetics, releasing up to 100% of entrapped drug within 1 month, wherein dexamethasone released at a steady rate of 4.83 µg/day after an initial burst release period. These findings imply that these polyelectrolyte-coated alginate microspheres show promise as release systems to improve biocompatibility and prolong lifetime of implantable glucose sensors.

Keywords

Alginate; dexamethasone; polyelectrolyte coating

Introduction

Blood glucose monitoring is an integral part of diabetes management and the maintenance of near-normoglycemia (Evans et al., 2005). Currently, patients measure their own blood glucose concentrations by intermittent finger-prick capillary blood sampling, a method that is painful and uncomfortable. The development of technology for minimal or non-invasive and continuous glucose sensing is, therefore, considered a priority in diabetes care. However, there are significant problems with the commercially available minimally invasive in vivo glucose sensors which are based on subcutaneously implanted amperometric enzyme electrodes (Pickup, 2004) or reverse iontophoresis (Pickup et al., 2005) including inaccurate results, low precision, and frequent calibration. On the other hand, fluorescence-based sensors that are implanted intradermally (Russell et al., 1999; McShane, 2002; Brown, Srivastava, & McShane, 2005; Brown et al., 2006) to measure the interstitial glucose level that can then be correlated with blood glucose levels may be an ideal solution (Thennadil et

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Address for Correspondence: Dr Rohit Srivastava, Assistant Professor, School of Biosciences and Bioengineering, IIT Bombay, Powai, Mumbai 400076, India. Tel: 91-22-25767746. Fax: 91-22-25723480. rsrivasta@iitb.ac.in.

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al.,2001). The added advantage with such biosensors is the ease of monitoring using simple optical instrumentation (McShane et al., 2000). Significant results have been demonstrated for such 'Smart-Tattoo' glucose biosensors (Russell et al., 1999; McShane, 2000; McShane, 2002; Chinnayelka & McShane, 2005, 2006; Brown & McShane, 2006; Chaudhary & Srivastava, 2008; Chinnayelka, Zhu, & McShane, 2008) that performs optimally in vitro. However, these results can not be related in vivo, since upon implantation such sensors are not capable of reliable glucose measurements. Implantation of biosensors typically causes tissue injury, which triggers a cascade of inflammatory responses that compromise device functionality and ultimately lead to device failure (Sharkaway et al., 1998; Hickey et al., 2002). This continuous immunos-timulatory reaction consists of the following sequential biological events: acute inflammation, angiogenesis, temporary matrix synthesis, collagen deposition, and fibrous capsule formation (Dungel, P et al. 2008). Also, calcification and protein fouling (Wisniewski, Moussy & Reichert, 2000) of implanted biosensors may also occur in vivo (Patil, Papadmitrakopoulos, & Burgess, 2004). It is generally believed that inflammation-initiated fibrous encapsulation, calcification, and protein bio-fouling (Sharkawy et al., 1997) are the leading causes of in vivo sensor failure (Anderson, 2001). Fibrous encapsulation can also deprive the sensor of adequate fluid supply for accurate detection of glucose levels, further compromising device functionality (Sharkaway et al., 1998). It is therefore apparent that localized inflammation must be minimized to ensure sensor functionality and long lifetime (Koschwanetz et al., 2008). This can be achieved using immunomodulating agents. However, long-term use and high dose of immunomodulating agents like dexamethasone can cause severe systemic side-effects to complicate diabetes management; therefore, an ideal solution is controlled, continuous local delivery of dexamethasone at the implantation site using a suitable carrier system. This can be considered as a means to avoid undesirable side effects to aid in development of a long lasting implantable glucose biosensor. Such an approach to control acute inflammation has been reported earlier (Patil et al., 2004), wherein polyvinyl alcohol (PVA) hydrogels have been developed as coatings on the sensor device. However, problems with rapid release of drug were a concern; therefore a PLGA microsphere/PVA hydrogel composite drug delivery system for coating the implantable was developed (Galeska et al., 2005; Patil, Papadmitrakopoulos, & Burgess, 2006; Bhardwaj et al., 2007). However, this approach is limited to macroscale devices wherein the surface of the biosensor can be modified using dip coating procedures; this approach cannot be applied for the microparticles or capsules employed in 'smart tattoo' glucose biosensors. Therefore, in this work, we aimed to develop a novel approach for release of an immunomodulating drug from alginate microspheres. The layer-by-layer (LBL) self-assembly technique was used to coat the alginate microspheres in order to achieve controlled release of encapsulated drug that will aid in improving sensor functionality and extend in vivo operating lifetime. Nanofilms are formed by electrostatic interactions between oppositely charged polyions species to create alternating layers of sequentially adsorbed polyions (Lvov, Decher, & Mohwald, 1993; Decher, Lvov, & Schmitt, 1994; Lvov et al., 1999). A major advantage of the LBL technique is the possibility to tune the layer thickness to nanometer scale and thus control the mechanical properties and the permeability of the polyelectrolyte shell (Hammond, 2004; De Geest et al., 2006). The influence of drug concentration and type of polyelectrolyte coating on release kinetics was then investigated. Furthermore, we explored the possibility of combining systems of coated

and uncoated microspheres with different individual release profiles as a means of more precisely controlling the overall kinetics. Finally, to ensure that the fabricated drug delivery device fulfills the prerequisites for implantation as recommended by the regulatory bodies, cytotoxicity studies were completed on the uncoated and polyelectrolyte-coated dexamethasone-loaded microspheres using L929 mouse fibroblast cell line. The results presented here are the evaluation of a novel technology that capitalizes on the advantages of both of the above concepts for encapsulation and controlled release, and takes an integrative approach to engineering the analyte response properties.

Materials and methods

Low viscosity alginate and dexamethasone-21-phosphate di-sodium salt (MW-392.5) were purchased from Sigma (India). Polyelectrolytes, including sodium poly (styrene sulfonate) (PSS, 70 kDa), poly (allylamine hydrochloride) (PAH, 70 kDa), Poly (acrylic acid) (PAA, 45KDa) and Poly (diallyldimethylammonium chloride) (PDDA, 20–35 KDa) were also purchased from Sigma (India). Other chemicals, including 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS) and Phosphate buffer saline tablets (PBS tablets) were purchased from Sigma-Aldrich (India). Sodium azide was purchased from Loba Chemie (Mumbai, India). Calcium chloride and dialysis membrane (10–14kDa) were purchased from Merck (Mumbai, India) and Hi-Media Laboratories (Mumbai, India), respectively. All chemicals were reagent grade and used as received.

Instrumentation

A commercial Encapsulation Unit (J30, Nisco Engineering AG, Zurich) and syringe pump (Multi-Phaser™, model NE-1000, New Era Pump Systems, NY) were used for preparing alginate microspheres. A Scanning Electron Microscope (Hitachi S3400, Tokyo, Japan) was used for observing the morphology of uncoated and coated microspheres, whereas particle size analysis was performed using a Cuvette Helos (CUV-50ML/US, Sympatech Instrument, Germany). Zetaplus (Brookhaven Instruments, USA) was used to measure the surface charge of microsphere after the deposition of each polyelectrolyte coatings. FTIR spectra were obtained using a Nicolet Spectrometer Magna-IR 550 (USA) wherein microspheres were completely dried and the powdered microsphere sample was then mixed with potassium bromide salt before taking measurements. A UV-Vis Spectrophotometer (Helios Alpha, Thermoscientific, USA) was used to determine dexamethasone concentration during in vitro release studies. A Microplate Reader (Thermo Electron Corporation, USA) was used for biocompatibility studies.

Preparation of alginate microspheres

Dexamethasone-loaded calcium alginate microspheres were prepared using a commercially available droplet generator (Nisco Engineering AG, Zurich) using a standard process. Briefly, 10 ml of 2% w/v sodium alginate solution was mixed with previously weighed dexamethasone sodium salt. The mixture was then extruded into a vessel containing 250 mM calcium chloride solution at a flow rate of 20 ml/h under a pressure of 75 mbar using the Var J30 droplet generator equipment. Droplets added to the CaCl₂, were allowed to

react for external gelation under continuous stirring. The hardened drug loaded alginate microspheres were then separated by centrifugation (1000 rpm for 1 min). The microspheres were then characterized using SEM and particle sizing. For SEM imaging, samples were prepared by placing a droplet of an aqueous microsphere suspension onto a glass slide, samples were dried overnight, and then sputter-coated with gold prior to imaging at 5–10 eV. Particle size analysis was performed using wet particle suspensions in distilled water. Drug loading efficiency was determined by measurement of absorbance of drug present in the supernatant after centrifugation relative to the absorbance of the dexamethasone-alginate precursor (λ_{max} of 242 nm). Therefore,

$$\text{Drug loading efficiency (\%)} = \frac{\text{Experimental drug loading}}{\text{Theoretical drug loading}} \times 100$$

where experimental drug loading is drug entrapped after the microspheres preparation and theoretical drug loading is the initial amount of dexamethasone used for encapsulation. All the measurements were conducted in triplicate and the mean values and standard deviations calculated.

Application of polyelectrolyte coatings

Polycations, including PAH and PDDA and polyanions PSS and PAA were used for this study. As an example, solutions of PAH (poly allylamine hydrochloride, cationic) and PSS (sodium poly styrene sulfonate, anionic) initially used for assembling (PAH/PSS)₁ multilayers were prepared in distilled water at 2 mg/ml with 250 mM calcium chloride salt. As the core particles are negatively charged, they were dispersed in 2 ml of 2 mg/ml PAH solution for 20 min, followed by two consecutive washing steps in distilled water to remove excess polyelectrolyte and finally in 2 ml of 2 mg/ml of PSS solution for 20 min to complete one bilayer. Additional polyelectrolyte coatings were applied, but each additional bilayer is expected to significantly reduce the rate of release of the encapsulated drug (Srivastava & McShane, 2005), therefore, they were not used for in vitro drug release experiments. An additional composition comprising chemically cross-linked PAH and PAA was also used as a part of this study. The chemical cross-linking was accomplished using a protocol described in an earlier work (Srivastava et al., 2005). Further, the cross-linked LbL-coated microspheres were analyzed using FTIR, wherein the microspheres were completely dried and mixed with potassium bromide before making measurement. After this, confirmation of polyelectrolyte nanofilms assembly was confirmed using zeta potential analysis. The ζ -potential was calculated from the electrophoretic mobility using the Smoluchowski relation. In this experiment, 50 μ l samples containing the polyelectrolyte-coated microspheres were diluted in 2 ml of distilled water. Finally, SEM images were obtained to image the effect of polyelectrolyte nanofilm deposition on particle surface structure.

In vitro drug release study

Alginate microspheres were prepared as described above using dexamethasone sodium salt. The microspheres were introduced in dialysis membrane (molecular weight cutoff: 10–14kDa) and transferred to a glass beaker containing 100 ml of PBS (pH 7.4) and 0.01% w/v sodium azide. The samples were incubated at $37 \pm 0.5^\circ\text{C}$ with constant agitation of 250 rpm

for the release studies. One milliliter of buffer was periodically withdrawn from the beaker and replaced to maintain the drug concentration below 10% solubility (sink condition). The amount of released dexamethasone in the collected medium was determined spectrophotometrically at $\lambda_{\max}$ 242 nm. All the in vitro release studies were conducted in triplicate ($n = 3$), mean values and standard deviation were then calculated as descriptive statistics and used for hypothesis testing.

Effect of drug loading on burst release

Varying concentrations of dexamethasone including 0.25, 0.50, 0.75, and 1.0 mg/ml were used to study the burst release from the uncoated alginate microspheres in order to determine the initial drug loading for the lowest observed burst release. Each formulation was prepared in duplicate and each analysis was done in triplicate. Data were fitted to the zero and first-order model equations to identify the drug release mechanism.

Effect of polyelectrolyte nanofilm coating on in vitro drug release

Polycations including PAH and PDDA and polyanions including PSS and PAA were used for this study. Polyelectrolyte coating were deposited according to the protocol just described. In vitro drug release studies were then completed according to the protocol just described. An additional coating involving chemically cross-linked PAH and PAA was also used as a part of this study. The chemical cross-linking was accomplished using a protocol described in an earlier work (Srivastava et al., 2005). Briefly, EDC and NHSS were used at a ratio of 1:0.6 and added to 1 ml of (PAH/PAA)₁ coated microspheres under continuous stirring. Afterwards, the microsphere was centrifuged to separate the unreacted EDC/NHSS and washed twice with distilled water before being used for the release study. Confirmation of chemical conjugation of the polyelectrolyte coatings was provided by FTIR measurements.

Effect of different ratios of uncoated and (PAH/PSS)₁ coated microspheres on dexamethasone release

We explored the possibility of combining systems of coated and uncoated microspheres with different individual release profiles as a means of more precisely controlling the overall kinetics. Therefore, in order to achieve zero-order release profile with 100% release of encapsulated drug within 30 days, various combinations of uncoated and (PAH/PSS)₁ coated dexamethasone-loaded microspheres were prepared. Microspheres types were combined in different ratios, e.g. 25% coated to 75% uncoated; 50% coated to 50% uncoated and 75% coated to 25% uncoated. The combinations were used for additional in vitro release studies.

In vitro cytotoxicity studies

The cytotoxicity of the dexamethasone-loaded uncoated and (PAH/PSS)₁, (PDDA/PSS)₁, (PAH/PAA)₁, and EDC(l-Ethyl-3-[3-dimethyl amino-propyl] carbodiimide hydrochloride) and NHSS (N-Hydroxysuccinimide) cross-linked (PAH/PAA)₁ coated alginate microspheres were evaluated by using sulforhodamine-B (SR-B) semi-automated assay. An in vitro biocompatibility study of these samples was performed using L929 (Mouse fibroblasts) cell

lines obtained from National Centre for Cell Science (NCCS) (Pune, India). The cells were grown in modified DMEM (Dulbecco's modified essential medium, Sigma, USA) supplemented with 10% FBS (fetal bovine serum, Sigma, USA) and 1% antibiotic/antimycotic solutions (Himedia, India) and incubated at 37°C under 5% CO₂ and saturated humid environment. Nearly confluent cells in 25 cm² tissue culture flasks were trypsinized by trypsin-EDTA (ethylene diamine tetra acetic acid) solution and centrifuged at 1000 g for 10 min. The cell pellet was then resuspended in fresh media. Cells were counted and cell count was adjusted according to the titration readings so as to give an optical density in the linear range (from 0.5–1.8). Samples were tested in 96 well plates in three triplicates, each well receiving 90 µl of cell suspension with a concentration of 1×10^4 cells per well. The plate was then incubated at 37°C in CO₂ incubator for 24 h; 10 µL of diluted polyelectrolyte coated and uncoated alginate microspheres were added after 24 h incubation to the 96 well-plate and further incubated for 48 h. Finally, the experiment was terminated by gently layering the cells in the wells with 50 µl of chilled 50% TCA (Trichloroacetic acid) for cell fixation. Plates were kept in a refrigerator (4°C) for 1 h. The plates were washed thoroughly with tap water at least five times and air dried. For the assay, plates were stained with 50 µl of 0.4% SR-B for 20 min then washed with 1% acetic acid at least five times and air dried. Finally, the bound SR-B was eluted with 100 µl of Tris (10 mM, pH 10.5) for 10 min. Thereafter, the plates were shaken for 1 min using an automated shaker and the absorbance (O.D) of each well was read in a micro plate reader (Thermo Electron Corporation, USA) at 540 nm with reference to 690 nm against blanks culture media without any cells.

Results and discussion

Preparation and characterization of alginate microspheres

The particle size of the alginate microspheres was determined using optical microscopy, from which it was observed that particles were in the range of $60 \pm 10 \mu\text{m}$ ($n > 400$). SEM images of microspheres corroborated these estimates, as shown in Figure 1(a). Particle size analysis further provided confirmation that particles were in the range of mean diameter of $60 \pm 10 \mu\text{m}$ (Saurter mean diameter, SMD at 90% cumulative distribution), as demonstrated in Figure 2. The encapsulation efficiency of dexamethasone-loaded uncoated alginate microspheres was calculated to be $77 \pm 8\%$.

Layer-by-layer self-assembly on alginate microspheres loaded with drug

Polyelectrolyte pairs including PAH/PSS, PDDA/PSS, PAH/PAA, and cross-linked PAA/PAH were used to coat the drug loaded microspheres. To confirm successful deposition of nanofilms coatings on top of the alginate microspheres, zeta potential analysis was performed as shown in Figure 3, while SEM images were obtained for polyelectrolyte-coated alginate microspheres, as shown in Figure 1(b). The roughness on the surface of microspheres is attributed to the deposition of nanofilm coatings. The graph in Figure 3 clearly indicates reversal of surface charge with the deposition of up to four polyelectrolyte layers on alginate microspheres. The ζ -potential values clearly demonstrate that the charge of the microspheres reversed upon coating them with poly-electrolytes, indicating that multilayer build-up is taking place. For charged microspheres, electrostatic interactions between the microspheres and the polyelectrolyte are the main driving force for multilayer

build-up. The multiple ionic bonds formed between adsorbing polymer and microspheres substrate are sufficient to retain the adsorbed layers upon exposure to the next polyelectrolyte. This is expected to result in better control over the release profile and a decrease in surface-associated drug release. Polyelectrolyte layers further act as diffusion barriers to drug release and lead to a decrease in total drug release due to an increase in mass transfer resistance.

FTIR results indicate that the most significant peak in the infrared spectrum for alginate was at $\sim 1600\text{ cm}^{-1}$, which is attributed to carbonyl groups on alginate molecules. The peaks at 3400 cm^{-1} and 3150 cm^{-1} indicate the presence of $-\text{NH}_2$ groups, while the peaks at 1115 and 1170 cm^{-1} indicate presence of $-\text{SO}_2^-$ groups. These peaks can be used to study the interaction of polyelectrolyte coatings on alginate microspheres. The 3460 cm^{-1} peak observed for microspheres with cross-linked coatings indicated the presence of $-\text{NH}_2$ group, which confirms the chemical complexation of carboxylic acid groups on alginate with amine groups NHSS (results not shown here).

Effect of initial drug loading on burst release behavior

The release behavior is mainly dependent on the polymer type, particle size, particle surface, and specific drug-matrix interactions within the system. However, these parameters are not independent, having multiplicative effects on each part of the release profile. Therefore, achieving a desired release behavior involves identifying the main influences and manipulating the different parameters simultaneously to reach the desired release profile. Some parameters can be manipulated so that the entire drug is released in the induction period to reduce release to a single continuous profile. For this, the biggest problem is to reduce the initial burst so as to maximize the amount of drug to be released in the induction period. In order to achieve 100% drug release within a period of 1 month with zero-order release kinetics, different concentrations of dexamethasone (0.25, 0.5, 0.75, and 1 mg/ml) were used in the precursor alginate solution. The burst release of encapsulated drug is affected by changing the initial drug loading, as shown in Figure 4 (data shown for initial 1 day of drug release). The burst release of encapsulated drug for 0.25, 0.5, 0.75, and 1 mg/ml drug loaded alginate microspheres is 19%, 24%, 28%, and 33%, respectively; as expected, there was increase in the burst release of encapsulated drug observed with increasing initial drug loading. In order to achieve an approximate zero-order release profile and 100% drug release over a period of 1 month to combat localized inflammation, the drug loading demonstrating lowest burst release was chosen for further studies.

Effect of polyelectrolyte coatings on in vitro drug release

To achieve the objective of obtaining a zero-order release profile and 100% release over a period of 1 month, the layer-by-layer self-assembly technique was used to deposit nanofilm coatings and verified as described above. The in-vitro drug release profile of uncoated and $(\text{PAH/PSS})_1$, $(\text{PDDA/PSS})_1$, $(\text{PAH/PAA})_1$, and EDC and NHSS cross-linked $(\text{PAH/PAA})_1$ coated alginate microspheres are shown in Figure 5(a). The cumulative release from uncoated dexamethasone-loaded microspheres was 100% in 22 days and 79%, 68%, 59%, and 29% from coated microspheres in 30 days for $(\text{PAH/PSS})_1$, $(\text{PDDA/PSS})_1$, $(\text{PAH/PAA})_1$, and EDC and NHSS cross-linked $(\text{PAH/PAA})_1$ microspheres, respectively. There

was a significant difference ($p < 0.05$) in the rate and extent of drug release as observed in uncoated and coated microspheres. Only $\sim 30\%$ of encapsulated drug was released from two bilayer-coated alginate microspheres; therefore, these microspheres were not used in further release studies. Approximately 20%, 11%, 12%, 6.5%, and 5% of the drug was released during the initial burst phase from uncoated, (PAH/PSS)₁, (PDDA/PSS)₁, (PAH/PAA)₁, and cross-linked (PAH/PAA)₁ coated alginate microspheres in the first day, respectively (shown in Figure 5b).

Effect of different ratios of uncoated and (PAH/PSS)₁ coated microspheres on dexamethasone release

Based on the observed release profile of the coated and uncoated microsphere systems, it was hypothesized that it may be possible to modulate release kinetics in a desired fashion by mixing appropriate proportions of one or two different types of microspheres, each having a different release profile (Narayani & Rao, 199632). Thus, we chose to mix uncoated microspheres with (PAH/PSS)₁-coated particles in various ratios and perform in vitro release studies to compare the predicted release profiles. For this, different combination of uncoated and (PAH/PASS)₁ coated microspheres, e.g. 25CP:75P, 50CP:50P, and 75CP:25P (CP-Coated particles and P-Plain particles) were used to achieve zero order release behavior and 100% drug release in 30 days. For the uncoated microspheres, initial burst release was 19.25% and for 25CP:75P, 50CP:50P, and 75CP:25P, it was found to be 10.50%, 16.11%, and 6.54%, respectively, as shown in Figure 6(a). The release profile demonstrates zero order release kinetics after a burst release period, which lasted for 1 day. The cumulative release of uncoated and different combination of uncoated and (PAH/PASS)₁ coated dexamethasone-loaded microspheres including 25P:75CP, 50P:50CP, and 75P:25CP was 100%, 99.94%, 86.63%, and 75.71%, respectively, as shown in Figure 6(b). There was a significant difference in the rate and extent of drug release as observed in uncoated and different combination of uncoated and (PAH/PASS)₁ coated microspheres ($p < 0.05$). The 25P:75CP combination showed only 6.54% burst release as compared to 19.25% in the case of uncoated microspheres with $\sim 100\%$ dexamethasone release in 30 days as compared to 22 days with uncoated microspheres. Thus, dexamethasone released at a steady rate of 4.83 $\mu\text{g/day}$ from 25P+75CP combination for the initial 18 days, after an initial burst release period, which is sufficient to combat localized inflammation (Sharkaway et al., 1998).

Mathematical modeling for drug release kinetics

The drug release from polymeric micro/nano particulate systems is considered a combination of Fickian (diffusion) and non-Fickian movement of drug molecules through polymer chains (Kosmidis et al., 2003). Further, a biphasic pattern was observed, i.e. initial high release and zero order release afterwards, which is characteristic of matrix diffusion kinetics. The release data was fitted to zero-order kinetics release equation. The regression goodness of fit parameter (R^2) (shown in Table 1) indicated that the drug release pattern followed the diffusion control mechanism from the coated microspheres. This also serves to understand whether LBL self assembly alters the release mechanism. As hypothesized earlier, the release data of the combined system (coated and uncoated microspheres) must follow zero order kinetics for the entire 30 days as observed with the individual system. To achieve this, data of the combined system was fitted to the zero order kinetic to predict the

release behavior. It was observed that the zero-order kinetics was followed only for the initial period of 18 days, excluding the burst release period, as shown in Figure 6(c). Data from days 18–30 also showed a fit with the zero-order release kinetics, with the release rate of 1.4908 µg/day for 25P+75CP combination (Table 2). This dual behavior can be attributed to the fact that the release profiles of the combined microspheres system have three different release phases namely: Phase I: burst release (combined burst from both systems); Phase II: synergistic release from uncoated and coated microspheres, with dominant contribution from uncoated particles; and Phase III: release of drug from coated microspheres. Thus, it is difficult to fit the experimental data with the theoretical release kinetics and achieve zero-order release kinetics for the entire 30 day period. The kinetic profile for days 2–18 has $R^2 = 0.9955$ and follows the zero order kinetics, whereas the profile for days 18–30 has $R^2 = 0.9971$, suggesting that the overall system appeared to follow predicted zero-order kinetics. Release rates follow the rate equation given below.

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

C is the concentration of the released drug, C_0 the release rate coefficient, k the release constant, and t the time. Similar trends in release profiles were obtained by Hickey et al. (2002) by using pre-degraded and standard PLGA microspheres where two periods of release were studied, including 1 day to 2 weeks and 2 weeks to 1 month. In that work, both periods of release appeared to have linear or zero-order release rates. Once all the drug has been released over a period of 30 days, no localized inflammation is expected to arise out of the implanted microspheres (Sharkaway et al., 1998).

Analysis of diffusion model for dexamethasone release

To determine drug diffusion constant, we have used the Fick's second law of diffusion as shown in equation (2).

$$\frac{\partial c}{\partial t} = D \left(\frac{\partial^2 c}{\partial r^2} + \frac{2}{r} \frac{\partial c}{\partial r} \right) \quad (2)$$

where c denotes the concentration of drug, t the time, D the diffusion coefficient, and r is the radial coordinate. For calculating the diffusion constant, a boundary condition was applied to equation (2) (Kulkarni et al., 2000; Siepmann et al., 2005) to arrive at a final expression, as shown in equation (3).

$$\frac{Mt}{M_\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp \left(-\frac{n^2 \pi^2}{R^2} Dt \right) + \alpha \quad (3)$$

A theoretical fit of equation (3) to our experimental release data for dexamethasone-loaded microspheres coated with different polyelectrolytes along with drug diffusivity data corresponding to the best-fit lines are also given in Table 1. The value of dexamethasone diffusivity in alginate matrix for uncoated and coated particles was calculated and found to be in the range of 10^{-14} to 10^{-13} cm²/s. The decrease in drug diffusivity for coated

microsphere could be attributed to the incorporation of polyelectrolyte layers onto polymer matrix, which further leads to an increase in diffusional path length that the drug has to traverse.

In vitro cytotoxicity studies

Cytotoxicity studies were carried out using L929 mouse fibroblast cell line on uncoated (Plain microspheres) and polyelectrolyte-coated dexamethasone-loaded alginate microspheres. The percent viability of the cells was ~ 100% with uncoated and unloaded alginate microspheres as compared to the control, indicating that there was no cytotoxicity to cells. On the other hand, dexamethasone-loaded uncoated particles showed less cell viability (~ 86%) as compared to the blank, as dexamethasone has been shown to have a clear-cut and profound effect on the proliferation of L929 fibroblasts. The percent viability for (PAH/PSS)₁-coated particles was further reduced to ~ 80%. Other polyelectrolyte coatings demonstrated still lower cell viability compared to that of uncoated microspheres, as shown in Figure 7. This might be attributed to the toxic nature of the polyelectrolyte coatings being used which can be minimized or replaced with the use of biocompatible coatings for which studies are underway. The IC₅₀ value of dexamethasone solution was found to be 28 µg/ml, well above the amount of dexamethasone being released during initial burst release for uncoated and coated alginate microspheres. Thus, modulating the inflammatory response using dexamethasone may improve the longevity and detection of glucose sensors in-vivo. Cytotoxicity studies also confirmed that the system has acceptable biocompatibility for use in the smart tattoo glucose biosensor, though studies on a better system of coating are in process.

Conclusion

Dexamethasone-loaded uniform size alginate microspheres were produced by a commercially available droplet generator and tested for their in vitro release behavior to aid in development of a 'smart-tattoo' glucose sensor. By manipulating the drug concentration and use of different combination of coated and uncoated microspheres, 100% drug release was achieved in 30 days. LbL coating further helps in reducing the initial burst release and prolongs the period of drug release. In uncoated and (CL-PAH/PAA)₁-coated alginate microspheres, the initial burst release decreased from 20% to 5%. The main mechanism of drug release was confirmed to be diffusion controlled by the application of mathematical models (e.g. Zero order kinetics) and the corresponding drug diffusivities were also established. Cytotoxicity studies were also accomplished for uncoated and polyelectrolyte-coated dexamethasone-loaded alginate microspheres and results demonstrate acceptable percent cell viability. Therefore, the release behavior of dexamethasone-loaded uniform-size alginate microsphere can be tailored to achieve desired objectives by selective manipulation of certain properties towards a functioning implantable glucose biosensor.

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Figure 1.
SEM image of (a) uncoated calcium alginate microspheres; (b) polyelectrolyte-coated alginate microspheres.

Figure 2.
Particle size distribution of uncoated calcium alginate microspheres.

Figure 3.
Zeta potential of uncoated and polyelectrolyte-coated alginate microspheres.

Figure 4.
Effect of initial drug content on burst release of dexamethasone-loaded uncoated microspheres.

Figure 5.

(a) Comparative release profile of uncoated and polyelectrolyte-coated dexamethasone-loaded alginate microspheres in 0.01 M PBS (pH 7.4) at 37°C. Mean $\pm$ SD ($n = 3$); (b) Initial burst release profile for 24h.

Figure 6.

Comparative release profile of uncoated and polyelectrolyte-coated dexamethasone-loaded alginate microspheres in 0.01 M PBS (pH 7.4) at 37°C. Mean $\pm$ SD ($n = 3$). (a) Initial burst release profile for 24h; (b) Cumulative release for 30 days, and (c) Zero order release kinetic data of combined system (uncoated and polyelectrolyte-coated microspheres) for the initial 18 days.

Figure 7. Cytotoxicity results of uncoated and polyelectrolyte-coated dexamethasone-loaded alginate microspheres.

Table 1

Mathematical modeling on zero order release kinetics along with the drug diffusivities (D) from the uncoated and polyelectrolyte-coated alginate microspheres.

Microsphere (MS) type	Zero order			Diffusivity, D (cm ² /s)
	<i>K</i> (μg/day)	<i>R</i>	<i>R</i> ²	
Uncoated MS	7.5214	0.9912	0.9826	1×10 ⁻¹³
(PAH/PSS) ₁ coated	4.7271	0.9814	0.9686	2×10 ⁻¹⁴
(PDDA/PSS) ₁ coated	3.4540	0.9925	0.9852	7×10 ⁻¹⁴
(PAH/PAA) ₁ coated	3.7511	0.9957	0.9916	6×10 ⁻¹⁴
(CL-PAH/PAA) ₁ coated	1.7505	0.9827	0.9657	5×10 ⁻¹⁴

Table 2

Zero order release kinetics data fitting to the combined system of uncoated and (PAH/PSS)₁-coated dexamethasone-loaded alginate microspheres.

Microsphere combination ratio	2–18 days		18–30 days	
	<i>K</i> ($\mu\text{g/day}$)	<i>R</i> ²	<i>K</i> ($\mu\text{g/day}$)	<i>R</i> ²
25P+75CP	4.8281	0.9955	1.4908	0.9971
50P+50CP	4.2394	0.9898	0.9605	0.9978
75P+25CP	3.4051	0.9917	0.7753	0.9906