Dexamethasone loaded microspheres as carriers for biosensor inflammation control

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INTRODUCTION

Blood glucose monitoring is an integral part of diabetes management and the maintenance of near-normoglycaemia (Evans N 2005). Currently, patients measure their 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. But, there are significant problems with the commercially available minimally invasive *in vivo* glucose sensors (Pickup J 2004) *viz* inaccurate results, low precision and frequent calibration. On the other hand, fluorescence-based sensors that are implanted intradermally (Russell R 1999; McShane M 2002) to measure the interstitial glucose level which can be correlated to blood glucose levels can be an ideal solution. Significant results have been demonstrated for such "Smart-Tattoo" glucose biosensor that performs optimally *in vitro*. Implantation of biosensors typically causes tissue injury, which triggers a cascade of inflammatory responses that compromise device functionality and ultimately lead to device failure due to inflammation-initiated fibrous encapsulation, calcification, and protein bio-fouling (Sharkaway A 1998). Fibrous encapsulation can also deprive the sensor of adequate fluid supply for accurate detection of glucose levels. It is therefore apparent that localized inflammation must be minimized to ensure sensor functionality and long lifetime. Delivering anti inflammatory agents in the vicinity of implant can be an ideal solution. However, long term use and high dose of these agents can cause severe systemic side effects and also complicate the diabetes management; therefore, an ideal solution would be controlled, continuous local delivery of anti inflammatory agents at the implantation site using a suitable carrier system.

In this work, we aimed to develop a novel approach for release of anti inflammatory agents e.g. Dexamethasone 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. The influence of drug concentration and type of polyelectrolyte coating on release kinetics was also 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. To ensure that the fabricated drug delivery device fulfills the prerequisites for implantation as recommended by the regulatory bodies, cytotoxicity studies were completed using L929 mouse fibroblast cell line. Lastly, the suppression of inflammation and fibrosis associated with implantation were studied in a rat model. The results presented here is 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 engineer the analyte response properties.

MATERIALS AND METHODS

Low viscosity alginates, 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 (10-14 kDa) were purchased from Merck Mumbai (India) and Hi-Media Laboratories Mumbai (India) respectively. All chemicals were reagent grade and used as received.

Methodology

Dexamethasone loaded calcium alginate microspheres were prepared using a commercially available droplet generator (Nisco Engineering AG, Zurich). Briefly, 10 ml of 2% w/v sodium alginate solution was mixed with previously weighed dexamethasone sodium salt & Dicolofenac sodium. The mixture was then extruded at a flow rate of 20 ml/h under a pressure of 75 mbar using the Var J30 droplet generator equipment into a vessel containing 250 mM calcium chloride solution for external gelation under continuous stirring. The hardened drug loaded alginate microspheres were then separated by centrifugation (1000 rpm for 1 min).

All microspheres samples were characterized using "Cuvette Helos" (CUV-50ML/US) particle size analyser, shape, Hitachi S3400 scanning electron microscope, Nikon YS 100 optical microscope was used for optical microscopic studies. Zetaplus (Brookhaven Instruments, USA) was used to measure the surface charge of microsphere after the deposition of each polyelectrolyte coatings. UV-Vis spectrophotometer (Helios Alpha, Thermoscientific, USA) has used for *in-vitro* release studies. For LbL process, solutions of PAH (Poly allylamine hydrochloride, cationic) and PSS (sodium poly styrene sulfonate, anionic) initially used for assembling $(PAH/PSS)_1$ multilayer 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 minutes to complete one bilayer. Similar process was repeated for other pair of polyelectrolytes (Srivastava R 2005). *In vitro* release studies were conducted for both the drugs. In order to achieve zero order release profile with 100% release of encapsulated drug within 30 days, various combination of uncoated and $(PAH/PSS)_1$ coated microspheres were taken in different ratios and used for *in vitro* release studies.

In vitro cytotoxicity studies

The cytotoxicity of the drug loaded uncoated and polyelectrolyte coated alginate microspheres were evaluated by using Sulforhodamine-B (SR-B) semi-automated assay 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 and incubated at 37°C temperature under 5% CO_2 and saturated humid environment. 96 wells plate containing the samples was read in a micro plate reader at 540 nm with reference to 690 nm against blanks culture media without any cells.

In vivo Pharmacodynamics

Drug loaded microspheres were implanted into the subcutaneous tissue of rats using 18-gauge needles. Tissue response was determined by serial sacrifice to investigate acute (Day 1, 3, 7) and chronic (weeks 2, 3, and 4) inflammation specifically fibrotic deposition. A histopathological evaluation of excised tissue samples from the site of implantation was performed. Hematoxylin and eosin (H&E) staining was used to characterize and quantify the inflammation-mediating cells in the vicinity of the implant in response to the inflammation induced by tissue injury on implantation and by the continued presence of the implants.

RESULTS AND DISCUSSIONS

Dexamethasone loaded alginate microspheres were prepared using the droplet generator technique. The particle size of the microspheres was measured using optical microscopy and was in the range of 60±10 µm. SEM images also confirmed the same. Particle size analysis further provided confirmation that particles were in range of mean diameter of 60±10 µm (Saurter mean diameter, SMD at 90% cumulative distribution). The encapsulation efficiency of dexamethasone loaded uncoated alginate microspheres was calculated to be 77± 8%. 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 $(PAH/PSS)_1$, $(PDDA/PSS)_1$, $(PAH/PAA)_1$, and EDC and NHSS cross linked $(PAH/PAA)_1$ microspheres respectively. There was a significant difference ($P<.05$) in the rate and extent of drug release as observed in uncoated and coated microspheres. 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.

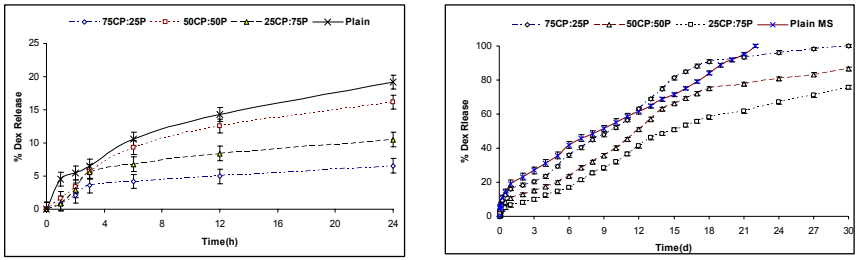

Figure. 1 Comparative release profile of uncoated and polyelectrolyte coated dexamethasone loaded microspheres in 0.01M PBS (pH 7.4) at 37ºC. Mean± SD (n=3). 2 A) Initial burst release profile for 24h; 2 B) Cumulative releases profile for 30 days

Uncoated microspheres, initial burst release were 19.25% and for 25CP:75P, 50CP:50P and 75CP:25P, it was found to be 10.50%, 16.11% and 6.54% respectively. Cumulative release of uncoated and different combination of uncoated and $(PAH/PASS)_1$ coated loaded microspheres including 25P:75CP, 50P:50CP and 75P:25CP was 100%, 99.94%, 86.63% and 75.71% respectively. There was a significant ($P<.05$) difference in the rate and extent of drug release as observed in uncoated and different combination of uncoated and $(PAH/PASS)_1$ coated microspheres. Data was fitted to Zero order kinetics model equation and results shows that coated and uncoated microspheres follow zero release kinetics. Dexamethasone was released at a steady rate of 4.83 µg/day from a combination of uncoated and polyelectrolyte coated microspheres, after an initial burst release period, which is sufficient to combat localized inflammation (Patil S 2004). The % viability of the cells was approximately 100% with uncoated and unloaded alginate microspheres as compared to the control, indicating that there was no cytotoxicity to cells. Whereas, drug loaded uncoated particles showed 86% cell viability as shown in figure 2. Localized elution of inflammation-mediating drugs can reduce the immunostimulatory cascade of events and facilitate implant functionality. To investigate the pharmacodynamic effect on the local tissue environment various formulation e.g. Plain microspheres, drug loaded and controls were studied. The inflammation mediating cells surrounding the implant site during the acute phase of inflammation are stained purple whereas normal tissue stained pink as shown in figure 3.

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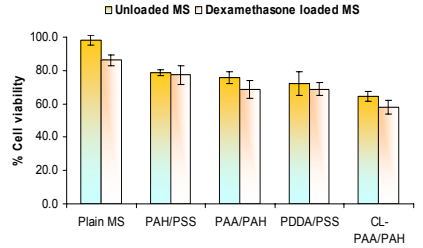

Figure2. Cytotoxicity results of uncoated and polyelectrolyte coated dexamethasone loaded alginate microspheres

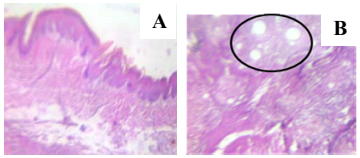

Figure 3: Pharmacodynamic changes subcutaneous tissue sections of rats implanted with Alginate microsphere (A) Plain skin (B) Dexamethasone loaded PAH/PSS coated MS (circled)

CONCLUSIONS

Drug loaded uniform size alginate microspheres were produced by a droplet generator and tested for their *in vitro* release behavior to aid in development of a "smart-tattoo" glucose sensor. By altering different combination of coated and uncoated microspheres 100% drug release was achieved in 30 days. LBL coatings help in reducing the burst release and prolong the period of drug release. The drug release mechanism was confirmed to be diffusion controlled by the application of mathematical models and the corresponding drug diffusivities were also calculated. Simultaneous site-specific elution of therapeutic agents to modify the immediate tissue environment strategies was used to improve biocompatibility and acceptability of implant. Thus, these findings imply that nanoengineered alginate microspheres show promise as release systems to improve biocompatibility and prolong lifetime of implantable "Smart-Tattoo" glucose sensor.

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