Fluorescent dissolved-core alginate microsphere glucose biosensors

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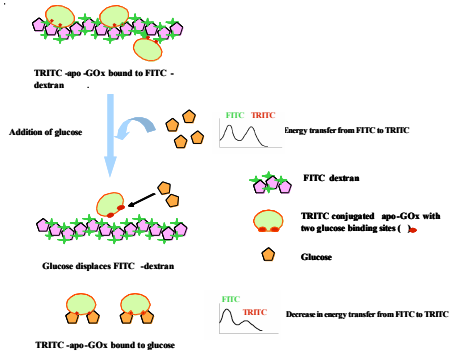
INTRODUCTION

The significance of diabetic control has resulted in worldwide efforts to develop implantable, non-invasive or minimally-invasive glucose sensors also known as "smart tattoos" (McShane, 2002, Chinnayelka et al., 2005, Brown et al., 2006, Chaudhary et al., 2008) intended for injection directly into the dermis, for continuous glucose monitoring. Such implants may be interrogated non-invasively using simple optical instrumentation (McShane et al., 2000) making their use all the more attractive. Several embodiments of potentially-implantable probes involving changes in resonance energy transfer (Chinnayelka et al., 2005, Chaudhary et al., 2008) that occur due to competitive binding of fluorescent ligand (FITC-dextran) and analyte (glucose) to occupy binding sites on a fluorescent receptor (Concanavalin A/ apo—glucose oxidase/ genetically engineered glucose-binding proteins (Tolosa et al., 1999, D'Auria et al., 2000) have been demonstrated. While the encapsulation of sensing reagents in the hollow nanoengineered capsules is elegant, and overcomes the major limitations of hydrogel-only entrapment (Russell et al., 1999, Rounds et al., 2007), the diffusion-limited post-fabrication loading of the sensing assay into the microcapsules is not efficient.

In this regard, nanoengineered dissolved-core alginate templated microsphere glucose biosensors containing the fluorescent sensing assay FITC (fluorescein isothiocyanate)-dextran/ TRITC (tetramethyl rhodamine)-apo-GOx have been fabricated and evaluated. The glucose sensitivity of these dissolved core microspheres was studied using fluorescence spectroscopy and characterized using confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM), fourier transform infra red spectroscopy (FTIR) and energy dispersive X- ray analysis (EDX).

The sensing scheme is explained by illustration Scheme 1. When FITC-dextran is bound to TRITC-apo-GOx, nonradiative energy transfer takes place from an excited donor dye (FITC) to a nearby acceptor dye (TRITC). When glucose is introduced into the system, it displaces FITC-dextran and binds to TRITC-apo-GOx resulting in a decrease in energy transfer, which appears as a change in the relative emission intensity of FITC and TRITC.



Scheme1. Principle of glucose sensing

MATERIALS AND METHODS

Materials

Alginate (Low viscosity, 2%), FITC-dextran (150kDa/500kDa), TRITC , glucose oxidase (160 kDa), β -D-Glucose(MW 180 Da), sodium poly (styrene sulfonate) (PSS, 70 kDa), poly (allylamine hydrochloride) (PAH, 70 kDa), phosphate buffer saline (PBS) tablets, dimethyl sulfoxide (FW 78.13), SPAN 85, TWEEN 85, 2,2,4-tri-methylpentane (iso-octane) and PD10 columns were purchased from Sigma-Aldrich (India). All chemicals were reagent grade and used as received.

Methods

Fabrication of alginate microsphere encapsulated with fluorescent sensing assay
Apo-glucose oxidase was prepared by the Swoboda protocol (Swoboda, 1969)as reported earlier (Chaudhary et al., 2008). A UV-Vis spectrophotometer was used to confirm the removal of FAD and to calculate to concentration of the apoenzyme (Chaudhary et al., 2008). Thereafter apo-GOx was conjugated with TRITC dye using a standard amine labeling procedure (Svensson et al., 1992). The fluorescent sensing assay (FITC-dextran and TRITC-apo-GOx) was then co-encapsulated inside alginate microspheres during their fabrication using the emulsification technique reported earlier with some modifications (Srivastava et al., 2005a). The prepared calcium alginate microspheres loaded with the fluorescent sensing assay were characterized using fluorescence microscopy. Layer by layer (LbL) self assembly technique was then used to fabricate multilayer thin film coatings over alginate micropsheres (Srivastava et al., 2005b) to prevent the leaching out of the sensing assay. The adsorption of LbL coatings was analyzed using FTIR studies.

Partial Dissolution of Alginate Microsphere Core
To allow free movement of the sensing chemistry required during competitive binding, partial dissolution of the alginate microsphere core was performed wherein 0.1M sodium citrate-TRIS HCl solution was added to a suspension of alginate microspheres and kept for 2-3 days. As the Ca^{2+} ions are removed, the crosslinking in the gel decreases and this leads to solubilization of the high molecular weight alginate polymers (Zhu et al., 2005). The polyelectrolyte coatings do not dissolve and thus stabilize the alginate microspheres simultaneously preventing the leakage of the sensing chemistry (Zhu et al., 2005). Visualization of core dissolution was confirmed using SEM, CLSM and finally EDX was used to analyze the resulting structures following core dissolution.

Stability of Encapsulation and Sensor response
To estimate the quantity of encapsulated material lost from the coated microspheres, leaching studies were performed on microspheres containing FD 150kDa-500kDa/TRITC-apo-GOx complexes. All samples were covered and stored under dark conditions at room temperature. Thereafter, the glucose response of alginate microspheres was tested in DI water using a fluorescence spectrophotometer.

RESULTS AND DISCUSSION

Fabrication of alginate microsphere biosensors
Representative fluorescent image of the LbL coated alginate microspheres loaded with both FITC-dextran(500kDa)/TRITC-apo-GOx sensing assay is shown in Fig 1. SEM images indicated that the microsphere were in the range of 10-20μm diameter as shown in Fig 2(a).

Partial core dissolution of alginate microsphere

The alginate microspheres appear to have collapsed partially after 3 days of citrate treatment particularly when compared to the undissolved particles shown in Fig. 2(a) and 2(b). Approximately 95% of the microspheres were observed to have a dissolved core after a 3-day citrate treatment (results not shown here) when examined in confocal microscope using line scans. It is noteworthy that the microspheres maintain integrity and the LbL coatings prevent rupture. EDX analysis was used to calculate the percentage composition of alginate microspheres before and after the citrate treatment. The percentage of calcium ions was found to decrease considerably from 4% to 0.5% after citrate treatment. Also, a very small decrease was observed in the carbon and oxygen percentages from 62% to 58% for carbon and 34% to 31% for oxygen. This may be due to fractional loss of alginate from the microspheres; however, clearly significant amount of the alginate remains inside the microsphere after dissolution of core.

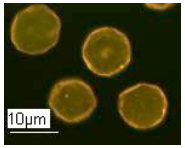


Figure 1: Fluorescence microscopy image of alginate microsphere loaded with fluorescent sensing assay

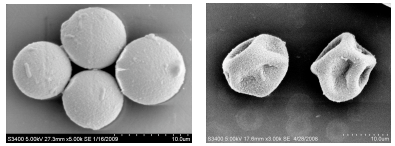


Figure 2: SEM images of (a) citrate untreated and (b) citrate treated microsphere

Stability of Encapsulation and sensor response

The release of the encapsulated molecules from the microspheres was observed as an increase in the fluorescence intensity of the supernatant. Most importantly, there was only a small amount of leaching of ~4% during the first 15 hours of the leaching studies for the sensor incorporating 500kDa FITC-dextran as illustrated in Fig. 3. Thereafter no change in the fluorescence intensities from the supernatant solutions was observed which proved the stability of the assay.

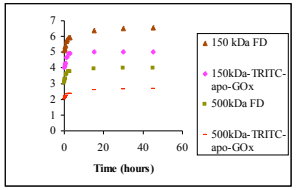


Figure 3: Leaching curve of alginate microsphere biosensors

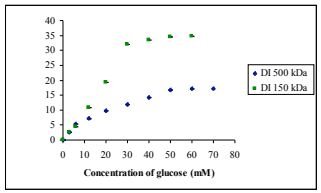


Figure 4: Glucose sensitivity curve of alginate microsphere biosensors

Microspheres loaded with FITC-dextran (500kDa)/TRITC-apo-GOx complex were tested for glucose sensing in DI water. It can be observed from Fig. 4 that the glucose sensitivity of the alginate microspheres loaded with FITC-dextran (500kDa)/TRITC-apo-GOx was observed to be 0.69%/mM glucose with a linear response in the range of 0-50mM glucose while that for alginate microspheres loaded with FITC-dextran (150kDa)/TRITC-apo-GOx was observed to be 0.84%/mM glucose with a linear response in the range of 0-40mM glucose. In addition, alginate microsphere

biosensors were observed to be completely reversible with a response sensitivity of 0.65%/mM glucose and a linear response over glucose range of 0-50mM (results not shown here) and the sensor response was observed to reach steady state within 2 min.

CONCLUSION

Preliminary glucose sensing studies with fluorescent dissolved-core alginate microspheres have demonstrated the feasibility of encapsulating working fluorescent reagents with a simple and efficient process. It can be concluded that these dissolved core alginate microspheres, used here for glucose sensing, are suitable as carriers for other assay chemistry. These novel systems have potential to be used as implantable glucose biosensors for in vivo glucose sensing.

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