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Enzymatic Fluorescent Microsphere Glucose Sensors: Evaluation of Response Under Dynamic Conditions

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Abstract

Most previous attempts at the development of a “smart tattoo” for glucose monitoring in diabetes—implantable fluorescent microspheres that can be implanted intradermally and interrogated transdermally using light—have focused on the encapsulation of a competitive binding assay for glucose within hydrogel microspheres or polyelectrolyte microcapsules. We recently reported on the development of a microsphere sensor based on an enzymatic scheme, combined with an oxygen-quenched fluorescent reporter element. A novel feature of this design is the use of polyelectrolyte multilayer nanofilms, which are assembled stepwise on the surface of the microspheres, for modulation of glucose and oxygen mass transport, allowing them to be designed for sensitive and safe operation within the “oxygen deficit” present in the skin. In this work, a flow-through sensor testing apparatus was used to demonstrate the reversible response of the sensors under controlled, dynamic conditions. The sensors were shown to be sensitive over at least 0–140 mg/dL glucose, with a response time of less than 2 min. This report also identifies some current problems with the approach, and gives potential solutions towards the development of a practical implantable sensor for glucose monitoring in diabetes.

INTRODUCTION

ONE INTERESTING POSSIBILITY for the minimally invasive monitoring of glucose for the management of diabetes is the concept of a “smart tattoo”—tiny implantable, color-changing fluorescent particles that could be implanted in the skin and interrogated transdermally.^{1–4} Previous¹ and current^{5–7} attempts at these devices have involved the use of competitive binding assays for glucose (involving lectins, glucose binding proteins, or deactivated glucose-specific enzymes) with signal transduction using resonance energy transfer (RET) methods. While some of these sensors show great promise as potentially useful implantable sensors, irreversible binding of glucose to the sugar-specific moiety is always a concern with competitive binding assays, and has the potential to limit the utility of sensors of this type if not prevented.

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We have recently reported on the development of a new type of enzymatic micrometer-scale fluorescent sensor intended for minimally invasive transdermal monitoring of glucose.⁸ These sensors comprise hydrogel microspheres that contain the enzyme glucose oxidase and the oxygen-quenched fluorescent indicator, tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) $[\text{Ru}(\text{dpp})_3]^{2+}$, co-immobilized throughout the interior of the spheres. A novel feature of this design is the use of ultrathin (2–100-nm) polyelectrolyte multilayer coatings, self-assembled on the surface of the hydrogel particles with high precision (2 nm) via electrostatic layer-by-layer assembly. These coatings allow for the introduction of a highly controllable barrier for mass transfer of glucose and oxygen into the enzyme-containing region, an aspect critical for proper function of the sensors,^{9,10} as well as a convenient and precise method for the application of biocompatible surface coatings. Figure 1 shows sample confocal micrographs of prototype sensors, illustrating the capability to construct particles with the desired structure with microscale dimensions and nanoscale features.

An advantage of sensors based on enzymatic action is that irreversible binding of glucose is not a problem since the glucose is consumed, making reversibility of the sensors less of a concern. Our previous work has demonstrated the stability of the sensors with regard to sensor assay chemistry leaching^{8,11,12} and long-term enzyme stability,^{11,12} as well as preliminary data on the glucose response of the sensors.⁸ In this report, we demonstrate the sensitive response of the sensors to glucose in the normal physiological range in a continuous fashion using a flow-through sensor testing apparatus, and describe ongoing efforts to improve the practicability of these sensors towards the development of actual implantable devices.

MATERIALS AND METHODS

All chemicals were acquired from Sigma Chemical Co. (St. Louis, MO) and used as received, with the exception of Alexa Fluor® 488 succinimidyl ester, which was obtained from Molecular Probes (Eugene, OR), and iso-octane, obtained from EMD Chemicals Inc. (Gibbstown, NJ). Alginate gel microspheres were prepared by a method modified from Wan et al.¹³ for encapsulation of macromolecules, and described previously.⁸ Following microsphere fabrication, the glucose oxidase was conjugated to the alginate matrix using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride with *N*-hydroxysuccinimide as a catalyst. The coupling chemistry is based upon the reaction of activated carboxylic sites with primary amine groups.¹⁴ This technique has been shown to drastically enhance the catalytic stability of the encapsulated enzyme over long periods of time (at least 6 months).¹⁵

After microsphere fabrication, the microspheres were placed in a solution of $3.5 \times 10^{-5} \text{ M}$ $[\text{Ru}(\text{dpp})_3]^{2+}$ ($\lambda_{\text{ex}} = 450 \text{ nm}$, $\lambda_{\text{em}} = 610 \text{ nm}$) in a 10% (vol/vol) methanol/deionized water solution at pH 12 for 30 min, which results in electrostatically mediated precipitation of the cationic, poorly water-soluble dye into the hydrated, anionic alginate matrix. After resuspension of the spheres in neutral-pH aqueous solutions, the entrapment of $[\text{Ru}(\text{dpp})_3]^{2+}$ is stable.⁸

The dye-doped microspheres were then rinsed five times with deionized water by successive centrifugation cycles. Poly(styrene sulfonate) (PSS) (molecular weight ~70,000) and poly(allylamine hydrochloride) (PAH) (molecular weight ~70,000) or PAH conjugated to Alexa Fluor 488 (PAH-AF488) ($\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 515 \text{ nm}$) were alternately assembled on the surfaces of the microspheres until 10 bilayer polyelectrolyte films (~40 nm thick) were realized. The fluorescence emission spectrum of the sensors can be tailored by varying the number of PAH-AF488 layers in the nanofilm; non-fluorescent PAH can be used after the desired spectrum is achieved if thicker nanofilms are required for control of stability or transport. The sensors were imaged using a Leica (Exton, PA) TCS SP2 confocal microscope with a 63X oil-immersion objective; the 458 nm Ar/Kr laser line was used for excitation, while emission was simultaneously collected from two channels, 500–550 nm for Alexa Fluor 488 and 600–650 nm for $[\text{Ru}(\text{dpp})_3]^{2+}$.

Since the sensors described here are analyte-consuming devices, it was necessary to construct a flow-through testing apparatus to accurately test the response of the sensors to changes in bulk glucose concentration. It is imperative that glucose and oxygen be supplied to the sensors at a rate faster than that of consumption; otherwise, erroneous measurements could be made because of global depletions of bulk substrate rather than local depletions inside the sensor structures. A sensor testing chamber was constructed of two opaque garolite plates, separated by a foam gasket, and secured together tightly with bolts and wingnut fasteners. The bottom plate was constructed with a recess to hold a standard microscope slide in its center; a through-hole was cut into the bottom of the plate in a position corresponding to the center of the slide to allow insertion of a fiber probe for spectral acquisition. A microscope slide with immobilized sensors on its surface was placed face-up into the recess in the bottom plate. A foam gasket with a cutout for the microscope slide was placed on top of the bottom plate, and then the top plate was fastened to the bottom two pieces. The top plate contained inlet and outlet ports machined through the plate thickness, such that fluids may be flown into the chamber at one end of the microscope slide and exit to a waste reservoir out of the other, with the sample chamber volume determined by the thickness of the foam gasket. Two 2-L beakers, one containing 0.01 M phosphate-buffered saline and the other 0.01 M phosphate-buffered saline plus 600 mg/dL ,8-D-glucose, were saturated with air using ceramic diffusers under constant stirring. Two peristaltic pumps with neoprene tubing (0.03 inch i.d.) were used to pump fluid from each of the reservoirs into a mixing tee and then through 1/8-inch-diameter PEEK tubing into and out of the sensor testing chamber. The relative flow rates of the pumps were then adjusted to obtain glucose concentrations in the testing chamber between 0 and 600 mg/dL glucose, with the overall flow rate maintained at 5 mL/min.

To assess the response of the sensors to glucose, some of the microspheres were first immobilized onto the surface of a glass microscope slide for use in the flow chamber. A 100- μL aliquot of sphere suspension (approximately 10^5 sensors) in deionized water was added to the surface of the slide and allowed to mostly dry. Then, 100 μL of a pre-polymer acrylamide cocktail (100 mg/mL monomer) with poly(ethylene glycol)-diacrylate cross-linker and Irgacure 184 (Ciba Specialty Chemicals, Basel, Switzerland) photoinitiator was pipetted onto the sensors and covered with a glass coverslip. The slide was placed under an

ultraviolet lamp for 10 min, resulting in polymerization of the acrylamide. The coverslip was then removed, leaving behind a thin polyacrylamide film ($\sim 2 \mu\text{m}$) covering the sensors. After the slide was loaded into the testing chamber, a 6-around-1 fiber probe with SMA connectors was interfaced through the bottom plate to the bottom of the microscope slide with refractive-index-matching gel. The probe was connected to an Ocean Optics (Dunedin, FL) USB 2000 charge-coupled device fluorescence spectrometer, with a blue light-emitting diode (450 nm) (Ocean Optics) used as an excitation source. Excitation light was supplied to the sensors through one optical fiber, and collected from the sensors with the other six fibers of the probe. After optimization of signal collection parameters to achieve a good signal-to-noise ratio, the spectrometer software was set to continuously monitor the ratio of the ruthenium peak (610 nm) to the Alexa Fluor 488 peak (520 nm), under constant irradiation of the sensors with the blue light-emitting diode. Plain phosphate-buffered saline buffer (0 mg/dL glucose) was first flowed through the chamber for 500 s. Then, a custom LabVIEW® (National Instruments, Austin, TX) Virtual Instrument was used to control the peristaltic pumps to mix the glucose and buffer solutions to achieve the desired concentration of glucose between 0 and 600 mg/dL glucose, which was then passed through the chamber for 500–800 s. The concentration was then returned to 0 mg/dL for 500 s before moving on to the next glucose concentration; this was done to demonstrate the reversible nature of the response, as well as to provide baseline data for photobleaching correction after data acquisition.

RESULTS AND DISCUSSION

Figure 2 is a continuous glucose sensing profile that depicts the response of the sensors under dynamic conditions. In Figure 2, the peak ratio (610/520 nm) is plotted as a function of time. In this experiment, the glucose concentration was alternated between 0 mg/dL and ~ 20 mg/dL multiples of glucose concentration up to 140 mg/dL, and then up to 600 mg/dL in the last step. The profile shown in Figure 2 demonstrates real-time continuous acquisition of spectra (with constant irradiation) under dynamic glucose concentration changes; for this experiment, the integration time was set at 1.2 s with five averages, resulting in acquisition of spectra every 6 s. Since the flow rate was maintained at 5 mL/min and the concentration of oxygen was held constant in the bulk reservoirs, any changes in fluorescence are due to local changes in oxygen within the sensors due to consumption of glucose and oxygen by the encapsulated enzyme. From Figure 2, it is apparent that the sensors exhibit a reversible, graded response to changes in glucose concentration under dynamic conditions, with response times on the order of <200 s. (Incidentally, the sensors used in this experiment had been stored under wet conditions for nearly 4 months, demonstrating the excellent stability of the devices.)

It should be noted that the peak ratio data depicted in Figure 2 were corrected for photobleaching after data acquisition, and therefore make the response appear somewhat better than what is observed in real-time. Photobleaching of the sensors under illumination by the milliwatt-emitting diode was found to be significant, with an overall ratio decrease at 0 mg/dL glucose of 55% after just over 4 h of constant irradiation. Photobleaching of the sensors is a complex phenomenon because of the very different photodegradation rates of each fluorophore (with the ruthenium compound bleaching much faster than the more

photostable Alexa Fluor 488 compound), and was further complicated by increasing spectral overlap of the scattered excitation light with the Alexa Fluor 488 signal as it decreased in intensity because of photobleaching. Over the course of the experiment, it was observed that the ratio at 0 mg/dL glucose decreased rapidly at first and then finally at a much slower rate after about 2 h of constant irradiation. In addition, photobleaching is an oxidative process, and as such, different oxygen levels elicit different photodegradation rates; this results in different photobleaching rates at different glucose concentrations also.^{16,17} For this study, photobleaching was compensated for by fitting individual lines to each subset of data at 0 mg/dL glucose, and additionally between the last and first points of consecutive 0 mg/dL data subsets. These curve fits were then subtracted from the data over the appropriate ranges, such that the 0 mg/dL glucose regions were effectively “zeroed” to create a baseline corresponding to zero glucose conditions. Efforts to decrease the effects of photobleaching are currently being investigated, including the use of alternative excitation sources with narrower bandwidth, lower output power, and pulsed excitation, as well as alternate oxygen indicators with enhanced photostability.

Figure 3 is a plot of percent change in peak ratio versus glucose concentration for the experiment of Figure 2. To construct this plot, the last 10 data points of each 0 mg/dL “trough” were subtracted from the last 10 points of each subsequent “crest” for each step in glucose concentration, then taken as a percentage of the average ratio at 0 mg/dL taken from the end of the experiment (when the photobleaching rates were very low). These values were then averaged and plotted versus glucose concentration, with error bars equal to ± 1 SD. From Figure 3, it is observed that the sensors exhibit a sensitive, nearly linear response to glucose up to at least 140 mg/dL, with no sign of saturation at that point. The percent change in ratio for the 600 mg/dL step was 4.4%; if a line is fit to the data of Figure 3, based on a 4.4% change in ratio for 600 mg/dL it is projected that the sensors (under these experimental conditions) will saturate at approximately 260 mg/dL glucose. Separate oxygen sensitivity experiments on these sensors resulted in a change in ratio of 42% from 0% to 100% dissolved O₂. Thus, it is apparent that, while the sensors have the potential to measure glucose with higher sensitivity than that observed here, the sensors are not able to remove oxygen from the sensing region fast enough to overcome the influx of oxygen from the bulk, resulting in decreased overall response to glucose.¹⁸

There are many factors that determine the sensitivity of the sensors. One factor that may have decreased the overall sensitivity and range of the sensors is possible forced mass transfer of oxygen to the sensors due to convection. Decreasing the flow rates used in the experiment should decrease or eliminate these effects and thereby increase the sensitivity,¹⁸ although care must be taken to ensure that the flow rates are sufficient to ensure that there is no depletion of substrate from the bulk solutions passing over the sensors. Future work will examine the effect of decreased flow rates on sensitivity to glucose; this clearly is a measurement variable that affects the evaluation of prototype sensors but is not expected to be a significant issue for sensors used in vivo, since convection is not normally present in the skin. In addition, the sensitivity of these sensors is dependent largely on the proper design of the sensors, i.e., the proper combination of enzyme concentration, sphere diameter, and nanofilm thickness, to create a sensitive, glucose-limited response over the physiological range.⁹ For the sensors used in the experiments described here, the enzyme

concentration in the spheres was approximately 20 mM, based on average sphere sizes and counts and protein mass determination by the Folin phenol reagent assay. However, conjugation of the enzyme to the alginate matrix results in a 70% decrease in the mass-normalized activity of the enzyme compared with that of the enzyme free in solution, such that the actual “effective” enzyme concentration is closer to 6 mM.¹⁵ However, the enzyme concentration in the spheres may be modulated by altering the amount of enzyme included in the alginate precursor, and is an easily controllable factor. Furthermore, the electrostatic self-assembly method used here enables the very repeatable deposition of ultrathin films with a precision of 2 nm; our previous work has shown that differences in film thicknesses on the order of several nanometers can have a dramatic effect on the sensitivity and dynamic range of sensors of this type.⁹ One area in need of improvement with regard to fabrication repeatability is the polydispersity of the spheres as they are currently produced; efforts to create more monodisperse spheres are currently underway in our laboratories.

However, the sensitivity of the sensors to glucose is equally dependent on the inherent sensitivity of the oxygen indicator used in the transduction mechanism. It is known that the sensitivity of metal–ligand complexes such as the ruthenium compound used here is greatly affected by the immobilization matrix. Matrices with high permeability to oxygen result in more sensitive responses, as do matrices that are more hydrophobic or polar in nature.¹⁹ Separate experiments to determine the response of the sensors described here to changes in oxygen alone demonstrated that $[\text{Ru}(\text{dpp})_3]^{2+}$ immobilized in alginate microspheres results in a sensitivity (percent change in ratio from 0% to 100% dissolved O_2) of 42%, whereas the highest reported sensitivity for this molecule is 50–80% (immobilized in ormosil and silica matrices).²⁰ Thus, without altering any other sensor parameters, increasing the sensitivity of the immobilized oxygen indicator would increase the sensitivity and range of the glucose response. Efforts to enhance the suitability of the alginate matrix for sensitive oxygen measurements using $[\text{Ru}(\text{dpp})_3]^{2+}$ are the subject of current investigations in our laboratories. In addition, alternate fluorophores with higher sensitivity than the ruthenium complexes and emission at longer wavelengths (e.g., metal porphyrins) are currently being considered for use in these sensors to improve light accessibility in turbid tissue.

CONCLUSIONS

The work presented here is the first demonstration of continuous glucose sensing under controlled, dynamic conditions using microspherical enzymatic fluorescent sensors. The sensors were shown to be sensitive with an approximately linear response over the range 0–140 mg/dL glucose. The response was found to be fully reversible, with response times (forward and reverse) on the order of 2 min, even with delays inherent in the testing apparatus. It is expected that reducing the overall flow rate of the solutions through the flow-through apparatus will result in increased apparent sensitivity because of a reduction in forced mass transfer of oxygen, and this factor is currently being investigated in continuous evaluation of prototypes. However, while these results are promising, some key factors must be addressed before these sensors could be practically used as implantable devices. First, photobleaching of the sensors was a problem, and required significant data processing efforts for correction. However, efforts are currently underway to reduce the effect of photobleaching on the sensors, and it is expected that changes in instrumentation and data

acquisition methods will significantly alleviate the problem. Methods to enhance the sensitivity of the oxygen indicator in the hydrogel matrix are also being considered, and may result in sensors with even higher sensitivity to glucose. In addition, the use of dyes with relatively short excitation/emission spectra may cause problems in actual application as implantable sensors due to the light attenuation and scattering properties of tissue at those wavelengths, although dyes with longer excitation/emission maxima (i.e., in the far-red/near-infrared) are currently being considered for use in these sensors. However, these sensors show great promise as potentially useful devices for monitoring in diabetes, and future work will describe the results of the above efforts to improve the practicability of the sensors towards this goal.

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REFERENCES

1. Russell RJ, Pishko MV, Gefrides CC, McShane MJ, Coté GL. A fluorescence-based glucose biosensor using concanavalin-A and dextran encapsulated in a poly(ethylene glycol) hydrogel. *Anal Chem.* 1999; 71:3126–3132. [PubMed: 10450158]
2. McShane MJ, Russell RJ, Pishko MV, Coté GL. Towards minimally-invasive glucose monitoring using implanted fluorescent microspheres. *IEEE Eng Med Biol Mag.* 2000; 19:36–45. [PubMed: 11103704]
3. McShane MJ, Rastegar S, Pishko MV, Coté GL. Monte Carlo modeling for implantable fluorescent analyte sensors. *IEEE Trans Biomed Eng.* 2000; 47:624–632. [PubMed: 10851806]
4. McShane MJ. Potential for glucose monitoring with nanoengineered fluorescent biosensors. *Diabetes Technol Ther.* 2002; 4:533–538. [PubMed: 12396748]
5. Chinnayelka S, McShane MJ. Glucose-sensitive nanoassemblies comprising affinity-binding complexes trapped in fuzzy microshells. *J Fluorescence.* 2004; 14:585–595.
6. Chinnayelka S, McShane MJ. Microcapsule biosensors using competitive binding RET assays based on apoenzymes. *Anal Chem.* 2005; 77:5501–5511. [PubMed: 16131059]
7. Ibey BL, Yadavalli BK, Thomas HR, Rounds RM, Pishko MV, Coté GL. Implantable fluorescence-based glucose sensor development. *Proc SPIE.* 2005; 5702:1–6.
8. Brown JQ, Srivastava R, McShane MJ. Encapsulation of glucose oxidase and an oxygen-quenched fluorophore in polyelectrolyte-coated calcium alginate microspheres as optical glucose sensor systems. *Biosens Bioelectron.* 2004; 21:212–216. [PubMed: 15967372]
9. Brown JQ, McShane MJ. Modeling of spherical fluorescent glucose microsensor systems: design of enzymatic smart tattoos. *Biosens Bioelectron.* 2005; 21:1760–1769. [PubMed: 16219457]
10. Leyboldt JK, Gough DA. Model of a two-substrate enzyme electrode for glucose. *Anal Chem.* 1984; 56:2896–2904. [PubMed: 6524662]
11. Srivastava R, Brown JQ, Zhu H, McShane MJ. Stable encapsulation of active enzyme by application of multilayer nanofilm coatings to alginate microspheres. *Macromol Biosci.* 2005; 5:717–727. [PubMed: 16096991]
12. Srivastava R, Brown JQ, Zhu H, McShane MJ. Stabilization of glucose oxidase in alginate microspheres with photoreactive diazo-resin nanofilm coatings. *Biotechnol Bioeng.* 2005; 91:124–131. [PubMed: 15849694]

13. Wan LSC, Heng PWS, Chan LW. Drug encapsulation in alginate microspheres by emulsification. *J Microencapsul.* 1992; 9:309–316. [PubMed: 1403481]
14. Hermanson, GT. Part I: 2. The chemistry of reactive groups. In: Hermanson, GT., editor. *Bioconjugate Techniques*. Academic Press; San Diego, CA: 1996. p. 169-176.
15. Zhu H, Srivastava R, Brown JQ, McShane MJ. Combined physical and chemical immobilization of glucose oxidase in alginate microspheres improves stability of encapsulation and activity. *Bioconj Chem.* 2005; 16:1451–1458.
16. Fuller ZJ, Bare WD, Kneas KA, Xu WY, Demas JN, DeGraff BA. Photostability of luminescent ruthenium(II) complexes in polymers and solution. *Anal Chem.* 2003; 75:2670–2677. [PubMed: 12948135]
17. Grant, PS. Novel sensors for neural glucose concentration based on enzyme fluorescent thin films fabricated on nanoparticle carriers using electrostatic layer-by-layer assembly, [Ph.D. Dissertation]. Louisiana Tech University; Ruston, LA: 2004.
18. Wolfbeis OS, Oehme I, Papkovskaya N, Klimant I. Sol-gel based glucose biosensors employing optical oxygen transducers, and a method for compensating for variable oxygen background. *Biosens Bioelectron.* 2000; 15:69–76. [PubMed: 10826645]
19. Xu W, McDonough RC, Langsdorf B, Demas JN, Degraff BA. Oxygen sensors based on luminescence quenching: interactions of metal complexes with the polymer supports. *Anal Chem.* 1994; 66:4133–4141. [PubMed: 7847622]
20. Koo YEL, Cao Y, Kopelman R, Koo SM, Brasuel M, Philbert MA. Real-time measurements of dissolved oxygen inside live cells by organically modified silicate fluorescent nanosensors. *Anal Chem.* 2004; 76:2498–2505. [PubMed: 15117189]

FIG. 1.
Confocal laser scanning micrographs of enzymatic fluorescent microsphere glucose sensors.
Red = $[\text{Ru}(\text{dpp})_3]^{2+}$; green = PAH-Alexa Fluor 488.

FIG. 2.

Continuous glucose sensing profile of glucose sensors under dynamic conditions, corrected for photobleaching. Baseline corresponds to 0 mg/dL glucose, while intermediate glucose concentrations in mg/dL are indicated in the graph. Total flow rate = 5 mL/min; constant irradiation; spectral acquisition every 6 s.

FIG. 3. Percent change in ratio from 0 mg/dL baseline as a function of glucose concentration. Error bars represent ± 1 SD.