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The role of H_2O_2 outer diffusion on the performance of implantable glucose sensors

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ABSTRACT

The performance of an implantable glucose sensor is strongly dependent on the ability of their outer membrane to govern the diffusion of the various participating species. In this contribution, using a series of layer-by-layer (LBL) assembled outer membranes, the role of outwards of H_2O_2 diffusion through the outer membrane of glucose sensors has been correlated to sensor sensitivity. Glucose sensors with highly permeable humic acids/ferric cations (HAs/Fe^{3+}) outer membranes displayed a combination of lower sensitivities and better linearities when compared with sensors coated with lesser permeable outer membranes (namely HAs/poly(diallyldimethylammonium chloride) (PDDA) and poly(styrene sulfonate) (PSS)/PDDA). On the basis of a comprehensive evaluation of the oxygen dependence of these sensors in conjunction with the permeability of H_2O_2 through these membranes, it was concluded that the outer diffusion of H_2O_2 is crucial to attain optimized sensor performance. This finding has important implications to the design of various bio-sensing elements employing perm-selective membranes.

1. Introduction

The development of electrochemical biosensors for continuous monitoring of metabolic analytes has become a vibrant area of research because of their potential in providing early indication of upcoming diseases and disorders (Wilson and Gifford, 2005). Moreover, implantable glucose sensors are rapidly progressing towards the ultimate goal of closed-loop artificial pancreas (Newman and Turner, 2005; Turner et al., 1999). Most of these biosensors are based on the activity of analyte-specific enzymes constituting the principle of Clark-type electrochemical detection (Heller, 1999; Wang, 2001; Wilson and Gifford, 2005).

First generation Clark-type glucose sensors employ the flavoenzyme glucose oxidase (GO_x), immobilized on top of a working electrode. The flavin adenine dinucleotide (FAD) redox cofactor of GO_x catalyzes the oxidation of glucose to glucarolactone, as shown in the following equations:

$$\text{Glucose} + GO_x(\text{FAD}) \rightarrow \text{Glucolactone} + GO_x(FADH_2) \quad (1)$$

$$GO_x(FADH_2) + O_2 \rightarrow GO_x(\text{FAD}) + H_2O_2 \quad (2)$$

The generated H_2O_2 is amperometrically assessed on the surface of working electrode (Eq. (3)), relating the current to glucose concentration:

$$H_2O_2 \xrightarrow{V} O_2 + 2H^+ + 2e^- \quad (3)$$

As evident from Eqs. (1) and (2), optimum sensor performance can only be attained when the ratio of glucose to O_2 is less than 1. If this is not the case, the lack of O_2 renders Eq. (2) oxygen limited, which is the case for *in vivo* applications. For example, in subcutaneous tissue the O_2 concentration is only 0.18 mM as compared to 5.6 mM of physiological glucose concentration (glucose/O_2 ratio ≅ 30). This leads to signal saturation at higher glucose concentrations. The onset of signal saturation is typically expressed as the apparent Michaelis–Menten constant ($k^{app}_{m,glu}$), which defines the upper limit of glucose range that the sensor can detect (Heller, 1999). In order to mitigate signal saturation, perm-selective membranes (based on either pore size or O_2 favorability to hydrophobic environments) have been employed, which decrease the glucose to O_2 ratio at the vicinity of GO_x (Fig. 1).

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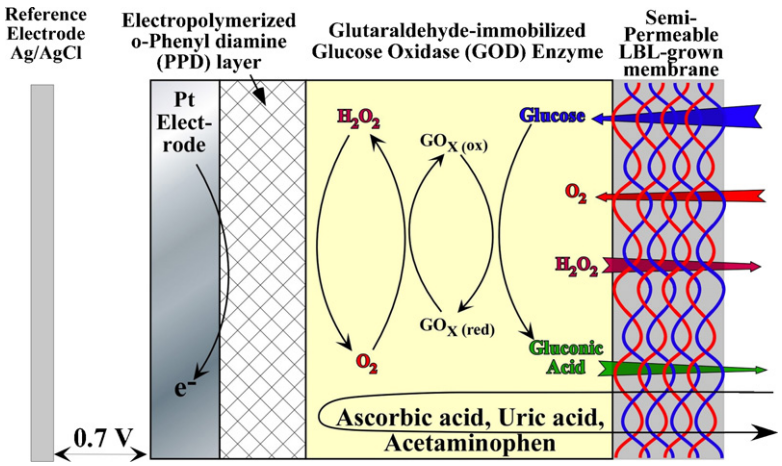

Fig. 1. Schematic cross-section of the glucose senor in study.

Second and third generation Clark-type biosensors employ redox mediators and direct 'wiring' of enzymes to electrodes in an attempt to minimize or completely eliminate the need for O_2. While research in this area is rapidly progressing, the use of outer membranes has also been proven effective in reducing biofouling (Wilson and Gifford, 2005). In addition, outer membranes help minimize temperature-induced variations in sensor responses arising from enzyme reaction kinetics (Jablecki and Gough, 2000). Such positive aspects of outer membranes come at the expense of an increase in sensor response time and decrease in sensitivity (Wilson and Gifford, 2005). Biosensor failure as a result of calcification-induced permeability changes and degradation of outer membranes has also been reported (Mercado and Moussy, 1998). In addition, precise control over the thickness and uniformity of outer membranes presents an added complexity for mechanistic studies towards deciphering sensor operation.

In lieu of the aforementioned limitations, our group has been developing semi-permeable outer membranes based on the layer-by-layer (LBL) assembly of multivalent polymers and/or small ions (Galeska et al., 2000, 2001, 2002; Tipnis et al., 2007; Vaddiraju et al., 2007). LBL assembly is a relatively inexpensive and efficient method for growing multi-component, conformal films with precise control of thickness and uniformity. Moreover, fine-tuning of microstructure and permeability characteristics of these membranes is also achieved by varying polyelectrolyte flexibility as well as their assembly conditions (*i.e.* pH and ionic strength) (Galeska et al., 2000, 2002; Hammond and Whitesides, 1995; Tipnis et al., 2007). The issue of calcification has also been mitigated with LBL assembly, where a stronger binding entity (*i.e.* Fe^{3+}) prevented the nucleation of calcium deposits (Galeska et al., 2000, 2002). When Fe^{3+} was LBL assembled with the naturally occurring humic acids (HAs), a reduction in tissue fibrosis was also observed following implantation (Galeska et al., 2001).

In this contribution, we present a comprehensive investigation of the role of outer membrane on the performance of glucose sensors. Using a series of LBL assembled outer membranes with different microstructures and permeabilities, we show that the outer diffusion of H_2O_2 through these semi-permeable outer membranes influences the sensitivity of the sensor. Sensors with highly permeable HAs/Fe^{3+} membranes displayed lower sensitivities and better linearities compared to sensors with less permeable (styrene sulfonate)/poly(diallyldimethylammonium chloride) (PSS/PDDA) membrane. By investigating the oxygen dependence of these sensors, it is proved that this unusual behavior (lower sensitivities from sensors with highly glucose permeable outer membranes) does not originate from the poor diffusion of O_2 through the outer membrane but rather from the pronounced outer diffusion of H_2O_2. To the best of our knowledge, this is the first report linking the outer diffusion of H_2O_2 to sensor performance, with important ramifications in lieu of H_2O_2 borne: (i) enzyme degradation (Valdes and Moussy, 1999), (ii) electrode saturation (Hall et al., 1998), (iii) potential for tissue irritation (Watt et al., 2004) and (iv) sensor crosstalk in multi-sensor architectures (Ward et al., 2004; Wilson and Gifford, 2005).

2. Materials and methods

2.1. Materials

Glucose oxidase enzyme (GO_x) (E.C. 1.1.3.4, 157,500 units/g, Aspergillus Niger), glutaraldehyde (25 wt.% solution in water), bovine serum albumin (BSA) and phosphate buffered saline (PBS) were purchased from Sigma and used without any further treatment. Sodium salt of humic acids (HA), sodium salt (lot no. 11909LR; molecular weight 169 kDa), ferric chloride hexahydrate (reagent grade), D-glucose (reagent grade), poly(sodium 4-styrenesulfonate) (PSS) (M_w: 70,000) and poly(diallyldimethylammonium chloride) (PDDA) (20 wt.% in water; M_w: 200,000–350,000) were used as received from Aldrich. *Ortho*-phenylenediamine (OPD) was obtained from Acros chemicals. The platinum and silver wires were purchased from Ladd Research. Deionized water was produced by a Millipore (Milli-Q) system with resistivity >18 MΩ and used to prepare all aqueous solutions.

2.2. Fabrication of working electrodes (Fig. 1)

Miniaturized working electrodes (sensors) were fabricated by skillfully winding a 50 μm platinum (Pt) wire on a 0.3 mm diameter monofilament nylon line, which served as the backbone. The total surface area of the working electrode is 3 mm^2. The sensors were electrochemically cleaned in a 0.5 M H_2SO_4 solution *via* potential cycling between −0.21 and 1.25 V, until a stable background has been reached (Bindra et al., 1991). Next, a film of poly(*o*-phenylenediamine) (PPD) was electropolymerized on the Pt working electrode from a 5 mM OPD solution in aqueous acetate buffer by applying a constant potential of 0.65 V *vs.* SCE for 15 min (Malitesta et al., 1990). This PPD layer was shown to block the oxidation of other endogenous species like ascorbic acid, uric acid and acetaminophen, which are likely to oxidize at the operating potential of the sensor (*vide supra*) (Kirwan