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THE ROLE OF H₂O₂ 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 H₂O₂ diffusion through the outer membrane of glucose sensors has been correlated to sensor sensitivity. Glucose sensors with highly permeable Humic Acids/Ferric cations (HAs/Fe³⁺) 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 through these membranes, it these sensors in conjunction with the permeability of H₂O₂ was concluded that the outer diffusion of H₂O₂ 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

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cofactor of GO_x catalyzes the oxidation of glucose to glucarolactone, as shown in equations (1) and (2).

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

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 equation (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 $\cong$ 30). This leads to signal saturation at higher glucose concentrations. The onset of signal saturation is typically expressed as the apparent Michaelis-Menten constant ($k_{m,glu}^{app}$), which defines the upper limit of glucose range that the sensor can detect. (Heller, 1999) In order to mitigate signal saturation, permselective 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 (Figure 1).

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 increased 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 semipermeable outer membranes based on the layer-by-layer (LBL) assembly of multivalent polymers and/or small ions. (Galeska et al., 2000; Galeska et al., 2002; Galeska et al., 2001; 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; Galeska et al., 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; Galeska et al., 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 semipermeable 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 169kDa), 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 $>18 \text{ M}\Omega$ and used to prepare all aqueous solutions.

2.2 Fabrication of Working Electrodes (Figure 1)

Miniaturized working electrodes (sensors) were fabricated by skillfully winding a $50 \mu\text{m}$ 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 to 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 et al., 2007; McMahon et al., 2004; Moussy et al., 1993) The GO_x enzyme was subsequently immobilized by dip coating the Pt/PPD electrode in a solution of 19.5 mg/ml glucose oxidase followed by immersion in an aqueous acetate buffer solution of 25% w/v glutaraldehyde and 73.2 mg/ml BSA to cross link the enzyme.

2.3 Layer-By-Layer (LBL) assembly of outer membranes

Three kinds of semi-permeable outer membranes were constructed on top of Pt/PPD/ GO_x electrodes by assembling 5 bilayers of: (i) HAs/ Fe^{3+} (Device A), (ii) HAs/poly

(diallyldimethylammonium chloride) (PDDA) (Device B), and (iii) poly (styrene sulfonate) (PSS)/PDDA (Device C). This was achieved by dipping the enzyme immobilized working electrode alternately in solutions of HAs or PSS and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ or PDDA, respectively. The concentrations of each solutions were fixed at 5, 1, 3, and 2 mg/ml, for HAs, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, PDDA, and PSS, respectively. The pH of HAs was adjusted to 5 in order to achieve an assembly of HAs with globular conformation, (Galeska et al., 2001; Tipnis et al., 2007) while the pH of all other solutions were left as is. The sensors were dipped in the respective solutions for 15 min, separated by a washing step in DI water for 5 min.

2.4 In Vitro Amperometric Experiments were formed in a stirred PBS solution ($\text{pH} \cong 7.4$) maintained at 37°C and under an applied potential of 0.7 V vs. an Ag/AgCl reference electrode using a CH Instruments (Model CHI660A) electrochemical analyzer. The sensor response current vs. various glucose concentrations were performed by raising the glucose levels in the test-cell by 2 mM every 100 seconds upto 130 mM, following an initial background stabilization period of ~ 8 min. The oxygen dependence of these sensors was performed in a PBS buffer filled test cell containing all three components of the glucose sensors (working, Ag/AgCl reference and Pt counter electrodes) along with a commercial oxygen sensor (Mettler Toledo, Model 4100e). Following the addition of a known amount of glucose, the test cell was sealed from the atmosphere and N_2 was bubbled until the desired oxygen concentration was reached (for ca. 20 min), and the response of the glucose sensor was obtained as a function of oxygen concentration for various glucose concentrations.

2.5 Atomic Force Microscopic (AFM) studies

5 bilayers of these LBL membranes were assembled on quartz substrates as described above. The surface topography of these membranes were assessed in air (dried), using an *Asylum Research MFP-3d* AFM in tapping mode.

2.6 Permeability Studies

The permeability of the aforementioned three membranes for glucose and H_2O_2 were performed as described elsewhere. (Tipnis et al., 2007; Vaddiraju et al., 2007) In brief, a dialysis chamber containing these membranes on top of a porous glass fiber membrane was used and filled with 2 ml of 1.0 M glucose or H_2O_2 solution, immersed in an outer chamber containing 200 ml of 0.1 M PBS solution maintained at 37°C , stirred at a fixed rate of 190 rpm and the outer diffusion of the two analytes were determined amperometrically. The amperometric response over time was converted to glucose or H_2O_2 concentration and the apparent diffusion coefficient (D_p) of the analyte species was calculated based on following equation: (Heitner-Wirguin, 1979; Lopez et al., 1976)

$$N_A = \frac{D_p}{L} \{ [C_G]_i - [C_G]_f \} \quad (4)$$

where, $N_A = \left(\frac{\Delta m}{A \cdot \Delta t} \right)$ is the flux over the area (A) of the membrane, Δm is the mass of analyte that diffuses out of the chamber in the time Δt , L is the thickness of the LBL assembled membranes (determined ellipsometrically by assembling the corresponding LBL films onto quartz substrates (Galeska et al., 2001; Tompkins, 1993), $[C_G]_i$ and $[C_G]_f$ are the concentrations of the analyte inside and outside of the diffusion chamber, respectively. In the calculation of D_p , we doubled the thickness L to account for membrane growth from both sides of the glass fiber substrate.

2.7 Determination of $K_{m,glu}^{app}$, K_{m,O_2}^{app} and Linear Range Sensitivity (LRS)

The basic Michaelis-Menten (MM) analysis for two substrates (glucose and O_2) shown in eq. (5). (McMahon et al., 2005)

$$J_{glu} = \frac{J_{max}}{\frac{K_{m,O_2}^{app}}{[O_2]} + \frac{K_{m,glu}^{app}}{[Glu]}} \quad (5)$$

where J_{glu} is the amperometric current density (Amp/cm²) for a particular concentration of glucose ([Glu]) and oxygen ([O₂]), J_{max} is the saturation J_{glu} , and $K_{m,glu}^{app}$, K_{m,O_2}^{app} are the apparent glucose and oxygen Michaelis-Menten constants, respectively. When either glucose or O_2 substrates remain constant, eq. 5 simplifies to eq. 6 or 7, respectively.

$$J_{glu} = \frac{J'_{max}}{1 + \frac{K_{m,glu}^{app}}{[Glu]}} \quad (6)$$

$$J_{glu} = \frac{J''_{max}}{1 + \frac{K_{m,O_2}^{app}}{[O_2]}} \quad (7)$$

Phenomenologically, both $K_{m,glu}^{app}$ and K_{m,O_2}^{app} represent the glucose and oxygen concentrations, at which the sensor's response is half of its maximum saturation value and define the upper glucose concentration or lower oxygen concentration where these sensors are usable for the respective substrates. As K_{m,O_2}^{app} approaches zero (*i.e.* optimum case for an oxygen independent sensor), eq. 7 reduces to eq. 8 where the maximum current at that glucose concentration (J'_{max}) remains constant for a given oxygen concentration.

$$\lim_{K_{m,O_2}^{app} \rightarrow 0} J_{glu} = \lim_{K_{m,O_2}^{app} \rightarrow 0} \left[\frac{J'_{max}}{1 + \frac{K_{m,glu}^{app}}{[Glu]}} \right] = J'_{max} \quad (8)$$

The values of $K_{m,glu}^{app}$ can be readily deciphered by the slope of the inverse of eq. 6 shown in eq. 9 (Lineweaver-Burk linearization (Lineweaver and Burk, 1934; Ramanathan et al., 2001)),

when $\frac{1}{J_{glu}}$ is plotted vs. $\frac{1}{[Glu]}$.

$$\frac{1}{J_{glu}} = \frac{1}{J'_{max}} + \frac{K_{m,glu}^{app}}{J'_{max}} \frac{1}{[Glu]} \quad (9)$$

The K_{m,O_2}^{app} can be determined following the similar method as above or by taking the oxygen concentration where the J_{Glu} is half of J_{max}'' for a particular glucose concentration.

The linear range sensitivity (LRS) of these sensors, are determined by eqs. 10 and 11 as the

initial slope of the J_{Glu} vs. glucose concentration (i.e. $\lim_{[Glu] \rightarrow 0} \frac{(J_{Glu})}{[Glu]}$). (McMahon et al., 2005)

$$\lim_{Glu \rightarrow 0} J_{glu} = \frac{J_{max}}{1 + \frac{K_{m,glu}^{app}}{[Glu]}} = \frac{J_{max} [Glu]}{[Glu] + K_{m,glu}^{app}} \approx \frac{J_{max}}{K_{m,glu}^{app}} [Glu] \quad (10)$$

$$\Rightarrow LRS = \frac{J_{max}}{K_{m,glu}^{app}} \quad (11)$$

2.8 Statistical Analysis

At least three sensors of each kind were fabricated and tested. Values of J_{glu} and K_{m,O_2}^{app} have been plotted as mean $\pm$ S.E.M (standard error mean). The LRS, $K_{m,glu}^{app}$ and slope of K_{m,O_2}^{app} vs. oxygen concentration ($K_{m,O_2}^{app} / [glu]$) are presented as mean $\pm$ S.D (standard deviation).

3. RESULTS

Figure 2 depicts representative AFM micrographs of 5 bilayer assembled films of HAs/Fe³⁺ (Device A), HAs/PDDA (Device B), and PSS/PDDA (Device C). The root mean square (RMS) roughnesses of these films, which is indicative of their compactness, varies from 40.4 nm for HAs/Fe³⁺, to 13.5 and 2.1 nm for HAs/PDDA and PSS/PDDA, respectively.

Figure 3 illustrates the amperometric response of devices A, B and C as a function of glucose concentration. The initial rise in response current with increasing glucose concentration saturates at concentrations higher than the physiologically-observed glucose levels (hatched box in Figure 3). Here, it is noteworthy to mention that the trends in the amperometric response of these three sensors are inversely proportional to the RMS roughness of their outer membrane. Based on the results of Figure 3 and the Michaelis-Menten analysis described in the

experimental section, the $K_{m,glu}^{app}$ and the linear range sensitivity (LRS) for all the sensors have been determined and shown in Table 1. Device A displayed a 3 fold higher $K_{m,glu}^{app}$ as to devices B and C. Such high linearity of device A comes at an order of magnitude lower LRS compared to devices B and C.

Figure 4A illustrates the oxygen dependence of Device A (right ordinate) following the addition of 6 mM of glucose at point (1), as a function of time (abscissa) and oxygen concentration (left ordinate), initiated at point (2). Following the addition of glucose, the sensor response saturates within 30–40 seconds, indicative of a rapid partitioning of glucose within the GO_x layer. Following the removal of oxygen from the test cell, the sensor response decreases in accordance with eq. 2. The rate at which the response of the sensor decreases appears also to depend on the concentration of glucose in the test solution.

Figure 4B depicts the current density of Device A (left ordinate) and Devices B and C (right ordinate) as a function of oxygen concentration for a constant glucose concentration of 6 mM, a value close to normal physiological levels. Similar to Figure 3, the amperometric response of Device A is almost an order of magnitude smaller than Devices B and C. In addition, while the response of Devices B and C are linear for the oxygen range tested, the response of Device A largely departs from linearity towards a hyperbolic fit. While the response of Devices B and C are far from oxygen saturation, Device A approaches saturation around 200 μM of O_2 . It has been suggested that the critical oxygen limit of a sensor is the point below which the response of sensor changes from its saturation point. (Dixon et al., 2002; Zhang and Wilson, 1993) Based on this and the results of Figure 4B, the critical oxygen limit for Device A to 6 mM of glucose is around 200 μM of O_2 , while for Device B and C such limit is much higher.

The $K_{m,\text{O}_2}^{\text{app}}$ value of a sensor for a particular glucose concentration forms the basis of quantitative assessment of sensor dependence on oxygen for different glucose concentrations. The smaller the $K_{m,\text{O}_2}^{\text{app}}$ value of a sensor, the lower is its dependence on oxygen. When comparing sensors with similar configurations, yet different outer membranes, the $K_{m,\text{O}_2}^{\text{app}}$ provides a quantitative account of the relative permeability of O_2 through the specific outer membrane. Figure 5 depicts the $K_{m,\text{O}_2}^{\text{app}}$ as a function of glucose concentration (within the physiological observed glucose range) for all three devices. The natural convergence of all three curves in Figure 5 to zero $K_{m,\text{O}_2}^{\text{app}}$ for zero glucose concentration is in agreement with previous studies. (Dixon et al., 2002) In accordance with the $K_{m,\text{glu}}^{\text{app}}$, Device A displayed a three to six times lower $K_{m,\text{O}_2}^{\text{app}}$ as opposed to Devices B and C. The significant variation in the measured $K_{m,\text{O}_2}^{\text{app}}$ values of Devices B and C precludes one from making a quantitative assessments between these two devices. From an analytical point of view the $K_{m,\text{O}_2}^{\text{app}}$ (eq. 6) should vary linearly, which is almost the case for Device A for high glucose concentrations. On the other hand, in order to attain a zero $K_{m,\text{O}_2}^{\text{app}}$ for zero glucose concentration, a hyperbolic fit is needed for glucose concentrations below 1 mM. In the case of Devices B and C, since they are so far below their critical oxygen limit (*vide supra*), a hyperbolic fit extends all the way to 10 mM of glucose. For comparison purposes, the slope of these curves (*i.e.* $K_{m,\text{O}_2}^{\text{app}} / [\text{Glu}]$) below 4 mM glucose is presented in Table 1. As expected, the slope for Device A is the lowest followed by devices B and C, respectively.

4. DISCUSSION

The interdependence between sensor performance and the permeability of various electrochemical species (*i.e.* H_2O_2 and O_2), enzymatic substrates (*i.e.* glucose and O_2) and enzymatic byproducts (*i.e.* glucorolactone, gluconic acid, H_2O_2) and various other endogenous species presents a complex multi-parameter system that is not completely understood. A number of mechanistic studies and theoretical modeling have been published, elucidating: (i) the glucose to O_2 mismatch and means to decrease its large offset, (Allen et al., 1994; Maines et al., 1996) (ii) effect of enzyme layer thickness, (Baronas et al., 2003) and (iii) glucose, O_2 and H_2O_2 concentration profiles within the sensor configuration. (Baronas et al., 2004; Cambiaso et al., 1996; Tse and Gough, 1987) The flexibility afforded by LBL assembly methodology (Galeska et al., 2000; Galeska et al., 2002; Galeska et al., 2001; Tipnis et al., 2007) has now allowed us to produce well defined, ultra-thin membranes with various permeabilities (Figure 1) in order to conduct mechanistic studies and further improve our understanding of sensor operation. As part of our investigations on layer-by-layer (LBL) assembled outer membranes, we have recently reported an interesting and counterintuitive behavior, where a sensor coated with a more glucose-permeable membrane produced lesser amperometric current for any given glucose concentration than a sensor with a less-permeable

membrane. The surprisingly higher linearities observed for the more permeable outer membrane prompted us to reexamine these results in a more systematic fashion, in order to decipher the nature of this unusual behavior.

For this we elected to focus on three semipermeable membranes where their glucose and H_2O_2 diffusion spanned over nearly two orders of magnitude. Structurally rigid entities like HAs, when assembled with micro-cations (*i.e.* Fe^{3+}) have been previously shown to result in globular microstructured assemblies (RMS of 40.2 nm, Figure 2A). (Galeska et al., 2001) On the other hand, flexible polyelectrolytes such as poly (styrene sulfonate) (PSS) and poly (dimethyl diallyl ammonium chloride) (PDAA) produce highly compact structures (RMS of 2.1 nm, Figure 2C). (Mendelsohn et al., 2003) The combination of rigid (HAs) and flexible (PDAA) structures result in an intermediate roughness (RMS of 13.5 nm, Figure 2B). Keeping in mind, the 2-dimensional (2D) nature of AFM-derived RMS values versus the 3D character of diffusivity, the glucose permeability results should scale to $3/2$ power of the RMS values. As shown in Table 1, this is indeed the case where the ratio of glucose diffusivity to $(\text{RMS})^{3/2}$ is constant for all the three devices. Since the same is true for H_2O_2 as well, it is safe to assume that these LBL assemblies are ideal candidates for conformal, ultra-thin, semi-permeable membranes with tunable permeability.

While the sensor amperometric response depends not only on the production of H_2O_2 but also to its availability at the electrode site, the concentration profile of H_2O_2 within the sensor becomes vitally important. (Weibel and Bright, 1971) Since the production of H_2O_2 is in turn dependent on the availability of O_2 (eq. 2), the sensor dependence on O_2 can provide a vital insight to whether the observed behavior originates from O_2 deficiency. The lower $K_{m,\text{O}_2}^{\text{app}}$ values (for any glucose concentration) for devices A as shown in Figure 5 as well its lower oxygen slope (Table 1) clearly indicates the presence of comparatively larger amounts of O_2 in the sensor A when compared to sensors B and C. In addition, the lower oxygen dependence of Device A (or in other words the high permeability of O_2 through the HAs/ Fe^{3+} membrane) also agrees well with the higher $K_{m,\text{glu}}^{\text{app}}$ observed in Device A. These two arguments provide a full proof case to exclude any potential role of lower oxygen levels within Device A, which could explain its lower amperometric response (Figure 3).

Having excluded the possibility of lower oxygen levels in Device A that could have led to its lower response, the only remaining possibility is a pronounced outer diffusion of H_2O_2 through Device A. Considering that: (i) H_2O_2 levels are directly proportional to the sensor current values, and (ii) that the outer diffusion of H_2O_2 is driven only by concentration gradient across the membrane, it can be safely concluded that the highest amperometric response of Device C originates from a poor outer diffusion of H_2O_2 from the inner GO_x layer (Table 1 and Figure 1). Similarly, the highest outer diffusion of H_2O_2 in Device A is responsible for its lowest amperometric response.

The last remaining piece of the puzzle is the reason as to why H_2O_2 builds up within Devices B and C, when the poor permeability of their outer membranes should have, in the first place, prevented the inwards diffusion of glucose and O_2 . This could be explained considering that unlike H_2O_2 which experiences only a concentration gradient, the GO_x enzyme provides an extra chemical potential for the inward diffusion of glucose and O_2 as they constitute the enzyme's substrate and co-substrate. Moreover, the high turnover rate of GO_x , (Weibel and Bright, 1971) ensures that the increase concentration levels of H_2O_2 sustains adequate catalytic activity of GO_x .

5. CONCLUSIONS

A mechanistic investigation of glucose sensor performance has been performed using ultra-thin and conformal layer-by-layer (LBL) assembled outer membranes. By varying the rigidity of the individual assembly components, two orders of magnitude in analyte permeability was attained. Surprisingly, the sensor with more permeable outer membrane exhibited the least amperometric response. By determination of glucose and H_2O_2 diffusivities through the outer membrane, along with a careful analysis of the sensor's oxygen dependence, it is concluded that the outer diffusion of H_2O_2 plays a critical role to sensor performance. Membranes with large H_2O_2 diffusivities facilitate its higher escape from the sensor interior thereby lowering its amperometric response. This study elucidates the complex interrelation between sensor performance and diffusivities of participating species at high glucose concentrations. Furthermore, it links for the first time the outer diffusion of H_2O_2 to sensor operation, with important ramifications to H_2O_2 -induced enzymatic degradation, electrode saturation, potential for tissue irritation, and sensor crosstalk in multi-sensor architectures.

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References

- Allen, D. J., Johnson, K. W., Nevin, R. S., 1994. US patent 5322063
- Baronas R, Ivanauskas F, Kulys J. *Sensors* 2003;3(7):248.
- Baronas R, Ivanauskas F, Kulys J. *J Math Chem* 2004;35(3):199–213.
- Bindra DS, Zhang Y, Wilson GS, Sternberg R, Thévenot DR, Moatti D, Reach G. *Anal Chem* 1991;63(17):1692–1696. [PubMed: 1789439]
- Cambiaso A, Delfino L, Grattarola M, Verreschi G, Ashworth D, Maines A, Vadgama P. *Sens Actuators, B* 1996;33(1–3):203–207.
- Dixon BM, Lowry JP, O'Neill RD. *J Neurosci Methods* 2002;119(2):135. [PubMed: 12323417]
- Galeska I, Chattopadhyay D, Moussy F, Papadimitrakopoulos F. *Biomacromolecules* 2000;1(2):202–207. [PubMed: 11710101]
- Galeska I, Chattopadhyay D, Papadimitrakopoulos F. *J Macromol Sci Pure and Appl Chem* 2002;A39(10):1207–1222.
- Galeska I, Hickey T, Moussy F, Kreutzer D, Papadimitrakopoulos F. *Biomacromolecules* 2001;2(4):1249–1255. [PubMed: 11777399]
- Hall SB, Khudaish EA, Hart AL. *Electrochim Acta* 1998;43(5–6):579–588.
- Hammond PT, Whitesides GM. *Macromolecules* 1995;28:7569–7571.
- Heitner-Wirguin C. *Polymer* 1979;20:371–374.
- Heller A. *Annu Rev Biomed Eng* 1999;1:153–175. [PubMed: 11701486]
- Jablecki M, Gough DA. *Anal Chem* 2000;72(8):1853–1859. [PubMed: 10784153]
- Kirwan SM, Rocchitta G, McMahon CP, Craig JD, Killoran SJ, O'Brien KB, Serra PA, Lowry JP, O'Neill RD. *Sensors* 2007;7:420–437.
- Lineweaver H, Burk D. *J Am Chem Soc* 1934;56(3):658–666.
- Lopez M, Kipling B, Yeager HL. *Anal Chem* 1976;48:1120–1122.
- Maines A, Ashworth D, Vadgama P. *Anal Chim Acta* 1996;333(3):223–231.
- Malitesta C, Palmisano F, Torsi L, Zamboni PG. *Anal Chem* 1990;62(24):2735–2740. [PubMed: 2096737]
- McMahon CP, Killoran SJ, Kirwan SM, O'Neill RD. *Chem Comm* 2004;10(18):2128–2130. [PubMed: 15368008]
- McMahon CP, Killoran SJ, O'Neill RD. *J Electroanal Chem* 2005;580:193–202.

- Mendelsohn JD, Yang SY, Hiller JA, Hochbaum AI, Rubner MF. *Biomacromolecules* 2003;4:96–106. [PubMed: 12523853]
- Mercado RC, Moussy F. *Biosens Bioelectron* 1998;13(2):133–145. [PubMed: 9597730]
- Moussy F, Harrison DJ, O'Brien DW, Rajotte RV. *Anal Chem* 1993;65(15):2072–2077. [PubMed: 8372970]
- Newman JD, Turner APF. *Biosens Bioelectron* 2005;20(12):2435–2453. [PubMed: 15854818]
- Ramanathan K, Jönsson BR, Danielsson B. *Analytica Chimica Acta* 2001;427(1):1.
- Tipnis R, Vaddiraju S, Jain F, Burgess D, Papadimitrakopoulos F. *J of Diabetes Sci Tech* 2007;1(2):193–200.
- Tompkins, HG. *A User's Guide to Ellipsometry*. Academic Press Inc; San Diego: 1993.
- Tse PHS, Gough DA. *Anal Chem* 1987;59(19):2339–2344. [PubMed: 3674445]
- Turner APF, Chen B, Piletsky S. *Clin Chem* 1999;45(9):1596–1601. [PubMed: 10471674]
- Vaddiraju S, Tipnis R, Jain F, Burgess D, Papadimitrakopoulos F. *PMSE preprints* 2007;96:914–915.
- Valdes TI, Moussy F. *Biosens Bioelectron* 1999;14:579–585. [PubMed: 11459103]
- Wang J. *Electroanalysis* 2001;13(12):983–988.
- Ward WK, House JL, Birck J, Anderson EM, Jansen LB. *Diabetes Tech Therapeutics* 2004;6(3):389–401.
- Watt BE, Proudfoot AT, Vale JA. *Toxicological Reviews* 2004;23(1):51–57. [PubMed: 15298493]
- Weibel MK, Bright HJ. *J Biol Chem* 1971;246(9):2734–2744. [PubMed: 4324339]
- Wilson GS, Gifford R. *Biosens Bioelectron* 2005;20(12):2388–2403. [PubMed: 15854814]
- Zhang Y, Wilson GS. *Anal Chim Acta* 1993;281(3):513.

Figure 1.
Schematic cross section of the glucose sensor in study.

Figure 2. AFM images comparing the morphologies of HAs/Fe³⁺, HAs/PDDA and PSS/PDDA films after 5 bilayers assembly onto quartz substrates.

Figure 3. Saturation amperometric current vs. glucose concentration for the three sensor devices. Hatched region illustrates the observed physiological glucose concentration.

Figure 4. Sensor performance as a function of O_2 concentration. **(A)** Representative sensor scans utilized to deduce the apparent Michaelis-Menten constants for oxygen (K_{m,O_2}^{app}). At points 1 and 2, addition of glucose (6 mM) and oxygen removal were respectively introduced. **(B)** Amperometric response at 6 mM glucose concentration vs. oxygen concentration for the three sensor devices.

Figure 5.

Variation of K_{m,O_2}^{app} as a function of glucose concentration for all three sensors. The slope of each curve below 4 mM of glucose allowed the determination of $K_{m,O_2}^{app}/[Glu]$ term, which is inversely proportional to the amount of oxygen within the sensor.

Interrelation between membrane diffusivity and their RMS roughness, in conjunction with sensor performance criteria (*i.e.* Michaelis-Menten constants, linear range sensitivity (LRS) and slope of the $K_{mO_2}^{app}$ vs. glucose concentration).

Table 1

Sensor Device	RMS (nm)	$D_{p, Glu} (\times 10^9 \text{ cm}^2/\text{sec})$	$\frac{D_{p, Glucose}}{(RMS)^{3/2}}$	$LRS \left(\frac{mA \cdot mM}{cm^2} \right)$	$K_{m, Glu}^{app} \text{ (mM)}$	$D_{p, O_2} (\times 10^9 \text{ cm}^2/\text{sec})$	Oxygen Slope $K_{m, O_2}^{app} \left[\frac{[Glu](\mu M)}{O_2 \mu M} \right]^{-1}_{Glu}$
A	40.4	4.4	0.017	11.1 ± 0.4	11 ± 2	4.8	9.0 ± 1.3
B	13.5	0.83	0.017	72 ± 14	3.3 ± 0.3	1.2	23.0 ± 7.5
C	2.1	0.058	0.019	101 ± 30	3.0 ± 1.0	0.10	23.4 ± 3.3