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Brain-Friendly Amperometric Enzyme Biosensor Based on Encapsulated Oxygen Generating Biomaterial

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***Abstract*—A novel first-generation Clark-type biosensor platform that can eliminate the oxygen dependence has been presented. Sufficient oxygen to drive the enzymatic reaction under hypoxic conditions was produced by encapsulated oxygen generating biomaterial, calcium peroxide. The catalase immobilized in chitosan matrix was coated on top of the groove to decompose residual hydrogen peroxide to oxygen. A glucose biosensor was developed on the proposed platform as proof of concept. Under hypoxic conditions, developed glucose biosensors maintained their sensitivity response around 84% of their response at oxygen tension of 151mmHg. The sensitivity deviation was less than 5.3% with the oxygen tension traversed from 0 to 57 mmHg. Under oxygen tension of 8.3mmHg, the sensitivity of 37.130nA/mM and the linear coefficient of R^2=0.9968 were obtained with the glucose concentration varying from 0.05 to 10mM. This new platform is particularly attractive for injured brain monitoring.**

I. Introduction

The pathology of traumatic brain injury (TBI) is heterogeneous and often leads to secondary insults [1-2]. Direct monitoring of cerebral extracellular chemistry is a promising technique that can enhance our understanding of the pathophysiology of brain injury and also has the potential for early detection of metabolic derangements associated with secondary events [3-4]. Implantable biosensors based on various detection techniques (e.g., electrochemical, optical, etc.) have been developed [5-6]. Particularly, the specificity and selectivity of electrochemical-based detection rendered it a popular choice for brain biosensors.

To date, three approaches exist for amperometric enzyme biosensors including mediatorless-based, mediator-based and direct electron transfer-based detection of hydrogen peroxide (H_2O_2), with each approach having its own advantages and disadvantages. Most implantable biosensors rely on mediatorless-based detection of H_2O_2 due to potential leaching of the mediator and immature direct electron transfer mechanism. However, mediatorless-based biosensors suffer a high oxygen dependency, which is further compounded by inflammation and biofouling following implantation. Despite many efforts, the problem of oxygen acting as the limiting reactant still exists for practical and reliable analytical use [7-8].

Herein, we present a novel brain-friendly amperometric enzyme biosensor platform that can eliminate the dependence on oxygen for mediatorless biosensors. Sufficient oxygen was produced sustainably by encapsulated oxygen generating biomaterial to drive the enzymatic reaction. The biosensor platform itself can release the oxygen, making this platform particularly attractive for monitoring injured brains where the low oxygen tension restricts the usage of nominal amperometric enzyme biosensors.

II. Design and Working Principle

Glucose biosensor was designed on the novel brain-friendly biosensor platform as proof-of-concept. Glucose oxidase (GOD) was chosen as due to its superior glucose specificity. Glucose is enzymatically converted to glucarolactone and H_2O_2. The generated H_2O_2 is amperometrically measured on the surface of working electrode, as shown in the following equations:

$$\text{Glucose} + O_2 \rightarrow \text{Glucolactone} + H_2O_2 \quad (1)$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (2)$$

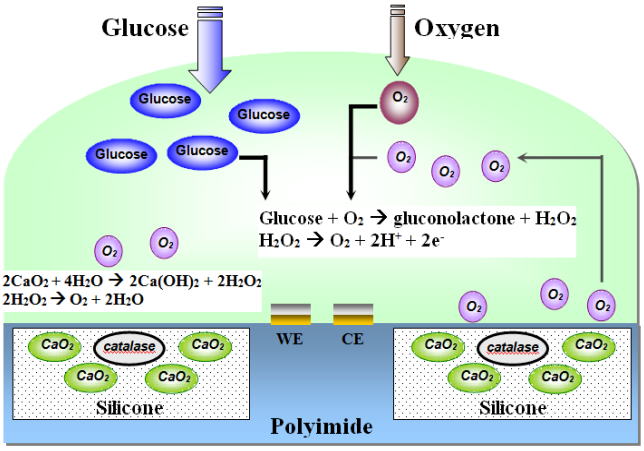

Figure 1: Concept for a novel injured brain-friendly amperometric enzyme biosensors. Calcium peroxide was encapsulated in the hydrophobic silicone membrane for sustained oxygen generation. Catalase entrapped in the chitosan matrix was coated on top of the groove for the decomposition of hydrogen peroxide byproducts.

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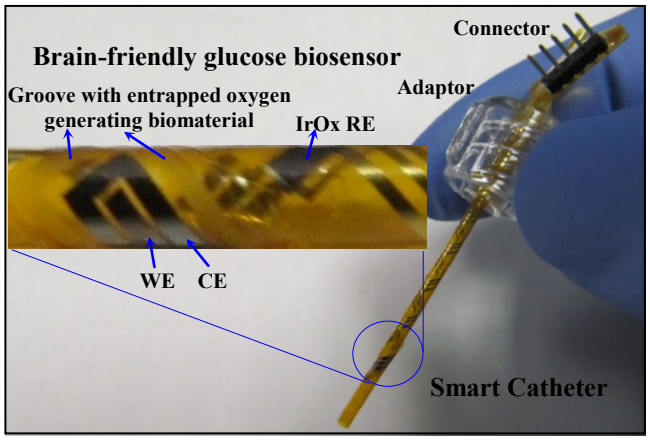
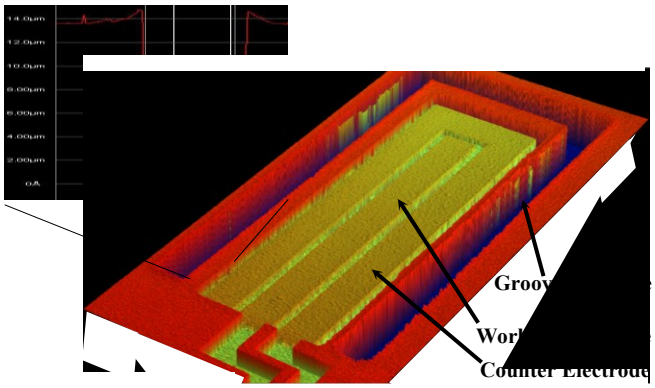


(a)



(b)

Figure 2: (a) Photographs of the fabricated smart catheter with brain-friendly glucose biosensor and (b) 3D view of the groove structure for the entrapment of calcium peroxide and catalase. The depth of the groove is around 12µm.

Optimum glucose sensor performance can only be achieved when glucose to oxygen ratio is less than 1. However, for *in vivo* applications, the 1 to 2 orders of magnitude lower oxygen vs glucose renders Eq. (2) oxygen limited.

To achieve no oxygen dependency, oxygen generating biomaterial, CaO_2, is encapsulated in the hydrophobic silicone matrix and immobilized in the groove structure as shown in Fig. 1. CaO_2 is hydrolytically activated to generate oxygen with H_2O_2 as an intermediate via the reaction shown in Eq. 3 and Eq. 4 [9].

$$2CaO_2 + 4H_2O \rightarrow 2Ca(OH)_2 + 2H_2O_2 \quad (3)$$

$$2H_2O_2 \rightarrow O_2 + 2H_2O \quad (4)$$

Residual H_2O_2 presents if CaO_2 hydration speed is not perfectly controlled. To avoid the accumulation of H_2O_2, catalase which decomposes H_2O_2 to water and oxygen, was coated on top of the groove.

III. Materials and Methods

A. Materials and Apparatus

Glucose oxidase (GOD) (Aspergillus niger, 136,100 U/g), Chitosan (MW ~ 10^5), Glutaraldehyde (GA, 25%), Nafion 117 (5% w/w), β-D-glucose, potassium platinum chloride (K_2PtCl_6), and Phosphate buffered saline (PBS, pH=7.2) were purchased from Sigma-Aldrich-Fluka. Ortho-phenylenediamine (OPD) and calcium peroxide (CaO_2) were obtained from Acros Chemical. RTV Silicone was obtained from M.G. Chemicals (USA).

All electrochemical experiments were performed in PBS (pH 7.2) using a PalmSens electrochemical workstation (PalmSens Instruments BV, the Netherlands), controlled by computer. The PBS was purged with calibration gases containing 1%, 2%, 3%, 5% and 8% oxygen concentrations for at least 30 min prior to each electrochemical measurement.

B. Microfabrication

Microelectrodes were fabricated by patterning E-beam evaporated Ti/Au (150/1500Å) thin film on the 15µm thick Polyimide film. The groove structure was developed by O_2 and CF_4 reactive ion etching (RIE) of Polyimide film. Platinum nanoparticles were electrochemically deposited on the working and counter electrodes [10]. The working electrodes were then electropolymerized in the 5mM OPD solution to yield a PPD membrane (0.7V vs. Ag/AgCl electrode for 900seconds). Iridium oxide was electroplated on the Au electrode to develop a reference electrode [11]. 2µl of GOD-chitosan solution (50U GOD, 1% chitosan, 1.25% GA) was applied on the working electrode. After curing at 4°C for 8 hours, 1µl Nafion solution was coated and cured at room temperature for 4 hours. 4µl Polyvinyl alcohol (PVA) solutions (5% w/v) were selectively coated and gelled by freeze-thaw cycles (n=5). 3µl CaO_2-silicone solution (30% w/w) was applied in the groove and cured at room temperature. 1µl of catalase-chitosan solution (2U catalase, 1% chitosan, 1.25% GA) was selectively applied on top of the groove. Finally, the Polyimide film was cut and spirally rolled based on our previous work [12]. The developed smart catheter with the glucose biosensor and 3D view of 12µm thick groove structure are shown in Fig. 2.

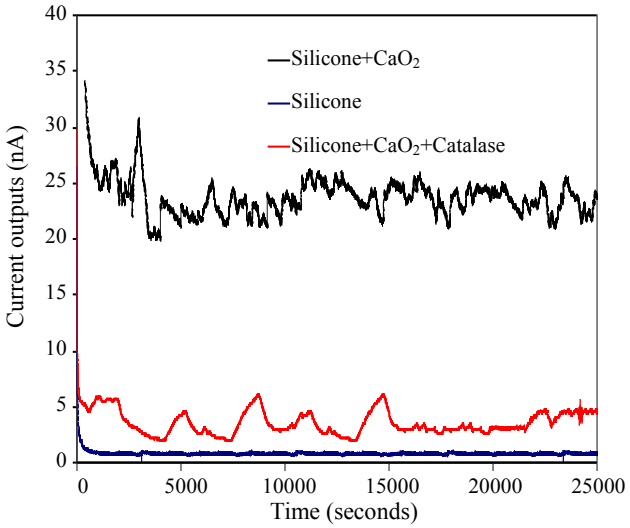
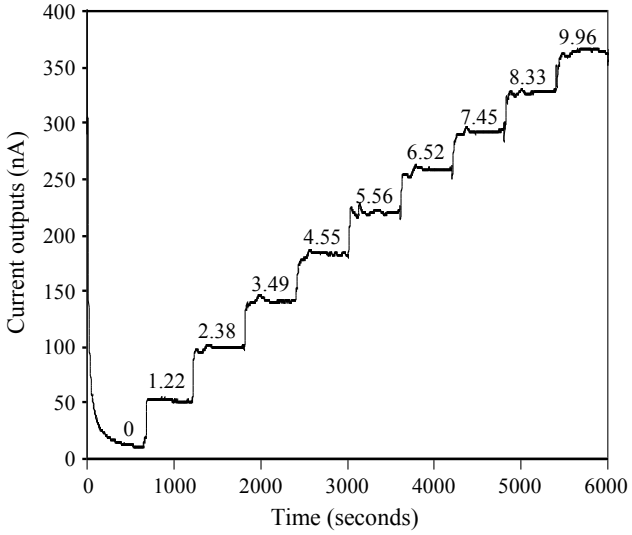
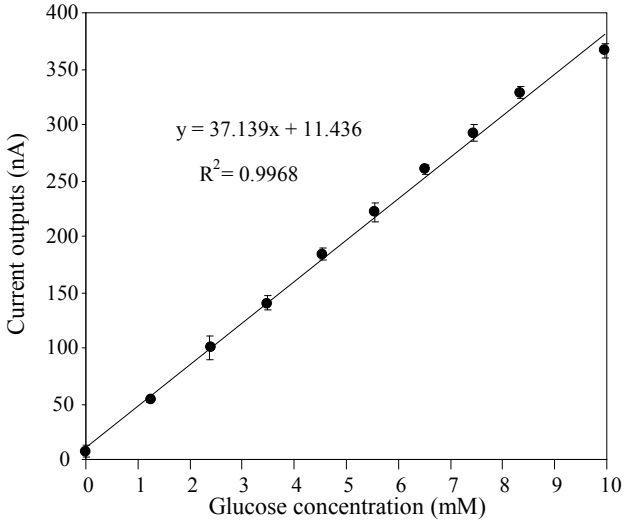


Figure 3: Oxygen generation characteristics: The generated H_2O_2 is assessed amperometrically on the surface of the working electrode for silicone, CaO_2 encapsulated silicone and CaO_2 and catalase encapsulated silicone.



(a)



(b)

Figure 4: Glucose biosensor characteristics: (a) Current-time curves recorded through the successive addition of glucose in PBS and (b) Calibration curve for glucose concentration in the range of 0.05 – 10mM at oxygen tension of 8.3mmHg.

IV. Results and Discussion

Developed glucose biosensors were placed into a closed container filled with PBS (pH=7.2). The solutions were kept in a temperature controlled waterbath (37 ± 0.2˚C) and equilibrated with calibration gases containing different oxygen concentration.

A. Hydrogen Peroxide Byproduct Generation

When CaO_2 decomposes, in addition to oxygen, H_2O_2 is produced as an intermediate. Accumulation of H_2O_2 leads to hyperoxide conditions and increased susceptibility of side reactions [13]. In addition, the migration of generated H_2O_2 to the biosensor working electrode will produce errors for biosensor performance. To solve this problem, the catalase immobilized in chitosan matrix was coated on the groove. Fig. 3 shows the H_2O_2 byproduct generation property for the biosensor with three different materials encapsulated in the groove, silicone, CaO_2 encapsulated silicone and both CaO_2 and catalase encapsulated silicone. The generated H_2O_2 is assessed amperometrically on the surface of the working electrode, which relates current to H_2O_2 concentrations. No H_2O_2 was detected for the biosensor with only silicone coating. For the biosensor with CaO_2 encapsulation, the H_2O_2 byproducts produced and migrated to the working electrode were measured. With additional encapsulation of catalase, around 90% of the produced H_2O_2 were decomposed into oxygen and water. Higher efficiency can be achieved once the catalase immobilization is optimized.

B. Amperometric Response of the Glucose Biosensor

In vitro calibration was carried out in PBS that has been equilibrated at 37±0.2˚C with 1% O_2 and 5% CO_2 (equivalent to oxygen tension of 8.3mmHg) to mimic hypoxic conditions. Fig. 4(a) illustrates the amperometric responses of the glucose biosensor to the successive addition of glucose solution at an applied potential of 400mV vs. the IrOx reference electrode. Developed glucose biosensors have the sensitivity of 37.130nA/mM in the linear range from 0.05 to 10mM with a linear coefficient of R^2=0.9968 as shown in Fig. 4(b).

C. Oxygen Dependence

The oxygen dependence of glucose biosensors was performed by sealing the chamber containing the biosensor along with a commercial oxygen sensor (OXY Micro, WPI Inc). Glucose was administered and the chamber was purged with specified calibration gas containing 1-8% oxygen. The response of the glucose biosensor as a function of oxygen tension was obtained for various glucose concentrations. Fig. 5 shows the performance of glucose biosensors with different outer membrane coatings. Due to the oxygen storing capability of PVA, the sensors with PVA coating are less sensitive to the environmental oxygen tension changes.

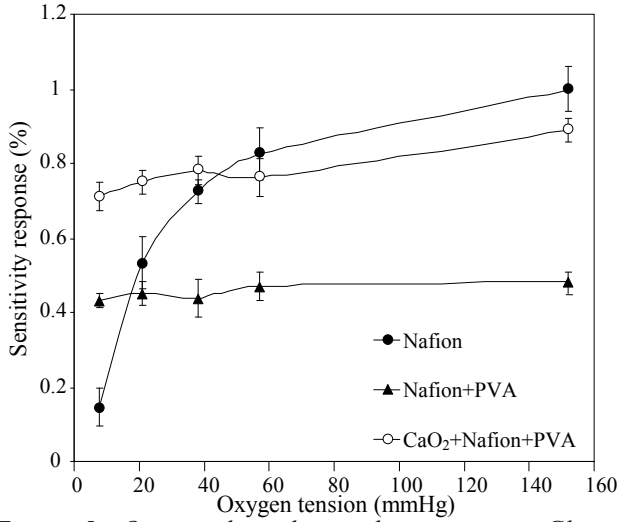


Figure 5: Oxygen dependence characteristics: Glucose biosensors with three different configurations were calibrated under different oxygen tensions. The one with CaO_2 encapsulation show the best sensitivity responses in the oxygen tension from 0 to 151mmHg.

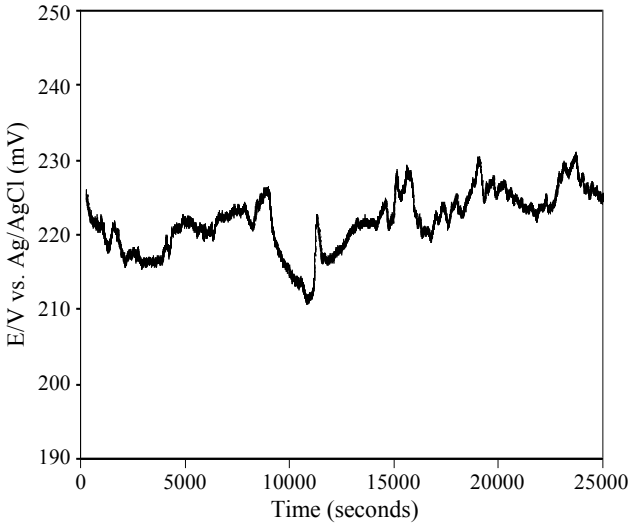


Figure 6: Effect of the glucose biosensor microenvironment pH on reference electrode stability: open circuit potential drifts of IrOx reference electrode vs Ag/AgCl reference electrode (3M) was less than 20mV.

However, the sensitivity response was decreased around 43% of their response of Nafion outer membrane at oxygen tension of 151mmHg. With additional CaO_2 encapsulation in the groove structure, the sensitivity response was increased up to 74%. Under hypoxic conditions, developed glucose biosensors maintained their sensitivity response around 84% of their response at oxygen tension of 151mmHg. The sensitivity deviation was less than 5.3% from oxygen tension of 0 to 57 mmHg.

D. Effect of the Microenvironment pH on Reference Electrode Stability

According to the Eq. (3), hydroxide ions are produced when calcium peroxide decomposes. It may influence the pH of the biosensor microenvironment. IrOx electrode exhibits minimal long-term drift of electrode potential in fixed pH solutions and demonstrates a linear super-Nernstian response with a sensitivity of -64.4mV/pH from pH 4 to pH 7 [10]. To eliminate the hydroxide ions induced pH change on the reference electrode surface, in glucose biosensor design, reference electrode is placed outside the semi-permeable outer membrane. Fig. 6 shows that open circuit potential drifts of IrOx reference electrode vs. Ag/AgCl reference electrode (3M) were less than 20mV. It introduces negligible errors for the glucose biosensors which has over 100mV diffusion dominant region.

V. CONCLUSIONS

We developed a novel brain-friendly amperometric enzyme biosensor platform that can eliminate the oxygen dependence. Sufficient oxygen to drive the enzymatic reaction in hypoxic conditions was produced sustainably by encapsulated oxygen generating biomaterial, calcium peroxide. The catalase immobilized in chitosan matrix was coated on top of the groove to decompose residual hydrogen peroxide to oxygen. The developed glucose biosensors maintained around 84% of their sensitivity response that at oxygen tension of 151mmHg under hypoxic conditions. Satisfactory performance of the glucose biosensor was observed obtaining low detection limit, wide linear operation range and good sensitivity. This new platform may serve as the most fundamental unit in an assembly of multiple enzyme-based biosensors. It may also exhibit therapeutic effect due to its oxygen generating capabilities.

ACKNOWLEDGEMENTS

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