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Evaluation of enzyme-based tear glucose electrochemical sensors over a wide range of blood glucose concentrations

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ABSTRACT

Miniature enzyme-based amperometric and coulometric glucose sensors were fabricated and applied to measure tear glucose concentrations in anesthetized rabbits. Without perturbing the eyeball, 3 μL of tear fluid was sampled from the marginal conjunctiva under the lower eyelid of anesthetized rabbits at various time points via a microliter glass capillary tube, and the miniature sensors were then inserted into the volume of collected tear fluids within the capillaries for detection. Intravenous bolus doses of insulin were administrated to the rabbits to lower the elevated blood glucose concentrations caused by anesthesia over the 7 h test periods. A significant correlation was found between tear and blood glucose levels for multiple rabbits, suggesting that electrochemical sensor-based tear glucose measurements may be a potential supplementary method for point-of-care glucose monitoring.

1. Introduction

It is well recognized that diabetes is one of the major causes of death and disabilities in developed countries, and the mortality rate of individuals diagnosed with diabetes is expected to double by 2030 (http://www.who.int/mediacentre/factsheets/fs312/en/index.html). Early diagnosis and tight glycemic management are critical in helping to prevent and control diabetes and its complications, including cardiovascular disease, kidney failure, and blindness (Muggeo, 1998; Zhao et al., 2009).

Conventional point-of-care glucose monitoring systems usually involve pricking a finger to obtain a drop of blood for analysis via a strip-based electrochemical glucometer. A small volume of blood is drawn into a test strip loaded with glucose oxidase or glucose dehydrogenase, which reacts with sample glucose and reports a reading of plasma glucose concentrations in a few seconds. For Type 1 diabetes, it is recommended that blood glucose concentration be monitored up to eight times a day. These repeated finger pricks may result in patient discomfort and, therefore, less frequent blood glucose checks that can lead to further complications (Gay et al., 2006; Greene, 2002).

Tear fluid has unique properties as an accessible body fluid and is known to contain elevated glucose concentration in diabetes (Iwata, 1973). Compared to other surrogate fluids, for example, saliva and urine, which may have a highly variable dilution effects, tear fluid is maintained at a miniscule and relatively stable volume (~4 μL). Tear fluid is continuously replenished by the production of the lacrimal gland and other accessory glands at a rate of production in the range of 0.5–2.2 μL/min (Ohashi et al., 2006). A thin film of tear fluid (~8 μm thick) keeps the cornea and conjunctiva continuously moist, without any stimulation (Lane et al., 2006).

Based on the above knowledge, tear glucose measurements may provide the possibility of developing a relatively simple and minimally invasive method of indirectly monitoring blood glucose levels, provided that the tear glucose concentrations can be shown to correlate with blood glucose concentrations in individuals. If a good correlation between the two types of samples can be established over a wide range of glucose concentrations, tear glucose monitoring could be an attractive supplement to conventional blood glucometer measurements.

There has been considerable research focused on the determination of glucose in tears with different methods. For any technique to be analytically useful, it requires a very low detection limit (since glucose is present in tear fluid at levels of 50–100 times lower than in blood) (Lane et al., 2006; Zhu and Chauhan,

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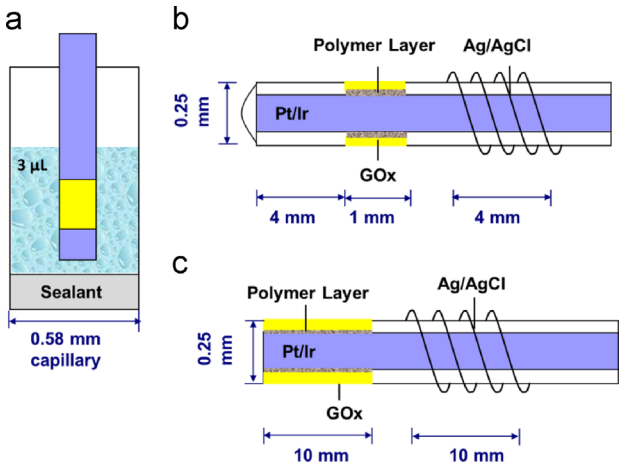

Fig. 1. (a) Schematic representation of tear glucose detection in a glass capillary. (b) Configuration of the amperometric sensor. (c) Configuration of the coulometric sensor.

2007), high selectivity over active interferences, and the ability to quantitatively measure very small sample volumes in a short time period. LeBlanc et al. (2005) reported a tear glucose concentration analysis system using high-performance liquid chromatography with a pulse amperometric detector (HPLC-PAD). After measurement of 44 paired samples from 5 different patients, they concluded that poor correlation exists between tear and blood glucose in critically ill patients, and that glucose measurements in tear fluid fails to provide an alternative method to blood glucose monitoring. Later, Lane et al. (2006) applied a similar chromatographic (LC-PAD) system for measurements of glucose in 121 diabetic and non-diabetic patients and found the mean values of tear glucose concentrations to be 0.35 ± 0.04 mmol/L and 0.16 ± 0.03 mmol/L, respectively. At the same time, Baca et al., (2007) employed liquid chromatography coupled with electrospray ionization mass spectrometry (LC–ESI–MS) to detect glucose concentrations in 1 μL samples of tear fluid from 25 normal fasting patients. They were able to obtain significant correlations between this method and a novel contact lens-based sensor device. In a more recent paper, LaBelle et al. (2010) introduced a disposable microfluidics system capable of measuring glucose to levels as low as 43.4 μM using an integrated electrochemical sensor. The authors proposed this device to be a future prototype for a non-invasive glucose testing, but did not provide any real sample data. In other work, an enzyme immobilized Pt electrode on the surface of a poly(dimethylsiloxane) (PDMDS) film was applied for measuring tear glucose levels in a rabbit, and a value of 0.12 mmol/L was found (Chu et al., 2011); however, no blood samples were collected to validate any relationship between tear and blood glucose levels. Despite these and other research efforts, there are significant discrepancies regarding the existence of a clinically useful correlation between tear glucose and blood glucose concentrations, and furthermore, the true concentration ranges of tear glucose in normal and diabetic subjects is still a subject of debate. Surprisingly, even though conventional hand-held glucometers are primarily based on the electrochemical/enzymatic detection of glucose in tiny volumes of whole blood, to date, there are very few reports on applying electrochemical devices for tear glucose measurements. Our group has previously proposed a small amperometric sensor for tear glucose measurement in 5 μL tear fluids and found a very positive correlation between tear and blood glucose levels using 12 anesthetized rabbits over a period of 8 h (Yan et al., 2011). However, the correlation could only be validated in the high blood glucose concentrations range due to the anesthesia inducing higher than normal blood glucose concentrations in all tested animals.

Herein, we now employ a similar needle-type enzyme-based amperometric sensor as well as a newer coulometric enzyme electrode configuration for the detection of tear glucose within a micro glass capillary tube requiring only 3 μL of tear fluid. The sensors were applied to detect tear glucose concentrations in anesthetized rabbits, but now with insulin administration to achieve a much broader range of blood glucose values (2–20 mM). Using the miniature electrochemical devices, good correlation in glucose levels between the two types of samples is observed, suggesting that tear glucose measurement may provide an attractive supplementary method to current blood glucose self-monitoring devices.

2. Materials and methods

2.1. Materials

Glucose oxidase (Type VII, from *Aspergillus niger*), D-(+)-glucose, glutaraldehyde, bovine serum albumin, sodium chloride, sodium phosphate dibasic, potassium hydroxide, potassium chloride, potassium phosphate monobasic, L-ascorbic acid, uric acid (sodium salt), acetaminophen, 1,3-diaminobenzene, resorcinol, Nafion (5 wt% solution in a lower aliphatic alcohols/H_2O mix) were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Platinum/iridium and silver wires with Teflon® coatings were obtained from A–M Systems (Sequim, WA). Heat shrinkable tubing was product of Advanced Polymers (Salem, NH). Micro glass capillary tubes were obtained from Drummond (Broomall, PA).

2.2. Tear glucose sensor fabrication

The sensor devices are based on immobilizing glucose oxidase on a Pt/Ir wire and anodically detecting the liberated hydrogen peroxide from the enzymatic reaction (Fig. 1). Because the amount of hydrogen peroxide produced from lacrimal glucose is very low (μM), it will not likely affect the immobilized enzyme activity with repeated use. In addition, the fast consumption of hydrogen peroxide by electrochemical oxidation can prevent its increase to detrimental levels to cause enzyme deactivation. Indeed, many commercial glucose electrodes based on hydrogen peroxide detection are used to measure much higher levels of glucose in many hundreds/thousands of discrete blood samples before significant degradation of enzyme activity is observed. It is also noted that since oxygen (ca. 300 μM) will be always present in excess to the low concentrations of glucose in tear fluid (20 -200 μM), there is no need for an electron transfer mediator, as often used in blood glucometer type devices. Inner selective layers of Nafion® and an electropolymerized film of 1,3-diaminobenzene/resorcinol greatly enhance the selectivity for glucose over known electroactive interferents, including ascorbate, urate and acetaminophen (Choy et al., 2000). Specifically, the underlying cation exchanger layer of Nafion® can effectively block anionic species such as ascorbic and uric acid from the platinum electrode surface. The electrochemically polymerized diaminobenzene/resorcinol layer forms a dense polymer matrix to retard the diffusion of neutral interference species such as acetaminophen (that is larger than hydrogen peroxide) to the working electrode.

The amperometric sensor was fabricated as previously described (Yan et al., 2011), with 1 mm active length of the exposed Pt wire of a total working area of 0.40 mm^2. At the same time, a newer coulometric sensor configuration was designed similarly except that a larger surface area on the Pt/Ir working electrode was exposed and covered with active layers (including enzyme) so that substantial consumption