A Noninvasive Fluorescent Biosensor for Transdermal Glucose

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ABSTRACT

Glucose determination in neonates require painful blood draws. Our group has been developing a painless, noninvasive method of collecting glucose passively diffusing through the skin. The transdermal glucose collected in this manner has a concentration in the µM range. Here we describe a fiber optic biosensor based on the glucose binding protein (GBP) labeled with either BADAN or acrylodan and immobilized on Ni-NTA agarose beads. The portable, low-cost biosensor system consists of an optical fiber with the sensitized beads trapped on one end, and appropriate optics and electronics on the other end. The control software and the visual interface for the optical sensor is designed and implemented in LabVIEW and runs on a tablet computer. To collect the transdermal glucose, an automated system with a sampling head attaches to the skin surface and executes a wash, dry and sampling protocol. Data collected from several human subjects using our noninvasive glucose monitoring system are presented.

Keywords: glucose, noninvasive sensing, transdermal diffusion, glucose binding protein

1 INTRODUCTION

Hypoglycemia (< 45 mg/dL) is the most common metabolic problem in neonates occurring in as many as 5-15% of normal newborn infants and as high as 73% in the high-risk intrauterine growth restricted/small for gestational age preterm infants [1]. Hyperglycemia (>125 mg/dL in term infants and >150 mg/dL in preterm infants) is less frequently observed in full term infants, but is the most commonly observed perturbation of glucose metabolism in low birth weight infants in the neonatal intensive care unit (NICU). Both extremes of blood glucose in newborns pose significant challenges in the clinical management of the sick infant, requiring careful and vigilant monitoring to minimize impact on infant morbidity and mortality [2]. It is widely accepted that tight glycemic control will improve clinical outcome [3,4]. But in the NICU, blood glucose readings are often too few and taken too far in between that glycemic swings can be missed. The difficulties associated with current blood glucose monitoring (pain, breaking the skin, blood overdraw, risk of infection) limit testing to about four times a day in the NICU or up to one every 1 - 2 hours in high risk neonates.

The search for a noninvasive glucose sensor is imperative for the NICU, but has been a difficult process for various reasons, such as a large complex background, interferences from other glucose-like substances, and differences in skin quality between subjects. Understandably, some skepticism follows claims of noninvasive glucose sensing. In more recent studies, continuous glucose monitoring systems (CGMS) have been tested on neonates with some success [5]. Hypoglycemic and hyperglycemic episodes missed by intermittent blood glucose readings have been detected in CGMS. However, CGMS involves the transcutaneous insertion of a needle probe (generally designed for adults not neonates) and requires a blood glucose reading for calibration. Thus, our method of passive diffusion of glucose through intact skin is a very significant development in this field [6-9]. A small volume of water is simply allowed to sit on the skin surface for a short period of time allowing spontaneous permeation of glucose from the blood/subcutaneous tissues to the sample water. The glucose extracted by diffusion is then directly sensed by a biosensor (fluorescent glucose binding protein, GBP). In our method, there is practically no background contribution from the skin matrix. In fact, the skin acts as a semi-permeable filter allowing mainly glucose, small molecules and ions to pass through while keeping proteins, glycoproteins, polysaccharides and lipids in.

Figure 1 Sample head for the noninvasive glucose sensing system on the thigh of the neonate.

The long term objective of this project is a painless, automated noninvasive glucose monitoring system for the NICU (Figure 1). This method directly measures passively

diffusing glucose through neonatal skin (transdermal glucose, TG) and obviates the need for blood-draws. Our method does NOT require skin pretreatment for permeabilization, as it relies on a unique high-sensitivity glucose sensor. It should be noted that the transdermal glucose concentrations we collect by passive diffusion are 10^{-3} to 10^{-4} of corresponding blood and interstitial fluid glucose levels. To use current glucose oxidase based biosensors will require expensive instrument modifications to detect these low concentrations. In contrast, the GBP biosensor's sensitivity (Figure 2) falls exactly within this range and is ideal for detecting these trace glucose amounts (K_d ~ 1 – 20 µM). In addition, the fluorescence-based GBP biosensor is easily integrated into a low-cost, miniature fluorimeter for ambulatory or bedside applications.

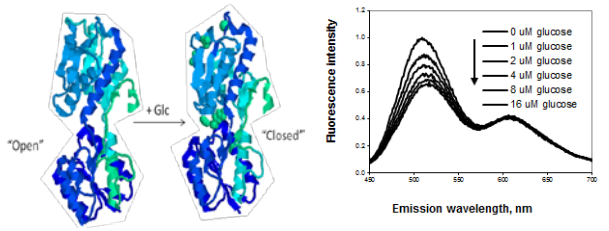

Figure 2 (Left) The glucose binding protein (GBP) undergoes conformational change in the presence of glucose. (Right) The fluorescence spectra of the glucose binding protein (GBP) labeled with acrylodan and a ruthenium metal ligand complex at increasing concentrations of glucose. The fluorescence of acrylodan decreases at increasing glucose concentrations while the ruthenium fluorescence is unchanged and serves as a standard.

2 METHODS

2.1 Preparation of the GBP Biosensor

GBP H152C or L255C was overexpressed in E. coli by induction with IPTG. Bacterial cells were lysed and the clarified cell extract was purified using affinity chromatography Ni-NTA agarose column. A 10-fold excess of Tris(2-carboxyethyl)phosphine hydrochloride (TCEP in 10 mg/mL) in deionized water was added to the protein solution to keep thiol groups in the reduced form. BADAN (for H152C) or acrylodan (for L255C) in dimethyl sulfoxide (DMSO) was added to the protein solution and allowed to stand for 2 hours at room temperature. Unreacted dye was removed from labelled protein by size exclusion chromatography, (Sephadex G-25) and by dialysis in 100 mM phosphate buffer, pH 7.5. The final product was 0.22 µm filter-sterilized and stored at 4ºC.

To prepare the immobilized GBP, triple washed Ni-NTA agarose beads were incubated with dye-labelled GBP in PBS and mixed in a rotary shaker at 25 rpm for 24 hours at 4ºC to ensure maximum binding of protein to Ni-NTA agarose beads. The beads were washed, centrifuged, separated from the supernatant and finally re-suspended in PBS, for a total volume of 120 µL. The Ni-NTA-GBP beads in PBS were stored in the refrigerator at 4ºC until use.

2.2 Instrumentation for fiber optic glucose biosensor system

The GBP optical probe was made by confining the Ni-NTA-GBP microbeads within nylon mesh at the tip of a fiber optic (Figure 3). The optical fiber is then connected to a mini-fluorimeter that was customized specifically for this application. The mini-fluorimeter in Figure 4 utilizes a violet LED (400 nm + 20 nm) for fluorescence excitation and employs a lock-in detection approach to allow measurement under ambient light conditions. The emission intensity of the dye (emission maximum 520 nm) is monitored, digitized and sent to the tablet via RS232 serial interface. The electronics consists of 3 main blocks: LED drivers, lock-in photodetectors, and system-on-a-chip controller (SOC, MSP430F4270, TI) that fits on a 5 x 5 cm board. The control software and the visual interface for the optical sensor was designed and implemented in LabVIEW and runs on the tablet PC.

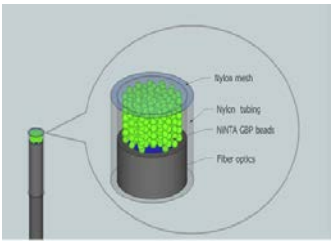

Figure 3. His-tagged GBP immobilized on Agarose-Ni-NTA microbeads and the microbeads confined at the tip of the fiber optic [8].

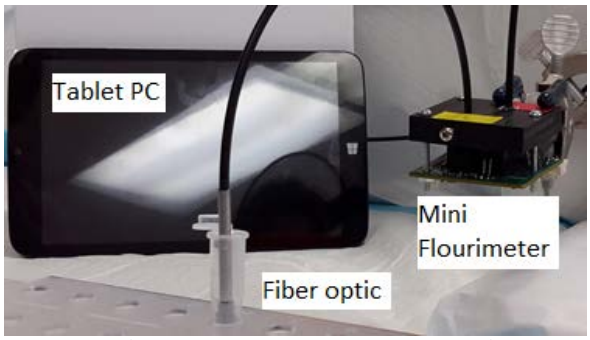

Figure 4 The fiber optic biosensor is attached to a mini-fluorimeter and a tablet PC [8].

2.3 *In Vitro* Tests of Transdermal Glucose Sampling in Neonates

In vitro flow-through diffusion cell experiments were conducted using a PermeGear™ flow through diffusion cell using a skin model for neonates and glucose solutions in the bottom reservoir equivalent to blood glucose concentrations. Intact Yucatan miniature pig skin with a transepidermal water loss (TEWL) of ~10 g/m^{2}h was tape-stripped until TEWL was 20 g/m^{2}h, equivalent to the stratum corneum of a 190 day post-conceptional age (PCA) infant. Glucose samples were collected in the small amount

of water applied to the sample chamber and then measured using GBP.

2.4 Automated Transdermal Glucose Sampling

The automated sampler (Figure 5) is comprised of two pumps, one for liquid and one for air, a heater and a sampling head. The sampling head is kept in place on the skin with medical grade silicon adhesive. First, the sampling chamber is flushed with buffer or DI water to wash glucose contamination from the skin. A heater module is installed to warm the wash water to 37 ºC both for patient comfort and to speed up removal of glucose contamination. After the wash, the valve switches to a piezo air pump/blower to completely dry the skin surface. The air is also heated to 37ºC by passing it via the heater module. For the sampling step, water or buffer is pumped into the sampling head and held there for a specified time period. The sample is then collected and analyzed using GBP.

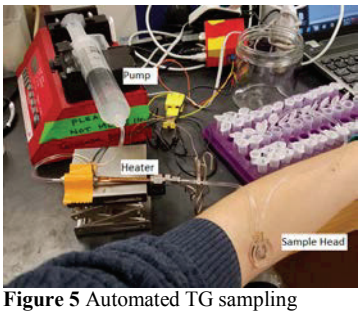

Figure 5 Automated TG sampling

2.5 Testing the Noninvasive Glucose Sensing System on Adult Human Subjects

Healthy adult subjects were subjected to an oral glucose tolerance test (OGTT) to induce glycemic excursions. The OGTT involves an overnight fasting by the test subject after which the blood glucose (BG) and TG were determined. This is followed by ingestion of 75 g of glucose and determination of BG and TG multiple times over a period of three hours.

3 RESULTS AND DISCUSSION

3.1 Characterization of the GBP Optode

Immobilization of the dye-labeled GBP onto Ni-NTA microbeads had the favorable effect of increasing the stability and reusability of the protein such that the microbeads can be stored at room temperature for a few days and can be washed and reused several times. The beads can also be illuminated continuously for >16 h with no noticeable decrease in performance. The response and reversibility of the sensor is shown in Figure 6, which indicates that the Ni-NTA-GBP can be used in continuous glucose monitoring or in frequent intermittent glucose determination. The response time of the fiber optic system is ~ 15 s (i.e. attains 70% signal change in 15 s when the glucose concentration is 6 µM). The linear range for the calibration curve is 4 – 20 µM glucose (r^2 = 0.9517) and the sensitivity is 0.0023 μM^{-1}.

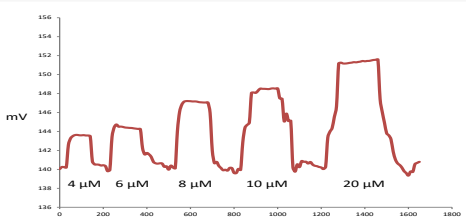

Figure 6 Typical response profile of the biosensor for standard glucose solutions 4 – 20 µM alternating with blank (n = 3) [8].

3.2 *In Vitro* Tests of Transdermal Glucose Sampling in Neonates

The plot in Figure 7 left shows the linear correlation between the TG collected and the glucose concentration in the bottom reservoir of the PermeGear™ flow through diffusion cell (2, 5 and 20 mM to mimic hypo-, normo- and hyperglycemic conditions). The linear correlation confirms the in vivo results in previous work [7]. These results also confirm a significant increase in TG concentration collected (>10x) at the higher TEWL (stripped skin vs. intact skin) for the same glucose concentration. Figure 7 right shows that the first measurement matched the glucose measurements repeated every 30 minutes, which means that equilibrium is reached in the first measurement. Thus, <30 min is required for a reading in the NICU. These results are also important because glucose samples are constant for the same TEWL value, which confirms that TEWL can be used as a standard to account for differences in skin diffusivities. Note as well that the sensor can detect neonatal hypoglycemic conditions, that is, the signal for samples collected at 2 mM (36 mg/dL) are significantly different from zero. The recommended range for most hand-held glucometers are at the tail end of this value. And because the CGMS uses the same glucose oxidase biosensor, readings below 40 mg/dL are considered ambiguous, which can lead to uncertainties at neonatal hypoglycemic

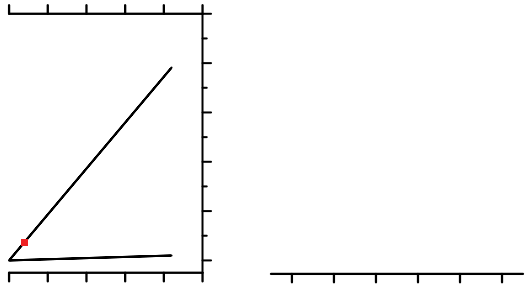
Figure 7. TG concentration measured as a function of three glucose concentrations (mimicking hypo, normal and hyperglycemic conditions) in contact with dermatomed Yucatan minipig skin that was stripped with tape to give TEWL values comparable to 190-day PCA neonate skin [9].

conditions.

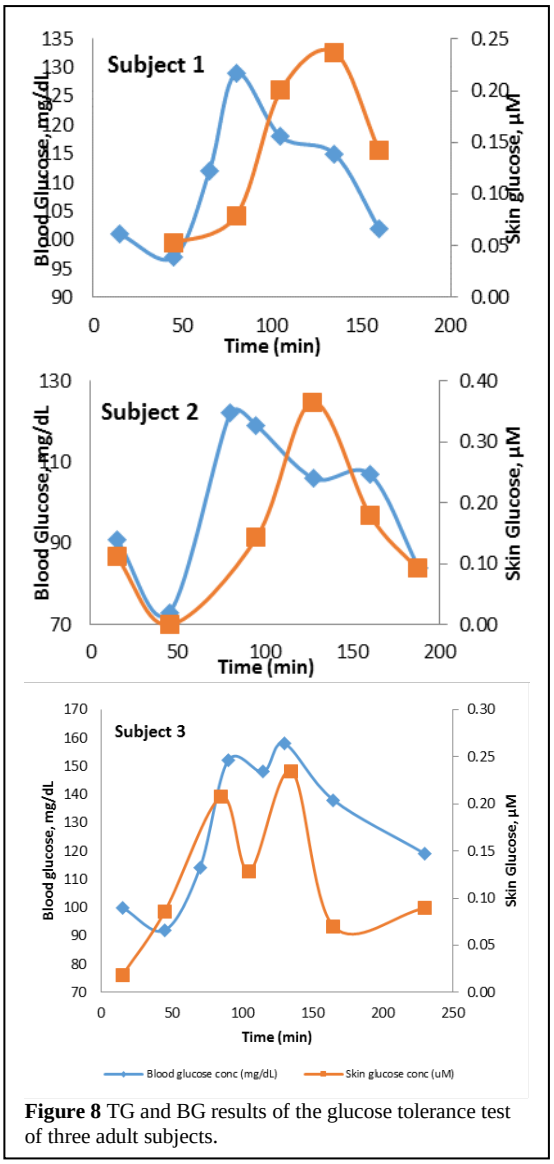

Figure 8 TG and BG results of the glucose tolerance test of three adult subjects.

3.3 Testing the Noninvasive Glucose Sensing System on Adult Human Subjects

The results of the oral glucose tolerance tests for 3 subjects are shown in Figure 8. Overnight fasting of the subjects was followed by ingestion of 75 g glucose and their TG and BG levels were monitored. The TG tracks the changes in BG for all 3 subjects but there is a clear lag in TG in Subjects 1 and 2. The blood glucose levels peaked at 129, 122 and 152 mg/dL approximately 30 min after the intake of glucose for the 3 subjects, respectively. The transdermal glucose concentration peaked at an average of 0.24, 0.37 and 0.24 µM respectively, 75 min after glucose consumption for Subjects 1 and 2. A lag between BG and TG readings is expected in skin glucose samples because of the time required for ingested glucose to circulate to the blood, the tissues and then through the skin and may be a function of skin diffusivity or TEWL. Subject 3 appears to have no to minimal lag time consistent with previous results [7]. Further investigation is needed to account for these individual differences. Nevertheless, the *in vitro* data for the thinner neonate skin showing the linear correlation between TG and BG is encouraging.

4 CONCLUSION AND FUTURE WORK

We have shown the potential of noninvasive glucose monitoring by measuring the passively diffusing transdermal glucose with a highly sensitive glucose biosensor. Further work is needed to determine a reference method, such as TEWL that can correct for differences in skin diffusivities between subjects. In terms of the instrumentation, we plan to combine the fluidics in Figure 5 with the analytics in Figure 4 for testing on neonatal subjects in the NICU.

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