Dual Glucose and Lactate Self-powered biosensor

Ankit Baingane, Naomi Mburu, Christopher Animanshaun and Gymama Slaughter

Bioelectronics Laboratory, Department of Computer science and Electrical engineering,
University of Maryland Baltimore County,
1000 Hilltop circle,
Baltimore MD 21250, USA

ABSTRACT

We report on the development of a dual self-powered biosensing system for temporary placement underneath tissue bed to continuously monitor glucose and lactate metabolites non-invasively for periods varying from several minutes to hours. The self-powered biosensing system is constructed from two 4 mm x 4 mm massively dense mesh network of multi-walled carbon nanotubes (MWCNTs) bioelectrodes employing pyroquinoline quinone glucose dehydrogenase (PQQ-GDH) as the biocatalyst for the glucose oxidation and D-Lactate dehydrogenase (D-LDH) as the biocatalyst for lactate oxidation in a biofuel cell arrangement. A common laccase modified-MWCNTs electrode served as the cathode for the reduction of molecular oxygen. Two 0.1 mF capacitors functioning as transducers were coupled with an energy amplification circuit to amplify the power output from each of the biofuel and charge the corresponding capacitor. The charging/discharging frequencies of the capacitors constituted the biosensing. The biosensors displayed the levels of lactate and glucose and these results showed very good sensitivity.

Keywords: glucose, lactate, biofuel cell, voltage boosting, biosensing

1 INTRODUCTION

When soldiers lose limbs on the battlefield, there is a very short time frame that the limb remains viable. In such conditions, it may be desirable to monitor more than one metabolic analyte concurrently. Lactate, a key biomarker of stress, increases and is the main source of metabolically-produce acid responsible for tissue acidosis. Therefore, when a limb is detached from the body, the flow of nutrients and oxygen to that limb ceases, which causes deterioration of the transplant. This process is described as ischemia [1]. Ischemia can be identified by the coupled and accelerated production of lactate acid and oxidation of glucose due to elevated glycolysis in the oxygen deprived tissue [2]. Thus, glucose and lactate concentrations are excellent biomarkers for monitoring the health of organs.

Here we develop dual analyte self-powered biosensor that can generate its own electrical power to drive its internal circuits. Such self-powered biosensor comprise of enzymatic biological fuel cells and capacitor circuits functioning as transducers [3-5]. This way the sensor generates an electric power proportional to the analyte concentration and senses analyte concentration via the charging frequency of the capacitor circuit. These types of biosensors are an improvement to traditional, battery powered biosensors because this self-powered biosensor does not rely on external power sources, such as batteries and completely eliminate the potentiostat circuit employed in amperometric biosensors by deriving electrical energy from its surroundings. The system continuously generates power and senses analytes as long as there is a continuous supply of biomarkers (fuel) to the system.

2 EXPERIMENTAL SECTION

2.1 Materials and method

Buckypaper was purchased from NanotechLabs (Yadkinville, NC). Pyrenebutanoic acid, succinimidyl ester (PBSE) was purchased from AnaSpec Inc. D(+) glucose, lactic acid, potassium phosphate monobasic, laccase, dimethyl sulfoxide (DMSO), isopropyl alcohol and d-lactate dehydrogenase (D-LDH) were purchased from Sigma Aldrich and were used as received. Polyamide HD-2611 was purchased from HD Microsystems (Parlin, NJ, USA). PQQ-GDH was purchased from Toyobo Co. Ltd. 10 mM phosphate buffered solution pH 7.0, 10 mM phosphate buffered solution pH 6.0, 10 mM phosphate buffered solution pH 7.0 with 1 mM $CaCl_2$, 100 mM phosphate buffered solution pH 7.4, and 100 mM phosphate buffered solution pH 6.0 were prepared in the laboratory.

2.2 Sensor Fabrication and Enzyme Immobilization

In an effort to miniaturize the sensors while maintaining high electrical output, roughly 4 mm x 4mm buckypaper squares were used at the electrode substrates. A 200 μm tungsten wire was sandwiched and sealed along the top edge of each square using polyimide and an additional 4 mm x 1 mm strip of buckypaper. Subsequently, polyimide was applied around the side edges to enhance the structural integrity of the buckypaper. The bioelectrodes were baked in a convection oven at 150 °C for one hour in order to cure the polyimide. Upon curing, the electrodes were flipped and polyimide was

applied on the backside and side edges of the bioelectrodes and cured again at 150 °C for one hour.

The tungsten wires attached to the bioelectrodes were bent so that they were perpendicular to the top edge. This enabled easy handling and connection to measurement equipment. These electrodes were washed with isopropyl alcohol to remove impurities from the surface. The buckypaper was then placed in 0.83 mg/ml PBSE/DMSO heterobifunctional cross-linking solution, where noncovalent π-π stacking occurred between the aromatic ring on the PBSE molecule and the series of aromatic rings that compose buckypaper. This crosslinking procedure was carried out in the dark with moderate shaking for 1 hour 30 min at room temperature. Afterwards, the electrodes were rinsed with DMSO for five minutes to remove excess PBSE, followed by a five minute 10 mM PBS (pH 7.0) rinse to remove excess DMSO. 5 mg/ml of PQQ-GDH in 10 mM phosphate buffered solution (PBS) with 1 mM $CaCl_2$ (pH 7) was immobilized onto one of the prepared electrodes in the dark at room temperature with moderate shaking. This electrode served as the bioanode for glucose analyte. The biocathode was prepared in a similar fashion, using 5 mg/ml of laccase in 10 mM PBS (pH 6). The lactate bioanode was modified with 1.4 mg/mL D-LDH in a solution containing 10 mM PBS with 1 mM $CaCl_2$ (pH 7) . The immobilized bioelectrodes were preserved by coating the active surface with 2 µL of Nafion, and placed in a desiccator for 15 minutes to dry at room temperature. The resulting bioanodes were then stored in 100 mM PBS (pH 7.4) and the biocathodes in 100 mM PBS (pH 6.0) in the refrigerator.

2.3 Voltage amplification circuit

The anode and cathode were assembled together to realize a biofuel cell. The electrical voltage produced by this single biofuel cell was supplied as the input voltage for the charge pump integrated circuit (IC) to excite the nominal input voltage from as low as 300 mV up to 1.8 V via the capacitor functioning as the transducing element. The charging/ discharging frequency of the capacitor is correlated to the changes in glucose concentration. All experiments were performed at 37°C and pH 7.0 to model the physiological conditions.

3 RESULTS AND DISCUSSION

Buckypaper, a dense mesh network of three-dimensional, multi-walled carbon nanotubes (MWCNTs), was selected as the substrate material for the construction of biofuel cells because its three-dimensional structural configuration provides high surface area for enzyme immobilization. The driving reaction behind the enzyme immobilization reaction is the formation of a peptide bond between the amino group on the respective enzyme and the carboxyl group found in PBSE [4]. During this reaction, the double bond between the carbon and oxygen on PBSE is broken, causing the oxygen to develop a negative formal charge as the electrons migrate towards the more electronegative atom. An unstable intermediate is formed when the nitrogen from the enzyme's amino group donates electrons to the now electron deficient carbon on the PBSE. This intermediate is unstable because the nitrogen atom now has a positive formal charge, and the oxygen atom retains its negative formal charge. The π bond electrons on the oxygen reform the double bond with the carbon atom on the PBSE. Simultaneously, the ester group also attached to the carbon breaks off, along with one of the hydrogen atoms attached to the nitrogen atoms, thus leaving behind a neutral, immobilized enzyme.

Pyrroloquinoline quinone glucose dehydrogenase (PQQ-GDH) enzyme was selected as an alternative enzyme to glucose oxidase to catalyze the oxidation of glucose via direct electron transfer as illustrated in the redox equations for the anode (Eq. 1), cathode (Eq. 2) and the overall reaction (Eq. 3):

$$Glucose \rightarrow Gluconolactone + 2H^+ + 2e^- \quad (1)$$

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (2)$$

$$2Glucose + O_2 \rightarrow 2\,Gluconolactone + 2H_2O \quad (3)$$

Since blood is saturated with oxygen, PQQ-GDH was selected because it does not react with oxygen or produce toxic hydrogen peroxide as its byproduct, which can subsequently foul the electrode surface [6]. PQQ-GDH is completely independent of dissolved oxygen, thereby enabling it to serve as a more effective enzyme in oxidizing glucose in vivo [7]. Additionally, common interfering analytes of glucose oxidase and lactate oxidase reactions are decomposed at the same +700 mV potential required for the decomposition of hydrogen peroxide byproduct [8]. Interestingly, the voltages generated by the biofuel cell are below this potential, thereby enabling glucose and lactate to be sensed with a high degree of selectivity. Lactate dehydrogenase was selected over lactate oxidase to avoid the production of hydrogen peroxide [9]. In this system the buckpaper served as the final electron acceptor in the developed system.

The power produced by the dual glucose and lactate biofuel cell was coupled with the voltage amplification circuit, which employed a charge pump integrated circuit (IC) that can increase a nominal voltage of 300 mV to 1.8 V. The sensing ability of the system was determined by measuring the charging/ discharging frequency of the capacitor connected to the voltage amplification circuit in response to glucose and lactate concentrations.

For indicating glucose concentration, the charging/discharging frequency of the 0.1 mF capacitor could be controlled by the performance of the glucose biofuel cell, specifically the glucose responsive bioanode because the bioelectricity (power) generated by the biofuel

cell is used to charge the capacitor via the charge pump IC. Once the capacitor is fully charged, the charge pump IC discharges the capacitor until the potential reaches 1.4 V. This charging/discharging of the capacitor continues and is observed to be directly proportional to the biocatalytic reaction at the glucose anode. Thus, by monitoring the charging frequency of the capacitor, the glucose analyte concentration can be determined. Testing of the charging frequency to various glucose concentrations yielded a dynamic linear range of 1.8 mg/dL to 360 mg/dL glucose.

Figure 1 shows a calibration curve in which the average frequency of charge/ discharge cycle of the capacitor was observed for glucose analyte. A linear correlation with a regression coefficient of 0.993 was achieved, which confirms that the system can be used to sense a wide range of glucose concentrations, including glucose concentrations that are at and below the normal glucose range of 79.2 mg/dL -110 mg/dL [4]. Since organ transplants undergo ischemia, this process further leads to decreased glucose levels. Therefore, a self-powered glucose biosensor that is sensitive to glucose levels below and above the normal levels is desired. The self-powered glucose biosensor exhibited a sensitivity of 0.559 Hz/ mM cm^2.

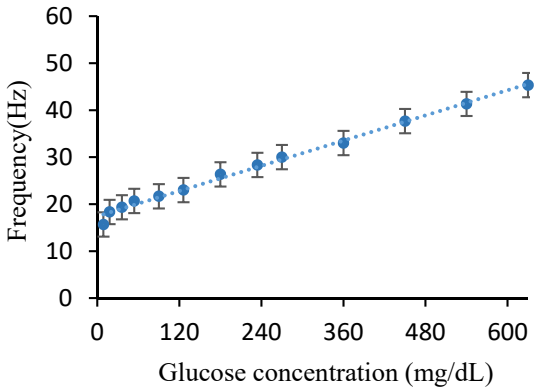

Figure 1: Calibration curve for self-powered glucose biosensor (error bars indicate RSD); 37 °C, pH 7.

In terms of lactate sensing, the frequency of charging/ discharging of the capacitor transducer was used to monitor lactate concentration levels. The system was tested in a variety of lactate solutions while monitoring the charging/ discharging frequency of the 0.1 mF capacitor. Figure 2 shows a calibration curve in which the frequency of charge/ discharge cycle of the capacitor was observed for the lactate analyte. An improved linear correlation with a regression coefficient of 0.999 was achieved with an extended linear dynamic range of 18 mg/dL to 450 mg/dL and an enhanced sensitivity of 9.869 Hz/ mM cm^2.

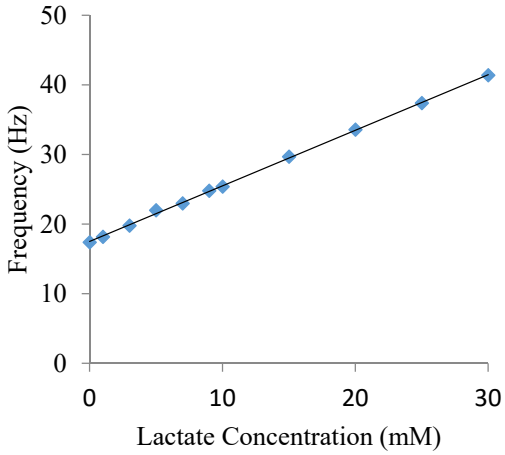

Figure 2: Calibration curve for self-powered lactate (37 °C, pH 7).

The performance of the dual self-powered glucose and lactate biosensor could be further improved by improving the performance of the individual glucose and lactate biofuel cells [10-13], which could potentially extend the lifetime of the biosensor. Additionally, the dual self-powered biosensor described here could greatly reduce the need for device recalibration. This sensing system's stability along with its stable operation at various pH and temperature demonstrated in our prior work [5] allow this dual self-powered glucose and lactate biosensing system to serve as a strong candidate for a potential metabolic biosensor.

4 CONCLUSION

We demonstrated a dual glucose and lactate self-powered biosensor capable of non-invasive, real-time monitoring of key metabolites in vitro. The self-powered biosensing system is constructed from two 4 mm x 4 mm buckypaper biofuel cells employing PQQ-GDH and D-LDH as the biocatalysts for glucose and lactate oxidation, respectively. A common laccase MWCNTs electrode served as the cathode for the reduction of molecular oxygen. By employing two 0.1 mF capacitors functioning as transducers, the biosensing system was able to generate distinct charging/discharging frequencies of the capacitors corresponding to the various levels of glucose and lactate. The realization of the complete system could enable its deployment in clinical settings to simultaneously monitor key biomarkers to access organ viability and thereby extending the heath outcome of the organ.

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