

Simultaneous Monitoring of Glucose and Lactate by Self-powered Biosensor

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Abstract: A dual self-powered biosensing system integrated with energy amplification circuit is described, for simultaneously monitoring glucose and lactate. The self-powered biosensing system is based on the conventional enzymatic biofuel cell equipped with three 4 mm x 4 mm massively dense mesh network of multi-walled carbon nanotubes (MWCNTs) bioelectrodes in parallel configuration. The bioelectrodes employed 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. A common laccase modified-MWCNTs bioelectrode served as the cathode for the reduction of molecular oxygen. Two charge pump circuits were coupled with 0.1 μF capacitors functioning as transducers. The advantages of employing capacitors were coupled with the efficient energy amplification of the charge pump circuit to amplify the power output from each of the biofuel and charge/discharge the corresponding capacitor. Under operating conditions, the open circuit voltages and short circuit current densities for 180 mg/dL glucose and 25 mM lactate were 339.2 mV and 228.75 $\mu\text{A}/\text{cm}^2$ and 370 mV and 66.17 $\mu\text{A}/\text{cm}^2$, respectively. The responses for glucose and lactate were linear up to 630 mg/dL and 30 mM with sensitivities of 20.11 Hz/ mM cm^{-2} and 9.869 Hz/ mM cm^{-2} , respectively. The potential of the described system was demonstrated to provide stable voltage and current output that was capable of driving the charge pump circuit integrated with the capacitor for simultaneously monitoring glucose and lactate. These results were in good agreement with those previously reported.

Keywords: Glucose, Lactate, Biofuel cell, Voltage boosting, Biosensing.

1. Introduction

Approximately 2 million people in the US are currently living with a limb loss and nearly 25 % of all amputations and limb loss are due to traumatic accidents and/or vascular diseases, such as diabetes [1]. Of the more than 50,000 Warfighters wounded in action in Iraq and Afghanistan, 2.6 % have lost a major limb (a leg or arm) as a result of being in combat and exposed to improvised explosive device attacks. When

Warfighters lose a major limb in combat due to the fact that there is no body armor for legs and arms, 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-produced 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 limb. This process is described as ischemia [2]. 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 [3]. Thus, glucose and lactate concentration levels can serve as excellent biomarkers for monitoring the viability of limbs.

In this work we develop a dual analyte self-powered biosensor that can generate its own electrical power to drive its internal circuits [4]. Such self-powered biosensor comprises enzymatic biological fuel cells and capacitor circuits functioning as transducers [5-7]. This enables the sensor to generate an electric power proportional to the analyte concentration and senses analyte concentration via the charging frequency of the capacitor circuit. These types of biosensors first reported by Slaughter and Kulkarni [6] 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 [8-9]. Thereby, the system continuously generates electrical power and senses analyte as long as there is a continuous supply of analyte.

2. Experimental

2.1. Materials

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 buffer pH 7.0, 10 mM phosphate buffer pH 6.0, 10 mM phosphate buffered saline pH 7.0 (1 mM CaCl₂), were prepared with 18.2 MΩ cm Milli-Q water.

2.2. Bioelectrode Construction

In an effort to miniaturize the sensors while maintaining high electrical output, roughly 4 mm × 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 × 1 mm strip of buckypaper. Subsequently, polyimide was applied around the side edges to enhance the structural integrity of the buckypaper. The bioelectrodes were cured at 150 °C for one hour. 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 2-propanol to remove impurities from the surface. The buckypaper was then placed in 0.83 mg/ml PBSE/DMSO 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.

A solution of 5 mg/ml of PQQ-GDH prepared in 10 mM phosphate buffered solution (PBS) with 1 mM CaCl₂ (pH 7) was used for enzyme immobilization at 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 CaCl₂ (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. Energy 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). The nominal input voltage as low as 300 mV was excited up to 1.8 V via the capacitor functioning as the transducing element as shown in Fig. 1. The charging/discharging frequency of the capacitor is correlated to the changes in glucose concentration. The charge pump circuit consists of S882Z charge pump IC. By monitoring the capacitor frequency, the exact concentration of the analyte can be deduced.

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.

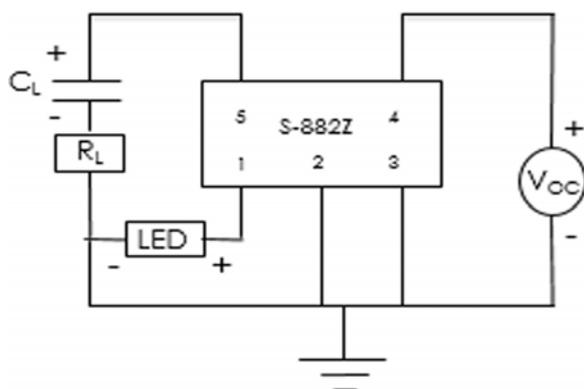
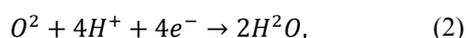


Fig. 1. Biofuel cell supply electrical power to drive the charge pump circuit consisting of a 0.1 μF capacitor functioning as an analyte transducer 'CL'.

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 [6]. 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 now has a positive formal charge, whereas the oxygen 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 attached to the nitrogen, 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):



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 [10]. PQQ-GDH is completely independent of dissolved oxygen, thereby enabling it to serve as a more effective enzyme in oxidizing glucose in vivo [11]. Additionally, common interfering analytes of glucose oxidase and lactate oxidase reactions are decomposed at the same potential of +700 mV, such as hydrogen peroxide byproduct [12]. 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 [13]. 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 energy amplification circuit, which employed a charge pump IC that can increase the 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 energy amplification circuit in response to glucose and lactate concentrations. The open circuit voltages for 180 mg/dL and 360 mg/dL glucose were 339.2 mV and 524 mV, respectively. The corresponding short circuit current densities were observed to be 228.75 $\mu\text{A}/\text{cm}^2$ and 337.5 $\mu\text{A}/\text{cm}^2$ in the presence of 180 mg/dL and 360 mg/dL, respectively.

For determining the analyte concentration, the charging/discharging frequency of the 0.1 μF capacitor is monitored. The glucose or lactate responsive bioanode generates electrical power that 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 ~ 1.2 V. This charging/discharging of the capacitor continues and is observed to be directly proportional to the biocatalytic reaction at the bioanode. Thus, by monitoring the charging frequency of the capacitor, the analyte concentration can be determined. Fig. 2 shows the calibration curve in which the average frequency of charge/discharge cycle of the capacitor was observed for glucose analyte.

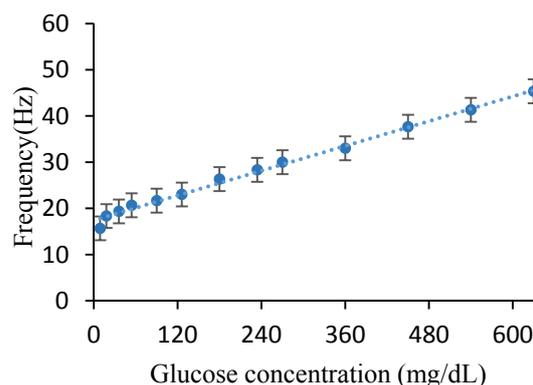


Fig. 2. Calibration curve for self-powered glucose biosensor; 37 $^{\circ}\text{C}$, pH 7.

The glucose self-powered biosensor exhibited a dynamic linear range of 1.8 mg/dL to 360 mg/dL glucose with a linear correlation regression coefficient of 0.993, thereby confirming the utility of the system for sensing a wide range of glucose concentrations. This includes glucose concentrations that are at and

below the normal glucose range [6]. Since limbs 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 20.11 Hz/mM cm².

Additionally, the lactate self-powered biosensor was characterized in the presence of 15 mM lactate at 37 °C (pH 7.4). The IV and power characteristics are shown in Fig. 3 and Fig. 4, respectively. An open circuit voltage of 370 mV and a short circuit current density of 532 μ A/cm² were obtained when employing the laccase biofuel cell in 15 mM lactate.

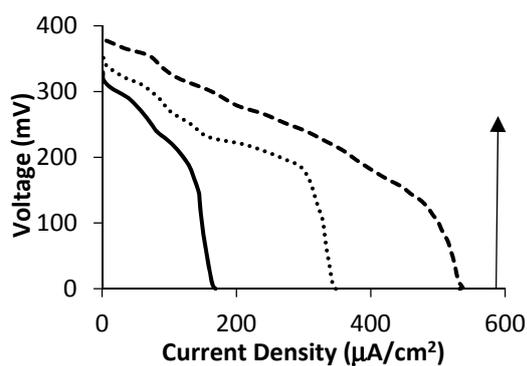


Fig. 3. Polarization curves as performed in 1 - 15 mM lactate.

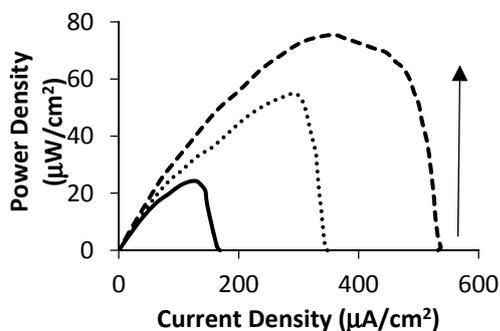


Fig. 4. Power density curves as performed in 1 - 15 mM lactate.

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 μ F capacitor. Fig. 5 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 1 mM to 30 mM lactate and an enhanced sensitivity of 9.869 Hz/mM cm² was achieved.

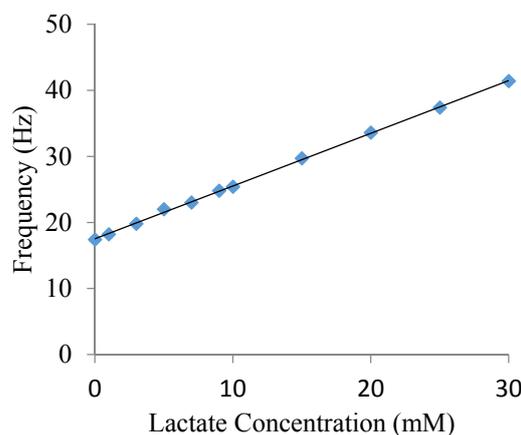


Fig. 5. Calibration curve for self-powered lactate (37 °C, pH 7).

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 [14-16], 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 [6] allow this dual self-powered glucose and lactate biosensing system to serve as a strong candidate for a potential metabolic biosensor.

4. Conclusions

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 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 μ F 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 health outcome of the organ.

Acknowledgements

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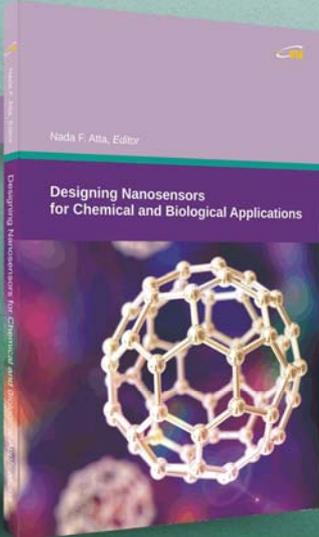
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Nada F. Atta, Editor

Designing Nanosensors for Chemical and Biological Applications



The present book aims at providing the readers with some of the most recent development of new and advanced materials and their applications as nanosensors. Examples of such materials are ferrocene and cyclodextrines as mediators, ionic liquid crystals, self-assembled monolayers on macro/nano-structures, perovskite nanomaterials and functionalized carbon materials. The emphasis of the book will be devoted to the difference in properties and its relation to the mechanism of detection and specificity. Miniaturization on the other hand, is of unique importance for sensors applications. The chapters of this book present the usage of robust, small, sensitive and reliable sensors that take advantage of the growing interest in nano-structures. Different chemical species are taken as good example of the determination of different chemical substances industrially, medically and environmentally.

The book will be useful for scientists and researchers, doctors and students working in medical research, engineers and students working in environmental research, professionals working in industrial field.

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