# A self-powered glucose biosensor based on pyrolloquinoline quinone glucose dehydrogenase and bilirubin oxidase operating under physiological conditions

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***Abstract*—A novel biosensing system capable of simultaneously sensing glucose and powering portable electronic devices such as a digital glucometer is described. The biosensing system consists of enzymatic glucose biofuel cell bioelectrodes functionalized with pyrolloquinoline quinone glucose dehydrogenase (PQQ-GDH) and bilirubin oxidase (BOD) at the bioanode and biocathode, respectively. A dual-stage power amplification circuit is integrated with the single biofuel cell to amplify the electrical power generated. In addition, a capacitor circuit was incorporated to serve as the transducer for sensing glucose. The open circuit voltage of the optimized biofuel cell reached 0.55 V, and the maximum power density achieved was 0.23 mW/ cm$^2$ at 0.29 V. The biofuel cell exhibited a sensitivity of 0.312 mW/mM.cm$^2$ with a linear dynamic range of 3 mM – 20 mM glucose. The overall self-powered glucose biosensor is capable of selectively screening against common interfering species, such as ascorbate and urate and exhibited an operational stability of over 53 days, while maintaining 90 % of its activity. These results demonstrate the system's potential to replace the current glucose monitoring devices that rely on external power supply, such as a battery.**

## I. Introduction

Biological fuel cells have gained significant attention as an alternative power source to battery since the advent of the very first conventional fuel cell. Unlike batteries, fuel cells continue to generate electrical energy as long as there is a continuous supply of fuel. Due to the limitations of batteries, they have become an inconvenient source of power for implantable application since the must be frequently replaced once the active mass is depleted. Even then, repeated surgeries must be performed to replace the battery-operated device in implantable applications. Hence, there is a need to design and develop an alternative power source that has an open loop operating configuration, in which continuous power is generated from the fuel.

In a fuel cell assembly, the anode and a cathode are modified by their respective biocatalyst to oxidize and reduce fuel whose chemical energy is converted into electricity by the catalytic action of the biocatalyst. However, upon oxidation of the fuel in a conventional fuel cell, poisonous metal oxides are formed as byproduct which has been shown to foul the fuel cell electrodes, thereby impacting the cell's stability and performance [1]. Other than non-conventional fuel cells, microbial fuel cells have also been extensively researched and implemented in various applications [2,3]. Initial research on various metal catalyst such as Platinum, Palladium, Gold and ZnO was conducted since these materials were shown to have high affinity towards glucose [4-6]. Although these devices produced higher electrical power, they produce poisonous metal oxide (with ZnO being the exception) as the byproduct of glucose oxidation reaction. Since then naturally occurring enzymes have been employed to optimize the conversion of the biochemical energy of glucose into electrical power [7].

Enzymatic glucose biofuel cell employs naturally occurring glucose selective and oxygen reducing enzymes derived from living organisms as clean, economical and easily renewable source of power. Various enzymes have been used both at anode and cathode to achieve optimal parameters. Anodic substrates are traditionally modified with glucose selective enzymes such as glucose oxidase or glucose dehydrogenase enzymes while the cathode is modified with oxygen reducing enzyme such as laccase, bilirubin oxidase or peroxidase enzyme [8-10]. However, glucose selective oxidase enzyme produces hydrogen peroxide byproduct upon oxidation of glucose, which affects the cell stability and longevity. Additionally, a potential of +700 mV is necessary to break hydrogen peroxide at which other chemical species such as ascorbate and urate decomposes, thereby leading to false output signal. As a result, glucose selective dehydrogenase enzymes are used and laccase enzyme are commonly used as a cathodic enzyme [11], however, for in vivo applications, such a system ceased to operate due to its low operating pH range. Recently, Slaughter's group implemented self-powering biosensing microsystem using a charge pump circuit with a capacitive element connected in series [12]. This transducer element was then used to sense glucose by monitoring the charge/ discharge frequency across of the capacitor. Although it showed promise, it ceased to operate at physiological conditions due to the use of laccase enzyme. Bilirubin oxidase on the other hand has an operating pH range that cover the physiological pH 7.4 making it an ideal candidate for physiological conditions [13]. In this paper, we demonstrate a novel self-powered glucose biosesor capable of sensing glucose and powering a glucometer in various glucose concentration solutions at physiological conditions by supplying a steady DC power.

*Research supported by National Science Foundation, United States (Award ECCS-1349603).

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## II. AMPLIFICATION CIRCUITS

### A. Charge pump circuit

A single enzymatic glucose biofuel cell produces insufficient electrical power and can neither sense glucose nor power any microelectronic device. Therefore, multiple glucose biofuel cells are typically connected in series which increases the circuit complexity along with the size of the system. In this work, an amplification circuit is used to amplify electrical power generated from a single enzymatic glucose biofuel cell by incorporating a charge pump IC S882z (Seiko electronics). This IC requires a minimum input supply of 250 mV to operate the circuit as shown in Fig. 1.

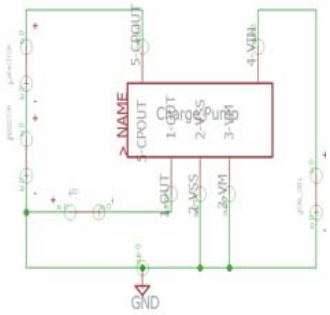


Figure 1. Charge pump circuit and wiring diagram

### B. Step up DC converter circuit

The charge pump amplification circuit above can amplify the input voltage from a single glucose biofuel cell up to 2.4 V and supply burst of power which is inconvenient to power any electronic device. To convert the burst supply into a steady DC power supply with minimum circuit complexity, a step-up DC converter circuit (Fig. 2, LTC 3105 IC, Linear technologies) was interfaced to the charge pump circuit to realize a two-stage power amplification circuit.

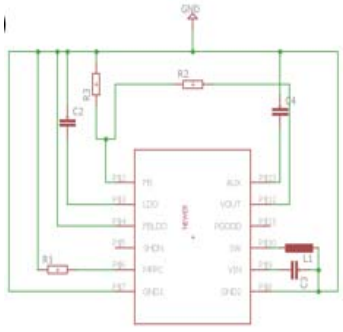


Figure 2. Step-up DC converter and wiring diagram

## III. METHODS

### A. Materials

A dense mesh network of multi-walled carbon nanotubes (MWCNTs) was purchased from Nanotech Labs. 1-pyrenebutanoic succinimidyl ester (PBSE) was purchased from AnaSpec. Inc. PQQ-GDH was purchased from Toyobo. Co. Ltd. Bilirubin oxidase derived from Myrothecium and all other solvents such as dimethyl sulfoxide (DMSO), potassium phosphate, D-(+)-glucose, calcium chloride and Nafion® were purchased from Sigma Aldrich.

### B. Electrode preparation

Two 500 µm x 500 µm dense mesh network of MWCNTs were sandwiched together with a tungsten wire between the strips of MWCNT using polyimide 2611 (HD Microsystems) and cured at 150 °C as shown in Fig. 3 (A and B) to form the electrode substrate. The tungsten wire was used to enable easy handling and connection to measuring instrument. The active surface area of the electrodes prepared was formed to be 0.04 $cm^2$.

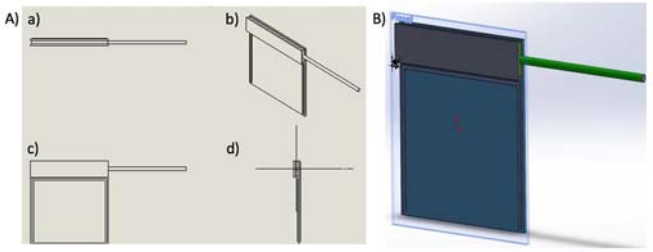


Figure 3. A) 2-dimensional representation of the mesh network of MWCNT electrodes a) Top view b) Front view c) Back view d) Side view. B) 3-dimensional representation of as prepared electrode.

### C. Surface modification

The as prepared electrodes were rinsed with isopropyl alcohol to remove any unbound surface impurities from the electrode surface. 1 mM PBSE crosslinker was dissolved in 10 mM DMSO and used to treat the IPA rinsed electrodes. Such treatment enables the PBSE to bind to the electrode surface via the '$\pi - \pi$' bonding [14]. After which, the respective electrodes were treated with 1 mg/ml PQQ-GDH dissolved in 10 mM PBS (pH 7.0) + 1 mM CaCl2 (pH 7.0) and 1 mg/ml bilirubin oxidase (BOD) to immobilize the enzymes by forming amide bonds at bioanode and biocathode, respectively [15]. These bioelectrodes were further coated with 2 µL Nafion® to enable selective screening of interfering species [16]. The resulting bioelectrodes were used to construct the biofuel cell.

## IV. RESULTS

### A. Biofuel cell characterization

The electrode design allowed for better handling of the bioelectrodes and protected them from tearing during experiments. The conductive MWCNT provided a large surface area for enzyme immobilization. The biofuel cell comprising of PQQ-GDH and BOD enzymes at anode and cathode, respectively allowed their characterization at physiological conditions (pH 7.4 and 37 °C). The biofuel cell was characterized in various glucose concentration and the open circuit voltage along with short circuit current density and peak power density was calculated using the geometrical surface area of the active region of the bioelectrode.

The characterization of glucose biofuel cell was performed under physiological conditions with continuous supply of increasing glucose concentration by measuring the current and voltage across the variable load resistor. Fig. 4 shows the power curves of the biofuel cell, wherein both the open circuit voltage and the short circuit current density was observed to be increasing with the increase in glucose concentration. In 20 mM glucose solution (37 °C, pH 7.4) an open circuit voltage and short circuit current density along

with a peak power density of 0.55 V, 1.29 mA/cm$^2$ and 0.23 mW/cm$^2$ at a cell voltage of 0.29 V, respectively. These results are comparable or superior to the already published data [17-18]. A linear dynamic range from of 3 – 20 mM glucose was observed with the following regression equation ($r^2$ = 99.7%):

$$Cell\ response\ \left(\frac{\mu W}{cm^2}\right) = 12.47\ [glucose](mM) + 12.38\ \left(\frac{\mu W}{cm^2}\right) \ldots (4.1.1)$$

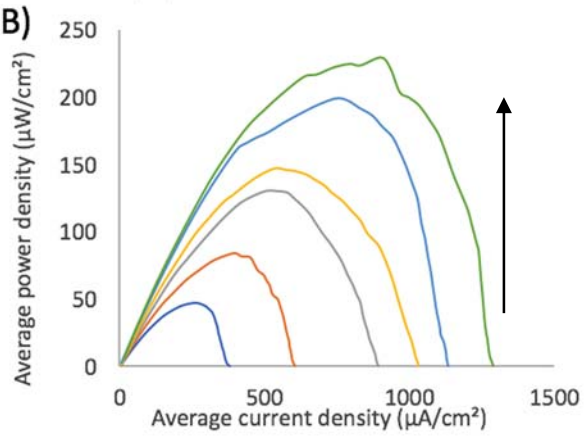


Figure 4. Glucose biofuel cell power curves at varying glucose concentrations (0.1 M PBS, 37 °C and pH 7.4).

As observed from Fig. 4, the peak power density of the biofuel cell increased with increase in glucose concentration. The biofuel cell stability was investigated by monitoring the power generated in various glucose concentrations. Fig. 5 shows the constant discharge curve acquired over 53 days. At the end of week one, the initial peak power density drops by 5.25 % and 0.56% in the presence of 3 mM and 10 mM glucose, respectively. At the end of day 53, the peak drop in the performance of the glucose biofuel cell was observed to be 31.75 % and 21.92 % in 3 mM and 10 mM, respectively. Although the electrical power generated decreased with time, the sheer ability of the biofuel cell to perform continuously over 53-day period is attributed to the effective immobilization of enzymes onto the MWCNTs, which prevented the enzymes from leaching off thus, improving the durability of the glucose biofuel cell. The performance of the glucose biofuel cell improved tremendously when glucose is continuously supplied (dynamic environment) compared to static environment at physiological conditions. The drop in the performance over time was due to the potential degradation of the enzymes.

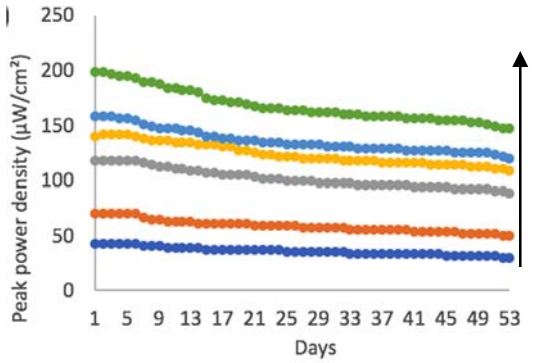


Figure 5. Stability profile in the presence of various glucose concentrations (3, 5, 7, 10, 15 and 20 mM) (37 °C, pH 7.4).

### B. *Self-powered biosensing system characterization*

The electrical power generated by the single biofuel cell can be improved by either stacking multiple biofuel cells in series or parallel depending on the applications. However, this approach makes the overall design bulky and increases the circuit complexity [19]. An alternative approach is to use a power amplification circuit to amplify the power generated. Using the 2-stage power amplification circuit described previously. The charge pump circuit is excited by the input voltage of at least 0.25 V from the biofuel cell and amplified to 1.8 V. The amplified power is stored in a 0.1 µF capacitor and supplied in bursts to power a device. The power produced by the biofuel cell was observed to increase with increasing glucose concentration. Thus, the power stored in the capacitor also increased with glucose concentration. This resulted in the increase in charge/ discharge frequency of the capacitor. The varying charge/ discharge frequencies was observed to be linearly correlated with the level of the glucose.

Since the charge pump circuit supplies burst of power which is not ideal for powering any devices, a step up DC convertor circuit was implement to further amplify the 1.8 V to 3.2 V. The output of the step-up DC convertor circuit is a steady DC voltage supply, which was then applied to various digital devices such as digital thermometer and digital glucometer device. The block representation of the system powered by a single glucose biofuel cell which simultaneously senses glucose and powers a digit*al* device is shown in Fig. 6.

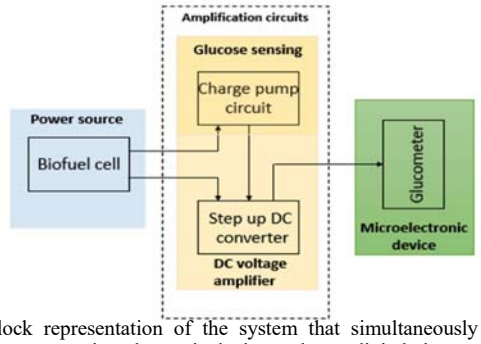


Figure 6. Block representation of the system that simultaneously senses glucose and powers a microelectronic device such as a digital glucometer.

### C. *Simultaneous glucose sensing and powering of glucometer device*

Interfacing the charge pump circuit and the step-up DC convertor circuit to the biofuel cell played a vital role in the successful implementation of the self-powered system that simultaneously senses glucose and powers a digital device. The interfacing provided the necessary trigger that step-up convertor circuit to begin its operation. Following the initial trigger, the circuit works on the voltage generated by the glucose biofuel cell. The DC voltage output can be changed per application requirement since different devices have different voltage requirements. This can be easily fulfilled by changing the resistances in the voltage divider circuit connected to the step-up convertor circuit. Fig. 7 shows the experimental set up for system that simultaneously senses glucose and powers a digital glucometer (FreeStyle Lite)

requiring a minimum voltage supply of 3 V to operate. This system operates over the 3 – 20 mM glucose range.

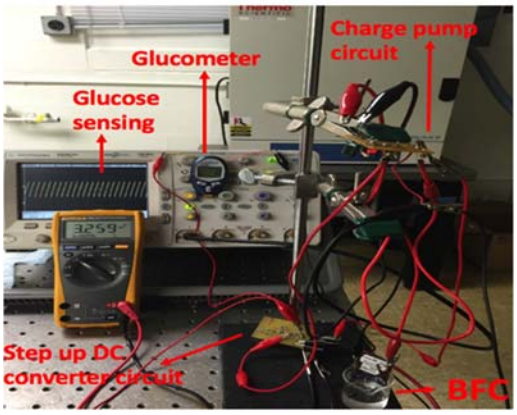


Figure 7. Experimental set up of the system to sense glucose and power digital glucometer simultaneously.

### C. *Self-powered glucose sensor selectivity*

One of the essential qualities of a glucose sensing system is that it needs to be highly selective. Interfering species such as ascorbate, urate and acetaminophen have been shown to affect the performance of glucose biosensors [20-22,23]. The physiological glucose concentration in serum is 5 mM, whereas the physiological concentration of these interfering is ~0.2 mM [24]. To determine the selectivity of this self-powered glucose biosensor, we monitored the charge/ discharge frequency of the capacitor for these interfering species. We observed that both ascorbate and urate had no impact on the charge/ discharge frequency of the capacitor. This is because ascorbate and urate decomposes at +700 mV. Also a single biofuel cell is incapable of producing such high potential, thereby these interfering species fail to have any impact on the overall electrical signals generated by the system. Therefore, this self-powered glucose sensor exhibits excellent selectivity.

## V. Conclusion

This work demonstrated a novel system powered by a single glucose biofuel cell and is capable of simultaneously sensing glucose and powering a glucometer. Future work will focus on the performance of this novel system in serum. Such a self-powered glucose sensor system shows promise in replacing the current glucose monitoring systems that rely on external power supply and potentiostat circuits.

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