Carbon Nanotube-Cellulose Pellicle for Glucose Biofuel Cell

Md Qumrul Hasan, Jonathan Yuen, and Gymama Slaughter, *Senior Member, IEEE*
Bioelectronics Laboratory and University of Maryland Baltimore County
Department of Computer Science and Electrical Engineering
1000 Hilltop Circle, Baltimore, MD 21250, USA

***Abstract*— Carbon nanotube (CNT)-cellulose pellicle was developed to create a conductive CNT network on 20 μm nanostructured cellulose film. The flexible and electrically conductive film was prepared by the modification of bacterial nanocellulose pellicle with multi-walled carbon nanotubes (MWCNTs). The composite film was further modified with redox enzymes including pyroquinoline quinone glucose dehydrogenase (PQQ-GDH) and bilirubin oxidase (BODx) functioning as the anodic and cathodic catalyst, respectively with glucose as the biofuel source. The enzyme functionalized MWCNT-cellulose based glucose/O_2 biofuel cell system harnessed the biochemical energy of glucose via the oxidation of glucose and reduction of molecular oxygen to generate electrical power in the microwatt range. The biofuel cell system exhibited an open circuit voltage and power density of 470 mV and 46.25 μW/cm^2, respectively, with a current density of 381 μA/cm^2 in the presence of 25 mM glucose. At physiological glucose concentration, the biofuel cell exhibited an open circuit voltage and power density of 418 mV and 24.975 μW/cm^2 respectively, with a current density of 293.75 μA/cm^2. As a result, we expect that this facile strategy to prepare flexible conductive bioelectrodes for the development of glucose biofuel cell system using synthesized bacterial nanocellulose crosslinked with MWCNTs and enzyme can be readily extended to diverse applications in enzymatic biofuel cell and biosensor technology.**

I. Introduction

The pursuit of an in vivo power source for implantable devices that can be deployed in plant and animal models, and eventually in humans is attracting significant research attention to the development of electrochemical power sources, in particular biological fuel (biofuel) cells. There are different types of electrochemical power sources currently available on the market (i.e., batteries), however these power sources are bulky, rigid, used toxic metals and chemicals, and exhibit short lifetime. On the other hand, biofuel cell generally utilizes analyte(s) present in their corresponding physiological environment as fuels and oxygen as an oxidizing agent to generate electrical power via enzymatic catalysis. These environmentally-friendly enzymes enable the selective oxidation of biofuels such as glucose, lactate, etc., which are abundantly present in diverse groups of living organisms, coupled with the reduction of molecular oxygen to form water as the byproduct of the enzyme catalysis. Additionally, biofuel cells can be operated under mild reaction environments (i.e., pH 6 – 7 and 20 °C – 40 °C), thereby making them a good alternative power source to conventional fuel cells and batteries for powering today's ultra-low powered bioelectronic and implantable devices [1].

We demonstrated the feasibility of a glucose biofuel cell as a power source for small portable electronic devices, as well as a self-powered biosensor [2-3]. Other research groups have also demonstrated biofuel cells as a potential power source in animal models, insects, and crustaceans [1,4]. However, enzymatic biofuel cell systems are stilled plagued by their low power density and short lifetime, which further limit their practical in vivo/ implantable applications. This shortcoming may be attributed to the low enzymatic activity at the electrode surface and the long-term stability of immobilized enzymes. Moreover, research on the utilization of nanostructured electrodes that can provide a microenvironment in favor of enzyme stability are being widely explored in the fabrication of bioelectrodes [1,4], in order to improve the enzyme loading and stability, and hence the overall power density of the biofuel cell system, while at the same time reducing the form factor of biofuel cell system for successful implantable applications.

Additionally, with the advent of modern light-weight, flexible, stretchable, and biodegradable microsystems, it has become necessary to adapt biofuel cell technologies to incorporate flexible and conductive nanostructured materials in similar form factors in attempt to address some of the current limitations. In particular, mesh dense network of carbon based nanostructures have been widely utilized as electrode material for enzyme immobilization due to their high surface to volume ratio for achieving high enzyme loading capacity, high conductivity and ability to enable direct electron transfer between the active center of the enzyme and the current collector. Here we developed an innovative strategy to prepare a multi-walled carbon nanotube (MWCNT)-cellulose composite material system for the immobilization of pyroquinoline quinone glucose dehydrogenase (PQQ-GDH) and bilirubin oxidase (BODx) to enable enzyme wiring and direct electron transfer at the anode and cathode, respectively. We evaluated the performances in terms of glucose dependent polarization and

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Md. Q. Hasan; Bioelectronics Laboratory; Department of Computer Science and Electrical Engineering; University of Maryland, Baltimore County, Baltimore MD 21250 USA.

J. Yuen; Center for Bio/Molecular Science and Engineering, U.S. Naval Research Laboratory, Washington D.C. 20375, USA

G. Slaughter is with the Bioelectronics Laboratory; Department of Computer Science and Electrical Engineering; University of Maryland, Baltimore County, Baltimore MD 21250 USA. (corresponding author phone: 410-455-8483; fax: 410-455-3969; e-mail: gslaught@umbc.edu).

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power density curves. The MWCNT-cellulose based biofuel cell system generated electrical power utilizing glucose as the biofuel source. This work is the first to fabricate and successfully demonstrate enzymatic biofuel cells operation by preparing both the bioanode and biocathode using flexible, electrically conductive MWCNT-cellulose composite. The enzyme functionalized MWCNT-cellulose-based biofuel cell system holds great promise for providing a unique environment to improve enzyme stability and enhance biofuel cell performance, in addition to providing new avenues for the development of electrochemical biosensors.

II. Materials and Methods

A. Preparation Carbon Nanotube-Cellulose Composite

· Cellulose is the most abundant natural organic biopolymer comprising a linear chain of glucose monomers linked by β(1 →4) glycosidic bonds [5]. Unlike starch, no coiling or branching occurs and the polymer adopts an extended and rather stiff rod-like conformation, aided by the equatorial conformation of the glucose residues [6]. The multiple hydroxyl groups on the glucose from one chain form hydrogen bonds with oxygen atoms on the same or on a neighbor chain, holding the chains firmly together side-by-side and forming nanofibrils with high tensile strength. Briefly, the bacterial nanocellulose utilized was synthesized by Gluconacetobacter xylinum bacteria in a static medium and allowed to dry. MWCNT (Nink-1000, Nano-Lab, MA USA) modified with carboxyl groups was used as a conductive agent and cetyltrimethylammonium bromide (CTAB) was used as the surfactant (Fluka, assay ≥ 96%) for the modification of bacterial nanocellulose and dispersion of MWCNT, respectively. The dispersion process consisted of placing dried bacterial nanocellulose pellicle in a 1 mg/ml MWCNT dispersion solution overnight under mild shaking. Afterwards, the MWCNT treated pellicle was washed carefully with deionized water in order to remove any unbound surfactant and MWCNT. · The as prepared MWCNT-bacterial nanocellulose pellicle was subsequently dried in a fume hood. An average total thicknesses of 25 ±1 µm were obtained by a micrometer. A scanning electron microscope (SEM) was used to characterize the surface morphology.

B. Bioelectrode Fabrication

The as prepared MWCNT-cellulose pellicle was cut into 10 mm × 4 mm strips. Polyimide was applied around the edges of the composite film to define the electroactive area. This process was achieved as using a two-step method, wherein each side on the top surface was treated with polyimide and allowed to set at 150° C for 30 minutes. This process was then repeated for the back surface. Subsequently, the surface of the MWCNT-cellulose composite was decontaminated by isopropyl alcohol treatment for 15 minutes, followed dimethyl sulfoxide (DMSO) for 30 minutes at room temperature. Prior to enzyme immobilization, the cleaned MWCNT-cellulose composite was treated with the heterobifunctional crosslinker, 1-pyrenebtanoic acid succinimidyl ester (PBSE) for an hour in the dark with mild shaking. The electrodes were subsequently rinsed with DMSO to remove any unbound PBSE followed by phosphate buffer solution (PBS) rinse to remove DMSO residues on the electrode surface. Two MWCNT-cellulose strips were immersed in a solution of pyroquinoline quinone glucose dehydrogenase (PQQ-GDH – 1 mg/ml in 10mM PBS, 1mM $CaCl_2$) and a solution of bilirubin oxidase (BODx – 1 mg/ml in 10mM PBS) at a pH of 7.4 to prepare the bioanode and biocathode, respectively. The enzymes immobilized on the surface of the composite film due to the strong amide bond formation between the lysine group on the enzyme and PBSE, which in turn binds readily to the MWCNTs via pi-pi stacking between aromatic rings interacting with each other (Figure 1). The enzyme modified electrodes were further coated with Nafion and dried in the desiccator for fifteen minutes. The bioanode and biocathode were assembled in a beaker containing glucose as the biofuel, wherein glucose was oxidized and molecular oxygen was reduced at the bioanode and biocathode respectively. Figure 2 shows the biofuel cell set up and the half-cell reactions that take place in the system. When not in use, the bioanode and biocathode were kept refrigerated in 100mM PBS at pH 7.4.

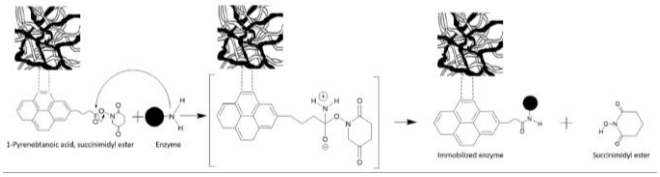
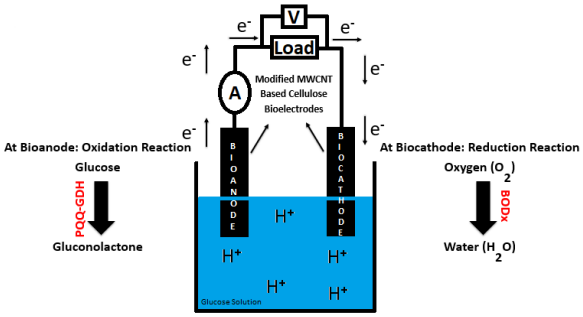


Figure 1. MWCNT-cellulose based glucose biofuel cell system.



$$\textit{At Bioanode:}\quad 2Glucose \xrightarrow{PQQ-GDH} 2Gluconolatone + 4H^+ + 4e^-$$

$$\textit{At Biocathode:}\quad O_2 + 4H^+ + 4e^- \xrightarrow{BODx} 2H_2O$$

$$\textit{Overall:}\quad 2Glucose + O_2 \rightarrow 2Gluconolactone + 2H_2O$$

Figure 2. MWCNT-cellulose based glucose biofuel cell system.

III. Result and Discussion

A. Morphology of MWCNT-cellulose pellicle

The MWCNT-modified cellulose pellicles were characterized by the same flexibility as the unmodified cellulose pellicles. The porosity of the bacterial nanocellulose pellicle (Figure 3a) enabled the incorporation of the MWCNTs among the cellulose nanofibrils (Figure 3b). As a result, a conductive layer was formed on the surface of the cellulose pellicles. The MWCNTs appears to be homogeneously distributed on the surface of cellulose

pellicle and is well infused, whereas the surface of the unmodified cellulose pellicles appears dull and smooth upon optical inspection.

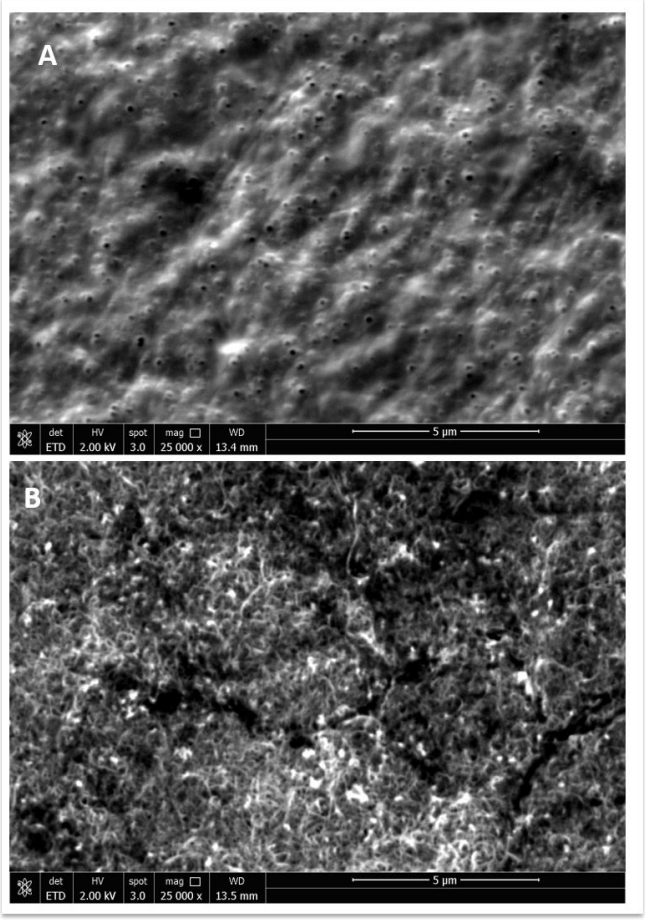


Figure 3. SEM micrographs of bacterial nanocellulose. (a) unmodified bacterial nanocellulose and (b) MWCNT modified bacterial nanocellulose.

B. MWCNT-cellulose based glucose/O_2 biofuel cell system

In the assembled biofuel cell system, glucose biofuel is oxidized at the bioanode to produce gluconolactone along with the electrons and protons. The ionic species traveled toward the cathode, whereas the electrons travel through the external circuitry to recombine at the cathode where molecular oxygen is reduced by BODx to produce water as illustrated in the half-reactions in Figure 2. The MWCNT-cellulose based glucose biofuel cell was characterized by measuring the open circuit voltage and short circuit current, in addition to acquiring the current-voltage (I-V) and power characteristics. The polarization curve displayed in Figure 4 depicts the current and voltage characteristics at varying load resistance. As the load increases, the voltage drops and the current increases. The power available from the biofuel cell at any point along the curve is the product of current and voltage at that point. Moreover, it was important that the system exhibit at least an open circuit voltage between 280 mV and 300 mV in order for it to provide enough electrical power to drive an energy amplification circuit. We previously demonstrated an open circuit voltage between 400 to 560 mV utilizing bucypaper [7]. The open circuit voltage of the MWCNT-cellulose based glucose biofuel cell was 470 mV in 25 mM glucose, and is attributed to the difference between the onset potentials of the oxidation and reduction of molecular oxygen at the bioanode and biocathode, respectively.

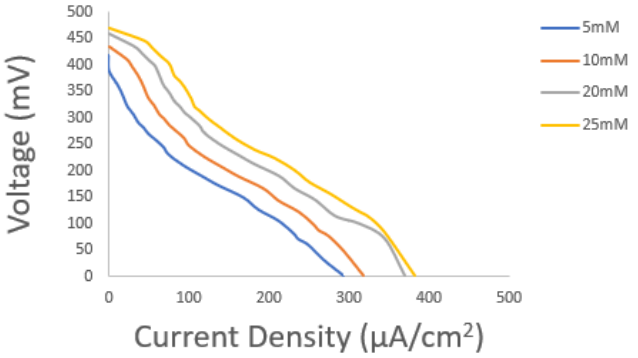


Figure 4. Current-voltage curves of the MWCNT-cellulose based glucos biofuel cell measured at pH 7.4 and 25 °C in a phosphate buffer containing various glucose concentrations.

Figure 5 shows the power curve of the assembled cell, wherein the performance of glucose biofuel cell improves as the glucose concentration increases. The maximum power density achieved was 46.25 µW/cm^2 at a cell voltage of 223.4 mV in the presence of 25 mM glucose with a current density of 381.25 µA/cm^2. At physiological glucose concentration, 5 mM glucose, an open circuit voltage of 418 mV was observed with a maximum peak power of 24.975 µW/cm^2 and a current density of 293.75 µA/cm^2. The open circuit at all glucose concentrations evaluated were well above the 280 mV required by the energy amplification circuit.

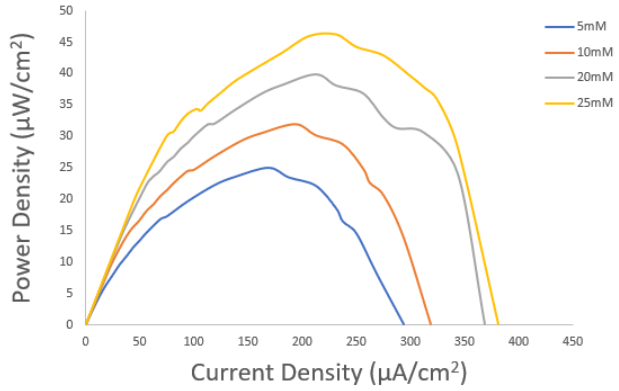


Figure 5. Power curves of the MWCNT-cellulose based glucos biofuel cell measured at pH 7.4 and 25 °C in a phosphate buffer containing various glucose concentrations.

Figure 6 shows the peak power dependency on concentration. The MWCNT-cellulose based glucose biofuel cell system exhibited a linear dynamic range of 5 mM – 25 mM glucose, wherein the performance of glucose biofuel cell assembly improves as the glucose concentration increases.

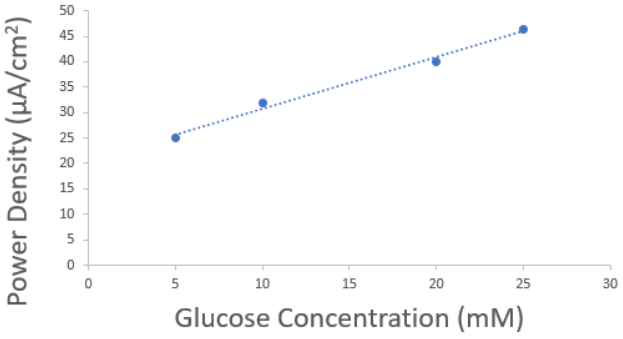


Figure 6. Calibration curve illustrating the relation between glucose concentration and power output of the MWCNT-cellulose biofuel cell.

Figure 7 demonstrates the MWCNT-cellulose glucose biofuel cell interfaced to a charge pump switching regulator circuit to amplify the nominal power generated by the biofuel cell to power an LED. This MWCNT-cellulose based glucose/O_2 biofuel cell has the potential to serve as a self-powered biosensing system by monitoring the glucose concentration. Future work will focus on the evaluation of the biofuel cell stabilities and responses to various levels of glucose over an extended period of time.

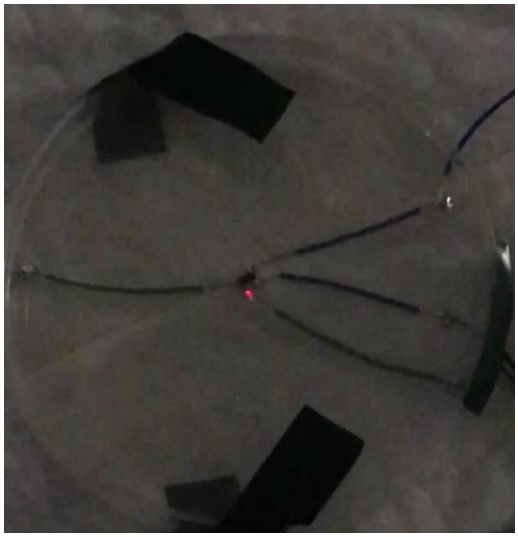


Figure 7. Turn on the LED diode light along with charge pump circuit using biofuel cell as a power source

IV. Conclusion

This work demonstrates the development of the first MWCNT-cellulose based glucose/O_2 biofuel cell system. Flexible, electrically conductive bacterial nanocellulose were prepared via the treatment of bacterial nanocellulose with dispersion of MWCNTs in the presence of CTAB. The as prepared conductive pellicle served as the electrode material for the construction of the bioanode and biocathode. Upon functionalization of the electrodes with their respective enzymes, the glucose biofuel cell generated enough power via an energy amplification circuit to drive an LED circuit.

Acknowledgment

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References

[1] Gymama Slaughter and Tanmay Kulkarni, "Enzymatic Glucose Biofuel Cell and its Application," *Journal of Biochips & Tissue Chips*. 5 (1) 1000111 (2015).

[2] Slaughter, T. Kulkarni, "A self-powered glucose biosensing system." *Biosensors & Bioelectronics.* 78 (2016) 45-50.

[3] Gymama Slaughter and Tanmay Kulkarni "Highly Selective and Sensitive Self-Powered Glucose Sensor Based on Capacitor Circuit," *Nature Scientific Reports*. 7(1471), 1-9, 2017.

[4] R. A. Bullen, T. C. Arnot, J. B. Lakeman, F. C. Walsh. Biofuel cells and their development. Biosensors and Bioelectronics. 21 (2006), 2015-2045G.

[5] C.W. Villarrubia, K.A. Narváez, S. O. Garcia, P. Atanassov. "NAD+/NADH Tethering on MWNTs-Bucky Papers for Glucose Dehydrogenase-Based Anodes." *Journal of The Electrochemical Society*. 161(2014), H3020-H3028.

[6] Vijay Kumar Thakur, "Cellulose-Based Graft Copolymers: Structure and Chemistry" CRC Press Taylor and Francis Group.

[7] Tanmay Kulkarni and Gymama Slaughter "Characteristics of two self-powered glucose biosensors," *IEEE Sensors Journal*. 17 (12), 3607-3612 (2017).