Development of a Bio-Energy Generation System Based on Microfluidic Platform

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***Abstract* – This paper reports the development of a bio-energy generation system - using microfluidic platform for processing the electrochemical energy conversion and storage inside a cell. The cell is designed to consist of two modified gold electrodes and glucose as the electrolyte. Electrical energy is generated by redox reaction through the process of glucose oxidation and oxygen reduction. The entire chemical reaction process is designed to take place between the cathode and anode without a proton exchange membrane. A thickness-controlled PDMS is used to form the fluidic channel for transporting the glucose solution. We have thus far completed the fabrication of the micro-bio-reaction system, and the electrodes were characterized secondary ion mass spectrometry (SIMS) to prove the adhesion of the necessary chemicals and proteins for bio-electrical-reaction.**

Index Terms* – *biofuel cell, micro fuel cell, microfluidics, bioelectrochemistry

I. Introduction

Recently, biofuel cells have been explored as a possible future renewable energy source and have drawn immense focus due to their various potential applications such as implantable electronic devices [1], biosensing device [2], low power applications for electronic systems by IMEC and CFDRC, and the military applications [3]. Biofuel cells can be classified into two categories, namely microbially catalysed [4] and enzymatically catlysed [4]. In this work, we explore a bio-energy generation process where glucose is enzymatically catalysed on the electrode surface to generate electrical energy by electrons shuttled from the redox centre to the electrodes using mediated transfer method [5-6].

The working principle of an enzymatic biofuel cell is based on the biocatalysts and enzymes to catalyze oxidation of biomass, such as glucose or ethanol, to generate the electrical energy. This is the redox process within the cell which converts biochemical energy to electrical energy. Glucose is typically used as the fuel at the anode. It is oxidized to form gluconic acid in the presence of glucose oxidase (GOx), which is a type of enzyme [Eq. (1)]. At the cathode, oxygen molecules are reduced to water by biocatalyst cytochrome *c* (Cyt. *c*) and cytochrome oxidase (COx) [Eq. (2)]. It is reported that most of the redox enzymes lack of direct electrical communication between the electrode supports [7], thus, mediators such as ferrocenemethanol [8], dimethyl-aminomethylferrocene [9], ferrocene monocarboxylic acid [10] and gold nanoparticles [11] have been investigated as the bridge and the path of transporting the electrons from the redox center to the electrodes. Different mediators would result in different electron transfer rate. In this project, Eugenii Katz *et al.*'s work [7] is adopted since they have shown that by using pyrroloquinoline quinine (PQQ) as a mediator for the anode, the electrical communication between the redox center and the electrode support is extremely effective, indicating that the electron turnover rate is extremely high [7]. Figure 1 shows the mechanism of the enzymatic mediated transfer adopted for the biofuel cell discussed in this paper:

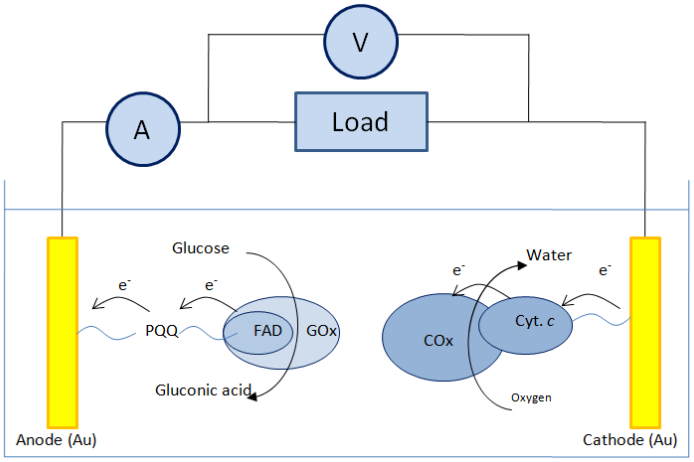

Fig. 1 Mechanism of the biofuel cell.

At the anode:

$$\text{Glucose} \xrightarrow{GO_x} \text{Gluconic acid} + 2H^+ + 2e^- \qquad (1)$$

At the cathode:

$$O_2 + 4H^+ + 4e^- \xrightarrow{\text{Cyt.}c,\,CO_x} 2H_2O \qquad (2)$$

Apart from the mediator concern, another factor that affects cell energy output is the electrode distance. The overall cell voltage (E_O) is determined by the difference of the electrode potential between cathode (E_C) and anode (E_A) and also the ohmic resistance loss (E_Ω), i.e.,

$$E_o = E_c - E_a - E_\Omega \quad (3)$$

The ohmic resistance is dependent on the conductivity of the electrolyte, the distance between the electrodes and the temperature of the entire environment. Naga S. Korivi *et al.* [12] reported that the smaller the distance, the larger the current. Hence, another key design factor is to reduce the gaps of two electrodes by controlling the thickness of PDMS microfluidic interface.

II. System Fabrication

A. Electrode Preparation

The electrodes with size of 0.5cm x 1cm were fabricated on a glass substrate by sputtering chromium and gold accordingly. The height of the sputtered gold was 7000 Å and the sputtered chromium was 500 Å. After fabrication of the desired pattern of the electrodes by traditional lithographic process, the electrodes were modified by immersing them into the biocatalysts and chemicals to form monolayers. Since gold and sulfur have a very strong attachment force, both electrodes were therefore first soaked with amino-acid-functionalized thiolate called cystamine ($SC_2H_4NH_2$) to form the thiolate monolayers. These self-assembling layers serve as conductive support between the gold surface, the enzymes and redox proteins. The resulted electrodes were made into anode and cathode by two different modification processes. The cathode was prepared by coupling with N-4-maleimidobutytyloxy-succinimide ester for ~12 hours. This ester compound acts as the electrical communication between the cytochrome *c* (Cyt. *c*) and the electrode. Then the Cyt. *c* was attached to the electrode and followed by the adhesion of cytochrome oxidise (COx). The anode was prepared by assembling a monolayer of PQQ on the flavin adenine dinucleotide phosphate (FAD) to form a PQQ-FAD monolayer. Lastly, apo-glucose oxidase was attached onto the PQQ-FAD monolayer, i.e., the same process as discussed in [13].

B. Device Fabrication

The fabrication of the biofuel cell involves two 2cm x 2.5cm glass substrates and one thin PDMS layer with controlled thickness to form a glass-PDMS-glass microfluidic platform. Both glass substrates were cut to the desired size and one of them was drilled with two 1mm diameter holes for the fluidic inlet and outlet ports. The thin PDMS layer was patterned with a spacer as a channel to allow glucose solution to pass through. The PDMS layer was prepared by mixing the PDMS prepolymer and the curing agent from SYLGARD® 184 silicone elastomer kit in a volume ratio of 5:1. The uncured PDMS was spin-coated on a SU-8 2100 master mold.

To make the 200μ thick master mold, we first spin-coated the SU-8 2100 onto a polymethyl methacrylate (PMMA) substrate at 1500 rpm for 30 sec. Then, it is pre-braked at 65°C for 30 minutes and 80°C for 90 minutes. Next, the substrate was exposed under UV for 4 minutes followed by post-braking at 65°C for 15 minutes and 80°C for 30 minutes. The substrate was then allowed to relax under room temperature for one hour. Finally, the patterned mold was created by rinsing it in a developer solution for 15 minutes.

The then PDMS layer was prepared by spinning uncured PDMS at 1000 rpm for 60 sec on the master mold. The mold with the spinned-on PDMS was then cured at 80°C for 2 hours. The patterned PDMS layer was then removed from the mold carefully. Finally, the PDMS layer was adhered to the two cover glass substrates by oxygen plasma to form the microfluidic platform.

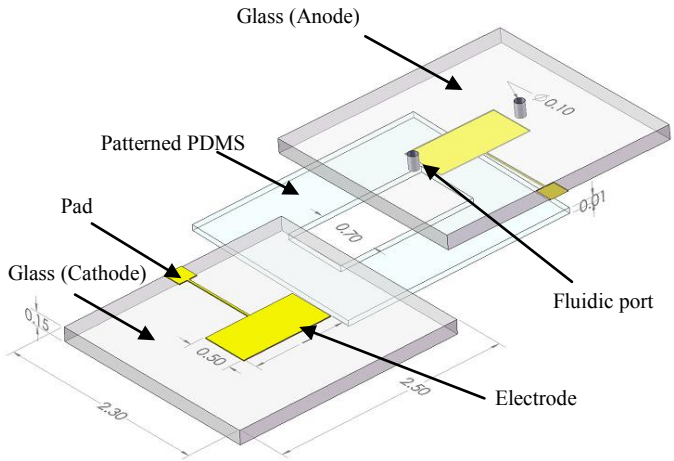

Fig. 2 Biofuel cell design based on the microfluidic platform (dimensions shown are in cm).

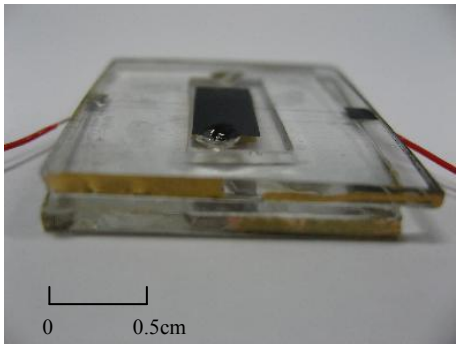

Fig. 3 Prototype of the biofuel cell. Three layers were adhered after oxygen plasma process.

III. Results and Discussion

After each self-assembly process, the modified electrodes were characterized by secondary ion mass spectrometry (SIMS) in order to ascertain the necessary chemicals that had been attached on to the surface of the electrodes. Since the substrate is glass, the surface is therefore very rough and hence it could not be characterized by an AFM or STM. In addition, the monolayers are in nanometer scale such that they could not be detected using an SEM. Therefore, the SIMS method was used and some of the test results are shown as follow. After the

first modification step, i.e., immersing the electrodes into the cystamine ($SC_2H_4NH_2$) solution, the final product should be $AuSC_2H_4NH_2$ theoretically. From the negative ion mass spectra of the sample (Figure 4), the signals of necessary compounds such as S^-, AuS^- and $AuSC_2H_4NH_2^-$ were very strong. Also, from the positive ion mass spectra (Figure 5), S^+, $SC_2H_3^+$ and $AuSC_2H_4^+$ signals were also strong. Hence, we confirmed that the $AuSC_2H_4NH_2$ monolayers were attached on the electrodes successfully.

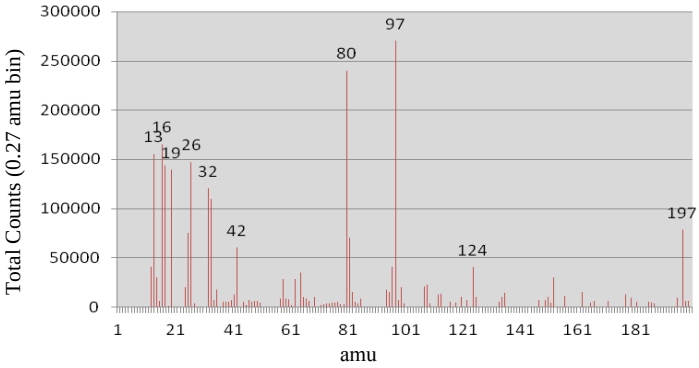

Fig. 4 Negative ion mass spectra of $AuSC_2H_4NH_2$

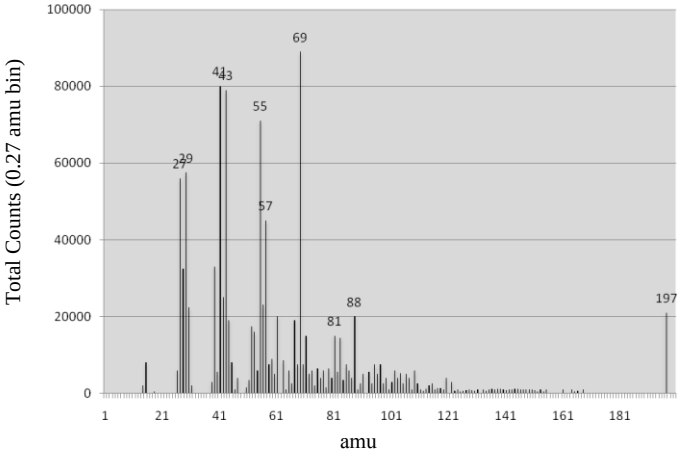

Fig. 5 Positive ion mass spectra of $AuSC_2H_4NH_2$ (switch to Fig 5)

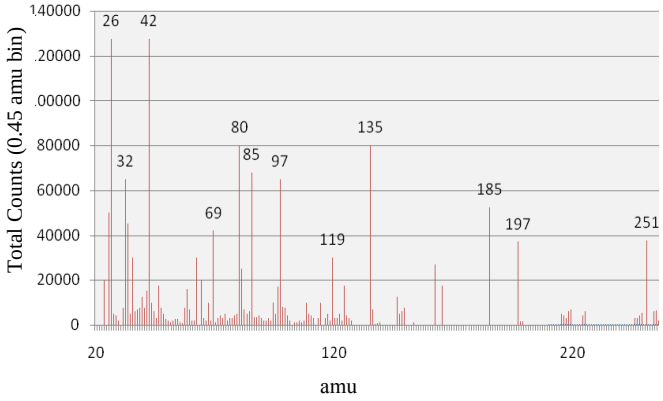

Fig. 6 Negative ion mass spectra of $AuSC_{10}H_{13}N_2O_3$

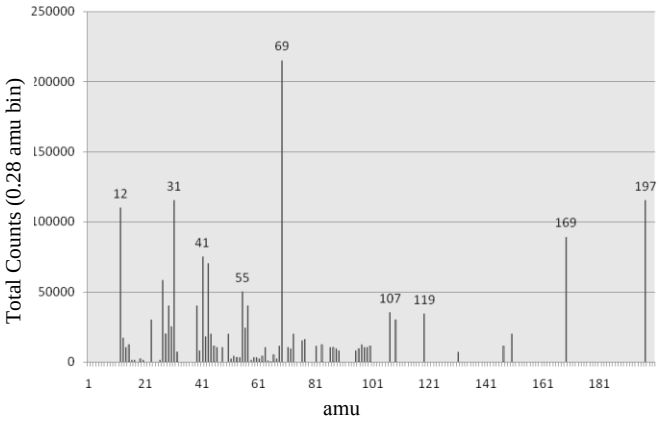

Fig. 7 Positive ion mass spectra of $AuSC_{10}H_{13}N_2O_3$

Another substrate ($AuSC_{10}H_{13}N_2O_3$) which was coated with N-4-maleimidobutytyloxy-succinimide on the cystamine layer was also tested (Figure 6 and 7). For the negative ion analysis, the S^- and AuS^- signals were very strong. In addition, although $SC_2H_4^-$, $SC_2H_5N^-$, $SC_3H_5NO^-$, $SC_{10}H_{13}N_2O_3^-$ and $NC_4O_2H_2^-$ signals were not clear enough, the mass spectra plot does indicate their presence on the sample. The positive ion mass spectra also showed similar results. The chemical noise signals indicated that the targeted substance may have some impurities or it was fragmented so that the result was compromised. For example, Figure 8 shows the mass spectra of the product of the second modification process, $SC_{10}H_{13}N_2O_3$. Its peak was centred at 241.0568amu for $SC_{10}H_{13}N_2O_3^+$ and 241.0659amu for $SC_{10}H_{13}N_2O_3^-$.

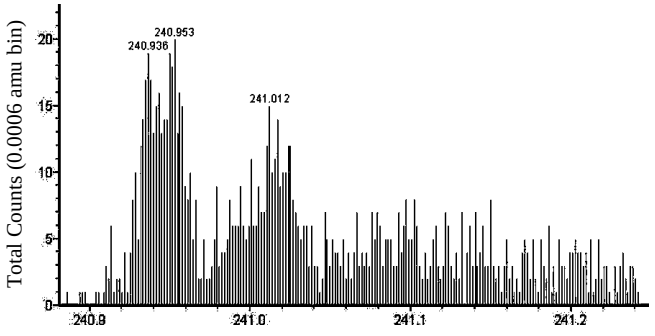

Fig. 8 Mass spectra of $SC_{10}H_{13}N_2O_3^-$

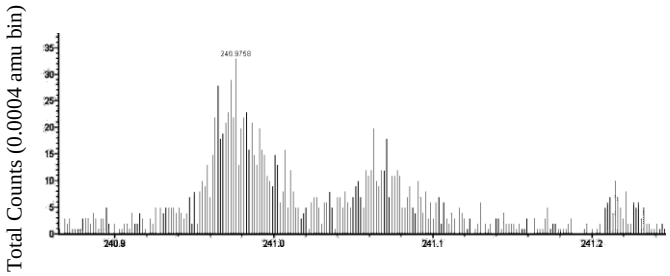

Fig. 9 Mass spectra of $SC_{10}H_{13}N_2O_3^+$

V. Conclusion

The working principles and the fabrication procedures of an energy generation system based on bio-electro-chemical reactions in a microfluidic platform have been presented. Surface characterization by SIMS was done after each fabrication step to ensure that the necessary chemicals were attached on the surface of the electrodes. Our next step is to test the durability and power density of the cell by varying the height of the PDMS channel. In addition, further studies involving the improvement of the self-assembly of monolayer are expected so that the chemical attachment process can be improved.

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