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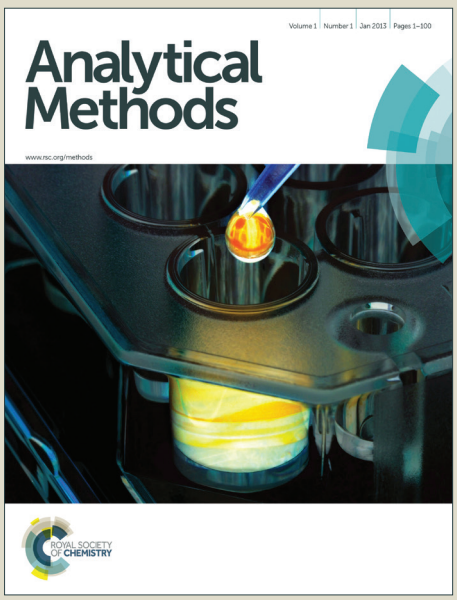

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COMMUNICATION

Microbial Biofuel Cell with Air-breathing Cathode for *In vivo* Glucose Sensing Application

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A hybrid biofuel cell employing baker's yeast and an abiotic cathode was designed and experimented. Using customized membrane electrode assembly and simple fabrication techniques, the prototype device was capable of detecting various glucose concentrations in human blood plasma, showing a strong potential for patch type sensor development.

For diabetic patients, monitoring blood sugar level is an essential daily routine and the demand for home glucose monitoring systems has been growing rapidly. At present, the finger-prick procedure along with disposable glucose strips overwhelmingly prevails in the market. The recommended sampling frequency is four times per day, which makes it cumbersome and pricey, not to mention the pain related with finger pricking.[1] Therefore, less invasive, continuous, and *in vivo* sensing of glucose is highly desirable. In this context, a patch type glucose sensor incorporated with an array of micro needles and built-in wireless signal transmitter could be a strong candidate. Most of the commercially available glucose sensors, including the glucose strips, utilize redox enzymes such as glucose oxidase or glucose dehydrogenase to catalyze glucose to gluconolactone.[2-6] The electrons released from the enzymatic redox reactions are measured amperometrically to estimate the glucose contents in blood. One intrinsic drawback of using enzymes as a biocatalyst is the relatively short lifetime, which is in the order of few days.[7, 8] A human body-attached, continuous glucose monitoring system (branded as MiniMed®) was commercialized more than a decade ago.[9] The needle shaped sensor is inserted subcutaneously and can continuously operate for up to six days using glucose oxidase for the redox reaction [http://www.diabetesforecast.org]. On the other hand, microbe-based electrochemical devices are more suitable for long term operation, albeit substantially low current generations mainly due to severe mass transport limits across the living cell bodies. Microbes or microorganisms are "live" and can live as long as proper metabolic conditions are met, while showing little degradation over time. Siu and Chiao reported a soft lithography-based, miniaturized biofuel cell using cultured yeast (*S. cerevisiae*) assisted by an electron mediator (methylene blue), which converts the chemical energy of blood glucose into electrical power. Potassium ferricyanide was used as an oxidant in the cathode compartment.[10] Sayed et al. studied the catalytic activity of yeast extract in a phosphate buffer as an anode medium, which was combined with an open air cathode. The behaviors of the yeast extract in their biofuel cell with and without glucose were investigated and an open circuit potential of as high as 1 V was achieved..[11]

With an intention of developing a patch type, *in vivo* glucose sensor having longer life, this communication presents a hybrid biofuel cell that couples a biological anode and an abiotic, air-breathing cathode. The prototype device utilized baker's yeast (*S. cerevisiae*), as a biocatalyst for glucose in human plasma at the anode, while a conventional noble metal catalyst (Pt) was chosen for the oxygen reduction reaction at the air-breathing cathode. Yeasts can use glucose (sugar) as a nutrient source to obtain the carbon skeletons needed to synthesize cellular constituents and the energy necessary for biosynthetic reactions. Once transported into the yeast cell, glucose undergoes multiple enzymatic reactions (glycolysis) and finally converts into simple molecules, such as carbon dioxide and water.[12] Residing in yeast, nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide dehydrogenase (NADH) are coenzymes that are repeatedly oxidized and reduced through the electron transport chains during the metabolic pathway of yeast[10]. The prototype cell operation is based on the following anodic and cathodic reactions at 1 atm, room temperature, and pH of 7 (vs. SHE):[13]

$$NADH \leftrightarrows NAD^+ + H^+ + 2e^- \qquad E^\circ_{anode} = -0.315\ V \qquad (1)$$

$$O_2 + 4H^+ + 4e^- \leftrightarrows 2H_2O \qquad E^\circ_{cathode} = +0.815\ V \qquad (2)$$

Combined (1) and (2), the overall redox reaction is:

$$2NADH + O_2 + 2H^+ \leftrightarrows 2NAD^+ + 2H_2O \qquad (3)$$

This proof-of-concept biofuel cell consists of a polydimethylsiloxane (PDMS) housing, two wires, and a membrane electrode assembly (MEA) as shown in Figure 1. In the PDMS housing, the anode chamber (22 × 22 × 3 mm) where the fuel and yeast are stored during the measurement was formed by an elastomeric stamp method. Made by a 3-D printer (Stratasys, Dimension 1200), an acylonitrile butadiene styrene (ABS) structure was used for the stamping mold. The two fluidic connection holes were intended for fuel supply and purge from the anode chamber. The wires through which the electron flows from the anode to cathode were directly attached to the anode/cathode by applying silver epoxy paste (MG Chemicals, 8331-14G). The customized MEA is a sandwich structure, comprised of two pieces of porous carbon cloth (20 × 20 × 0.4 mm) and a proton exchange membrane (PEM, Nafion 117, 40 ×

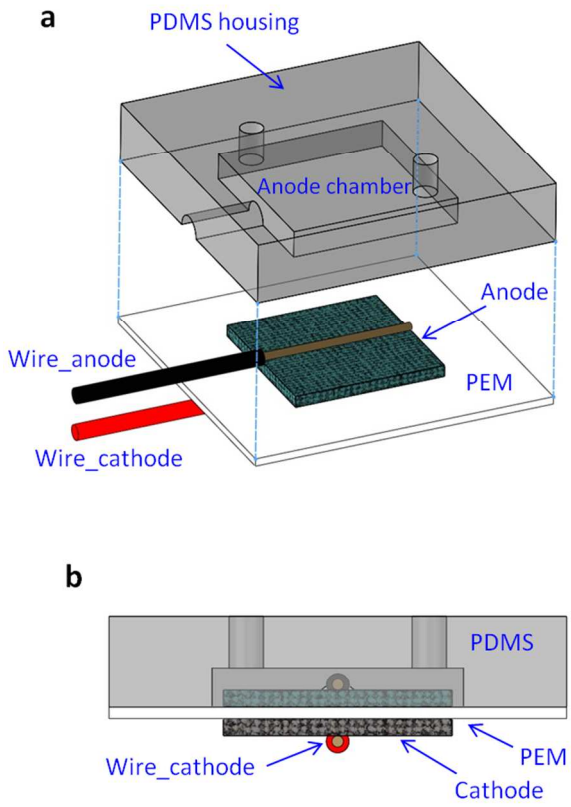

Figure 1. Schematic of prototype cell design: (a) explored view; (b) side view.

40 × 0.2 mm). At the anode, hydrophilic carbon cloth (ELAT®) was employed for better wetting, while plain hydrophobic carbon cloth with Pt catalyst (0.3 mg/cm^2 loading) was the air-breathing cathode [http://fuelcellsetc.com]. The MEA and PDMS housing were glued and clamped together to minimize any leaking during the experiment.

To prepare the fuel and working fluid, 0.43 g of dry yeast (Sigma-Aldrich, Type-I, YSC1) was cultured by adding 5 ml of distilled water at 37 °C on a hot plate for 18 hours. Secondly, a bottle of dry human plasma (Sigma-Aldrich, P9523-5 ml) was mixed with 5 ml of distilled water with its glucose concentration measured by a portable glucose meter (Walgreens, TRUEtrack®). Additional water was added to dilute the plasma to reach desired glucose concentrations. Four different glucose levels were prepared and measured in the experiment: 46, 92, 184, and 318 mg/dl. The cultured yeast, approximately 0.5 ml, was then pumped in the anode chamber, followed by injecting the fuel (0.5 ml of human plasma) to fill up the anode chamber completely. The chamber was tightly sealed to minimize any unwanted yeast respiration.

For each glucose level, the electrochemical characterization was performed using an in-a-timely-manner protocol established by the author to reduce the uncertainties associated with yeast reproduction (budding) and fermentation: (a) after the fill-up, wait for 10 min before the open circuit potential (OCP) measurement for complete mixing of the fuel and the yeasts; (b) continuous OCP measurement for 20 min to ensure stable readings; and (c) immediately after, start a linear sweep

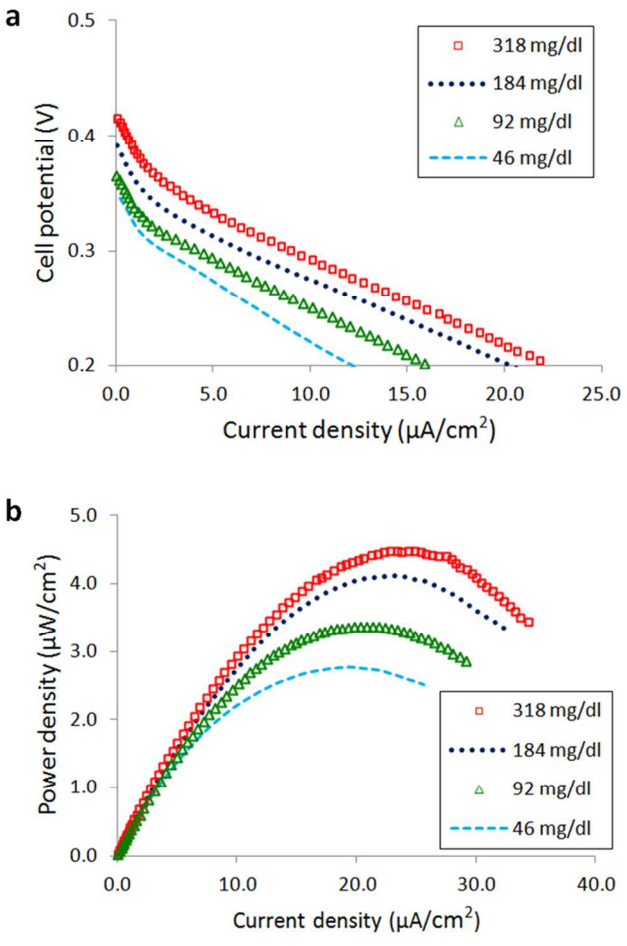

Figure 2. Results from the linear potential sweep starting from the OCPs with scan rate of 5 mV/s: (a) polarization curves; (b) the corresponding power density curves.

voltammetry to create polarization curves. Electrochemical impedance spectroscopy (EIS) at OCP with perturbation magnitude of 10 mV (0.2 ~ 10^5 Hz) was conducted separately after fresh fuel was refilled and reached a stable OCP. All the measurements reported in this communication were done by potentiostat (Gamry, Reference 3000) and the measured pH was close to 7.

The measured polarization and power density curves from the linear sweep voltammetry indicate that each glucose level has very unique and distinct performance characteristics (Figure 2a - b). Note that the current and power densities are normalized by the net electrode area, which is 4 cm^2. Figure 3 presents a comparison of each glucose concentration with respect to normalized OCPs and normalized currents at the cell potential of 0.2 V. The data demonstrated that, compared to 46 mg/dl, 318 mg/dl increased by approximately 86% in the cell current and had 20% higher OCP value (solid green square mark). Therefore, it was concluded that by monitoring OCPs or currents at a certain potential, one can estimate relative glucose concentrations, if the device is properly calibrated. The EIS results gave the overall cell resistance values for multiple cells, which was consistently in a range of 2 to 4 Ω.

Finally, to evaluate the performance of the bioanode towards the NADH oxidation, a standard-three-electrode (half cell) measurement was performed using hydrophilic carbon cloth as

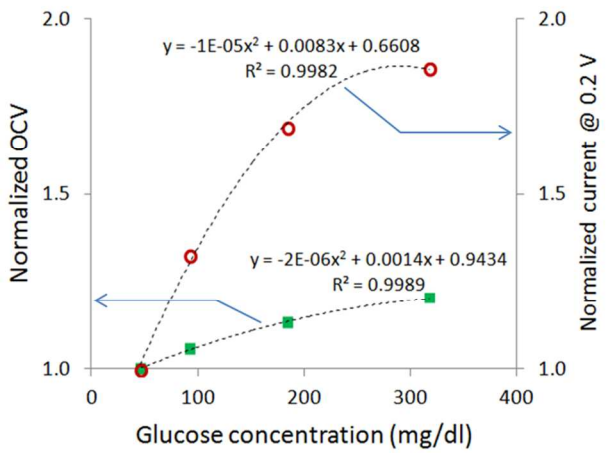

Figure 3. Comparison of each glucose concentration with polynomial fitting equations and its R-square values. The OCVs (first y-axis) and electric currents at 0.2 V (second y-axis) are normalized by the measured values at the lowest concentration (46 mg/dl).

working electrode, a platinum wire as counter electrode, and a Ag/AgCl reference electrode. The measured OCP values from two glucose concentrations (153 and 282 mg/dl) were -0.043 and -0.068 V, respectively. As seen in Figure 4, cyclic voltammetry reveals that there is no anodic or cathodic peak near the OCP, which is indicative of irreversible reaction and therefore sluggish kinetic rates for both concentrations. The slow kinetic rates can be greatly alleviated by employing electron cofactors or mediators.

Conclusions

In summary, as a part of developing a home blood glucose monitoring system, a hybrid biofuel cell was proposed and the prototype device was experimentally investigated for a proof of concept. This preliminary study will open up tremendous research opportunities with strong potentials for a patch type, *in vivo* glucose sensor system. The key technical challenges to be addressed include: (1) better understanding on the yeast metabolism especially for long-term operation; (2) search for other microbe candidates that can be controlled practically to maintain metabolic conditions; (3) immobilization technique to prolong the life of yeasts; and (4) employing electron cofactors or mediators, such as methylene blue, for high signal output and power density. Furthermore, ongoing research efforts in our group are now being focused on identifying basic sensing capabilities such as the response time, detection sensitivity, and sensing limits.

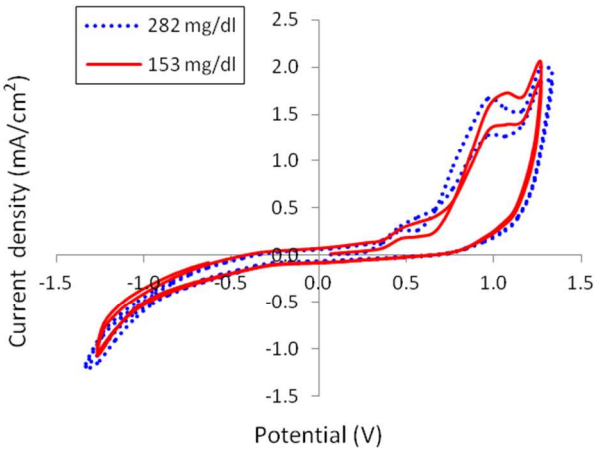

Figure 4. Evaluation of the bioanode was evaluated by cyclic voltamettary with scan rate of 30 mV/s. Two cycles of each glucose concentration are shown and the current density is normalized by the active working electrode area (0.4 cm^2).

Notes and references

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