Insect biofuel cell using an electrode with gold nanoparticles deposited by sputtering

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A high-stability living battery that uses a metallic catalyst for glucose oxidation instead of enzymes is investigated. In this battery, glucose in insect haemolymph is oxidised by a gold nanoparticle (AuNP)-modified electrode, which is utilised as the anode of the battery. The AuNP-modified electrode was fabricated by sputtering gold onto a carbon cloth in a low-vacuum state. First, the oxidation performance values of glucose were evaluated and the oxidation current of glucose was obtained by the AuNP-modified electrode from cockroach haemolymph (CHL). Then, the electrocatalytic stability of the AuNP-modified electrode was compared with an enzymatic electrode. Furthermore, a glucose biofuel cell consisting of the AuNP-modified electrode and a bilirubin oxidase-modified electrode was constructed; the respective power densities obtained from 100 mM glucose solution and CHL were 22.4 and 15.1 μW/cm^2, respectively. It is concluded that AuNPs are a useful catalyst for living batteries.

1. Introduction: Insect cyborgs controlled by electric stimulation to their muscles and neurons have been developed as a search robot at disaster sites and as an environmental monitoring robot. For example, Holzer and Shimoyama [1] analysed the locomotion reaction of an insect and controlled its walking by electric stimulation of its neurons, and Kanzaki's group [2, 3] utilised moth antennas as a sensitive sensor for their robot. In other studies, Sato *et al.* [4] and Bozkurt *et al.* [5] succeeded in controlling the flight of insects by electrical stimulation of the insect brain and neurons. Insect cyborg can move flexibly and utilise their highly sensitive sensors, because living insects are utilised as microrobots. However, coin batteries which are currently used for insect cyborgs have to be charged or replaced to drive an integrated circuit for a long time and they sometimes interfere with the insect's movement owing to their size and weight. It is desirable that the battery mounting on an insect is small and light and has a semi-permanent lifetime.

Biofuel cells (BFCs) which generate electric power from glucose found in blood have received attention as a new power source for implantable sensors and cardiac pacemakers. BFCs which generate electricity from blood sugar of living organisms have been developed [6–15]; for example, Cinquin *et al.* [8] succeeded in generating electric power from rat blood by implanting a glucose BFC protected by dialysis membranes. Zebda *et al.* [9] implanted the glucose BFC into a rat and succeeded in driving a temperature sensor powered by the BFC. Rasmussen *et al.* [10] implanted a trehalose BFC into an insect and generated electric power from trehalose found in the insect. Szczupak *et al.* [11] implanted glucose BFCs into clams and a motor was driven by three BFCs connected in series. Halámkova *et al.* [12] implanted glucose BFCs into a snail and confirmed the possibility of refuelling by feeding. Shoji *et al.* [13–15] also developed a BFC using trehalose found in insect haemolymph and succeeded in driving a melody integrated circuit and a piezo speaker by connecting five BFCs in series. Unfortunately, the power output decreased to several per cent of the initial output in several hours due to inactivation of enzymes modified for the electrodes by organic compounds included in insect haemolymph.

In this Letter, we propose a living organism BFC using gold nanoparticles (AuNPs) as a catalyst for glucose oxidation instead of enzymes (Fig. 1). AuNP has the most powerful monosaccharide oxidation reactions among metallic catalysts and it has been applied as the catalyst of glucose BFCs [16, 17]. The conventional method to fabricate AuNP-modified electrodes, however, is time consuming; all processes from the purification of AuNPs to their modification for the electrodes take about ten steps (several hours). Therefore, we propose a one-step (2 minutes) process for the fabrication of the AuNP-modified electrode by sputtering gold onto the electrode.

In our previous Letter, we developed a multi-enzyme-based trehalose BFC which generated electric power by a trehalose decomposition step and a glucose oxidation step [14]. Differing from our previous BFC with trehalose, we directly use glucose which is the energy source of most animals and is the building block of such polysaccharides as trehalose, sucrose, cellulose and starch. Therefore, the AuNP-modified BFC represents a basic technology for BFCs powered with sugar and it can be applied to most living batteries by combining it with any other catalyst which decomposes polysaccharides. First, we fabricate the AuNP-modified electrode by sputtering gold onto a carbon electrode. In the conventional method, the solution casting method is utilised for the modification of AuNPs for electrodes. By fabricating AuNPs onto the electrode directly, the adherence property between the AuNPs and the electrode will be improved. Then, we evaluate the glucose oxidation of the AuNP-modified electrode by cyclic voltammetry (CV) measurements and compare the electrocatalytic stability of the AuNP-modified electrode and a conventional enzymatic anode. Finally, we construct a glucose/O_2 BFC consisting of the AuNP-modified electrode and an O_2 reduction electrode and measure the power output of the BFC with insect haemolymph.

2. Materials and methods

2.1. Materials: Glucose was purchased from Wako Pure Chemical Industries. *N* methyl-2-pyrrolidone (NMP) and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid ammonium salt) (ABTS) were purchased from the Tokyo Chemical Industry Co. Ferrocene, poly-L-lysine hydrobromide (PLL), poly(sodium 4-styrenesulphonate) (PSS) and poly (vinylidene fluoride) (PVDF) were purchased from Sigma-Aldrich. Glucose oxidase (GOD) (160 U/mg) (E.C. 1.1.3.4) was purchased from the Toyobo Co. Ltd. Bilirubin oxidase

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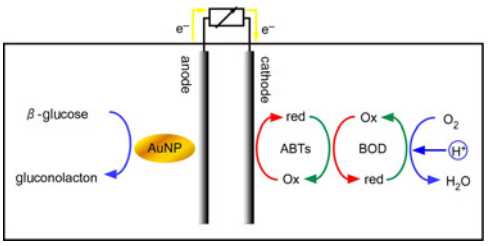

Fig. 1 *Power generation mechanism of the insect BFC consisting of the AuNP-modified electrode and the BOD/ABTS electrode. On the anode, AuNPs oxidise glucose molecules found in insect haemolymph and electrons and protons are produced. On the cathode, an enzyme which reduces oxygen causes the electrochemical conversion of oxygen and hydrogen into water to occur*

(BOD) (2.39 U/mg) (E.C. 1.3.3.5) was purchased from Amano Enzyme Inc. Ketjen black EC600JD (KB) was purchased from the Lion Corporation.

2.2. Fabrication of the AuNP-modified electrode: The AuNPs modified electrode was fabricated by sputtering gold onto the electrode material which was carbon cloth (CC) (EC-CC1-060, Toray Industries Inc.). Gold was sputtered onto both sides of the CC for 1 min at 4 mA in a low-vacuum condition. A quick coater device (SC-701, Sanyu Electron Co. Ltd) was used for sputtering. The surface of the electrode was observed with an atomic force microscope (AFM) (AFM5000, Hitachi High-Tech Science Corp.). An AFM image of the AuNP-modified electrode is shown in Fig. 2. The particle size of the AuNPs was measured by image J. It was confirmed that AuNPs with 30.9 ± 10.2 nm particle size were deposited on the CC by sputtering Au in the low-vacuum condition.

2.3. Electrochemical measurement of the AuNP-modified electrode: Madagascar hissing cockroaches (ca. 7 cm long, weighing 6 g) were used and they were given trehalose-rich feed (Pro Jelly, Ketjen black (KB) Farm) for a week before we collected their haemolymph. Cockroach haemolymph (CHL) is contained along with fatty bodies in the body. CHL was collected along with the fatty bodies from the cockroaches and purified by centrifugation (6000*g*, 15 min).

The electrochemical properties of the AuNP-modified electrode were analysed by CV measurement. Electrochemical experiments were carried out using an electrochemical measurement system (SI 1280b, Solartron). The AuNP-modified electrode, a platinum wire and an Ag|AgCl (3 M NaCl) electrode were used as the working electrode, the counter electrode and the reference electrode, respectively. All the potentials cited in this Letter are referenced to the Ag|AgCl (3 M NaCl) electrode, with a potential

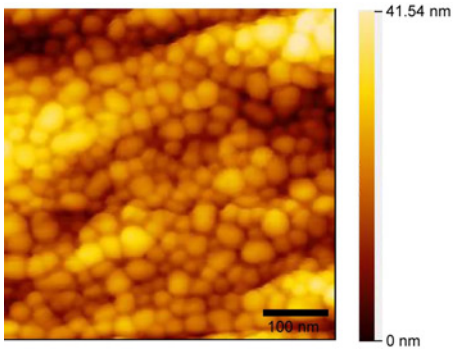

Fig. 2 *AFM image of AuNPs modified onto the CC electrode. Scale bar is 100 nm*

of +205 mV *against* the normal hydrogen electrode. All measurements were carried out at room temperature.

2.4. Comparison with the enzymatic anode: We analysed and compared the electrochemical stability of the AuNP-modified electrode and the conventional enzymatic anode by CV measurements. The enzymatic anode was made by successively dropping 4 µl of 10 mM ferrocene in acetone and 20 µl of 50 mg/ml GOD solution (0.1 M phosphate potassium buffer, pH 7.0) onto the CC electrode (width, 5 mm; length, 5 mm). Then, 40 µl of PLL solution (50 mM monomer unit) and 20 µl of PSS solution (60 mM monomer unit) were cast on the electrode in order to immobilise the enzyme and mediator onto the electrode by forming the insoluble PLL–PSS complex film (poly ion complex).

2.5. Fuel cell measurements: The AuNP-based BFC consisted of the AuNP-modified electrode (width, 5 mm; length, 5 mm) and a BOD-modified electrode (width, 5 mm; length, 5 mm). The cathode used in the BFC measurements was prepared as follows. First, 20 µl of a mixture (4:1, v/v) of 5 mg/ml KB in NMP and 10 wt.% PVDF in NMP was dropped on the CC electrode and the NMP was allowed to evaporate away at room temperature. After evaporating the NMP, the electrode was first anodised at +2.0 V for 900 s and then cathodised at −1.1 for 15 s in 100 mM phosphate potassium buffer (pH 7.0) in order to obtain a hydrophilic surface. Then, 10 µl of 100 mM ABTS solution and 20 µl of 50 mg/ml BOD solution were dropped onto the electrode; the solvent was allowed to evaporate away at room temperature after each solution was dropped. Finally, 40 µl of PLL solution (50 mM monomer unit) and 20 µl of PSS solution (60 mM monomer unit) were cast on the electrode.

The voltages and currents of the AuNP-based BFC from the glucose solution and CHL were measured by the electrochemical measurement system.

3. Results and discussion

3.1. Electrochemical reaction of the AuNP-modified electrode: CV measurements of the AuNP-modified electrode in 0.1 M phosphate potassium buffer (pH 6.5), 100 mM trehalose solution (0.1 M, phosphate potassium buffer pH 6.5), 100 mM glucose solution (0.1 M, phosphate potassium buffer pH 6.5) and CHL were performed (see Fig. 3*a*). The potential sweep range was from −0.8 to 0.6 V (*against* Ag|AgCl) and the scan rate was 50 mV/s.

In the buffer solution and trehalose solution, no redox response of AuNP was observed. Although the main sugar of insect haemolymph is trehalose, trehalose was not oxidised by the AuNPs. In the glucose oxidation process by the AuNPs, the hydroxyl group at position 1 is replaced by a ketone group and gluconolactone is produced. Since trehalose is a disaccharide formed by a 1,1-glucoside bond between two α-glucose units, trehalose was not oxidised by AuNPs. In the glucose solution and CHL, catalytic currents were observed. These results indicated that AuNP oxidised glucose found in CHL and transferred electrons to the electrode. As shown in Fig. 3*a*, the catalytic oxidation currents in 100 mM glucose solution and CHL began to increase at a potential around +200 and +300 mV (*against* Ag|AgCl). The oxidation peaks of the AuNP-modified electrode in 100 mM glucose solution and CHL were obtained at +400 mV (*against* Ag|AgCl) and +500 mV (*against* Ag|AgCl), respectively.

The substance diffusion in CHL is slower than that in the buffer solution because CHL is more viscous than the buffer solution. Many ions such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^- and organic acid ions found in CHL have been reported to influence the electrochemical reactions [18–20]. As a result, the oxidation peak of the AuNP-modified electrode in CHL shifted +100 mV from the peak in the glucose solution. We saw that the maximum current density of CHL was about 0.5 mA lower than that of the glucose

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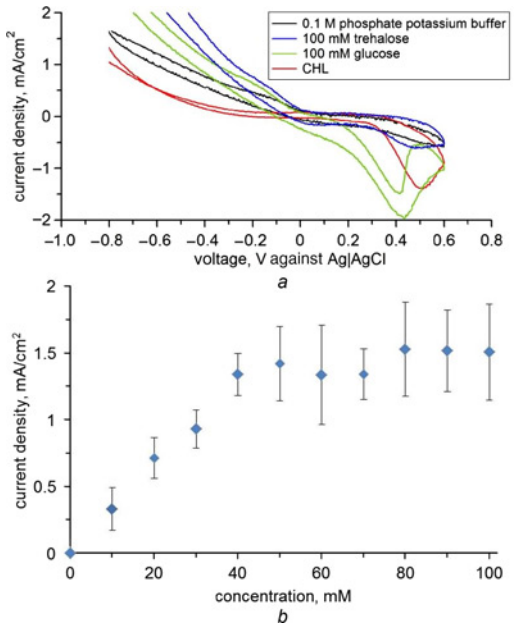

Fig. 3 *Cyclic voltammograms (Fig. 3a) of AuNP-modified electrodes in 0.1 M phosphate potassium buffer, pH 6.5 (black line), with 100 mM trehalose (blue line), 100 mM glucose (green line) and CHL (red line) (scan rate was 50 mV/s); dependence of current density of AuNP-modified electrodes on glucose concentration at 0.4 V against Ag/AgCl (Fig. 3b), (n = 5)*

solution. The main sugar of insect haemolymph is trehalose, and insects produce glucose by decomposing trehalose. Glucose found in insect haemolymph was oxidised by AuNPs and the oxidation current was observed.

Shoji *et al.* [14] measured the trehalose concentration of CHL by liquid chromatography mass spectrometry and reported that 100 mM trehalose was included in CHL. We determined, however, that the glucose concentration was lower than the trehalose concentration of insect haemolymph. Thus, the maximum current density of CHL became lower than that of the glucose solution.

The dependence of the current density on the glucose concentration for the electrode at 0.4 V *against* Ag|AgCl is shown Fig. 3*b*. The current density increased with the glucose concentration up to 50 mM, where a plateau of 1.4 mA/cm^2 was reached. The maximum current density of the AuNP-modified electrode in CHL was nearly equal to that of the 30 mM glucose solution. As a result, we could assume that about 30 mM glucose was included in CHL. Since the glucose concentration in mammal blood is about 5 mM, the cockroaches have a higher level of glucose in their body fluid compared with mammals. It follows from what has been said that glucose BFCs can be applied to insect BFCs.

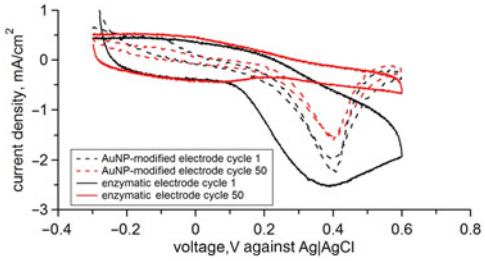

Fig. 4 *Comparison of the electrocatalytic stability of the AuNP-modified and the enzymatic electrodes. First and 50th cycles of the AuNP-modified (dotted line) and enzymatic (solid line) electrodes in 100 mM glucose solution (0.1 M phosphate potassium buffer, pH 6.5). Scan rate was 50 mV/s*

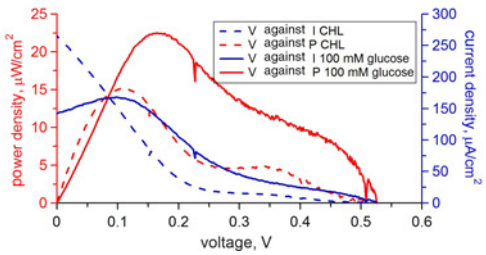

Fig. 5 *Performance values of the glucose/O_2 BFC with 100 mM glucose solution and CHL. The perpendicular axes on the left and the right show power density and current density, respectively*

3.2. Electrochemical catalytic stability: Fig. 4 compares the first and 50th (after 30 min) CVs of the AuNP-modified electrode and the enzymatic electrode with the same surface area in 100 mM glucose solution (0.1 M phosphate potassium buffer, pH 6.5). The potential sweep range was from −0.3 to 0.6 V (*against* Ag|AgCl) and the scan rate was 50 mV/s. The CVs clearly showed that the current degradation of the AuNP-modified electrode was lower than the enzymatic electrode. At the first cycle, the current densities of the AuNP-modified electrode and enzymatic electrode at 0.4 V (*against* Ag|AgCl) were about 2.5 and 2.0 mA/cm^2, respectively. At the 50th cycle, while no oxidation current of the enzymatic electrode was obtained, the current density of the AuNP-modified electrode was about 1.5 mA/cm^2. The oxidation current of the enzymatic electrode decreased because the enzyme modified for the electrode was deactivated by the influences of pH and temperature and peeled from the electrode. While the power output of the previous enzyme-based BFC decreased to about 25% of initial power within 30 min [14], the oxidation current of our present AuNP-modified electrode was maintained at more than 70% of the initial value for 30 min. These results lead us to conclude that we can construct a long lifetime insect BFC by using the AuNP-modified electrode.

3.3. Performance of the metallic catalyst-based BFC: Maximum power densities of 22.4 and 15.1 μW/cm^2 were obtained from 100 mM glucose solution and CHL, respectively (Fig. 5). Maximum current densities from 100 mM glucose solution and CHL reached 168 and 265 μA/cm^2. Open-circuit voltages from 100 mM glucose solution and CHL were 503 and 526 mV, respectively. We succeeded in the construction of an insect BFC by using the AuNP-modified electrode as the anode. The power density from CHL was lower than that of the 100 mM glucose solution due to the glucose concentration of CHL. Approaches to increase the electrode surface area on which gold is sputtered by using carbon-MEMS technology [21] or forming oriented carbon nanotubes [22] on the electrodes can possibly improve the output of insect BFCs.

4. Conclusion: We fabricated the AuNP-modified electrode which oxidises glucose by sputtering gold onto the CC electrode in the low-vacuum condition. The oxidation performance of the AuNP-modified electrode in glucose solution and CHL was evaluated and the oxidation current of glucose was obtained from CHL. The oxidation current of the AuNP-modified electrode was maintained at 70% of the initial value for 30 min, whereas the oxidation current of the enzymatic electrode was not obtained after 30 min. Furthermore, we constructed the glucose/O_2 BFC composed of the AuNP-modified electrode and the BOD-modified electrode and the maximum power density of 15.1 μW/cm^2 was obtained from CHL.

To utilise the insect BFC for the battery of insect cyborgs instead of the coin battery used currently, further improvements in

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increasing the power output by fabricating nanostructural electrodes and smaller AuNPs are necessary.

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