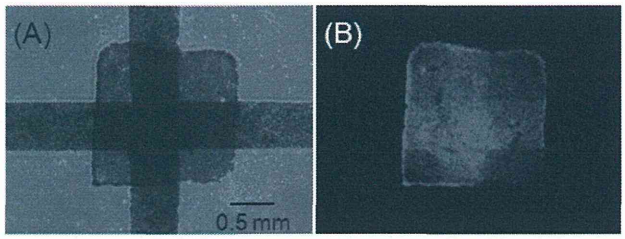

Figure 5. Phase-contrast (A) and fluorescent micrographs (B) of the oxygen imaging sheet attached to the cross-formed GOD-modified microparticles.

(an oxygen scavenger) was added, fluorescent intensity increased (Fig. 4B). 100% response was observed in 6.3 ± 1.2 s after addition of Na_2SO_3 (n = 3, Fig. 4C). Figure 4D shows the inverse relationship between the relative fluorescent intensity of the imaging sheet and the dissolved oxygen concentration measured with a DO meter (Horiba Ltd.). The relative fluorescent intensity indicates the ratio between the fluorescent intensity detected in the absence and the presence of each concentration of oxygen. This result indicated that the gel-sheet can serve as an imaging sensor for dissolved oxygen. The stability of the sensor response was evaluated by measuring the relative fluorescent intensity for PBS(−) every day for a week, showing only 4.2% decrease after 1 week.

Figure 5 demonstrates fluorescent imaging of a GOD-modified microparticle pattern using the oxygen imaging sheet. The imaging sheet was directly attached onto the GOD pattern (Fig. 5A). As can be seen in Fig. 5B, the cross-formed fluorescence displayed corresponds to the GOD pattern. We confirmed there was no response of the imaging sheet against pH change which will be induced by H_2O_2 generation during GOD-catalyzed glucose oxidation. These results suggested that GOD activity was successfully imaged using this patch-type imaging sheet. Non-uniform cross image would be attributed to non-uniform distribution of GOD beads or oxygen sensor beads. Now we are carrying out to optimize electric manipulation of the beads to obtain uniform images.

As mentioned in Introduction, oxygen sensing can be applicable to the study of type 2 diabetes using skeletal muscle cells. Our previous study of scanning electrochemical microscope (SECM) imaging using HeLa cell, which has similar basal respiratory activity with skeletal muscle myoblast,[10] has shown that the difference between the oxygen concentration at the cell surface and the bulk solution far from the cell was ~40 μM.[11] This value corresponds to 0.03 of detectable change in relative fluorescent intensity from the value at bulk oxygen concentration (~200 μM) as estimated from Fig. 4D. The muscle cells enhance their respiratory activities depending on their contractile activities, promising to obtain clear image of contraction-dependent respiratory activity of the cells. During cellular contraction, the sensor beads on the flexible imaging sheet would contract synchronously with the motion of the cells.[12] This characteristic enables continuous monitoring of respiratory activity at same position on the cell surface without concern for disturbance of oxygen concentration gradient around the contracting cells that can cause adverse effect on several oxygen imaging techniques such as SECM.

4. Conclusion

In this study, a patch type oxygen imaging hydrogel sheet was developed. For effective detection of the metabolites in the vicinity of target cells sustaining original hydrogel characteristics, the oxygen sensor beads were locally patterned on the surface of the hydrogel using the electric manipulation technique. We successfully imaged oxygen-consumption activity of GOD pattern as a cellular model, suggesting applicability of the imaging sheet to metabolic bioassays. This flexible sensor sheet is useful for glucose metabolic activity imaging of contracting skeletal muscle cells with supporting cellular contraction to study the relationship between exercise and metabolic activity of muscle in type 2 diabetes.[13]

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Sheet-shaped biofuel cell constructed from enzyme-modified nanoengineered carbon fabric

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ABSTRACT

A strip of carbon fabric (CF) electrode modified with multiwalled carbon nanotubes and subsequently fructose dehydrogenase (FDH) showed an oxidation current density of ~11 mA cm^{-2} in stirred 200 mM fructose solution. Obtaining a sufficient dispersion of the nanotubes during its modification was found to be critical to ensure such a performance of the FDH anode. For use with this anode, a CF strip modified with ketjenblack (KB) and bilirubin oxidase (BOD) served as a gas-diffusion cathode for the reduction of O_2 from air at a current density of ~2 mA cm^{-2}. The FDH-modified CF strip and the BOD-modified CF strip were stacked with an agarose film that retained an electrolyte solution and fuel (fructose) to construct a totally flexible sheet-shaped biofuel cell. This assembly allowed bending of 44° without affecting the maximum output power density, 550 μW cm^{-2} obtained at 0.4 V.

1. Introduction

Enzyme-based biofuel cells that generate electricity through enzymatic oxidation of biological fuels like sugars and alcohols have attracted attention as ubiquitous, safe power sources [1–23]. Recent rapid improvements in their power performance up to mW cm^{-2} levels by employing nanostructured carbon electrodes [24–28] have motivated various applications including a sheet-shaped cell that can be combined with advanced flexible film electronics [29,30]. However, the brittle carbon electrodes, which are generally aggregates of particulate or tubular nanocarbons, often limit the design and uses of such biofuel cells.

In the present work, we have prepared a totally flexible, sheet-shaped biofuel cell by using a carbon fabric (CF) as the flexible, conductive base for the enzyme electrodes. We modified the CF strip with multiwalled carbon nanotubes (CNTs) and fructose dehydrogenase (FDH) for the oxidation of fructose, and with ketjenblack (KB) and bilirubin oxidase (BOD) for the reduction of oxygen in the ambient air. Both FDH and BOD are capable of efficient "direct electron transfer" with common electrode materials including carbon [10,16,21,31,32]. The pre-modifications with CNT or KB increase the specific surface area of the CF electrodes, resulting in effective enzyme immobilization and, ultimately, higher power. The FDH anode strip and the BOD cathode strip are stacked with a hydrogel film that retains the electrolyte solution and fuel (fructose), as shown in Fig. 1. This assembly provides a stand-alone, sheet-shaped power source that can be bent without loss of output power.

2. Experimental

2.1. Preparation of carbon fabric anode

A 5 mm × 5 mm strip of carbon fabric (CF) (TCC-3250, donated from Toho Tenax Co.) was first modified with multiwalled carbon nanotubes (Baytubes, donated from Bayer Material Science Co.) to increase the specific surface area. The carbon nanotubes (CNTs) were pretreated by heating at 400 °C for 11 h and by immersing in mixed acid ($H_2SO_4 + HNO_3$ in a 1:3 ratio) for 5 h. The treated CNT were dispersed in water containing Triton X-100 surfactant (0.05, 0.1, 0.5 or 1%). A 40 μl aliquot of the 10 mg ml^{-1} CNT dispersion was dropped on a CF strip (0.32 mm thickness, 0.25 cm^2 geometric area) and dried in air, followed by thoroughly washing out the surfactant by soaking in a pure McIlvaine buffer solution for more than 1 h with stirring. Then, the CNT-modified CF strip was immersed in a 5 mg ml^{-1} solution of D-fructose dehydrogenase (FDH) (EC1.1.99.11, 169.9 U mg^{-1}, ca. 140 kDa, from Gluconobacter, purchased from Toyobo Enzyme Co.) for FDH immobilization [28]. It has been reported that FDH works as a electrocatalyst for oxidation of fructose without electron transfer mediators [10,16,21,31]. The flavin-containing subunit of FDH accepts electrons from fructose, and transfers these electrons to the heme C-containing subunit that can electrically communicate with electrode [31].

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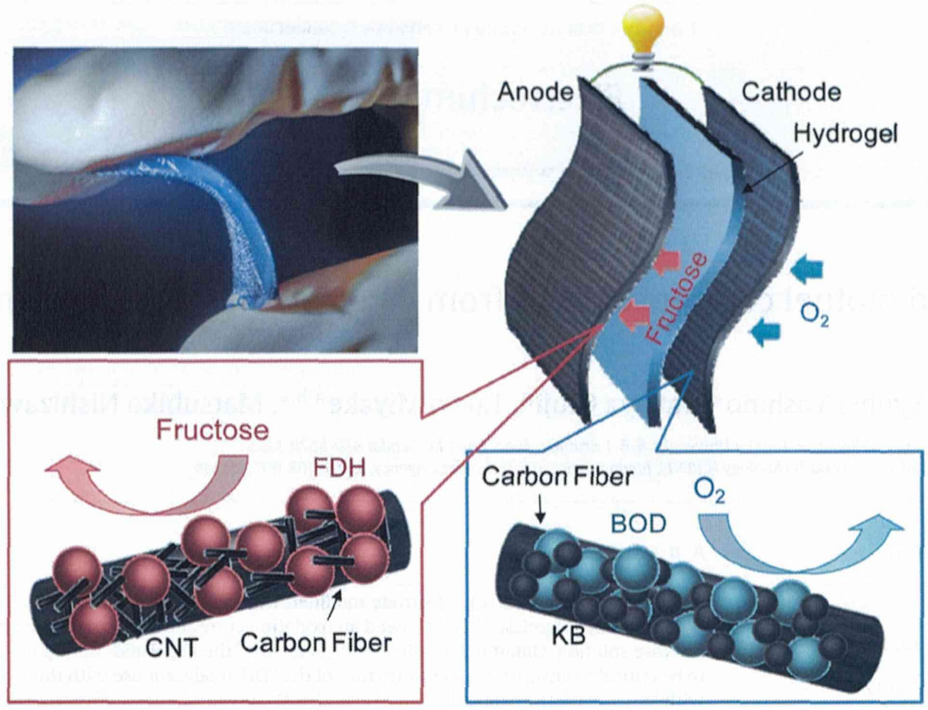

Fig. 1. A sheet-shaped biofuel cell constructed by stacking enzyme-modified nanoengineered carbon fabric strips with a hydrogel film that retains electrolyte solutions and fructose fuel.

2.2. Preparation of gas-diffusion carbon fabric cathode

The preparation of the cathode basically followed the procedures used for our previous carbon paper-based BOD cathode [23]. BOD is one of multi-cupper oxidases that can directly catalyze four-electron reduction of O_2 to H_2O even without electron transfer mediators [10,16,32]. A 25 μl aliquot of a 8 mg ml^{-1} solution of ketjenblack (KB)/poly(tetrafluoroethylene) (PTFE) (1: 1) was put on a CF strip and dried in air. The surface of the KB-modified CF electrode was further modified with a 0.1 ml solution of 5 mg ml^{-1} bilirubin oxidase (BOD, EC 1.3.3.5, 2.5 U/mg, from *Myrothecium*). After drying in air, the strip was additionally coated with the KB solution to make surface hydrophobic. The geometric size was the same as the anode (0.32 mm thickness, 0.25 cm^2 area).

2.3. Electrochemical measurements

The performance of the CF electrodes was analyzed by a three-electrode system (BSA, 730C electrochemical analyzer) in solution using a Ag/AgCl reference and a platinum counter electrode. The FDH-modified anodes were evaluated in stirred McIlvaine buffer (pH 5.0) containing 200 mM fructose, while the BOD-modified cathodes were in air-saturated McIlvaine buffer (pH 5.0). The performance of a biofuel cell constructed with the FDH-modified CF anode, the BOD-modified CF cathode, and the fructose-containing agarose film (3 mm thick) was evaluated from the cell voltage upon connecting with a variable external resistance between 180 Ω and 10 kΩ. For preparing the fructose-containing agarose films, a 150 mM McIlvaine buffer containing 200 mM fructose was first warmed to dissolve 1.5 wt% agarose, and molded with cooling. The current and the power were derived from the detected cell voltage and the resistance. Unless otherwise indicated, the electrochemical measurements were carried out at room temperature, around 25 °C.

3. Results and discussion

3.1. Performance of FDH/CNT/CF bioanodes

Fig. 2 shows cyclic voltammograms of the FDH/CNT/CF electrodes (solid plots) at 10 mV s^{-1} in a stirred buffer solution containing 200 mM fructose. In comparison with the FDH/CF electrode prepared without CNTs (broken line plot), the increased specific surface area produced by CNT-modification obviously increased the current density by at least an order of magnitude. In fact, the measured double-layer capacitance of the CNT-modified electrodes has a 2 orders larger value (ca. 6.5 mF cm^{-2}) than that of the original CF (0.07 mF cm^{-2}). Importantly, the oxidation current

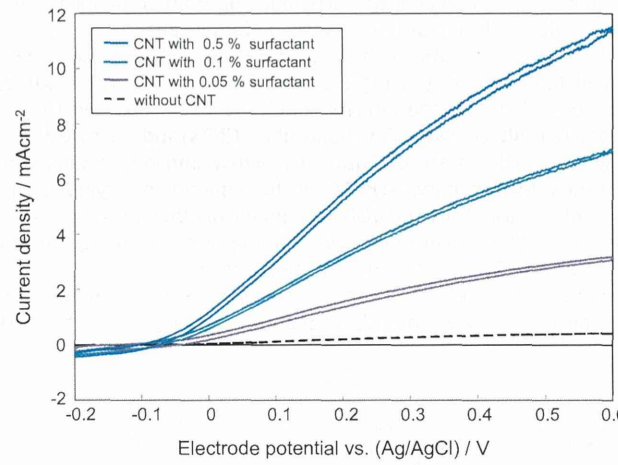

Fig. 2. Cyclic voltammograms of FDH-immobilized CF strip electrodes at 10 mV s^{-1} in a stirred buffer solution (pH 5) containing 200 mM fructose. The CF electrodes were pre-modified with CNTs dispersed with different concentrations of Triton X-100 surfactant.

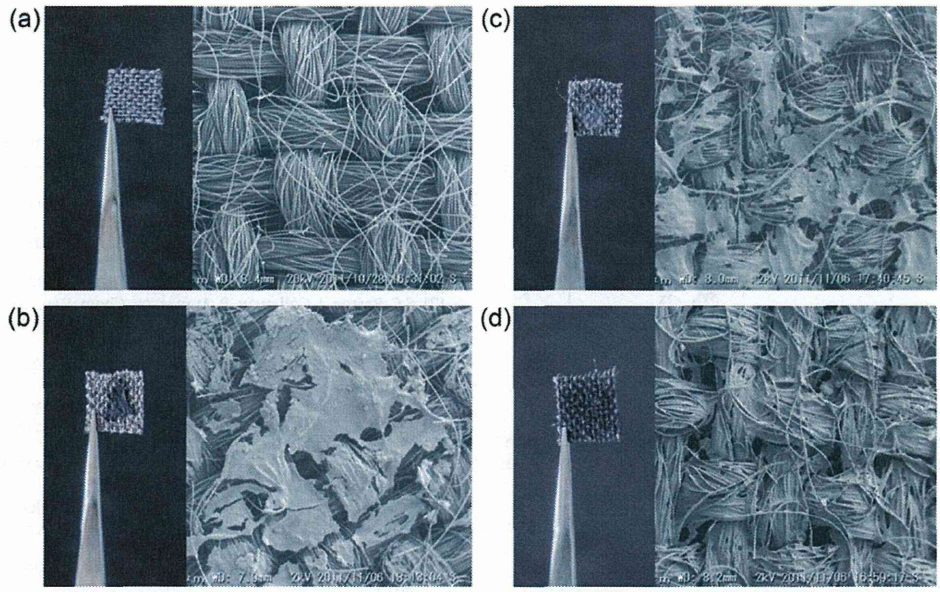

Fig. 3. Photographs and SEM images of (a) a bare CF strip and (b–d) CNT-modified strips. The CNT dispersions were prepared with (b) 0.05%, (c) 0.1%, (d) 0.5% Triton X-100 surfactant.

density depended on the concentration of the Triton X-100 surfactant used for the CNT dispersion (0.05, 0.1 and 0.5%), despite the fact that all these CNT-modified electrodes had similar capacitance (similar specific surface area). The use of 1% surfactant brought no significant further change over that from 0.5% surfactant. Fig. 3 shows the surface structure of the CNT-adsorbed CF strips observed by optical and scanning electron microscopies. The CNT dispersions with 0.05% and 0.1% surfactant are found to precipitate on the CF surface (Fig. 3b and c). In contrast, the CNT dispersion with 0.5% surfactant seems to entirely penetrate into the CF strip. This uniform modification with CNT would be a main reason of the enhanced anode performance, up to a value of 11.5 mA cm^{-2} at 0.6 V.

3.2. Performance of gas-diffusion biocathodes

Fig. 4 shows cyclic voltammograms of a BOD-modified CF cathode at 10 mV s^{-1}. The electrode strip was put on an oxygenic pH 5.0 buffer solution so as to contact the solution by the BOD-modified face (purple solid plot). The reduction current density reaches ~0.76 mA cm^{-2} (at 0 V), which is 1.5 times larger than that measured by the biocathode immersed into the solution (broken line plot). This increase of current density is a result of a better oxygen supply from the ambient air through the CF. Moreover, an additional KB coating onto the BOD-modified face of the CF strip enhanced the performance further to 2.0 mA cm^{-2}, which was reproducible within 10% variation (1.8–2.2 mA cm^{-2}) for four independent electrode specimens. Presumably, the hydrophobic nature of that coating controls excess penetration of solution into the CF electrodes [23]. The reduction current density at 0 V varied 1.8–2.2 mA cm^{-2}.

3.3. Performance of the flexible biofuel cell

A biofuel cell was constructed with the FDH/CNT-modified CF anode and the KB/BOD/KB-modified gas-diffusion CF cathode. These electrodes were attached to both sides of an agarose hydrogel (3 mm thick) made with 150 mM McIlvaine buffer solution (pH 5.0) containing 200 mM fructose. The enzyme-modified hydrophilic anode appeared to become moistened by blotting of the solution from the hydrogel layer. On the other hand, the O_2 reduction at the hydrophobic cathode proceeded at the three-phase boundary of the hydrogel–electrode interface. Fig. 5 shows typical examples of the cell performance. The open-circuit voltage of the cell was 0.7 V, which is similar to the difference between the potentials at which fructose oxidation and oxygen reduction start to occur in cyclic voltammograms (−0.1 V in Fig. 2 and 0.6 V in Fig. 4, respectively). The maximum values of current and power densities are determined by the BOD-cathode because of its comparatively lower performance than FDH-anode. Even as a stand-alone assembly with the fuel (fructose)-containing gel sheet, the maximum power density reached 550 μW cm^{-2} at 0.4 V. Importantly, this device could be repeatedly bent to a 44° angle without significant loss of output power. Bending in excess of this value caused fracture of the agarose hydrogel sheet; our device can be made more resistant to mechanical stress by using more elastic hydrogels such as polyvinylalcohol.

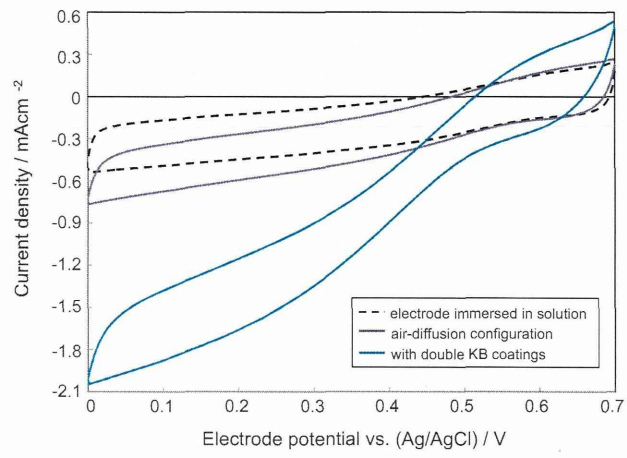

Fig. 4. Cyclic voltammograms of O_2 reduction at a BOD/KB-modified CF strip measured at 10 mV s^{-1} in the solution (broken line) and on the solution (air-diffusion configuration, solid lines). The activity of the CF electrode was enhanced by further modification with KB after the BOD immobilization.

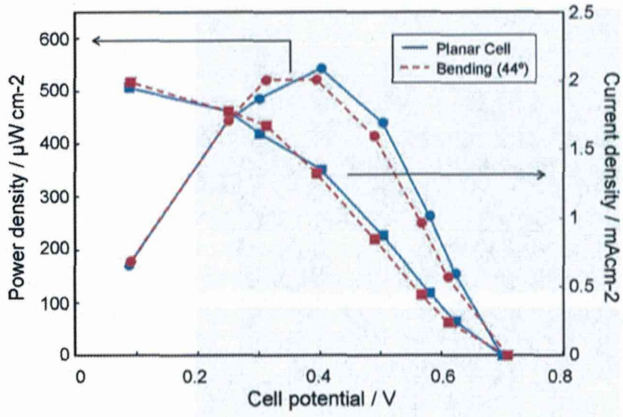

Fig. 5. Performance of the sheet-shaped biofuel cell (1 cm × 0.2 cm) with and without bending. The internal agarose layer was made with 150 mM McIlvaine buffer solution (pH 5.0) containing 200 mM fructose.

4. Conclusions

We have developed a totally flexible, sheet-shaped biofuel cell device by stacking a FDH/CNT-modified CF strip, a KB/BOD/KB-modified gas-diffusion CF strip, and an agarose hydrogel film that retains electrolyte solution and fuel (fructose). The results presented include two strategies to improve the performance of the device. (1) A CF anode modified with an appropriate CNT dispersion showed higher activity. (2) The gas-diffusion biocathode was improved by optimizing its hydrophobicity. The improved biofuel cell sheet produced a maximum power density of 550 $\mu W\,cm^{-2}$ at 0.4 V even when bent. Such a flexible, sheet-shaped power source could be combined in the future with flexible electronic to make wearable devices.

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Conducting Polymer Microelectrodes Anchored to Hydrogel Films

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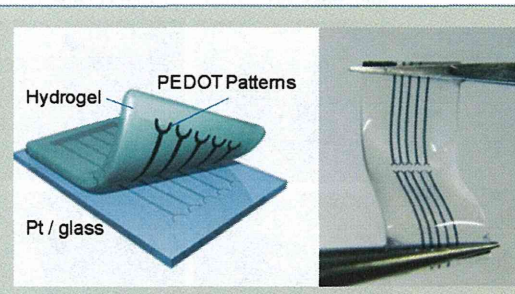
Supporting Information

ABSTRACT: We report the fabrication of totally organic hydrogel-based microelectrodes of poly(3,4-ethylenedioxythiophene) (PEDOT), which exhibit a lowered sheet resistivity of about 100 Ω/□. The preparation process starts with the electrodeposition of conductive PEDOT (ca. 20 S cm^{-1}) on Pt microelectrodes. After laminating hydrogels onto the PEDOT-modified Pt electrode substrates, a second PEDOT (low conductivity) layer was electrodeposited to anchor the first PEDOT film to the hydrogel. Finally, the hydrogel sheet with PEDOT micropatterns was peeled off by taking advantage of the electroactuation property of PEDOT. The process proved to be versatile, allowing the use of most natural and synthetic hydrogels including agarose, collagen, polyacrylamide, and so on.



Conducting polymers (CPs) such as poly(3,4-ethylenedioxythiophene) (PEDOT) are attractive electrode-coating materials, having the advantages of biocompatibility and low electrical impedance.[1−3] They have been utilized for implanted electronics[4−6] and in vitro devices for culturing cells.[7−11] In contrast to these conventional metal-supported CP electrodes, we have attempted to prepare an autonomous CP microelectrode on a hydrogel substrate that contains ~80% H_2O in order to develop a totally organic, flexible, and molecularly permeable electrode. All of the existing printing methods using screens, inkjet systems, or microstamps, require the drying of fluid inks and, thus, cannot be used for printing on a moist gel substrate. Recently, we found that the electrodeposition of PEDOT into an agarose film produces such a gel-based electrode, which is soft enough to contract synchronously with the motion of muscle cells.[12] However, the sheet resistivity of that PEDOT electrodes formed in the agarose (ca. 10 kΩ/□) was unfortunately higher than expected.[13] Apparently, dendritic growth through the hydrogel matrix[5] resulted in a larger surface area (manifested by a larger double layer capacitance) but a lower electrical conductivity due to the sparse structure. An improvement in the conductive property of the PEDOT/hydrogel electrodes should expand their possible applications.

We report herein an improved process to prepare more conductive PEDOT micropatterns on hydrogels. As shown in Figure 1a, the dense PEDOT film was first electropolymerized on Pt microelectrodes. Owing to the absence of hydrogel, we can freely employ appropriate polymerization conditions. For example, the use of CH_3CN as solvent leads to highly conductive PEDOT, as described later; the polymerization from aqueous EDOT solutions would have advantages for the biofunctionalization of PEDOT such as enzyme immobilization.[3,14] Next, as illustrated in Figure 1b,c, after forming agarose or laminating a precured other hydrogel onto the PEDOT-modified electrode substrates, a second PEDOT layer was electropolymerized from aqueous EDOT solution to anchor the first conductive PEDOT film to the hydrogel matrix. The process we previously reported[12] depended only on this sparse PEDOT for electrode preparation. Finally, the hydrogel film with PEDOT micropatterns was peeled from the Pt electrode substrate (Figure 1d) by taking advantage of the electrochemical elastic actuation of PEDOT (±0.5 V vs Ag/AgCl).[15,16]

Figure 2 shows photographs of typical specimens after the peeling process with different polymerization charges of the second PEDOT, proving its importance for nondisruptive peeling. The 1 × 1 cm Pt electrodes on glass substrates were first coated with a 300 mC PEDOT film. Next, a melted 2.8 wt % agarose solution was poured over the substrate and gelated by cooling in room temperature (2 mm thickness). Then a second PEDOT layer was electropolymerized at charges of (a) 0, (b) 100, and (c) 200 mC. Finally, twin cycles of electrochemical elastic actuation was applied for inducing stress at the polymer/electrode interface, leading to eventually

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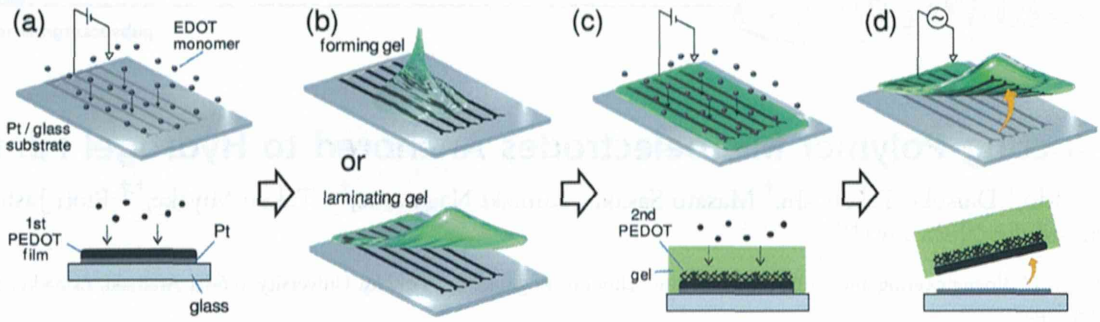

Figure 1. Schematic illustrations of the fabrication process for a conducting polymer/hydrogel electrode: (a) PEDOT was electropolymerized on a Pt microelectrode substrate; (b) a hydrogel sheet was formed or laminated on the substrate; (c) PEDOT was again polymerized; (d) then a PEDOT/hydrogel electrode was peeled from the substrate after electrochemical elastic actuation.

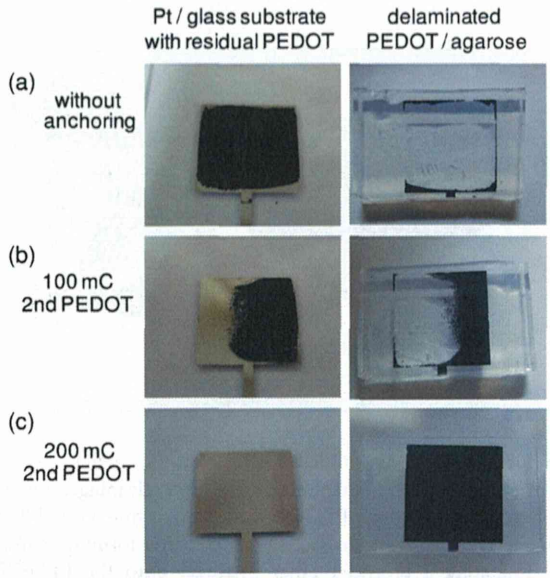

Figure 2. Photographs of Pt/glass substrates and agarose sheets after the peeling process with twin redox cycles (±0.5 V vs Ag/AgCl). The polymerization charge of the first PEDOT films was 300 mC and (a) 0, (b) 100, and (c) 200 mC for the second PEDOT layers. The polymerization was potentiostatic at 1.0 V vs Ag/AgCl in 0.1 M $LiClO_4$ aqueous solution of EDOT.

detachment. In the case without the second PEDOT deposition, a clean transfer of the pattern has never achieved (Figure 2a). The second PEDOT of 100 mC resulted in an irregular, partial transfer (Figure 2b). On the other hand, the second PEDOT of 200 mC ensured 100% transfer every time (Figure 2c), indicating that a sufficient amount of a second dendritic PEDOT layer (more than 200 mC) can serve as an effective anchor to connect the first PEDOT film and the hydrogel matrix. It is worth noting that a prior hydrophilic modification of the glass substrates with aminosilane is also necessary for nondisruptive peeling; we immersed Pt/glass substrates in 20 mM 3-aminopropyltriethoxysilane/heptane for 6 h for forming the self-assembling monolayer of aminosilane on the surface of the glass part of the substrates. Without these treatments, the naturally impure glass surface often causes anisotropic lateral growth of the polymer from the Pt electrode along the surface of the surrounding glass, resulting in adhesion between the CP and the glass substrate.[11]

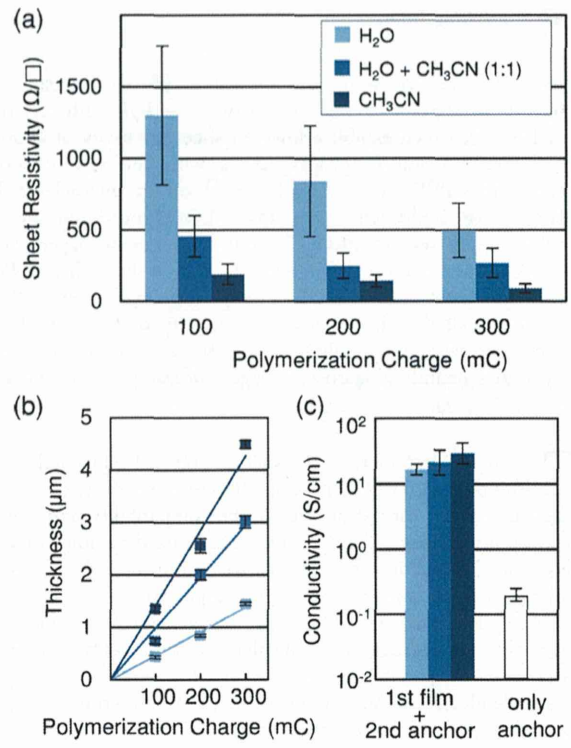

Figure 3. (a) Sheet resistivity of the PEDOT patterns (1 × 1 cm) transferred to agarose films as functions of polymerization charge of the first PEDOT film (100, 200, and 300 mC) and the solvents used for the polymerization (H_2O, CH_3CN, and their 1:1 mixture). The mean values (±standard deviation) of at least three independent specimens are given. The polymerization was potentiostatic at 1.0 V in each solution containing 50 mM EDOT and 0.1 M $LiClO_4$. The charge for second PEDOT layer was 300 mC. (b) Thickness of the first PEDOT films measured by a surface texture analyzer (DEKTAC 150). (c) Conductivity of the PEDOTs calculated by using their thickness. The conductivity value in the case without the first PEDOT film (only the second PEDOT anchor) is also shown.

With the polymerization charge of the second PEDOT fixed at 300 mC, we studied next the sheet resistivity of the peeled PEDOT patterns by changing the polymerization conditions of the first PEDOT films. The resistivity measurements were conducted under wet conditions by the two-point probe method around 0.4 V versus Ag/AgCl, where the PEDOT is in

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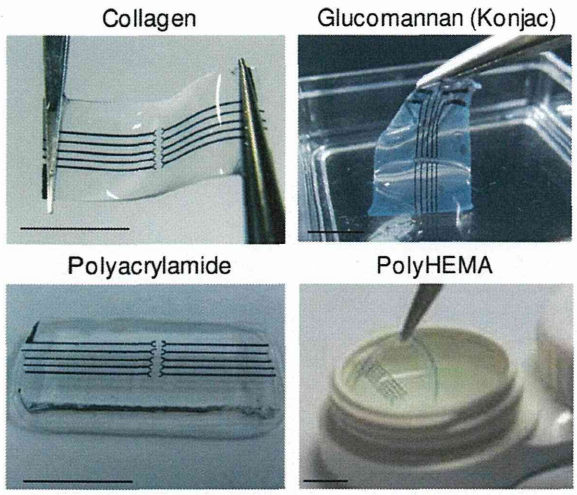

Figure 4. Photographs of the PEDOT microelectrodes anchored to the preliminarily molded hydrogel films of collagen (0.3 mm thick), glucomannan (1 mm thick), polyacrylamide (1 mm thick), and a commercial soft contact lens made of poly(2-hydroxyethyl methacrylate). Scale bar: 5 mm.

the oxidized form. The ohmic property was checked by varying the bias between the probes. Figure 3a shows that increasing the polymerization charge up to 300 mC decreased the sheet resistivity to less than 500 Ω/□, a value 2 orders of magnitude lower than that (ca. 10 kΩ/□) of the PEDOT electrode prepared by our previous process without the first PEDOT film.[12] In particular, the PEDOT film prepared using CH_3CN solvent showed the lowest resistivity, about 100 Ω/□. Presumably, polymerization at greater than 300 mC will further decrease the sheet resistivity. As shown in Figure 3b, the thickness of the first PEDOT film, measured by a surface texture analyzer, were found vary with solvents used, probably due to difference in the Coulombic efficiency of electrodeposition. The 300 mC/cm^2 polymerization led to a thickness of about 1.5 μm in H_2O, 3.0 μm in H_2O/CH_3CN, and 4.5 μm in CH_3CN, respectively. Figure 3c depicts the conductivity of the transferred PEDOTs calculated taking account of their thickness. The conductivity values of the present PEDOT electrodes reach the range of 10^1 S/cm, regardless of the kind of solvent, the value being comparable to the generally known conductivity of the PEDOT.[13] For reference, the second PEDOT layer grown in agarose showed a thickness of about 5 μm for 300 mC/cm^2,[12] as also judged from the cross section (Supporting Information, Figure S1). Because the conductivity of the second PEDOT layer grown in agarose was in the range of 10^{-1} S/cm, its contribution to the net conductivity is small; it functions simply as an anchor between the first PEDOT film and the hydrogel.

The process used to prepare PEDOT micropatterns was versatile, being also successful with precured films of other kinds of natural hydrogels (collagen, glucomannan) and synthetic hydrogels (polyacrylamide, poly(2-hydroxyethyl methacrylate)), as shown in Figure 4. In addition, the PEDOT patterning process is adaptive to the variations of elasticity, thickness and shapes of the hydrogels. For example, even a commercial soft contact lens can be used as the substrate for PEDOT electrodes. Although the structural and electrical characters of the second PEDOT would be somewhat different by the hydrogels used, they functioned well as the anchor for nondisruptive peeling of the first PEDOT, as with the case of agarose. Among the hydrogels we studied, only the fibrin gel could not be used as the substrate for PEDOT electrodes. The electrostatic and chemical conditions in fibrin may inhibit the polymerization of the second PEDOT.

The hydrogel-based CP micropatterns discussed here represent a totally organic, moist, flexible, and molecularly permeable electrode that can be combined with cells and tissues without disturbing the physiological conditions including the continuous supply of O_2 and nutrients. Such properties are ideal for use as in vivo and in vitro electrodes for stimulation and recording. Besides such cellular applications, these improved conductivity CP/gel electrodes should be applicable to a variety of hydrogel-based electronic systems such as iontophoretic drug delivery.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The optical microscope image of the cross section of PEDOT/agarose electrode. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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Flexible, layered biofuel cells

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ABSTRACT

Similar to conventional electrolyte batteries, biofuel cells often need to be stacked in order to boost their single cell voltage (< 1 V) up to a practical level. Here, we report a laminated stack of biofuel cells that is composed of bioanode fabrics for fructose oxidation, hydrogel sheets containing electrolyte and fuel (fructose), and O_2-diffusion biocathode fabrics. The anode and cathode fabrics were prepared by modifying fructose dehydrogenase and bilirubin oxidase, respectively, on carbon nanotubes-decorated carbon fiber fabrics. The total thickness of the single set of anode/gel/cathode sheets is just 1.1 mm. The laminated triple-layer stack produces an open-circuit voltage of 2.09 V, which is a 2.8-fold increase over that of a single set cell (0.74 V). The present layered cell (5 mm × 5 mm) produces a maximum power of 0.64 mW at 1.21 V, a level that is sufficient to drive light-emitting diodes.

1. Introduction

Enzyme-based biofuel cells (BFCs) that generate electricity through enzymatic oxidation of biological fuels like sugars and alcohols have attracted attention as ubiquitous, safe power sources (Heller, 2004; Barton et al., 2004; Cooney et al., 2008; Willner et al., 2009). In this decade, the output current of enzymatic BFCs have been dramatically improved from μA to mA level (Sakai et al., 2009; Gao et al., 2010; Zebda et al., 2011; Miyake et al., 2011a). In contrast, the cell voltage is determined by the coupling of redox reactions at anode and cathode, and is typically limited around 1.0 V, a value that is insufficient for many practical applications; for example, a single light-emitting diode (LED) requires a voltage larger than 1.6 V. Therefore, in a similar manner to electrolyte batteries, BFCs are often stacked for boosting the output voltage (Ferrigno et al., 2002; Sakai et al., 2009; Gellett et al., 2010; Holzinger et al., in press). When stacking with series-connections, each BFC should be isolated by proper packaging to prevent short-circuits via ion-conductive fuel solutions, and these packages are then interconnected electrically with metal lead. Such requirements, however, are often troublesome from the standpoint of exploiting the BFC's simplicity and disposability.

In this manuscript, we describe a layered biofuel cell constructed by laminating enzyme-modified carbon fabric (CF) strips and hydrogel film containing electrolyte and fuel as shown in Fig. 1. The hydrogel sheets ensure ion-conduction between anode/cathode fabrics, and also serve as the fuel tank that could eliminate the necessity of packaging. A BFC sheet using a conventional agarose (Haneda et al., in press) was thick and weak due to the fragile nature of agarose. In the present work, we employ a heavy-duty "double network (DN) hydrogel", resulting in a very flexible, thinner BFC (~1 mm thickness). The pre-modification of CF with carbon nanotubes (CNTs) was effective to improve the performances of both bioanode and biocathode. The laminated stack of the improved bioelectrodes was practical for LED lighting.

2. Experimental section

2.1. Preparation of carbon fabric anodes

A 5 mm × 5 mm strip (0.3 mm thickness) of carbon fabric (CF) (TCC-3250, donated from Toho Tenax Co.) was first modified with multiwalled carbon nanotubes (CNTs) (Baytubes, donated from Bayer Material Science Co.) to increase the specific surface area (Supplementary Fig. 1). The CNTs were pretreated by heating at 400 °C for 11 h and by immersing in mixed acid (H_2SO_4+HNO_3 in a 1:3 ratio) for 5 h. The treated CNT were dispersed in water containing 0.5% Triton X-100 surfactant. A 40 μl aliquot of the 10 mg ml^{-1} CNT dispersion was dropped on a CF strip and dried in air. After degassing the CNT-modified strip by immersion in a stirred McIlvaine buffer solution for more than 1 h under vacuum

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