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Exceptionally High Glucose Current on a Hierarchically Structured Porous Carbon Electrode with "Wired" Flavin Adenine Dinucleotide-Dependent Glucose Dehydrogenase

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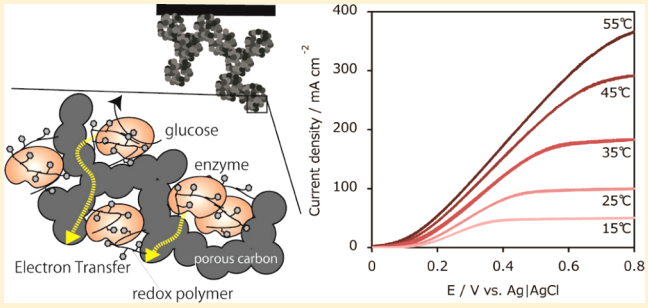
Supporting Information

ABSTRACT: This article introduces a carbon electrode designed to achieve efficient enzymatic electrolysis by exploiting a hierarchical pore structure based on macropores for efficient mass transfer and mesopores for high enzyme loading. Magnesium oxide-templated mesoporous carbon (MgOC, mean pore diameter 38 nm) was used to increase the effective specific surface area for enzyme immobilization. MgOC particles were deposited on a current collector by an electrophoretic deposition method to generate micrometer-scale macropores to improve the mass transfer of glucose and electrolyte (buffer) ions. To create a glucose bioanode, the porous-carbon-modified electrode was further coated with a biocatalytic hydrogel composed of a conductive redox polymer, deglycosylated flavin adenine dinucleotide-dependent glucose dehydrogenase (d-FAD-GDH), and a cross-linker. Carbohydrate chains on the peripheral surfaces of the FAD-GDH molecules were removed by periodate oxidation before cross-linking. The current density for the oxidation of glucose was 100 mA cm^{-2} at 25 °C and pH 7, with a hydrogel loading of 1.0 mg cm^{-2}. For the same hydrogel composition and loading, the current density on the MgOC-modified electrode was more than 30 times higher than that on a flat carbon electrode. On increasing the solution temperature to 45 °C, the catalytic current increased to 300 mA cm^{-2}, with a hydrogel loading of 1.6 mg cm^{-2}. Furthermore, the stability of the hydrogel electrode was improved by using the mesoporous carbon materials; more than 95% of the initial catalytic current remained after a 220-day storage test in 4 °C phosphate buffer, and 80% was observed after 7 days of continuous operation at 25 °C.



1. INTRODUCTION

Efficient bioelectrocatalytic reactions are of tremendous interest because of the selectivity of enzymes toward specific reactants and their very mild reaction conditions. For real applications, such as the mass production of fine chemicals (bioreactors), energy conversion (biofuel cells), and sensing (biosensors), extensive efforts have been made to construct efficient enzyme electrodes, so as to generate higher current densities with lower amounts of enzymes.[1−4] The catalytic current produced by a surface enzymatic redox reaction is generally proportional to the surface enzyme concentration and the enzyme kinetics; thus, one promising approach to increase the current production efficiency is to immobilize the enzyme and mediator on the electrode surface at high concentration.[1−5] In this study, we focused on the redox wiring technology pioneered by Heller's group, which permits enzymes to be electrically connected by an electron-conducting hydrogel that consists of a polymer containing an Os complex as a redox mediator.[6−8] This technique can produce high current densities on the order of milliamperes per square centimeter at 25 °C, even on a flat electrode.[9,10] The hydrogel formed on a flat electrode surface may normally be micrometer scale in thickness, and mediator concentration−polarization occurs in the redox hydrogel film. Thus, the enzymes located near the solution−gel interface would not contribute to the enzymatic electrode reaction;[11,12] rather, the only electrocatalytically active enzyme is located within the reaction layer, which is formed in a thickness of several tens or hundreds of nanometers at the gel−electrode interface.[11,12]

It is essential to increase the specific surface area of an electrode in order to increase its output current density per geometric surface area.[4,13−15] This may be achieved by employing porous carbon electrodes with large specific surface areas. The combination of the "wiring" technology and a porous carbon material would help to achieve a much higher and stable current output, and thus contribute a practical advancement in the field of bioelectrochemistry.

Three-dimensional (3D) carbon electrodes for use as redox hydrogel enzyme electrodes have been prepared using carbon fibers with diameters of several micrometers, such as carbon felt and carbon cloth.[16−18] The observed catalytic currents were in the range of several milliamperes per square centimeter, due to either the large average distance between the carbon fibers in a

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sheet of carbon fiber-based electrode material or the low effective total surface area of the fibers constituting the felt electrode.[19] Mano et al. reported a glucose oxidase (GOx) monolithic macroporous carbon electrode operating at pH 7.2 and 37 °C with a current density of 7.49 mA cm^{-2}.[20] However, this current density was much smaller than that expected from a total hydrogel loading of 2.2 mg cm^{-2}.

To increase the efficiency of enzyme utilization, an electrode material which has a high surface area and a specific pore structure controlled at the nanometer scale would be desirable. Also, given the increased electron exchange reaction in the vicinity of the electrode surface, the maximum catalytic current density would be suppressed by substrate consumption. To successfully satisfy the needs for both a large specific surface area for enzyme loading and the fast mass transport of fuel, a nano/micro hierarchically structured carbon electrode would be required.[21] Two strategies may be considered for the construction of hierarchically structured carbon electrodes, namely, "top-down" and "bottom-up" approaches. In the "top-down" method, the macroporous structure is constructed first, followed by mesopore formation on the surface of the macroporous materials. For example, in 2007, Calabrese Barton reported a carbon nanotube (CNT)-modified carbon paper electrode by chemical vapor deposition, which led to a 100-fold increase in surface area and a 10-fold increase in current density (22 mA cm^{-2} of glucose current at 37.5 °C).[22] CNT modification would be a promising technique for fabricating a tailored hierarchical structure; however, the process requires special equipment and might be impractical for mass production.

Here, our aim was to prepare a highly efficient enzyme electrode that simultaneously meets the criteria of a large surface area for efficient bioelectrocatalytic reactions (structure-controlled mesopores) and fast mass transport of the reactants (macropores). We selected a "bottom-up" approach, in which the macropore structure was built up on mesoporous carbon materials. In this study, we used a magnesium oxide-templated mesoporous carbon (MgOC; CNovel from Toyo Tanso, Japan) as an enzyme support, which has an average pore diameter of 38 nm and a Brunauer–Emmett–Teller (BET) specific surface area of 580 m^2 g^{-1}.[23] One of the striking advantages of MgOC is its simple production procedure, compared to previously reported mesoporous carbons such as carbon cryogels and carbon aerogels.[24−26] Macropores were formed by the electrophoretic deposition (EPD) method in this study,[27,28] in which carbon particles dispersed in a solvent are forced to migrate toward an electrode by applying an electric field. The MgOC particles are collected on the electrode and form a compact macroporous deposit. The macropore size (morphology) can be changed by varying the applied voltage and the distance between the electrodes.

In this study, deglycosylated flavin adenine dinucleotide-dependent glucose dehydrogenase (d-FAD-GDH) was used as the electrocatalyst on the anode, because this enzyme shows better catalytic performance in the redox hydrogel than GOx.[9] Used in many related studies, poly(1-vinylimidazole) with [$Os(bpy)_2Cl$] (PVI-$Os(bpy)_2Cl$, $E^{o\prime}$ = 0.23 V vs Ag|AgCl)[7,29] was chosen as a typical redox mediator to probe the effects of enzymatic activity and the morphology of the porous electrode. A hydrogel film was prepared by the deposition and cross-linking of the redox polymer PVI-$Os(bpy)_2Cl$ with d-FAD-GDH on the MgOC electrodes.

We found that the bioelectrocatalytic current density for glucose oxidation at 25 °C and pH 7.0 was 100 mA cm^{-2} on the porous carbon electrode with a total hydrogel loading of 1.0 mg cm^{-2}. On increasing the solution temperature to 37 °C, the catalytic current increased to 180 mA cm^{-2}. For the same hydrogel composition and loading, the current density on the MgOC-modified electrode was more than 30 times higher than that on a flat carbon electrode. Furthermore, the stability of the hydrogel electrode was improved by using mesoporous carbon materials; after 220 days, more than 95% of the initial catalytic current remained. The dependence of the catalytic current on hydrogel loading, reaction temperature, and buffer concentration is also discussed.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Materials. FAD-GDH from *Aspergillus terreus* was kindly donated by Ikeda Tohka Industries Co., Ltd. (Fukuyama, Japan).[30] The carbohydrate components on the surfaces of the FAD-GDH molecules were oxidatively removed: FAD-GDH (1 mg) was reacted with sodium periodate (0.044 mg) for 1 h at 25 °C in the dark in sodium hydrogen carbonate solution (0.053 mg/25 μL).[31] The reaction solution was replaced by distilled water using an Amicon Ultra centrifugal filter to remove unreacted periodate. Poly(ethylene glycol) diglycidyl ether (PEGDGE, molecular weight 500) was purchased from Sigma-Aldrich Japan. Polyvinylidine difluoride (PVDF) was obtained from the Kureha Corp. (Tokyo Japan). MgO-templated carbon (MgOC, CNovel) was donated by Toyo Tanso (Osaka, Japan).

2.2. Fabrication of Electrodes. The electrode fabricated by the EPD method (MgOC-E) was prepared as follows. An EPD bath was prepared by mixing 15 mg of MgOC powder and 90 mg of PVDF in acetonitrile (15 mL).[27] The carbon and polymer were dispersed by ultrasonication (SMT UH-50, Tokyo, Japan) for 10 min before deposition. A glassy carbon (GC) electrode, 3 mm in diameter, was used as the substrate and another carbon plate as the counter electrode. The MgOC particles were deposited by applying a DC voltage (Anatech, Japan) at 50 V between the two carbon plates for 60 s (electrode distance 1 cm). The electrodes were dried in air at room temperature. The morphology of the electrode surface was characterized by scanning electron microscopy (SEM, JSM-5510SEM, JEOL) at a beam voltage of 20 kV.

2.3. Preparation of the Hydrogel Film. The poly(1-vinylimidazole) complex of [$Os(bpy)_2Cl$] (PVI-$Os(bpy)_2Cl$) was synthesized according to an earlier report[9,29] and quaternized[32] as follows. To a solution of PVI-$Os(bpy)_2Cl$ (1.4 mg) in methanol (0.4 mL) was added 2-bromoethylamine (3.5 mg). The mixture was heated overnight at 60 °C. The solvent was evaporated, and the residue was dissolved in water. To prepare the hydrogel film, an anodic catalyst solution was made as follows: d-FAD-GDH solution (3.2 μL, 25 mg mL^{-1}) was mixed with solutions of PVI-$Os(bpy)_2Cl$ (6.7 μL, 12 mg mL^{-1}) and PEGDGE (6.4 μL, 5 mg mL^{-1}). For a hydrogel loading of 200 μg cm^{-2}, 1.2 μL of this solution was placed via syringe onto the MgOC-modified electrodes. Electrodes with different hydrogel loadings were prepared by simply changing the volume of the deposition solution while maintaining a constant composition. The respective electrodes were made hydrophilic by plasma oxidation (20 min) before coating. The film on the electrode was cured at room temperature and constant 20% humidity in a desiccator for 12 h prior to experimentation. The resulting anodic catalyst consisted of the cross-linked adduct containing 41.6 wt% d-FAD-GDH, 41.8 wt% polymeric PVI-$Os(bpy)_2Cl$, and 16.6 wt% PEGDGE.

2.4. Electrochemical Measurements. Unless otherwise specified, cyclic voltammetry (CV) was performed in a water-jacketed electrolysis cell containing 15 mL of phosphate buffer solution (PBS, 1000 mM, pH 7.0) at 25 °C by a circulator using a three-electrode potentiostat (BAS, CV-50W). A platinum wire was used as the counter electrode, and all potentials were referenced to a Ag|AgCl (saturated KCl) electrode.

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3. RESULTS AND DISCUSSION

Figure 1A shows a scanning electron micrograph of the MgOC-E, deposited at 50 V over 1 min. The surface shows significant

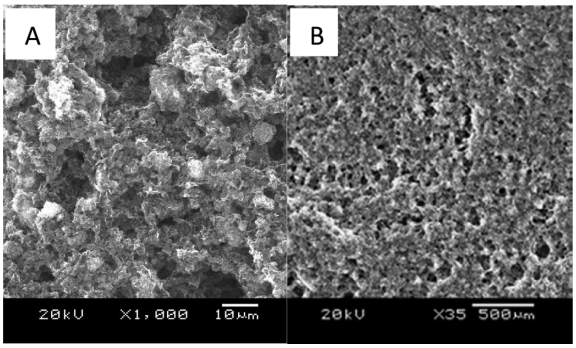

Figure 1. SEM images of the MgOC-modified electrode fabricated by the EPD method at 50 V over 1 min at (A) 1000× and (B) 35× magnifications.

roughness with several 10-μm-scale macropores. The macropore structure and carbon layer thickness can be controlled by tuning the applied voltage and distance between the two electrodes, as well as the electrophoresis time. For example, at lower applied voltage, no pores larger than 10 μm were observed (Supporting Information, Figure S1). Macropore formation is ascribed to the gas generated on the electrode surface.[33] The carbon layer deposited on the GC electrode was uniform, and no cracking was observed (Figure 1B).

Figure 2 shows the electrochemical characteristics of the MgOC-E modified at a loading of 1.0 mg cm^{-2}. CVs of the

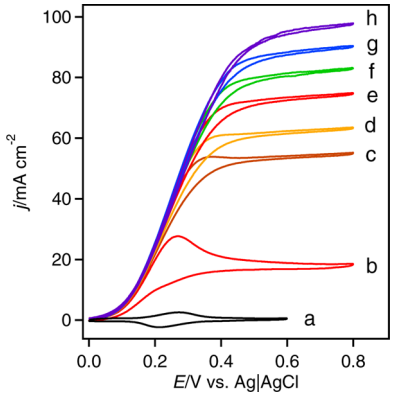

Figure 2. Cyclic voltammograms for glucose oxidation on d-FAD-GDH-modified MgOC-E in phosphate buffer (1000 mM, pH 7.0, and 25 °C) in the presence of 500 mM glucose (curves b–h). The rotation rates were (b) 0, (c) 500, (d) 1000, (e) 2500, (f) 5000, (g) 7500, and (h) 9000 rpm. Curve a was obtained in the phosphate buffer in the absence of glucose. The scan rate was 10 mV s^{-1}. The total hydrogel loading was 1.0 mg cm^{-2}.

hydrogel-coated MgOC-E in PBS at a scan rate of 10 mV s^{-1} in the absence of glucose displayed a well-defined surface redox wave (curve a, Figure 2, and Supporting Information, Figure S2), and the peak currents increased linearly with increasing scan rate (data not shown). These results indicate that a thin hydrogel layer was formed on the surface of the carbon mesopores. In contrast, the peak current density for a GC electrode is proportional to the square root of the scan rate (data not shown), as expected for semi-infinite diffusion limited by electron transport through the redox hydrogel film.[34]

In the presence of a quiescent solution of 500 mM D-glucose, the glucose electrooxidation current increased and reached its peak of 27 mA cm^{-2} at 0.3 V (curve b, Figure 2); the catalytic current decreased thereafter to a steady-state value of 18 mA cm^{-2}. The local depletion of glucose in the carbon layer and in the vicinity of the electrode surface would play a dominant role in the current decay during the electrocatalytic oxidation reaction. The steady-state current observed in the potential range from 0.5 to 0.8 V suggests a balance between the mass transfer of glucose and electroenzymatic consumption. Upon rotating the electrode, the catalytic current above 0.3 V increased with the rotation rate (curves c–h, Figure 2), indicating that the current is limited by the mass transport of glucose from the bulk solution to the electrode surface and the permeation of the glucose in the porous carbon structure. The current density for the oxidation of glucose reached as high as 100 mA cm^{-2} at 0.7 V, under conditions of 25 °C, pH 7, and a 9000 rpm electrode rotation rate. This is the highest catalytic current density ever reported for any enzymatic glucose anode operating at 25 °C. In comparison with the previously reported most efficient hydrogel electrode, based on CNT-modified carbon paper,[22] the electrode presented here shows 1.5-fold higher catalytic current under similar experimental conditions (50 mM glucose, 4000 rpm, 37 °C, and 0.8 mg cm^{-2} total hydrogel loading; Supporting Information, Figure S3). For the GC electrode, the catalytic current ratio of d-FAD-GDH to d-GOx was 1.6.[9] This fact indicates that the current increase in the MgOC-E electrode can be ascribed to an increase in the enzymatic activity in the hydrogel. Thus, the structure of this MgOC-E-based electrode is as effective as the CNT-modified carbon paper-based electrode.

The MgOC-E modified with 1.0 mg cm^{-2} hydrogel exhibited a 33-fold higher current density than a flat GC electrode (3 mA cm^{-2}; Supporting Information, Figure S4) with the same amounts of deposited enzyme and mediator. Polarization of the mediator concentration occurs in hydrogel films of micrometer-scale thickness formed on a GC electrode; thus, the enzymes located outside of the reaction layer would not contribute to the enzymatic electrode reaction. In addition, the catalytic current density on the GC electrode with 1.0 mg cm^{-2} hydrogel loading is lower than that with 0.2 mg cm^{-2} loading (4 mA cm^{-2}).[9] The current might be restricted by the mass transfer of glucose within the hydrogel film.[12] In order to preserve the stability of such high catalytic currents, one must consider the effects of the electrolyte (ion conductivity) and the pH changes in the vicinity of the electrode surface. Before the experiments described above, we had optimized the buffer and glucose concentrations. Figure 3 shows the effects of the buffer concentrations on the shapes of the CVs of the MgOC-E modified at a loading of 1.0 mg cm^{-2}. With increasing buffer concentration, the slopes of the voltammograms between 0.2 and 0.4 V became steeper, and the maximum currents above 0.6 V increased. The former result may be ascribed to a decrease in the ohmic resistance of the electrolyte solution by the increase in the phosphate ion content. When the buffer concentration was increased to 1500 mM, the slope did not change; however, the maximum current decreased to 80% of that observed in 1000 mM phosphate buffer (Supporting Information, Figure S5). The current magnitude was recovered when the buffer concentration was restored to 1000 mM. We may conclude that the maximum current increase is due to the changes in the

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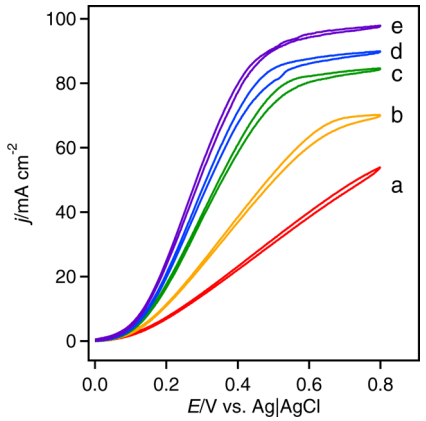

Figure 3. Effects of buffer concentration on cyclic voltammogram shape for hydrogel loaded at 1.0 mg cm^{-2} on MgOC-E at 25 °C. CVs are shown for phosphate buffer concentrations of (a) 100, (b) 200, (c) 500, (d) 750, and (e) 1000 mM. Each buffer solution was pH 7.0 and contained 500 mM glucose.

hydrogel structure and electrostatic interactions between the Os complexes and the enzyme, as affected by ionic strength. To minimize the ohmic resistance, the concentration of the buffer was fixed at 1000 mM in the following experiments. Figure S6 (Supporting Information) shows the glucose concentration dependence of the catalytic current for glucose oxidation by d-FAD-GDH at pH 7 in 1000 mM phosphate buffer at 37 °C. The catalytic current increased with increasing glucose concentration until 500 mM. Above 500 mM glucose, a slight decrease of catalytic current was observed, due to the increased viscosity of the electrolyte solution.

Figure 4A presents the dependence of the catalytic current on the amount of hydrogel loaded. The plateau catalytic current

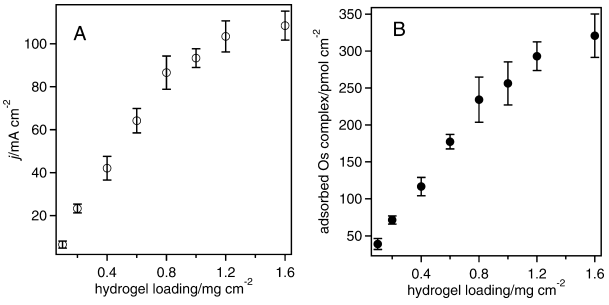

Figure 4. (A) Current densities at 0.7 V versus the amount of hydrogel. The conditions were 25 °C and 1000 mM phosphate buffer (pH 7.0) containing 500 mM D-glucose. (B) Dependence of the adsorption of the electroactive Os complex on the hydrogel loading.

at a high rotation rate increases linearly with increasing hydrogel loading until 0.8 mg cm^{-2}, and reaches a plateau of 110 mA cm^{-2} at 1.6 mg cm^{-2} loading. The MgOC-E containing macropores larger than 10 μm allows fast mass transfer of glucose throughout the hydrogel film formed on the mesopores. In the linear region, assuming that all of the enzyme loaded can work as an electrocatalyst, the reaction rate constant can be evaluated as 150 s^{-1}, which includes the k_{cat} value and $1/(1 + K_M/[Os])$ (where K_M is the Michaelis constant and [Os] is Os complex concentration).[11,12] When the hydrogel loading is high, we might account for the leveling-off of the current in three ways: (1) the substrate is depleted in the vicinity of the electrode surface; (2) concentration polarization of the mediator occurs within the redox hydrogel; and (3) the mesopores are filled with the maximum amount of hydrogel possible. To discern among these possibilities, we evaluated the dependence of the amount of electrochemically active Os complexes immobilized on the electrode surface on the loading (Figure 4B). The amount of the immobilized Os complex was measured by integrating the voltammogram obtained in the absence of glucose. The amounts of immobilized Os complexes increased linearly as the hydrogel loading increased until 0.8 mg cm^{-2}, and leveled off at a loading of 1.2 mg cm^{-2}. Thus, we can see a clear linear relationship between the catalytic current and the immobilized hydrogel (Figure 4). The leveling off at high hydrogel loading suggests that the surfaces of the mesopores were already filled with the hydrogel. Although electrodes made of 3D materials increase the specific surface area per projected area, the mass transfer of glucose through the thick 3D matrix can easily restrict current generation. The MgOC film achieved efficient diffusion of the glucose because of the relatively thin carbon layer (<0.1 mm) and the macroporous structure within it.

In contrast, for the MgOC applied on the GC electrode by the drop-casting technique (which lacked macropores larger than 10 μm), the maximum current was less than 40 mA cm^{-2}, even at 9000 rpm (Supporting Information, Figure S7). The maximum current was ascribed to the limitations of biocatalyst and glucose transport in the carbon layer structure on the GC support.

Upon increasing the solution temperature to 55 °C, the current density was enhanced to as high as 360 mA cm^{-2} (Figure 5). According to the Arrhenius plot, the activation

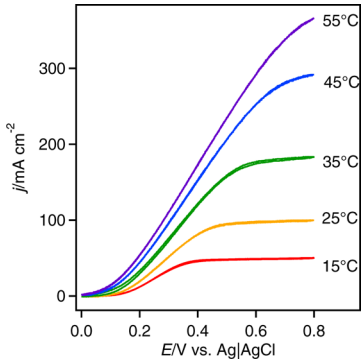

Figure 5. Temperature dependence of the catalytic current for glucose oxidation at pH 7 in 1000 mM phosphate buffer containing 500 mM D-glucose at 15, 25, 35, 45, and 55 °C. The electrode rotation rate was 9000 rpm and the scan rate was 10 mV s^{-1}. The total hydrogel loading was 1.6 mg cm^{-2}.

energy could be estimated as 42 ± 3 kJ mol^{-1}, which indicates that the limiting catalytic current is mostly determined by the enzymatic reaction.[35−37]

Figure 6 compares the CVs for glucose oxidation using hydrogel MgOC electrodes loaded with FAD-GDH or d-FAD-GDH. The total hydrogel loading was 1.0 mg cm^{-2}. The catalytic current density of the d-FAD-GDH-modified electrode was almost 3 times higher (100 mA cm^{-2}) than the FAD-GDH electrode (35 mA cm^{-2}) for the same hydrogel loading and composition. By removing the sugars grafted on the enzyme molecules, the hydrogel might have a more rigid, dense structure with enhanced interaction between the enzyme and redox polymer chain.[38,39] The catalytic current density of the d-

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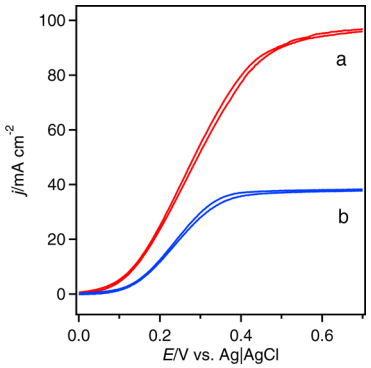

Figure 6. Rotating disk cyclic voltammograms for glucose oxidation by (a) d-FAD-GDH and (b) FAD-GDH electrodes, using 500 mM glucose in 1000 mM phosphate buffer, at pH 7.0, 25 °C, 9000 rpm, and 10 mV s^{-1}. The total hydrogel loading was 1.0 mg cm^{-2}.

FAD-GDH-modified GC electrode was 4.1 mA cm^{-2}, which is 1.6 times that of the FAD-GDH-modified GC electrode (2.6 mA cm^{-2}) for the same hydrogel loading (0.2 mg cm^{-2}) and composition.[9] The redox mediator concentration in the hydrogel layer formed on the GC electrode might be polarized because of the slow electron transfer between the Os complexes tethered to the poly(1-vinylimidazole) backbone, and this would depress the overall reaction rate (observed current density).[40−42] In the porous carbon electrode, a thin hydrogel is formed on the mesoporous carbon surface, and the catalytic current can be simply determined by the enzymatic reaction rates: roughly, the product of the enzymatic kinetics and the surface concentration of the enzyme.[11,12] The catalytic current for the FAD-GDH-modified electrode showed a glucose concentration dependence similar to that of the d-FAD-GDH-modified electrode (Supporting Information, Figure S6). The catalytic current ratio of the FAD-GDH/d-FAD-GDH-modified electrodes was 0.3−0.4 at each concentration point.

The stability of the continuous current responses of the hydrogel electrodes was investigated. Hydrogel-modified electrodes using d-FAD-GDH were prepared on MgOC and GC substrates, and operated at 0.5 V in an incubator controlled at 25 °C. Figure 7A shows the continuous current responses of the electrodes with 1.0 mg cm^{-2} hydrogel loading in 1.0 M quiescent PBS. The residual current response after 7 days of continuous operation was more than 80% for the d-FAD-GDH-modified MgOC-E. In contrast, the d-FAD-GDH-modified GC electrode retained more than ca. 80% of its initial catalytic current over 2 days of continuous operation and afterward gradually decreased. The d-FAD-GDH-modified MgOC-E was stored at 4 °C in pH 7.0 phosphate buffer when not in use. The magnitude of its catalytic current remained unchanged for 220 days, as determined by nearly weekly current measurements (Figure 7B). Adhesive interactions between the hydrogel and the electrode surface might be very strong (Supporting Information, Figure S8).

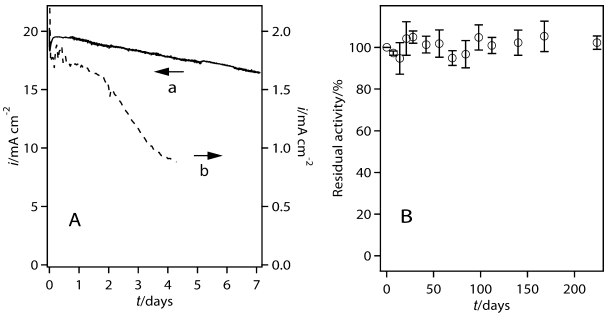

Figure 7. (A) Continuous operation of MgOC-E (curve a) and GC (curve b) electrodes modified with 1.0 mg cm^{-2} hydrogel loading in 1000 mM PBS containing 500 mM glucose at pH 7.0. (B) Stability of the electrode stored in phosphate buffer at 4 °C over time. The catalytic current was observed in 1000 mM PBS at pH 7.0 containing 500 mM glucose.

4. CONCLUSIONS

This article demonstrated that enzyme-modified MgO-templated porous carbon forms are promising candidates as electrode materials for the elaboration of efficient bioelectrochemical devices. A mesoporous MgO-templated carbon electrode with high surface area for mediated biocatalysis was fabricated by the electrophoretic deposition method. The electrode coated with d-FAD-GDH and a redox polymer showed a 33-fold increase in glucose oxidation current density (ca. 100 mA cm^{-2}) compared to that of the flat electrode. Immobilization of the enzyme in the mesopores with polymers can realize a significant increase in the stability of the enzyme.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

SEM image of prepared electrode, CV results under various conditions, and glucose concentration dependence of the catalytic current for glucose oxidation. 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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