Enhanced Performance of a Glucose/O₂ Biofuel Cell Assembled with Laccase-Covalently Immobilized Three-Dimensional Macroporous Gold Film-Based Biocathode and Bacterial Surface Displayed Glucose Dehydrogenase-Based Bioanode

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S Supporting Information

ABSTRACT: The power output and stability of enzyme-based biofuel cells (BFCs) is greatly dependent on the properties of both the biocathode and bioanode, which may be adapted for portable power production. In this paper, a novel highly uniform three-dimensional (3D) macroporous gold (MP-Au) film was prepared by heating the gold “supraspheres”, which were synthesized by a bottom-up protein templating approach, and followed by modification of laccase on the MP-Au film by covalent immobilization. The as-prepared laccase/MP-Au biocathode exhibited an onset potential of 0.62 V versus saturated calomel electrode (SCE, or 0.86 V vs NHE, normal hydrogen electrode) toward O₂ reduction and a high catalytic current of 0.61 mAcm⁻². On the other hand, mutated glucose dehydrogenase (GDH) surface displayed bacteria (GDH-bacteria) were used to improve the stability of the glucose oxidation at the bioanode. The as-assembled membraneless glucose/O₂ fuel cell showed a high power output of 55.8 ± 2.0 μW cm⁻² and open circuit potential of 0.80 V, contributing to the improved electrocatalysis toward O₂ reduction at the laccase/MP-Au biocathode. Moreover, the BFC retained 84% of its maximal power density even after continuous operation for 55 h because of the high stability of the bacterial surface displayed GDH mutant toward glucose oxidation. Our findings may be promising for the development of more efficient glucose BFC for portable battery or self-powered device applications.

Glucose biofuel cells (BFCs), which can convert glucose to electrical energy by enzymatic biocatalysts, have attracted substantial attention in recent years because they can work under mild conditions and are well-suited for device miniaturization with a membraneless fuel cell configuration for portable, or *in vivo*, power production.^{1–6} These prominent features render their potential applications in sugar-powered portable electronics, blood-powered wireless sensors, and implants.^{7–10} In the past decade, significant progresses concerned with enzymatic biofuel cells have been achieved.^{2,6,11–17} Many enzymatic biofuel cells can be successfully implanted in biological organisms such as rat,^{12,13} clam,¹⁴ lobster,¹⁵ insect,¹⁶ and snail.¹⁷ However, enzyme-based BFCs still experience great challenges in power performance, stability, and possible practical implantable use. As such, the critical issues to limit the power density include the low activity of immobilized enzymes, anisotropic electron transport properties, and poor stability.¹⁸

The power output of a BFC strongly depends on the performance of electrocatalysts employed at the bioanode and biocathode.¹ In the past decade, various approaches have been developed to improve the current density of glucose oxidation at the bioanodes including the use of unique nanomaterial and

exploring the strategy for facile enzyme immobilization.^{19–21} For example, Mao et al. used single-walled carbon nanotubes to immobilize glucose dehydrogenase (GDH), by which the efficiency of glucose oxidation was improved.²² The open-circuit potential (OCP) of that BFC reached 0.80 V, and the maximum power density was 9.5 μW cm⁻² at 0.52 V.²² Cross-linking of enzyme has also been widely used for bioanode fabrication.^{23–29} Willner et al. adopted the cross-linked composites of gold nanoparticles/GDH electrodes to assemble high-powered BFC, and a maximum power output of 32 μW cm⁻² in the air condition was obtained.²⁴ For the effective electrical communication of redox enzymes with electrodes, Willner and his co-workers also developed a versatile method of reconstituting electrically contacted enzyme electrodes, which was accomplished by the reconstitution of apoenzyme on a relay-cofactor monolayer on a thin film-functionalized electrode.²¹ The reconstitution of different apo-enzymes on these relay-cofactor-functionalized electrodes led to unprecedented efficient electrical contacting between the redox centers of the

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enzymes and the electrodes.^{26,27} In comparison with pure enzymes, enzyme displayed on the microbial surface showed improved stability.^{30–33} We also found that the bacterial surface displaying glucose dehydrogenase (GDH) mutant exhibited improved stability and substrate specificity compared with its wild-type GDH counterpart.^{32,33} When the enzyme-displaying microbes were used for BFC assembly, high power performance and stability was obtained.^{34–36} Our laboratory reported on the direct energy conversion from xylose achieved by using a xylose dehydrogenase surface displayed bacteria (XDH-bacteria) bioanode-based enzymatic biofuel cell, by which the maximum power density and the open-circuit potential of the cell is $63 \mu\text{W cm}^{-2}$ and 0.58 V, respectively, obtained at 30 mM xylose.³⁷ Versatile methods have been widely used for improving the bioanodes performance; nevertheless, the poor performance such as the lower BFC output, high substrate concentration, and eventually limited long-term stability stimulated researchers to develop alternative strategies.

However, to date, reports on the fabrication of biocathodes and study of the beneficial help of those biocathodes toward the assembled BFC performance are relatively rare.^{38–40} In this respect, novel biocathode materials may provide a feasible way to improve the BFC performance. Murata et al. reported a bilirubin oxidase (BOD)-based three-dimensional (3D) gold nanoparticle bioelectrode, which provided a current density as high as 5.2 mA cm^{-2} at a 4000 rpm electrode rotating rate.³⁹ The maximum power density of the final fructose/ O_2 BFC reached 0.87 mW cm^{-2} at an operating voltage of 0.3 V in stirring solution. Leech and his co-workers developed a laccase-based highly ordered macroporous gold bioelectrode to construct a glucose/ O_2 BFC.⁴⁰ Their BFC only showed OCP of 0.3 V and a maximum power density of $38 \mu\text{W cm}^{-2}$ in 10 mM glucose,⁴⁰ for which the BFC performance was probably hindered by the unromantic morphology of the gold nanostructure and, accordingly, resulted in the inefficient immobilization of enzyme. Therefore, novel macroporous materials with complex morphology and a high capability of immobilization of enzyme are highly desired.

Herein, we report a bottom-up protein templating approach to synthesize a 3D macroporous gold (MP-Au) film based on gold “supraspheres” to prepare laccase/MP-Au/FTO (FTO denotes fluorine-doped tin oxide electrode) biocathode by covalent immobilization. This film is interconnected with ligaments and discrete gold crystallites at the cross-section, forming a sponge-like architecture throughout the film. As expected, this biocathode showed a high current density of 0.61 mA cm^{-2} and an onset potential of 0.62 V (vs saturated calomel electrode, SCE) toward O_2 reduction. On the other hand, for the bioanode fabrication and for the improvement of the stability of glucose oxidation, we fabricated a novel bioanode based on cell surface displayed GDH (GDH-bacteria), which has been prepared previously by our group.³³ Subsequently, we assembled a novel compartment-less glucose/ O_2 BFC by using laccase immobilized macroporous gold film-based biocathode and GDH-bacteria-based bioanode, which showed considerable power output and stability at a low glucose concentration of 10 mM. The laccase/MP-Au/FTO biocathode-based BFC can significantly improve the BFC output by 4-fold compared with control biocathode-based BFC. Further, the use of the bacteria-GDH-based bioanode greatly improved the BFC stability.

■ EXPERIMENTAL SECTION

Chemicals and Reagents. Laccase from *Trametes versicolor* was purchased from Sigma-Aldrich and purified by the standard protocol. 4-Nitrophenyl diazonium tetrafluoroborate and tetrabutylammonium tetrafluoroborate (NBu_4BF_4) were synthesized according to our previous report.⁴¹ Sodium periodate (NaIO_4), chloroauric acid (HAuCl_4), ascorbic acid (AA), bovine serum albumin (BSA), 6-mercapto-1-hexanol (MH), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (EDC), *N*-hydroxysuccinimide (NHS), morpholino-ethanesulfonic acid (MES), and other reagents were purchased from Aladdin Reagent Company (Shanghai, China) and used without further purification. Multiwalled carbon nanotubes (MWNTs) were kindly gifted by Prof. Gebo Pan in Suzhou Institute of Nano-Tech and Nano-Biomics, Chinese Academy of Sciences.

Preparation of the 3D Macroporous Gold Film on FTO Electrode. *Synthesis of Gold Supraspheres.* Ten mL of HAuCl_4 (10 mM) was added dropwise into 10 mL of aqueous solution containing 50 mg of BSA under vigorous stirring. This solution was then continuously stirred for another 5 min. Subsequently, 50 mg of AA was quickly added. The color of the reaction solution changed from brownish-yellow to dark-gray within 1 min, indicating the successful reduction of HAuCl_4 to Au by this process. The final product was kept at 4 °C for further characterization and use.

Preparation of 3D Macroporous Gold Film. Twenty μL of the as-prepared gold supraspheres solution was dropped on a piece of cleaned FTO electrode and dried at room temperature. The dried product was directly calcined at 450 °C for 2 h with a ramping rate of 1 °C/min, followed by cooling down to room temperature. The obtained product was denoted as MP-Au/FTO.

Mutated GDH Bacterial Surface Display. The details for the construction of mutated plasmid pTInaPb-N/Gdh and the growth of Q252L/E170R/V149 K/G259A variant can be found in our publication.³³ Briefly, *E. coli* strain BL21 (DE3) was used as the host cell for the expression of recombinant protein INP-GDH. Cells bearing the expressing plasmids carrying mutant genes of Q252L/E170R/V149 K/G259A were grown in LB media with 30 mg/L kanamycin at 37 °C. Fusion protein was induced with isopropyl- β -D-thiogalactoside at a final concentration of 0.5 mM at 25 °C. Then, cells were harvested and resuspended in 75 mM Tris-HCl buffer (pH 8.0). The GDH activity of whole cells was $1.12 \pm 0.03 \text{ U/mg cells}$, suggesting that mutated GDH was successfully displayed on the surface of bacteria. One unit of activity was defined as the amount of enzyme necessary for the production of 1 μmol of NADH per mg of cell per minute. Q252L/E170R/V149 K/G259A variant showed excellent stability and substrate specificity. Specifically, the half-life of the Q252L/E170R/V149 K/G259A mutant was ~ 21 days at 60 °C and ~ 3.8 days at 70 °C.

Preparation of Laccase-Immobilized MP-Au Biocathode. The MP-Au/FTO electrode was activated by continuous electrochemical oxidative scanning for 25 cycles in 0.1 M H_2SO_4 from 0 to 1.5 V (vs SCE) at 0.1 V s^{-1} . The MP-Au/FTO electrode was then immersed in a solution of acetonitrile containing 2 mM *p*-nitrophenyl diazonium salt and 100 mM BF_4ClO_4 . One cyclic voltammogram from 0.6 to -0.6 V at scan rate of 0.1 V s^{-1} was recorded for each electrode. Afterward, the gold electrode was taken into a 0.1 M KCl solution, and the electrochemical modification was carried out by scanning two reductive cyclic voltammetric cycles from 0 to

-1.4 at 0.1 V s^{-1} . The amino-terminated modified electrode was then immersed overnight in 1 mM MH aqueous solution. The covalent immobilization of laccase was performed according to the literature.³⁸ Briefly, the laccase from *T. versicolor* was activated by NaIO_4 solution for 30 min , and the pH value was adjusted to 7 by adding Na_2HPO_4 solution. After the functionalized MP-Au/FTO electrode was incubated in the enzyme solution for 90 min and rinsed with MES buffer (pH 6.0), the EDC/NHS mixture was dropped onto the electrode surface and kept for 2 h for laccase immobilization. As a control, laccase/planar gold electrode was fabricated by the same procedure except that planar gold electrode rather than the MP-Au film electrode was used.

Preparation of GDH-Bacteria-Based Bioanode. The glassy carbon electrode (GCE, 3 mm in diameter) was thoroughly polished and cleaned. Four μL of 2 mg/mL MWNTs solution was dropped on the inverted GCE and dried at room temperature. The as-prepared MWNTs/GCE was immersed in 2 mM methylene blue (MB) solution for 3 h to adsorb the redox mediator monomer; a washing step was followed by immersing the as-prepared electrode in Milli-Q water for 5 min to remove the loosely bound dye molecules. Afterward, the as-prepared electrode was electropolymerized at 0.85 V in 0.1 M phosphate buffer (pH 6.0) for 60 min for the electropolymerization of MB to form a PMB-MWNTs nanocomposite. To immobilize GDH-bacteria onto the PMB/MWNTs/GCE, $10 \mu\text{L}$ of mutated GDH-bacteria aqueous dispersion was added to the modified electrode and dried overnight at room temperature. Finally, $5 \mu\text{L}$ of Nafion solution ($0.05 \text{ wt } \%$) was syringed to the electrode surface to obtain the GDH-bacteria/PMB/MWNTs/GCE bioanode. Before use, the modified electrode was washed repeatedly with Milli-Q water to remove the loosely combined modifiers.

Characterization. Morphologies and chemical components of gold supraspheres and 3D gold macroporous film were characterized by scanning electron microscopy (LEO 1525, Carl Zeiss; $3\text{--}12 \text{ kV}$) with an X-ray EDX instrument.

Electrochemical Measurements. Electrochemical experiments were carried out with a CHI 660D potentiostat (CH Instruments, Chenhua, Shanghai, China). The half-cell measurement was carried out in a conventional three-electrode system using the as-prepared bioelectrode as working electrode, Pt wire electrode as auxiliary electrode, and a saturated calomel electrode (SCE) as reference electrode. All potentials in this paper were recorded versus this reference.

RESULTS AND DISCUSSION

Synthesis of the Macroporous Gold Film. A bottom up approach, which involved the synthesis and calcination of gold “supraspheres”, was adopted to obtain the 3D macroporous gold film as illustrated in Figure 1. First, the gold “supraspheres” were obtained on the basis of a biomineralization method. BSA has a strong affinity to a variety of metal ions binding to different sites. When an excess of reducer was added into the mixed solution of BSA and AuCl_4^- , the coordinated metal ions were rapidly reduced and underwent a burst of nucleation process in solution. With the increasing crystallization time, the nucleation began to grow and became primary clusters. These clusters would further turn into small nanoparticles (ca. 20 nm in diameter). Owing to the existence of the denatured BSA and other molecules, the small individual nanoparticle was limited in size and a self-assembly process occurred at this stage. Thus, the metal “supraspheres” were

Figure 1. Schematic illustration of fabrication of the 3D macroporous gold film via a bottom-up approach.

formed via some nanoscale forces acting between nanoparticles. Finally, the 3D macroporous gold film was obtained by calcination of the “supraspheres”. Upon heating, organic molecules were removed from individual “supraspheres” and the naked nanoparticles fused into a 3D macroporous film. It is worth noting that BSA and self-assembled microsphere structures play a key role in forming 3D macroporous film. From the scanning electronic microscopic (SEM) images shown in Figure 2A,B, the gold “supraspheres” with an average

Figure 2. SEM images of gold supraspheres (A) and an individual gold suprasphere with higher magnification (B) synthesized by the protein-templated method; photograph of 3D macroporous gold film electrodes (C); the top view of a porous gold film surface (D).

diameter of 500 nm displayed spherical morphology and monodispersed characters. The enlarged image of an individual “suprasphere” reveals that the sphere surface is composed of numerous gold nanoparticles with an average size of 20 nm (Figure 2B), which are tightly connected with each other with the aid of proteins. Figure 2C shows a photograph of the resultant gold film on FTO obtained upon heating the gold

“supraspheres” at 400 °C for 2 h. It can be seen that the uniform layers of the gold film adhere well to the FTO surface. A top view SEM image shows the surface consisting of interconnected ligaments with an average width of 0.5 μm , which form irregularly distributed pores that are 1–3 μm in size (Figure 2D). The interconnected ligaments are composed of discrete gold crystallites, 300–800 nm in size, forming a sponge-like architecture throughout the sample. The SEM images of the sectional view and the top view of the 3D macroporous gold film with high magnification are shown in Figure S1 (Supporting Information). The compositional analysis using energy-dispersive X-ray spectroscopy (EDS) was also proved to contain a Au element (Supporting Information, Figure S2).

Biocathode Preparation. Laccase, one of the most interesting types of blue multicopper oxidases, was used to fabricate the biocathode because it is capable of selectively catalyzing O_2 reduction to H_2O at low overpotential.^{42,43} To acquire the largest current density of O_2 reduction, laccase was immobilized to the MP-Au/FTO by covalent bonding and with the optimal orientation for direct electron transfer (DET).³⁸ This was accomplished by forming mixed 4-aminophenyl and MH monolayers on the MP-Au/FTO surface. First, the 4-aminophenyl groups were grafted to the MP-Au/FTO by electrochemical reduction of aryl diazonium salt, which was widely used in conductive surface modification.^{44–47} The cyclic voltammograms (CVs) showed two characteristic reduction peaks at 0.37 and 0.02 V attributed to the reduction of the diazonium salt (Supporting Information, Figure S3), suggesting the successful formation of a strong Au-phenyl bond at the MP-Au/FTO surface. Afterward, the nitro groups were electrochemically reduced to amino and hydroxylamine groups by scanning two cycles from 0 to –1.4 V. The first CV curve showed the irreversible reduction of nitro groups to amino and hydroxylamino groups as a broad electrochemical wave ranging from –0.6 to –1.2 V and centered at –1.0 V. The second cycle showed the reversible oxidation of hydroxylamine groups to nitro groups at about –0.3 V.⁴⁸ Second, the assembled MH by the Au–S bond can cover the Au regions not grafted with 4-aminophenyl groups, and it was reported that the MH molecules could help keep an optimal orientation of laccase on Au electrodes for DET.⁴⁹ In this work, the laccase was immobilized on the functionalized MP-Au/FTO in two simple steps: (1) formation of imino bonds between the oxidized sugar residues of the laccase and the amino groups of the MP-Au/FTO surface and (2) formation of amide bonds between the activated carboxylic groups of the laccase and the amino groups of the electrode surface by carbodiimide chemistry.

Characterization of the Bioelectrodes. The electrocatalytic properties of the prepared laccase/MP-Au/FTO electrodes toward reduction of O_2 were evaluated by electrochemical measurements (Figure 3). As expected, the O_2 reduction at the laccase/MP-Au/FTO biocathode in the acetate buffer (pH 5.5) was observed at 0.62 V versus SCE (or 0.86 V vs NHE, normal hydrogen electrode), which was very close to the redox potential of laccase from *Trametes versicolor*, indicating that the DET of laccase was successfully achieved.^{50,51} However, a redox response of the T1 copper center of laccase on the MP-Au electrode in the absence of O_2 was not observed. A similar phenomenon was also observed for other laccase or bilirubin oxidase-based biocathode.^{39,52,53} The polarization curves of the laccase/MP-Au/FTO bioanode toward O_2 reduction under various conditions showed that the

Figure 3. Electrochemical reduction of O_2 at the laccase/MP-Au/FTO biocathode. (A) CVs of the laccase/MP-Au/FTO biocathode in 50 mM acetate buffer (pH 5.5) under saturated N_2 (red), ambient air (black), and saturated O_2 (blue) conditions. Scan rate, 20 mV s^{-1} . (B) Polarization curves of the laccase/MP-Au/FTO biocathode for O_2 reduction in 50 mM acetate buffer (pH 5.5) under saturated N_2 , ambient air, and saturated O_2 conditions. Scan rate, 1 mV s^{-1} .

catalytic reduction of O_2 started at 0.62 V (Figure 3B). The onset potential was quite close to the equilibrium value of $E'_{\text{O}_2/\text{H}_2\text{O}}$ (0.87 V vs NHE), suggesting that a much low overpotential was required for oxygen reduction at the as-prepared laccase/MP-Au/FTO biocathode. It is noteworthy that the potential for oxygen reduction reaction (ORR) obtained in this work is more positive than those values obtained with laccase as the biocatalyst when the mediators were used.^{54,55} Actually, to the best of the authors' knowledge, this is the highest potential to reduce O_2 reported so far. On the other hand, the catalytic current reached 0.61 mA cm^{-2} for the laccase/MP-Au/FTO electrode under O_2 -saturated conditions. For comparison, control experiments were done in which (1) laccase was not immobilized on the 4-aminophenyl modified MP-Au electrode and (2) the MP-Au electrode was replaced by a planar Au electrode to prepare a laccase/Au electrode. As shown in Figure S4, Supporting Information, in the absence of laccase, no cathodic current on the 4-aminophenyl modified MP-Au electrode was detected, demonstrating that the obtained catalytic current of O_2 reduction on the laccase/MP-Au/FTO biocathode was attributed to the active laccase rather than the 3D macroporous gold. The laccase/Au electrode prepared with the planar gold electrode showed a lower catalytic reduction current of 0.13 mA cm^{-2} under saturated O_2 conditions (Figure S4, red curve, Supporting

Information), suggesting that the significant improvement of biocathode performance toward O_2 reduction was originating from the MP-Au film.

The electrocatalytic performance of the laccase/MP-Au/FTO is superior to that of the latest reported laccase/nanoporous material-based biocathode.⁴⁰ In Leech's report, a highly ordered macroporous Au electrode was used to prepare the laccase-based biocathode. The final bioelectrode showed a catalytic current less than 0.1 mA cm^{-2} and onset potential of ca. 0.38 V versus Ag/AgCl electrode.⁴⁰ The great improvement in our biocathode performance should contribute to the covalent immobilization of laccase and the peculiar morphology of the 3D macroporous gold film. To further confirm this viewpoint, SEM images of the sectional view and the top view of the 3D macroporous gold film with high magnification were examined (Supporting Information, Figure S1). The MP-Au film surface is interconnected with ligaments with an average width of $0.5 \mu\text{m}$, forming irregularly distributed pores that are $1\text{--}3 \mu\text{m}$ in size (Supporting Information, Figure S1). As seen from the cross-section SEM image, interconnected 3D porous frameworks of gold film are composed of discrete gold crystallites, forming a sponge-like architecture throughout the film (Supporting Information, Figure S1). The 3D porous gold film can form biocomposites with laccase, to exhibit enhanced biocatalytic performance and stability,⁵⁶ which is not met for other films. CV of the MP-Au film electrode in $0.1 \text{ M H}_2\text{SO}_4$ solution was also recorded (Supporting Information, Figure S5). The effective surface area of the MP-Au film electrode, determined by the integration of the area under the gold oxide reduction peak, was about 45 times larger than the geometric surface area of the substrate. Thus, compared with planar gold electrode, the immobilization amount of laccase on the MP-Au film electrode should significantly increase. This high current density of O_2 reduction was attributed to the large surface area of the MP-Au film. The current densities of ORR at Pt/C or nanocarbon material-based electrodes were reportedly much higher, generally in several mA cm^{-2} ; however, those non-enzyme electrodes always underwent high overpotential and substrates crossed over, which would hinder their applications in biofuel cells.^{57,58}

The catalytic oxidation of glucose at the GDH-bacteria/PMB/MWNTs/GCE bioanode was performed. Generally, for a given fuel cell assembly, the OCP is determined by the difference between the onset potential for catalysis at the bioanode and biocathode. Therefore, PMB was used for decreasing the onset potential of the glucose electrocatalysis. The electropolymerization process of MB-MWNTs was under a constant potential of $+0.85 \text{ V}$ to generate the PMB-MWNTs adduct (Supporting Information, Figure S6). The peak currents around -0.3 V , according to the redox of MB, decreased, and a pair of new redox peaks at -0.1 V appeared as the time progressed, suggesting the formation of PMB/MWNTs/GCE. After the immobilization of GDH-bacteria onto the PMB/MWNTs/GCE, the obtained GDH-bacteria/PMB/MWNTs/GCE bioanode showed the onset potential of -0.1 V for glucose oxidation (Figure 4A). This phenomenon indicates the efficient electrocatalytic activity of the PMB-MWNTs mediator system toward the oxidation of NADH.²² The polarization curves were recorded using GDH-bacteria/PMB/MWNTs/GCE in different concentrations of glucose in the presence of 10 mM NAD^+ (Figure 4B). The electrocatalytic current enhanced dramatically with the increase in glucose concen-

Figure 4. (A) CVs obtained at the GDH-bacteria/pMB/MWNTs/GCE containing 10 mM NAD^+ in the absence (red) and presence (black) of 5 mM glucose. Scan rate: 20 mV s^{-1} . (B) Polarization curves of the GDH-bacteria/pMB/MWNTs/GCE bioanode in 10 mM NAD^+ and glucose with different concentrations of 5 mM (a), 10 mM (b), and 15 mM (c). Scan rate: 1 mV s^{-1} .

tration from 5 to 10 mM and reached its 0.059 mA cm^{-2} plateau at 0.1 V in 10 mM glucose solution.

Biofuel Cell Performance and Stability. Subsequently, a glucose/ O_2 biofuel cell was assembled by associating the bioanode and biocathode in one compartment (Figure 5). The OCP and the maximum power density of our titled BFC was

Figure 5. Schematic representation of the working principle of the glucose/ O_2 BFC composed of a laccase-based 3D macroporous gold film biocathode and bacterial surface displaying GDH mutant-based bioanode.

0.8 V and $55.8 \pm 2 \mu\text{W cm}^{-2}$ at 0.45 V, respectively, in 10 mM NAD^+ and 10 mM glucose in an O_2 -saturated atmosphere (Figure 6A, curve a). The OCP approximated the difference

Figure 6. (A) The polarization curves and power outputs of the glucose BFCs: GDH-bacteria/PMB/MWNTs/GCE bioanode vs laccase/MP-Au/FTO (a) or laccase/planar Au biocathode (b) in 50 mM acetate buffer (pH 5.5) containing 10 mM NAD^+ and 10 mM glucose under an O_2 -saturated condition. (B) The operational stability of laccase/MP-Au/FTO-based BFC. P is the power measured as a function of time, and P_{max} is the maximum power density.

between the onset potential for catalysis at the bioanode and biocathode. In contrast, when the laccase/MP-Au/FTO biocathode was replaced by the laccase/planar Au biocathode, a maximum power density was decreased to $6.8 \mu\text{W cm}^{-2}$ under the same condition. Moreover, the OCP of the control cell slightly decreased to 0.74 V, attributing to the higher overpotential of the reduction of O_2 at the laccase/planar Au biocathode. This suggests that the developed laccase/MP-Au/FTO biocathode did improve the power output of the assembled BFC several fold even at a low glucose concentration of 10 mM. Although the value of $55.8 \mu\text{W cm}^{-2}$ is still smaller than these values of 350 to $1300 \mu\text{W cm}^{-2}$ for BFC with compressed carbon nanotube-enzyme electrodes and a mediator-based wired enzyme bioelectrode,^{59,60} it is much higher than the values of $22.5 \mu\text{W cm}^{-2}$ reported for BFC with the wired enzyme PQQ-FAD/apo-GOx anode,^{26,27} $8.15 \mu\text{W cm}^{-2}$ for BFC at 0.31 V with the glucoamylase/GOx/MWNTs-modified anode,⁵¹ and $9.6 \mu\text{W cm}^{-2}$ for BFC with the GOx/nanomaterial anode.⁵⁴ Significantly, our system is even comparable to the recently reported nonenzymatic glucose-air fuel cell, which showed an OCP of 0.52 V and a maximum power density of $64.3 \mu\text{W cm}^{-2}$ at 0.28 V.⁶¹

To test the operational stability of the assembled BFC, the cell was operated continuously in a quiescent 10 mM glucose solution containing 10 mM NAD^+ under ambient air. After a 12 h operation, it retained 95% of its maximal power (Figure 6B), indicating a favorably stable power output process, and over 84% of the maximal power was kept even after continuous operation for 55 h. This high performance evidenced the applicability of our system to glucose biofuel cells.

CONCLUSIONS

In summary, a glucose/ O_2 biofuel cell was assembled with laccase-covalently immobilized three-dimensional macroporous gold film-based biocathode and bacterial surface displayed glucose dehydrogenase mutant-based bioanode, which exhibited improved power output and stability. The laccase/MP-Au/FTO biocathode could dramatically enhance the electrocatalytic current of O_2 reduction, while the bacterial surface expressing GDH mutant exhibited high stability toward glucose oxidation. Thus, our findings are promising for the development of more efficient glucose BFC for portable, or *in vivo*, power production applications.

ASSOCIATED CONTENT

Supporting Information

Additional figures as mentioned in the text. 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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