CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200903486

Three-Dimensional Conductive Nanowire Networks for Maximizing Anode Performance in Microbial Fuel Cells

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Microbial fuel cells (MFCs), which exploit microbial activities to generate electricity from organic matters, have attracted broad interest from fundamental research to industrial applications, particularly with respect to wastewater treatment, renewable energy recovery, electrical power sources for space shuttles, and self-powered robots.[1] However, due to relatively low power densities, practical application of MFCs still faces a number of challenges and requires extensive investigation.[2] Many factors influence the performance of MFCs, including electrode materials/structures, microbial source, electrolyte/substrate compositions, and reactor configurations.[3] Among them, MFC anodes, which serve to collect electrons released from electrochemically active microbes by various electron-transfer mechanisms (e.g., cell-surface proteins, excreted mediator compounds, and extracellular nanowires), are of crucial importance.[4] Although most MFC studies employ conventional carbon/graphite materials for anodes, some recent reports have attempted to modify carbon/graphite with functional materials, which include polypyrrole-coated carbon, carbon felts doped with quinone derivatives, and ammonia-treated carbon cloth.[5,6] Since the use of these materials is limited by the relatively small improvements in current density (two to three fold)[4] and complex preparation procedures, there is a strong desire to develop anodes with higher electron-collecting capacities.

In this study, a conductive polyaniline nanowire network (PANI-NN) with three-dimensional nanosized porous structures was developed on anodes, and its effect on performance was evaluated in both electrochemical cells (ECs) with a model bacterium and MFCs with self-organized microbial communities. Micrometer-scale network structures, such as graphite felts, provide suitable habitats for electricity-generating microbes (generally 0.2–1 microns in diameter and 2–5 microns in length). However, there have been few studies focusing on the nanostructure of MFC anodes.[3e,4] Herein, we describe the substantial improvements (10 to 100 fold) in current and power densities achieved by using a hierarchical porous structure comprised of graphite felt (microstructure) and PANI-NN (nanostructure) in microbial-community MFCs fueled with a model organic waste. An order of magnitude increase in power output through the use of PANI-NN represents a promising future for the application of MFCs towards energy recovery coupled to waste treatment.

To prepare the anode, indium tin oxide (ITO) conductive glass was used as a substrate and coated with PANI-NN (ca. 5 $mg\,cm^{-2}$)[7] (Figure 1). For comparison, a thin, flat structure of PANI (PANI-TF; less than 0.1 $mg\,cm^{-2}$) was also used as a control to confirm the effects of the nanostructure (Figure S1 in the Supporting Information,). An electron micrograph showed that rough PANI fibers ($\approx$100 nm in thickness) formed network structures with dense nanosized pores from 10 to 100 nm in diameter (Figure 1). The specific surface area of the PANI-NN electrode was estimated to be 820 cm^2 (per geometric area of 3.14 cm^2) based on data from nitrogen-adsorption experiments (5.6 $m^2\,g^{-1}$).

To examine the utility of PANI-NN for microbial electric-current generation, a dissimilatory metal-reducing bacterium, *Shewanella loihica* PV-4, which transfers electrons to anodes through cell-surface cytochromes and excreted redox

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200903486.

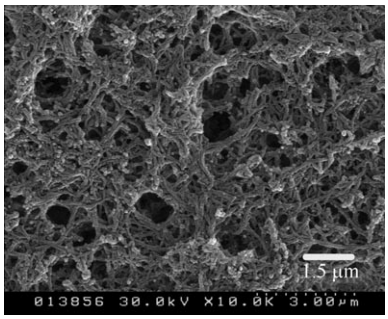

Figure 1. An SEM image of electrochemically synthesized PANI-NN on a conductive ITO glass slide. Pores ranging from 10–100 nm are randomly distributed in the film.

mediators,[8,9] was used as a model microbe. In the EC system (see Figure S2 in the Supporting Information for its configuration), either flat ITO, PANI-TF, or PANI-NN were used as anodes poised at 0.2 V versus an Ag/AgCl (saturated aqueous KCl) electrode. After microbial inoculation of ECs containing medium supplemented with 10 mM lactate as the sole electron donor for microbial catabolism, the current from systems with different anodes were compared (Figure 2). By using an ITO anode, the current density remained nearly constant at approximately 1 $\mu A\,cm^{-2}$ (per geometric surface area) (Figure 2, trace 1) for several days, which is similar to a previously described result.[9] When the ITO electrode was coated with PANI-TF, the current density increased to around 4 $\mu A\,cm^{-2}$ (Figure 2, trace 2); a level of improvement also previously reported for PANI-coated anodes.[10] However, when the anode was coated with PANI-NN, the current dramatically increased and reached 45 $\mu A\,cm^{-2}$ (per geometric surface area) within 18 h after inoculation with PV-4 (Figure 2, trace 3). The current decreased thereafter, but recovered quickly after lactate injection, indicating that lactate was rapidly depleted in association with the generation of the large current in the EC with the PANI-NN-coated anode. Repeated injections of lactate resulted in a highest peak current value of approximately 100 $\mu A\,cm^{-2}$ after two days of operation.

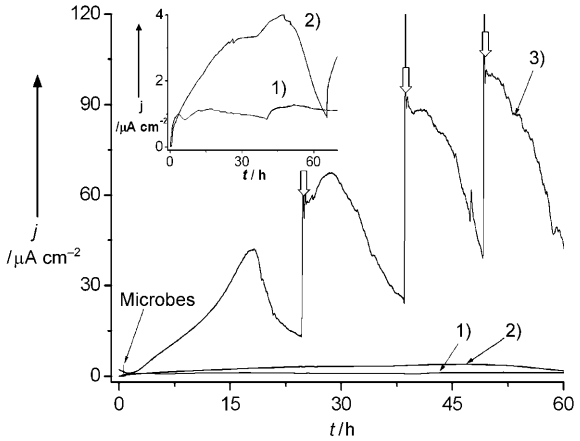

Figure 2. Current generation by *Shewanella loihica* PV-4 in ECs with the following anodes 1) ITO glass, 2) PANI-TF on ITO, and 3) PANI-NN on ITO. The anodes were potentiostatically poised at 0.2 V versus Ag/AgCl (saturated aqueous KCl). Lactate was injected into the EC with PANI-NN at the time points indicated with arrows.

The distinctive improvement in anode performance, represented by a 30-fold increase in current density compared with that of the PANI-TF anode, was likely to be due to the introduction of the nanoporous structures (Figure 1). Although it is possible the rough surface structure of PANI-NN contributed to an increased number of microbial cells directly attached to the electrode surface, the large surface area was mostly due to nanosized network structures with pores much smaller than microbial cells. We therefore speculated that the high current density was in large part attributed to enhanced electron collection by mediator molecules excreted from the microbes, which are efficiently recycled in the nanoporous structure. *Shewanella* species are known to produce two types of mediator compounds, flavins and quinones, which are distinguishable based on their fluorescence spectra.[8] To confirm the presence of these mediators, samples taken from the ECs were centrifuged and filtered to remove cells, and the resultant supernatants were subjected to florescence spectrum analyses (Figure S3 in the Supporting Information). The analyses confirmed that these two mediator species with small quantity (1–2 μM) were present in the ECs. As determined from the peak heights, a small difference in the concentrations of quinone between the ECs with PANI-NN and flat electrodes was observed. Nonetheless, the difference of mediator concentration between these ECs was very small compared with the large increase of current density with the PANI-NN anode. Taken together, the results suggest that electron collection mediated by electron shuttles in the nanopores and bacterial biofilms was strongly enhanced within the PANI-NN anode, which was much more efficient than that of flat anodes (Figure S4 in the Supporting Information, the research of whole-cell cyclic voltammograms).

To further explore the utility of PANI-NN for increasing microbial current generation, 3D microporous graphite felt was overlaid with PANI-NN and used as the anode for a MFC. SEM images of bare graphite felt (BGF) showed that microfibers were twisted and had smooth surfaces (Figure 3a and b). After coating the BGF with PANI-NN, both micro- and nanosized pores were formed (Figure 3c and d), indicating that PANI-NN and graphite microfibers produced hierarchical porous networks. It was anticipated that microsized pores in graphite felts would provide an increased area for microbial attachment, while nanoporous structures in PANI-NN would efficiently collect electrons from mediators excreted by microbes.

The performance of the PANI-NN anode was examined in a single-chamber MFC equipped with an air cathode[1f] (see Figure S5 in the Supporting Information for the MFC configuration). Rice paddy field soil provided the source of microbes, while a mixture of starch, peptone, and fish extract was used as the fuel. We used this particular mixture,

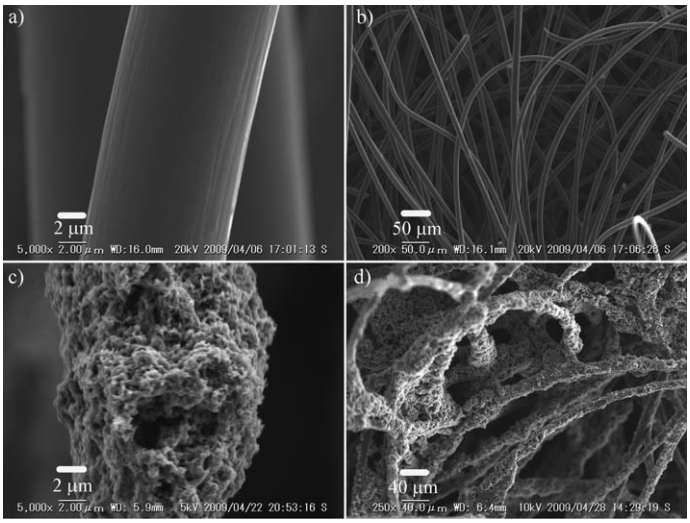

Figure 3. SEM images for bare graphite felt (BGF) (a, b) and BGF coated with PANI-NN (c, d).

since it has been used in many previous MFC and anaerobic-digestion experiments.[3d] MFCs with three different anodes (BGF, and graphite felt coated with either PANI-TF or PANI-NN) were operated for two weeks, and their performances were then compared by measuring polarization and power curves (Figure 4). For the BGF anode, a short-circuit current density (j_{sc}) of 214 $\mu A\,cm^{-2}$ and maximum power density (P_m) of 28 $\mu W\,cm^{-2}$ (geometric area) were obtained, whereas the PANI-TF anode had slightly increased values of 270 $\mu A\,cm^{-2}$ and 70 $\mu W\,cm^{-2}$, respectively. A similar two- to threefold increase after PANI coating has been described in a previous report.[10] In contrast, anodes coated with PANI-NN displayed j_{sc} and P_m values reaching 2.5 $mA\,cm^{-2}$ and 230 $\mu W\,cm^{-2}$ (the fill factor, 16%), respectively, which represents an approximately threefold increase relative to that of PANI-TF anode. After analyzing the polarization/power curve and fill factor of PANI-NN anode, it was suggested that ohmic limitation governs the MFC performance, while the increased performance was achieved. The open-circuit voltage of PANI-NN anode was almost identical to those of BGF and PANI-TF anodes, while the j_{sc} was increased largely. It was consistent with an EC experiment with model microbe *S. loihica* PV-4; this showed a substantial increase in current generation. The results indicate that the conductive PANI-NN structure assembled on the microsized graphite felts possesses high electron-harvesting capacity.

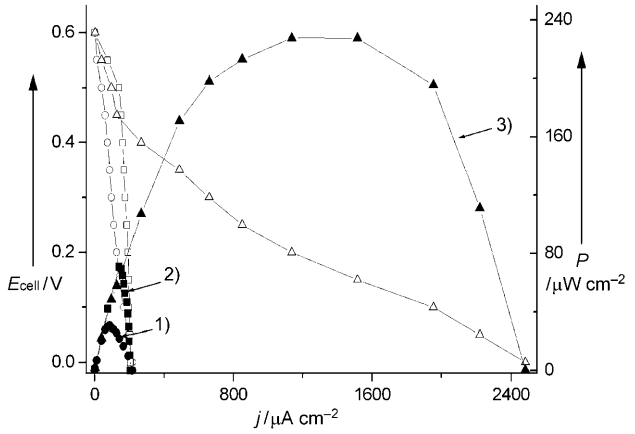

Figure 4. Polarization (open symbols) and power (closed symbols) curves for MFCs with 1) BGF, 2) PANI-TF, and 3) PANI-NN anodes. Current and power densities were calculated based on geometric surface areas.

In summary, the conductive nanowire networks assembled onto ITO and BGF served as efficient electron collectors in microbial fuel cell systems. A two orders of magnitude increase in current density was demonstrated in the *S. loihica* PV-4 system, while a single order of enhancement was achieved in the self-organized, mixed-culture MFC when using model organic waste as fuel. The large improvement in the mixed-culture MFC is particularly noteworthy because in most previous studies,[3a,e,10] the utility of functional materials for MFC anodes has been demonstrated only in pure-culture systems. Since PANI-NN can be easily added onto electrode substrates, we consider that this hierarchical conductive porous network will be a promising strategy for constructing highly efficient anodes for bioelectrochemical systems such as MFCs and microbial electrolysis cells.[11]

Experimental Section

Materials: PANI-NN was deposited onto ITO electrodes as described previously[10] with a few modifications. An Ag/AgCl (saturated aqueous KCl) electrode and platinum wire were used as reference and counter electrodes, respectively, and the electrolyte contained H_2SO_4 (1 $mol\,L^{-1}$) and aniline monomer (0.15 $mol\,L^{-1}$). Its deposition onto an area of 3.14 cm^2 was carried out by time potential cyclings from −0.3 to 1.3 V with the scan rate of 50 $mV\,s^{-1}$. A detailed fabrication method for PANI-TF is given in the Supporting Information. To deposit PANI-NN on BGF (geometric surface area of 1 cm^2), BGF was firstly cut into a 1×1 cm piece, soaked in sulfuric acid with ultrasonic treatment for one day, rinsed thoroughly with distilled water, and then dried. It was connected with a titanium wire, and its potential was cycled several times between −0.3 and 1.3 V (vs. Ag/AgCl (saturated aqueous KCl)). The surface area of the PANI-NN electrode was analyzed by using a Micromeritics Tristar 3000 machine (Shimadzu). *S. loihica* PV-4 was grown aerobically in defined inorganic medium (DM)[8] at 30 °C for two days with 10 mM of lactate as the carbon and energy source, pelleted by centrifugation, and resuspended in the DM medium prior to inoculation. The EC experiments are given in the Supporting Information (Figure S2).

MFC experiment: A single-chamber MFC was composed of an anode (1 cm^2 in geometric surface area) and an air cathode[3d] (7 cm^2) with a compartment capacity of 15 mL. The air cathode was carbon paper containing 8 $mg\,cm^{-2}$ Pt particles. A buffer solution (12 mL) containing K_2HPO_4/KH_2PO_4 (200 mM, pH 6.8) was injected into the MFC and then purged with pure nitrogen gas for 5 min. The MFC was inoculated with approximately 0.1 g of rice paddy field soil (Kamaishi, Japan) and incubated at 30 °C. The anode and cathode were connected through an external resistance of 10 kΩ, and the voltage across the resistor was monitored by using a data logger (HA-1510, Graphtec). After the voltage output was stable, a mixed substrate containing starch, peptone, and fish extract at a ratio of 3:1:1 (289 $g\,COD\,L^{-1}$, COD = chemical oxygen demand) was injected at 0.12 mL per day. To determine power and short-circuit current

densities, currents were measured at various cell voltages by using the potentistat system.

Acknowledgements

This work was financially supported by the Exploratory Research for Advanced Technology (ERATO) program of the Japan Science and Technology Agency (JST). We acknowledge G. Newton for critical reading of the manuscript.

Keywords: electrochemistry · electron transfer · microbiology · nanostructures · polymers

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Received: December 19, 2009
Published online: March 25, 2010

