MICROFLUIDIC FLOW-THROUGH MICROBIAL FUEL CELLS

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ABSTRACT

This paper reports a flow-through (FT) miniaturized microbial fuel cell (μ MFC) using three-dimensional graphene foam (GF) as a porous 3D anode. The FT design allows enhancing nutrient use efficiency for electrochemical reaction between electron-generating bacteria and bio-convertible and minimizing to sustain energy production of μ MFC. The built-in interconnected pore network of the GF anode can not only provide 3D scaffolds for bacterial attachment and colonization, but also serve as a microfluidic porous channel for flowing nutrient solutions over the biofilm formed on the scaffolds. The FT μ MFC provides a volume power density of $745 \mu\text{W}/\text{cm}^3$ based on the total volume of anolyte chamber.

INTRODUCTION

Microbial fuel cells (MFC) use bacteria to convert organic matter in bio-convertible substrate into electricity [1]. MFCs not only can be used to remove organic matter from wastewater and produce renewable energy [2], but also have been suggested as an in-field energy source to power sensors for environmental and process monitoring [3]. However, currently, the main applications remain confined to laboratory-scale devices. Factors limiting the application of MFCs to natural scale plants include high initial capital cost, especially for electrode construction, membranes, and limited power density that can be achieved [2, 4-9].

Miniaturized MFC (μ MFC) technologies have recently received increased attention, because of their potential to deliver significant insights into green power generation and to realize high-throughput screening of different bacterial strains for conversion of substrates to electricity [2, 4-6]. Most variations in μ MFCs have been to optimize anode materials, reduce substrates feeding fuel cell microbes, and optimize bacterial strains. Qian et al. developed a μ MFC using gold as an anode material with a remarkable $1.5 \mu\text{L}$ anode chamber [4]. Choi et al. reported their μ MFC using an optimal *Geobacter*-enriched mixed bacterial culture [5]. Ren et al. demonstrated another device with an increased surface-area-to-volume ratio that led to an improved power density [6]. Also, several three-dimensional (3D) micro/nanomaterials have been demonstrated as high performance anode materials of μ MFCs, due to their larger surface area for bacterial attachment and colonization, and high electrochemical catalytic activity [7-9]. Inoue et al. utilized carbon nanotubes (CNTs) to demonstrate the hypothesis that the electron transport is based on direct attachment of bacteria on the surface of anode [7]. Mink et al. applied multi-walled CNTs as anode to their μ MFC capable of harvesting stable and high power [8]. Our previous work resulted in another μ MFC with a $12 \mu\text{L}$ anode chamber and using electrospun conducting poly(3,4-ethylenedioxythiophene) nanofibers as a 3D porous anode. This device produced a power density of $423 \mu\text{W}/\text{cm}^3$ using *S. oneidensis* MR-1 as a biocatalyst [9]. Generally, these μ MFCs are featured by large surface-area-to-volume ratio, low material consumption, and short start-up time. Despite these considerable efforts, it still remains challenging in obtaining high-current and high-power intensity for μ MFCs, due to their small processing volume and insufficient biofilm formation.

Graphene foam (GF) is a porous conductive structure formed by vapor deposition of graphene onto a 3D mesh of metal filaments. GF has been utilized in electronic devices [10], energy storage and conversion devices [11], and neural tissue engineering. Recently, GF has been used as anode material of medium-to-large size MFCs [10, 12] since GF-based MFCs have relatively large dimensions and demonstrate that the conductive 3D scaffolds are favorable for bacteria colonization and electron transfer. In particular, the pore size of GF is approximately a few hundred micrometers, which enables efficient mass transfer of nutrients and easy access of bacteria to the interstitial space within the 3D anode in order to grow on the surface of scaffolds.

It should be noted almost all existing MFCs employs a similar device structure (with limited variations) where carbon-containing organic substrate solutions flow over the surface of a planar metal anode (e.g. gold and platinum) or 3D micro/nanomaterials based anode (e.g., carbon cloth, CNTs, and GF) laid on the bottom of anolyte chamber or attached to a proton exchange membrane (PEM). During the batch mode operation, mass transport of nutrients to the microbes colonized on the surface of anode is often implemented through a slow diffusion process from the bulk solution outside the anode to the surface or the inside of the anode. In a continuous flow mode, some of the bio convertible substrates are inevitably wasted as they directly flow out from the anolyte chamber through the freeway space outside the anode, without participating in the reaction with the colonized microbes on the surface of anode.

Thus, it is critical to improve the efficiency of electrochemical reaction between the bioconvertible substrates and the colonized microbes in miniaturized anolyte chambers. With continuing efforts in μ MFCs, the volume of substrate solutions decreases dramatically, thus necessitating increasing the efficiency by which the nutrients are made available to microbes colonizing the anode. Here we propose that a porous 3D GF enabled flow-through (FT) mechanism would increase nutrient use of μ MFC for microbial colonization and reduce response time of electricity generation (Fig. 1). The porous GF anode is embedded in the anolyte chamber and sandwiched between a PEM and a gold electron collector at the bottom of the anolyte chamber. With the built-in interconnected 3D pore network, the GF anode can not only provide 3D scaffolds for bacterial attachment and colonization, but more importantly serve as a natural microfluidic porous channel for flowing nutrient solutions over the biofilm formed on the scaffolds. The catholyte chamber located on the other side of the PEM contains carbon cloth, which acts as the cathode. A unique feature of this μ MFC design is that the porous GF anode provides numerous passages for nutritional media to flow through the anode. By minimizing freeway space, nutrient solutions are not wasted through flowing over the anode without interacting with the bacteria colonizing the porous scaffold anode. As a result, nutrient use efficiency should be improved as a consequence of efficient mass transport driven by pressure and fast diffusion of nutrients directly inside the interstitial pores of the GF. Over the micrometer length scale, molecular diffusion is fast because the diffusion time scales as the square of the distance.

Figure 1: (a) Schematic of the proposed FT (left) and non-FT μ MFC devices (right). (b) Schematic of the device components for the FT μ MFC. (c) Optical image of the porous anolyte chamber with an embedded 3D GF anode. Diverging microfluidic channels distribute the anolyte solution flow in a relatively even manner across the width of the chamber. (d) Photo of a $\sim 1 \times 3$ inch³ array of six FT μ MFCs. (e, f) Scanning electron microscopic images of the porous GF anode with a bacterial biofilm formed on the surface of the GF scaffolds. A close up view in image (f) shows the microbial colonization of the scaffolds in (e).

EXPERIMENTAL

The following materials were used for fabrication of the proposed μ MFCs: GF (multilayer graphene film on nickel foam; thickness: 1.2 mm; pore size: 580 μ m; Graphene Supermarket, Calverton, NY), PEM (Nafion 117; Fuel Cells, College Station, TX), carbon cloth (Fuel Cell Store, College Station, TX), acrylic sheets (TAP Plastics, Oakland, CA), glass slides (Corning, Oneonta, New York), a photopolymerizable precursor solution for making microfluidic channels.

Tryptic soy broth (TSB, Sigma-Aldrich, St. Louis, MO) was used as a culture medium for *S. oneidensis* strain MR-1. A lactate defined minimal medium used for electrochemical measurements. Potassium ferricyanide (Fisher Scientific, Fair Lawn, NJ) was used as catholyte solutions. For studying electrochemical activity of GF anode electrode, the phosphate buffered saline (PBS) containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (pH = 7.0) was used, where $[\text{Fe}(\text{CN})_6]^{3-/4-}$ acted as a redox mediator.

The μ MFCs were sterilized by filling all of compartments with a 70% ethanol in water and letting it sit for 20 min at room temperature, followed by flushing the device with sterile DI water for 5 min prior to TSB culture medium for 5 min. The TSB medium flowed into the anolyte chamber through the polyethylene tubing using a programmable syringe pump (210P, KD Scientific, Holliston, MA). To minimize possible oxygen contamination in the batch mode operation, the tubing was closed by steel clamps after the injection of bacterial suspension into the anolyte chamber. The

catholyte solution of potassium ferricyanide was supplied using a syringe pump.

An external resistor (R) was connected between the anode and cathode to form a closed circuit. The voltage potential (U) between the two electrodes was measured using a data acquisition device (Model DI-245; DATAQ Instruments, Akron, OH) and recorded once a minute via DATAQ Instruments Hardware Manager software. The current (I) flowing through the resistor was calculated via $I = U/R$ and the output power was calculated via $P = U \times I$. Electrochemical properties of the GF anode was measured in PBS containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ by an electrochemical workstation (SP1, Zive Potentiostat, Seoul, Korea). A platinum (Pt) wire and a silver/silver chloride (Ag/AgCl) wire were used as the counter and reference electrodes, respectively. Each electrical measurement result given in this paper is representative of the typical result obtained over three independent experiments on three devices.

RESULTS AND DISCUSSION

To illustrate how the FT design affected fluid flow in the anolyte chamber, we conducted hydrodynamic simulation using a finite element method based commercial software package (COMSOL Multiphysics). Fig. 2 shows simulated flow rate distributions of the FT design with the GF anode sandwiched by the PEM and the glass slide, and two non-FT counterparts with freeway space height of three and six times the thickness of the GF anode, respectively. Free- and porous-media flow models were used for the simulations. For the porous media, the porosity, permeability, and

Forchheimer coefficient were set as 0.9, $1 \times 10^{-7} \text{ m}^2$, and $4.35 \times 10^3 \text{ kg/m}^4$, respectively. In the settings for fluid properties, the density, dynamic viscosity, and flow rate were set as $1 \times 10^3 \text{ kg/m}^3$, $1.02 \times 10^{-3} \text{ Pa} \cdot \text{s}$, and $4.6 \times 10^{-4} \text{ cm/s}$ (calculated by a sample volumetric flow rate of $10 \text{ } \mu\text{L/hr}$ used in the experiment), respectively. The width and side length of the porous media were set to 1.2 mm and 5 mm , respectively, based on the geometric parameters used in the device.

Fig. 2a shows that as the pressure driven laminar flow was pumped through the FT porous anolyte chamber, the fluid velocity approaches zero the closer to the walls generating a parabolic velocity profile within the chamber in each case. The parabolic velocity profile has significant implications for the distribution of molecules transported within the anolyte chamber. Specifically, in the FT device all fluids would travel through the porous anode, while in the two non-FT systems (Fig. 2b, c), only a portion of the fluids directly interacted with the GF anode, with a majority of the fluids flowed through the freeway space channel as waste. The wider the freeway space, the smaller the flow rate inside the porous anode and the lower the efficiency use of the medium. Importantly, the FT design will allow for faster nutrient replenishment due to the presence of a higher flow rate inside the GF anode.

Figure 2: Simulated flow rate distributions over the vertical cross-section of the anolyte chambers of three sample μMFCs : a FT (a), and two non-FT devices with the freeway space height (above the GF anode) 3 (b) and 6 times (c) the thickness of the GF anode.

Electric current output of the FT device before and during cell inoculation was monitored using a closed circuit carrying an external resistive load of $11.5 \text{ k}\Omega$. When TSB culture medium was injected into the anolyte chamber the background current without *S. oneidensis* bacteria was as low as $12 \pm 10 \text{ nA}$. When TSB with *S. oneidensis* cell suspensions was injected into the anolyte chamber the output current increased to $\sim 43 \text{ } \mu\text{A}$ within five hours and then decreased gradually over time. Fig. 3 shows the polarization and power density curves of the FT device plotted by measuring the output voltage and current at different external resistive loads. According to the literature [1], a polarization curve can be divided into three regions reflecting activation loss, ohmic loss, and mass transfer loss. At the activation loss stage, the output current increased from zero (measured at the open circuit voltage of 1240 mV) to $18.1 \text{ } \mu\text{A}$. The voltage dropped as the external resistance decreased. In the current range of 18.1 to $44.2 \text{ } \mu\text{A}$, there was a near-linear drop in voltage with increasing electric current. As a result, an internal resistance was estimated to be $7.3 \text{ k}\Omega$ through linear fitting of the curve in the ohmic loss region. In addition, the surface power density reached a maximum of $89.8 \text{ } \mu\text{W cm}^{-1}$ in the ohmic loss region, while the output voltage decreased in the mass transfer loss region.

The device provided $745 \text{ } \mu\text{W/cm}^3$ volume power density based on the total volume of anolyte chamber, and $89.4 \text{ } \mu\text{W/cm}^2$ surface power density and surface current density of $91.8 \text{ } \mu\text{A/cm}^2$ based on the planar surface area of GF anode.

Figure 3: Polarization curve (black) and power density output (red) curves of the FT μMFC as a function of current.

To evaluate the influence of the feeding rate of culture medium on the electric current generation of the FT device, TSB medium was continuously injected into the FT anode at different flow rates after inoculating the chamber with *S. oneidensis*. As shown in Fig. 4a, the output currents at the low flow rates were similar ($\sim 5 \text{ } \mu\text{A}$) to each other. But, with increasing flow rates at or above $10 \text{ } \mu\text{L/hr}$, there were dramatic current increases. It is interesting that the electric currents of FT and non-FT at flow rates between 10 – $40 \text{ } \mu\text{L/hr}$ were very different which FT anode researched to the level of $50 \text{ } \mu\text{A}$, but only a small current increase as the flow rate increased on non-FT anode. Fig. 4b shows the time (t_i) that the FT device and six non-FT counterparts took to generate 80% of their corresponding peak current with respective flow rates of 20 , 40 , and $60 \text{ } \mu\text{L/hr}$. The result shows that the FT design allowed for significant reduction in t_i . The larger the freeway space volume of the non-FT device, the longer the time t_i of the device. Along the length direction of the GF anode in the FT device, mass transport of nutrients was mainly driven by pressure in the continuous flow mode. As a result, the higher the feeding flow rate the shorter the time t_i required. Inside the interstitial pores of the GF anode, nutrients could diffuse to the surface of scaffolds over a short length of approximately $290 \text{ } \mu\text{m}$ or half of the mean pore size of the GF, which also contributed to the short t_i of the FT device. In contrast, the non-FT devices required more time because the nutrients in the freeway space outside the anode diffused over a longer distance to the scaffolds of the GF anode. Although pressure-driven mass flow also occurred in the non-FT devices, the effective amount of nutrients delivered to the colonized microbes was actually less than that in the FT device. As shown in Fig. 4b, when the medium flow rate was set at 20 , 40 , and $60 \text{ } \mu\text{L/hr}$, the response time t_i of the FT device was 4.2 , 3.2 , and 2.6 times, respectively, shorter than that of the non-FT device with $V_{fr} = 6V_{gr}$. Fig. 4c shows the total medium volume consumed to obtain 80% of the peak current increases with increasing freeway space volume. The result demonstrates that with the FT design, the medium consumption reduces by up to 16.4 times and the response time of the device reduces by up to 4.2 times.

Lastly, to reveal the benefit of the FT design, the electrochemical properties of the GF anode are investigated by cyclic voltammetry and electrochemical impedance spectroscopy studies (no figure is shown here) with the lactate defined minimal medium. Compared to the non-FT device, the FT device has a

higher diffusion coefficient ($6.28 \times 10^{-9} \text{ cm}^2/\text{s}$ vs. $1.40 \times 10^{-9} \text{ cm}^2/\text{s}$), a desired lower charge transfer resistance R_{ct} (0.88 vs. 2.96 kohm), and a higher electron transfer rate k_o ($1.13 \times 10^{-10} \text{ cm}^2/\text{s}$ vs. $2.35 \times 10^{-11} \text{ cm}^2/\text{s}$).

Figure 4: (a) Maximum output current of the FT and non-FT devices at different TSB medium flow rates. The non-FT devices used here have the freeway space volume V_{fr} varying from V_{gf} to $6V_{gf}$ (V_{gf} represents the volume of GF anode). (b) Time required for the FT and non-FT devices to obtain 80% of the peak output current as a function of freeway space volume of the devices. (c) Total volume of TSB medium consumed to obtain 80% of the peak output current as a function of freeway space volume of the devices.

CONCLUSION

We have demonstrated a FT μMFC with a porous GF anode sandwiched by a PEM and an electron collector at the bottom of the anolyte chamber. Mass transport of nutrients was mainly driven by

pressure. In addition, molecular diffusion of nutrients to the biofilm on the scaffolds occurred directly inside the pores of the GF over a short length scale. Therefore, the present FT design allowed reducing bioconvertible substrate consumption, enhancing nutrient use efficiency to sustain energy production of the biofilm, and shortening response time of current generation.

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