A Stackable, Two-chambered, Paper-based Microbial Fuel Cell

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Abstract: We developed a stackable and integrable paper-based microbial fuel cell (MFC) for potentially powering on-chip paper-based devices. Four MFCs were prepared on a T-shaped filter paper which was eventually folded three times to connect these MFCs in series. Each MFC was fabricated by sandwiching multifunctional paper layers for two-chambered fuel cell configuration. One drop of bacteria-containing anolyte into the anodic inlet and another drop of potassium ferricyanide for cathodic reaction flowed through patterned fluidic pathways within the paper matrix, both vertically and horizontally, reaching each of the four MFCs and filling the reservoir of each device. Bacterial respiration then transferred electrons to the anode, which traveled across an external load to the cathode where they combined with protons. The MFC stack connected in series generated a high power density ($1.2\mu\text{W}/\text{cm}^2$), which is two orders of magnitude higher than the previous report on the paper-based MFC stack. This work will represent the fusion of the art of origami and paper-based MFC technology, which could provide a paradigm shift for the architecture and design of paper-based batteries.

Keywords: Paper-based batteries, microbial fuel cells, origami techniques, battery stack

1. Introduction

Microfluidic paper-based analytical devices (μ PADs) have been recently developed as a simple and low-cost paradigm for fluid manipulation and analytical/clinical testing (Martinez et al., 2010). Compared to traditional microfluidic technologies, paper has many distinct advantages that make it especially suitable for point-of-care and point-of-use testing with simple-to-use and low-cost solutions (Lisowski and Zarzycki, 2013). For example, μ PADs do not require external pumps/tubing to transport liquid and can be readily disposable via incineration (Yetisen et al., 2013). Therefore, μ PADs have attracted a growing interest for applications in many areas such as health diagnostics, environmental monitoring and food quality control. However, there are significant challenges in developing μ PADs at a system level that contains an integrated, paper-based power source (Thom et al., 2012; Thom et al., 2013; Choi, 2016). Most of the applications developed so far are still powered by conventional batteries, which are several times larger in size and weight, and are more expensive than the μ PAD itself. The paper-based power source is essential to creating a complete paper-based system that can work independently and stand-alone/self-sustainable μ PADs will provide the most effective analytical tools even in resource-limited settings.

Various paper-based power sources and energy storage devices have been demonstrated for μ PAD applications (Nguyen et al., 2014) such as Li-ion batteries (Wang et al., 2012; Leijonmarck et al., 2013; Hu et al., 2013), supercapacitors (Ge et al., 2013; Yuan et al., 2012), and galvanic cells (Thom et al., 2012; Thom et al. 2013). Due to their intrinsic characteristics, the paper-based power sources and energy storage devices show outstanding performances while retaining low-cost, flexibility and

1 disposability. Recently, Choi group pioneered the novel biofuel cell platform on paper
2 as an alternative power source for μ PADs (Fraivan et al., 2013; Fraivan et al., 2014a).
3 They fabricated microbial fuel cells (MFCs) on paper to harvest bioelectricity by
4 converting biological energy in biomass directly into electrons via microbial metabolism.
5 The paper-based MFC allowed for (i) simple fabrication/design for microfluidic and
6 microbial handling, (ii) rapid adsorption of liquid agents through capillary force of paper
7 and (iii) immediate redox reactions for rapid power generation. Since their first
8 demonstration of the MFC on paper, many other paper-based MFCs have been
9 proposed with further reduction of the fabrication complexity while showing their
10 remarkable applicability for μ PADs (Veerubhotla et al., 2015; Zuo et al., 2015; Winfield
11 et al., 2015). However, the obtainable current and voltage of a single paper-based MFC
12 unit was relatively low and insufficient for use in practical applications. Consequently, a
13 stack of MFCs was required to obtain the desired power performance. Recent
14 connection of four paper-based MFCs in series provided the desired current and voltage
15 outputs, powering a red LED for over 30 minutes (Fraivan et al., 2014b). However, the
16 simple electrical connections between the single MFC units were not suitable for
17 compact and easily operated paper systems since they required a large footprint with a
18 liquid introduction process. Moreover, the paper-based MFC had to use a non-paper
19 electrode (e.g. Carbon cloth), which hampered the easy systems integration onto paper.
20 Furthermore, the recent development of a paper-based MFC stack with a single-
21 chambered air-cathode configuration still suffered from (i) extremely low power
22 performance due to high cathodic over-potential and (ii) complicated operation
23 procedures requiring repeated paper folding/unfolding processes (Lee and Choi, 2015).

1 In this work, we created a practical, easy-to-use, high performance paper-based
2 MFC by using two-chambered fuel cell configuration. By folding the MFC array pre-
3 defined on the T-shaped filter paper, four MFCs were connected in series and ready for
4 operation without further manual operation. When one drop of the anolyte and catholyte
5 was applied onto the anodic and cathodic inlets, respectively, 1.6V was generated as an
6 open circuit voltage and $1.2\mu\text{W}/\text{cm}^2$ of maximum power density was produced under
7 $500\text{k}\Omega$ external resistor, which is more than two orders of magnitude larger than the
8 recent paper-based MFC stack (Lee and Choi, 2015).

10 **2. Materials and Methods**

11 *2.1 Operating Principle*

12 Most microorganisms use respiration to convert biochemical energy stored in organic
13 matter into biological energy, adenosine triphosphate (ATP), where this process
14 involves a cascade of reactions through a system of electron-carrier biomolecules in
15 which electrons are transferred to the terminal electron acceptor (Torres et al., 2010;
16 Choi, 2015). Most forms of respiration use a soluble compound as an electron acceptor,
17 such as oxygen, nitrate and sulfate; however, some microorganisms are able to respire
18 solid electron acceptors to obtain biological energy. These microorganisms can transfer
19 electrons produced via metabolism across the cell membrane to an external electrode.
20 MFCs are typically comprised of anodic and cathodic chambers separated by a proton
21 exchange membrane (PEM) so that only H^+ or other cations can pass from the anode to
22 the cathode. A conductive load connects the two electrodes to complete the external

circuit. Unlike other MFC experiments that use a sole carbon source for bacterial metabolism, we utilized a common rich medium known as Luria-Bertani (LB) broth. This medium allows for robust growth of all bacteria used in our study. The electrons travel to the cathode, where the redox couple is completed when the captured electrons reduce ferricyanide $[\text{Fe}(\text{CN})_6]^{3-}$ at the cathode (eqn (1)) (Choi et al., 2011).

In the meantime, protons pass from the anode to the cathode across a proton exchange membrane to maintain the electro-neutrality in the system.

2.2 Anolyte and catholyte

Shewanella oneidensis MR-1 were grown from -80°C glycerol stock cultures by inoculating 20mL of L-broth medium with gentle shaking in air for 24h at 35°C. The L-broth media consisted of 10.0g tryptone, 5.0g yeast extract and 5.0g NaCl per liter. The culture was then centrifuged at 5,000rpm for 5min to remove the supernatant. The bacterial cells were re-suspended in a new LB medium and used as an anolyte for the device. Growth was monitored by measurement of the optical density at 600 nm (OD_{600}) and the culture we used reached an OD_{600} of 2.0. The catholyte was 50 mM potassium ferricyanide in a 100 mM phosphate buffer in which pH was adjusted at 7.0 with 0.1 M NaOH. 50 μ L of anolyte and catholyte solutions were dropped on the anodic and cathodic inlet windows, respectively.

2.3 Device Fabrication

Figure 1. Schematic diagrams of the paper-based MFC array. The MFC used (i) screen printed carbon electrodes on paper for bacterial attachment/cathodic reaction and (ii) paper reservoirs for holding the anolyte and catholyte.

Four paper-based MFCs were prepared on a single paper sheet (Whatman #1 filter paper) by sandwiching multifunctional paper layers (Fig. 1); (i) screen-printed carbon anode/cathode on paper substrates, (ii) paper anodic/cathodic reservoirs, and (iii) parchment paper based proton exchange membrane (PEM). Each one of the paper layers were patterned with printed wax boundaries with controlled precision using a commercially available solid-wax printer (XeroxColorQube 8570dn). The wax patterns were then heat treated at 150°C for 2min to allow for complete penetration of the wax through the paper matrix. AutoCAD software was used to design the wax patterns on the paper sheet. The PEM minimized the anolyte and catholyte transfer while maintaining efficient proton transfer (Fraivan et al., 2014b). The electrical contacts for each MFC were prepared by screening-printing carbon paste through the holes (Fig. 1),

which allowed the series connection of the MFCs when folded. All cutting and mask patterning processes were executed using a laser machine (Universal Laser Systems, VLS 3.5). Each paper layer was combined using a spray adhesive (3M Super 77 Spray Adhesive) that offers very simple, mass production-friendly fabrication. The paper reservoir layers were extended toward the windows, which formed stair-type inlets for anolyte and catholyte introduction. The circular anode/cathode has 1.7cm diameter and about 2.27cm² area. Each square MFC has 3.5cm x 3.5cm which is also the final size of the folded MFC stack.

Figure 2. Schematic diagrams of the origami paper-based MFC stack showing the folding steps. The carbon electrodes of each MFC were connected by folding. The four MFCs were stacked in series.

2.4 Device operation

Fig. 2 shows the folding steps of the assembled paper-based MFC array. MFC 3 was first folded to connect the cathode of MFC 3 and the anode of MFC 4. Then, MFC 2 was

folded over MFC 3. Finally, MFC 1 was folded over MFC 2 to alternatively connect the anodes and cathodes of all the MFCs. The anodic and cathodic inlets for each MFC appeared in the peripheral windows, respectively. To maintain the structure of the stacked MFC and assure strong connections between each individual MFC, two paper clips were used to hold it together.

2.5 Measurement Setup

We measured the voltage difference between the anode and the cathode with a data acquisition system (NI, USB-6212) and recorded the results every 20 seconds via a customized LabVIEW interface. An external resistor was used to close the circuit. The current was calculated via Ohm's law, and the power output was calculated via $P = V \times I$.

2.6 Bacterial fixation and SEM imaging

The individual MFCs in the stack were disassembled, rinsed, and adherent bacteria on the anodic paper reservoirs and the surface of the anode carbon electrodes were immediately fixed in 2% glutaraldehyde solution overnight at 4°C. Samples were then dehydrated by serial 5 min transfers through 50, 70, 80, 90, 95, and 100% ethanol. Fixed samples were examined using a FESEM (Field Emission SEM) (Supra 55 VP, Zeiss).

3. Results and Discussion

3.1. Sample Introduction

After the MFC stack was fully folded and connected in series, two inlets offered access to the anode and cathode reservoirs through which the bacteria-containing anolyte and ferricyanide catholyte were introduced, respectively (Fig. 3).

The solutions wicked through the paper reservoirs and across the surface of the electrodes

without the need for any external pumps/tubing. This absorption promoted bacterial attachment to the anode and immediate electron transfer from bacterial metabolism, resulting in a short start-up time. The solutions were able to be transported horizontally and vertically, filling the reservoir of each MFC and reaching the different MFC's at the same time. The large pore sized filter paper (Whatman #1; ~10 μm) allowed bacterial movement through the paper matrix and a higher volume of sample to be absorbed into the reservoir, consequently providing a longer duration of stable current generation. Our previous research showed that the bacterial cell transportation through the paper matrix was highly dependent on the pore size and the sample volume (Choi and Choi, 2015). A small sample volume along with a small pore sized filter paper will not

Figure 3. Photo images of (a) the assembled MFC array, (b) folded MFC stack and (c) sample injection process. We introduced *Shewanella oneidensis* MR-1 as an anolyte and potassium ferricyanide as a catholyte. The potential difference between electrodes was measured by a data acquisition system.

1 generate enough capillary force to move the liquid and bacterial cells to the end of each
2 layer. Therefore, optimizing pipetting volume of solutions and selecting proper pore size
3 of the paper is critical. Whatman #1 has large pore size (10 μ m) enough for moving the
4 2 μ m-sized *Shewanella* sp. Also, the rapid and full sufficiency of the sample over all the
5 MFC reservoirs was obtained with the pipetting volume of 50 μ L.

8 3.2 Open circuit voltage (OCV)

9 The open circuit voltage (OCV) of the
10 MFC stack was first measured (Fig. 4).
11 Once the anolyte and catholyte were
12 introduced, the stack instantly generated
13 1.15V and then gradually increased up to
14 1.6V within 3 minutes, which is equal to
15 the sum of each MFC's OCVs ($\sim$ 0.4V).
16 This OCV value is lower than those of

Figure 4. Open circuit voltage measured from the MFC stack.

17 typical non-paper-based MFCs ($\sim$ 600mV), which indicates that oxygen diffusion into the
18 paper-based layers was more severe than the conventional MFC device and the oxygen
19 abiotically reacted with the anode, decreasing the OCV to some degree (Choi et al.,
20 2011). However, the MFC stack was systematically stable generating a steady OCV,
21 which indicates that the device can be used as a reliable power source for real world
22 applications.

3.3 Current/power generation

Following the OCV measurement, we connected a 100k Ω resistor between the anode and the cathode to close the circuit (Fig. 5a). A stable current of approximately 5 μ A was produced for more than 20 minutes. This is due to the fact that the paper reservoirs enabled the device to retain the anolyte and catholyte for extended time intervals allowing for more bacterial metabolic reactions inside the MFC. A cell polarization curve and power output of the MFC stack were derived and calculated based on the saturated current value at a given

external resistance (1M, 700k, 500k, 300k, 100k, and 10k Ω) (Fig. 5b). The maximum power was 2.88 μ W (1.27 μ W/cm²) at 500k Ω , which is two orders of magnitude larger than our previous paper-based MFC stack (Lee and Choi, 2015). The significant power improvement is mainly due to the two-chambered configuration using potassium ferricyanide as an electron acceptor instead of oxygen from ambient air. This chemical provided fast cathodic reaction and low overpotential, ultimately leading to power enhancement. Moreover, using the catholyte avoided manual folding/unfolding processes to expose air-cathodes to the air for their cathodic reactions (Lee and Choi,

Figure 5. (a) output voltage measured from the MFC stack with or without the external resistor (100k Ω) and (b) polarization curve/output power as a function of current.

2015). Using the polarization curve in Fig. 5b, we estimated the internal resistance, which is equal to the external resistor values where the maximum power density is obtained (Fan et al., 2008). The internal resistance of the MFC stack was about 500k Ω , which is several orders of magnitude higher than that of other conventional MFCs (several Ω) (Choi et al., 2011). The high internal resistance observed in the experiments is due to the vertical discontinuity between layers. Although paper clips mechanically clamped the layers, the stacked layers might not be tightly maintained. A more efficient clamping method would enhance the power performance of the MFC by decreasing the internal resistance between the layers.

3.4 Bacterial electron transfer through paper-based MFC

Electric current production mechanisms of the paper-based MFC can be explained by analyzing electron microscope examinations of the anode and paper reservoir (Fig. 6). Very

Figure 6. SEM image of the (a) paper reservoir and (b) carbon anode used in the paper-based MFC after current generation. A large number of bacteria cells were found on both layers. Inset: individual bacterial cells. Scale bar is 2 μ m.

densely packed bacterial cells were observed on the paper reservoir layer (Fig. 6a), while a less number of cells was found on the anode surface (Fig. 6b). The strong capillary force of the paper reservoir allowed for rapid adsorption of the anolyte and promoted attachment of the cells onto the paper matrix and then anode. *Shewanella oneidensis* used in this experiment can be found simultaneously as planktonic cells and biofilms, and both types are capable of generating electricity alongside each other

(Borole et al., 2011). Theoretically, *Shewanella oneidensis* can conduct extracellular electron transfer (EET) via three mechanisms: (i) direct electron transfer, where the cells adhere physically to the anode surface and transfer electrons to the anode, (ii) shuttle transfer, where the electrons are transferred to the anode via electron mediators, and (iii) nanowire transfer, where a solid conductive wire is biosynthesized and used for electron transfer (Torres et al., 2010; Lovley, 2012). Bacterial electron transfer mechanisms in this paper-based MFC are mainly based on direct electron transfer. This is because other electron transfer mechanisms such as shuttle transfer and nanowire transfer are unlikely. All shuttling chemical compounds were removed by centrifugation before the anolyte injection and nanowire production is expected to require a relatively long period of time compared to the total experimental time. Furthermore, no biofilm formation was observed on the anode surface. If we can promote more bacterial cells on the anodic surface or increase the interaction between the cell and anode, we believe that the performance of the paper-based MFC will increase substantially.

4. Conclusions

We developed a stackable and integrable paper-based MFC as an alternative power source to expensive and bulky batteries. The 3D MFC stack was created from 2D paper sheets through high degree of folding and multifunctional layers sandwiched for two-chamber configuration. The MFC stack generated a $1.27\mu\text{W}/\text{cm}^2$ maximum power density and 1.6V open circuit voltage from microbial metabolism with a 50 μL drop of anolyte and catholyte, respectively. This novel MFC stack will be a simple, low-cost, and easy-to-use power source for potentially powering on-chip μPADs .

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Figure Captions

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