A SALIVA-POWERED PAPER BIOBATTERY FOR DISPOSABLE BIODEVICES

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ABSTRACT

We created a paper-based microbial fuel cell (MFC) which generated power from human saliva. Upon adding one drop of saliva, the dried exoelectrogens, pre-inoculated in a conductive paper reservoir of the MFC, activated their respiration by oxidizing organic substrates (e.g. glucose) in the saliva and transferring electrons to the anode. The device generated the maximum current density of 10.5µA/cm^2 and power density of 1.1µW/cm^2 with the glucose concentration of 19.4mg/dl in the saliva, which is enough to be directly used as an energy harvester in portable point-of-care (POC) devices. The biobattery fuses the art of paper microfluidics and the technology of biofuel cells and has the potential to shift the paradigm for disposable POC diagnostic tools by providing a practical power supply readily accessible everywhere. Furthermore, its electron harvesting capability as a quantitative indicator for the presence of organic substances (e.g. glucose) in saliva can potentially be utilized as a self-powered biosensor for monitoring the matter levels.

INTRODUCTION

Paperfluidics and papertronics have recently emerged as a simple and low-cost paradigm for disposable point-of-care (POC) diagnostics [1]. Stand-alone and self-sustained paper-based POC devices are essential to providing effective and life-saving treatments in resource-limited settings [2]. Such areas generally have weak laboratory infrastructure and inadequately serviced equipment, lack essential consumables and reagents, and have limited access to clean water. Above all, the electricity grid is not well established and the use of commercial batteries is not cost-effective. A stable power supply is the most critical factor in developing practical paper-based POC devices because their diagnostic performance and portability depend significantly on power availability [2]. A realistic and accessible power source is needed for actual paper-based POC applications in those challenging areas. Among many flexible and integrative paper-based batteries and energy storage devices with a large upside potential [3, 4], paper-based microbial fuel cell (MFC) technology is arguably the most underdeveloped [5-9]. Even so, excitement is building, as microorganisms can harvest electrical power from any type of biodegradable sources (e.g. wastewater) that are readily available in resource-limited settings [10, 11]. However, the promise of this technology has not been translated into practical power applications because of its low performance (~nW/cm^2) and lack of battery activation methods (limited to wastewater or biomasses). The environmental liquid for the battery activation may not be available, especially in dry, desert climates and their absence would prevent the MFC from on-site operation. Therefore, we need to develop a fundamentally different strategy to effectively use MFCs to power POC diagnostic devices in resource-limited settings. If one of the biggest arguments for MFC technology is the potential for energy accessibility in resource-constrained settings, then there is a clear and pressing need to discover powerful yet simple methods for on-demand bacterial power generation.

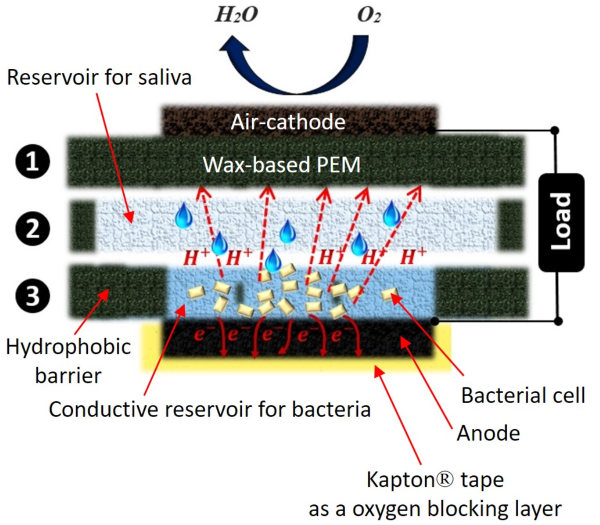

Figure 1: Schematic diagram of a cross section of the device.

In this work, a more abundant resource, human saliva, was demonstrated to be an easily accessible fuel for electricity production in MFCs. The saliva includes biodegradable organic substrates (e.g. glucose) that contain chemical energy convertible to electoral energy by MFCs. This novel saliva-powered MFC will be a simple, low-cost, easy-to-use, and disposable power supply for potentially one-time use POC diagnostic devices even in the most resource-limited settings. Moreover, the saliva-based MFC itself can be a self-powered biosensor for a diagnosis of diabetes by measuring changes in current generation from the glucose oxidation.

METHODS AND MATERIALS

We used a novel origami technique for fabricating the paper-based MFC. The 3-D battery was created by folding a 2-D sheet of paper having several functional components; an anode, a conductive reservoir, a proton exchange membrane (PEM), and an air-cathode (Fig. 1). To increase the electrical conductivity for efficient electron transfers from the bacterial cells, the anode reservoir was treated with conductive poly(3,4-ethylened ioxythiophene): polystyrene sulfonate (PEDOT:PSS). The PEDOT:PSS inks covered the paper fibers that retain the porosity and hydrophilicity [12]. Therefore, we could obtain the collective and thus high electricity generation harvested from all the bacterial cells placed throughout a paper (Fig. 1).

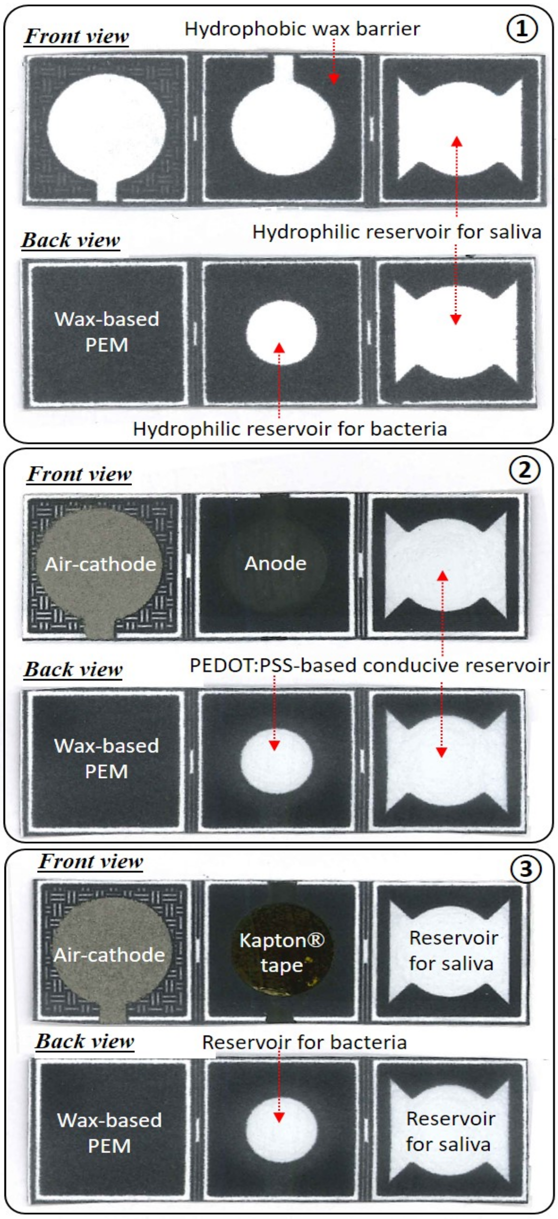

Figure 2: Fabrication processes of the paper-based MFC.

Device fabrication and operating principle

The 2-D sheet of paper was designed and patterned to have folding tabs on which a conductive reservoir with PEDOT:PSS, a graphite ink-based anode with another conductive reservoir for bacterial inoculation, and an air-cathode with a wax PEM (Fig. 1 & Fig. 2). The hydrophilic regions were prepared with hydrophobic wax boundaries. Kapton® tape was used to minimize the oxygen invasion from the anode. By folding the tabs along pre-defined creases, an anode/reservoirs/PEM/air-cathode sandwiched construct was well aligned (Fig. 3). When one drop of organic liquid was added, it passed through patterned conductive reservoir within the paper matrix and activated the bacterial cells pre-defined in the device, triggering their respiration by oxidizing the organic substrates in the organic fuels (e.g. saliva) and transferring electrons to the anode.

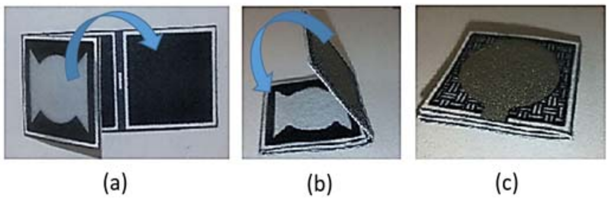

Figure 3: Photo-images of the 3-D origami battery showing the folding steps.

Anode and conductive anode reservoir

A mixture of 1wt% PEDOT:PSS (Sigma-Aldrich), 5wt% dimethyl sulfoxide (DMSO, Sigma-Aldrich) and deionized (DI) water was pipetted into the defined anode reservoir to make it conductive and porous and 3-glycidoxypropy-trimethoxysilane was added to improve hydrophilicity (Figure 2). After the reservoir was air-dried in a fume hood, graphite ink with activated carbon (AC) was screen printed on the electrode side of the reservoir, and subsequently baked in a ventilated oven.

Air-cathode

An AC-based air-cathode was constructed on one side of the paper with carbon spray to provide structural support and to function as a current collector. Then, the AC catalysts (CABOT Corporation) with a binder solution was applied on top of the carbon-sprayed paper zone, which was subsequently air-dried for 24h. The binder solution was prepared by adding a mixture of (i) 1200µL of 5 wt% Nafion solution, (ii) 150µL of DI water, and (iii) 600µL of isopropanol into a beaker, followed by ultrasonication for 1 min. Due to the porous structure of the paper, the other side of the paper allowed the oxygen to reach carbon to complete the redox reaction for the MFC operation.

Inoculum Preparation

Shewanella oneidensis MR-1 were grown from -80ºC glycerol stock cultures by inoculating 20 mL of L-broth (LB) medium with gentle shaking for 24 h at 37ºC. 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 5000 rpm for 5 min to remove the supernatant, and resuspended in fresh L-broth medium. Growth was monitored by measurement of the optical density at 600 nm (OD_{600})

Test Setup

We measured the potentials between the anodes and the cathodes with a data acquisition system (National Instrument, USB-6212), and recorded the readings every 1 min via a customized LabView interface. An external resistor connected between the anode and the cathode closed the circuit. The current through this resistor was calculated using Ohm's law.

RESULTS AND DISCUSSION

Bacteria inoculation

In our previous work on paper-based MFCs, we used bacterial cells cultured only for several hours for the MFC operation [8]. Although this simple culture method can be applicable for power generation, the device cannot be made operable as a rapid and easy-to-use power source for

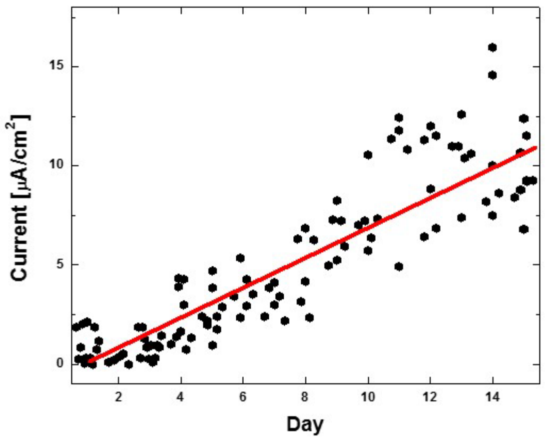

Figure 4: Current generation from devices with pre-inoculated bacteria in the reservoir for different days

the urgent POC testing. Furthermore, a local environment may not host many electrogenic bacterial strains needed for the efficient operation of MFCs. Even worse, the environmental liquid may not be available, especially in dry, desert climates. Therefore, we need a new approach to useing MFCs for POC diagnostic devices in those challenging field conditions. In this work, we pre-inoculated *Shewanella oneidensis MR-1* in the paper reservoir up to 2 weeks and sealed the device to prevent the evaporation of the media. The device was air-dried for an hour right before the use for power generation with additional organic fuel (i.e. saliva). As shown in Figure 4, bacterial cells were able to survive long enough and gradually increased current outputs under a 1kΩ external resistor even at 15 days from inoculation. This indicates that the bacterial cells were still growing and proliferating in the sealed paper reservoir.

Current and power generation

While several macro-sized MFCs activated with urine have been proposed [13], reported work about other body fluids such as saliva for the MFCs and especially for portable power supplies is either unavailable or quite limited. Furthermore, we lack fundamental knowledge about bacterial electrogenicity in the human saliva. A new kind of MFCs that can turn the saliva into electricity could revolutionize the way we produce power particularly in resource-limited environments. If this new MFC validated, a self-powered POC testing device will be replicated, allowing patients to self-test and self-manage care at their home, accident sites and battlefield. For a proof-of-concept demonstration of our hypothesis, paper-based MFCs were developed for harnessing energy from artificial saliva. Various artificial saliva samples with different glucose concentrations were tested and compared to that of the most common rich organic fuel, LB media. Four different organic fuels were introduced onto the dried reservoir with pre-inoculated bacteria; (i) LB media, (ii) artificial saliva without glucose, (iii) artificial saliva with 7.8mg/dl glucose (the normal concentration in people without diabetes) [14], and (iv) artificial saliva with 19.4mg/dl glucose (the abnormal concentration in patients with diabetes) [14]. The polarization curves and power outputs from the four

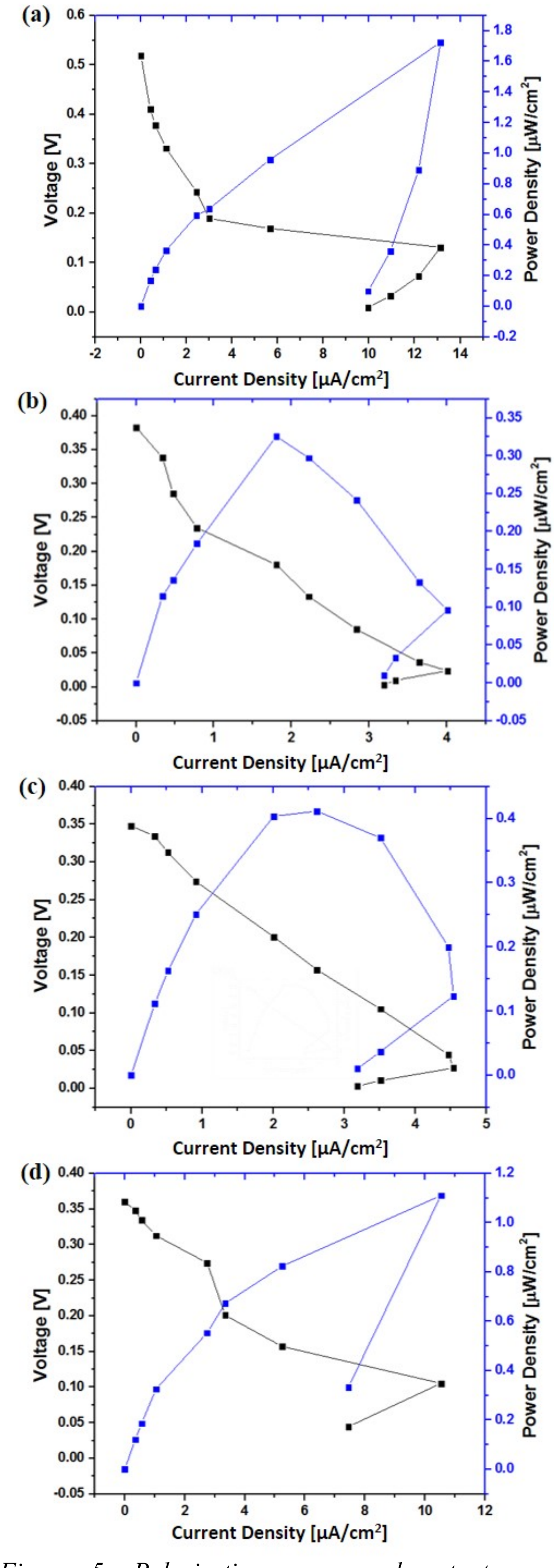

Figure 5: Polarization curve and output power measured as a function of current. (a) LB media, (b) artificial saliva without glucose, (c) artificial saliva with 7.8mg/dl glucose, and (d) artificial saliva with 19.4mg/dl glucose.

different organic fuels were derived and calculated based on the saturated current value at a given external resistance (Figure. 5). Measured OCVs varied between the different fuels. The devices with (i) LB media generated the highest

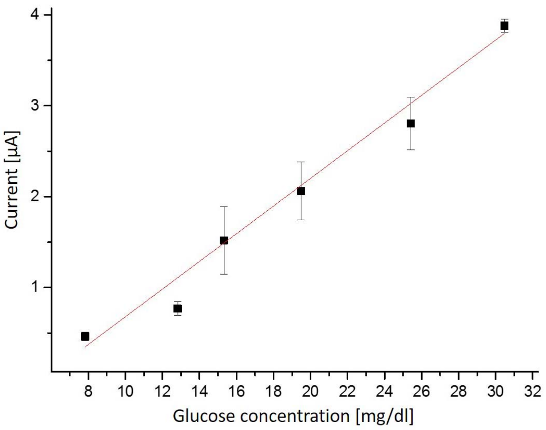

Figure 6: Calibration curve of the MFC's output current vs. the concentration of glucose

OCVs among all the other samples. The highest power and current density (1.72µW/cm^2 and 13.4µA/cm^2) was obtained by (i) LB media, followed by (iv) artificial saliva with 19.4mg/dl glucose (1.1µW/cm^2 and 10.5 µA/cm^2). The results demonstrate that the saliva can be an accessible fuel for electricity production in MFCs. This novel energy harvesting technique will revolutionize the application of the paper-based MFCs for portable on-demand power generation, delivering on-chip energy to the next generation of paper-based POC platforms.

Glucose monitoring

The electrical outputs of the MFC proportional to the glucose concentration in saliva can create a novel low-cost, self-powered paper-based biosensor platform for glucose monitoring. This is because the MFC can monitor an electrochemical energy conversion as a transducing element for glucose monitoring in the sample, thus obviating the requirement of external power sources and readout instrumentation. Our MFC generated enough current to sensitively resolve varying glucose concentration. Figure 6 shows the MFC's calibration curve. A high linearity is shown (R^2=0.943) with a high sensitivity. Given that the glucose levels in people with diabetes ranged from 10mg/dl to 32mg/dl while the non-diabetic glucose ones ranged from 4.3mg/dl to 12.9mg/dl, our MFC can be used to diagnose diabetes from human saliva.

CONCLUSION

In this work, we created a simple bacteria-powered biobattery on paper as an alternative power source for interfaced, paper-based POC devices, that can be readily operated by one drop of human saliva. This work provided novel approaches to revolutionarily activate the MFC and significantly improved its performance for the practical applications in resource-limited settings. An air-cathode and PEDOT:PSS treated conductive anode reservoirs were used for the paper-based MFC technique, which improved the MFC fabrication on paper and provided higher electrical performance. The saliva-based MFC can also be viable as a self-powered glucose monitoring biosensor.

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