A Papertronic Sensing System for Rapid Visual Screening of Bacterial Electrogenicity

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Abstract. In this work, we report a papertronic sensing system with the ability to achieve easy, rapid, and sensitive characterization of bacterial electrogenicity from a single drop of culture. Paper was used as a device substrate that inherently produces favorable conditions for easy, rapid, and sensitive and potentially high-throughput controlling of a microbial liquid sample. Through an innovative microscale device structure and a simple transistor amplifier circuit directly integrated into a single sheet of paper substrate, a powerful sensing array was constructed, resulting in the rapid and sensitive characterization of bacterial electrogenicity from a microliter sample volume. The microbial current generations were amplified by the transistor providing power to a 4-wide LED circuit board indicator bar for the direct visual readout with the naked eyes. Depending on bacterial electrogenicity, the LED intensity was changed. We validated the effectiveness of the sensor using two known bacterial electrogens (wild-type *S. oneidensis* and *P. aeruginosa*) and hypothesis-driven genetically modified *P. aeruginosa* mutant strains.

1. Introduction

Electromicrobiology, which uses the electricity-producing capability, or "electrogenicity" of bacteria, promises novel technologies that address pressing societal concerns about energy security, environmental protection and remediation, and economic development [1]. The bidirectional bacterial electron exchange generates environmentally sustainable bioelectricity from organic waste [2]. The process produces value-added chemicals, or biofuels, and can perform many other environmentally important functions, such as water desalination, bioremediation, and toxicity detection [3]. These bacterial capabilities have entered a new phase of development with biotechniques in synthetic biology that genetically regulate bacterial metabolic pathways and improve their electrogenic potential [4]. Microbial synthetic biology will develop a fundamentally different strategy to advance electromicrobiology by maximizing the inherent electron-transferring capability of bacteria, translating the technology from laboratory novelty to practical applications. What is needed is a rapid and highly sensitive test array for the electrogenicity of the newly discovered, genetically engineered, bacterial species [5-7]. This is by no means a simple challenge, as the accurate quantitative measurements of bacterial electrogenicity require a long measurement time, continuous introduction of organic fuels, complex device architectures, and labor-intensive operation.

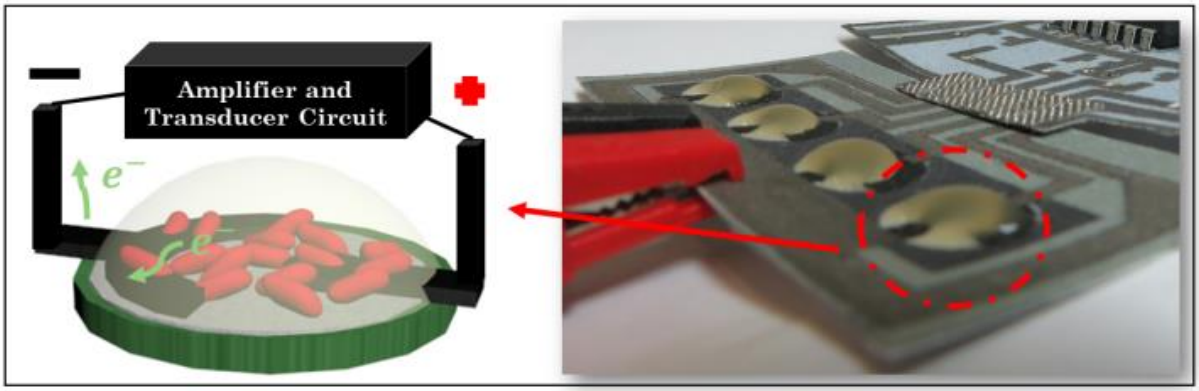

Figure 1: Device working principle. The two-electrode microbial fuel cell is directly incorporated to the amplifier circuit with LED readout.

Even the latest colorimetric method that indirectly measures microbial extracellular respiration suffers from low-sensitivity for quantitative measurements and needs to be further validated as a measure for bacterial electrogenicity [8]. The overall objective of this work is to create the ability to achieve easy, rapid, and sensitive characterization of bacterial electrogenicity from a single drop of culture.

2. Materials and Methods

2.1. Materials

Graphite ink and Ag/AgCl ink were purchased from Ercon Inc. Nickel conductive spray was received from MG Chemicals and Grade 3MM chromatography paper substrates were obtained from Whatman. MPQ7093 IC and SSB-LX2620IW LED assay were purchased from Digikey.

2.2. Inoculum

Wile-type *Shewanella oneidensis* MR1, wild-type *Pseudomonas aeruginosa* PAO1, bdlA (PA1423, Biofilm Dispersion Locus), pmpR (PA0964, PqsR-Mediated Pseudomonas Quinolone Signal (PQS) Regulator PmpR), and fliC/pilA (PA1092, Flagellin Type B, PA4525 Type IV Fimbrial Precursor PilA) were cultured from −80 °C bacterial glycerol stock by inoculating 15 mL of LB medium with gentle shaking in air for 24 h at 35 °C. The LB media consisted of 10.0 g tryptone, 5.0 g yeast extract, and 5.0 g NaCl per liter. The culture was then centrifuged at 5000 rpm for 5 min to remove the supernatant. The bacterial cells were resuspended in a new medium and used as an anolyte for the device.

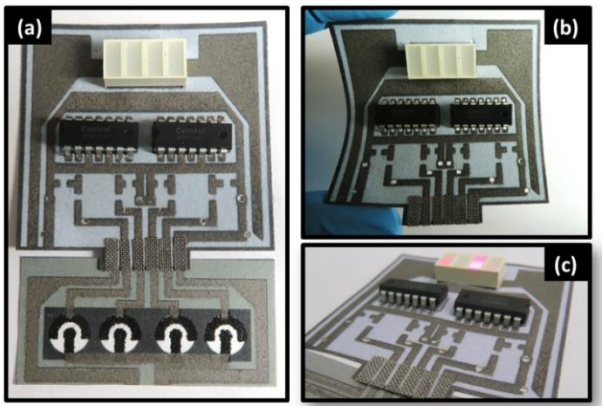

Figure 2: Photos of our papertronic sensing system.

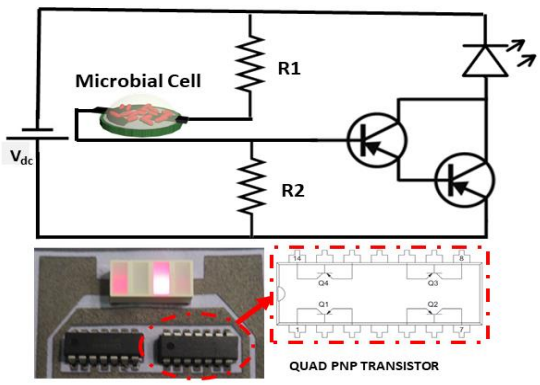

Figure 3: Schematic of the electric circuit and photo of the transistor and the 4-wide LED.

2.3. Fabrication and working principle of two electrode-sensing array

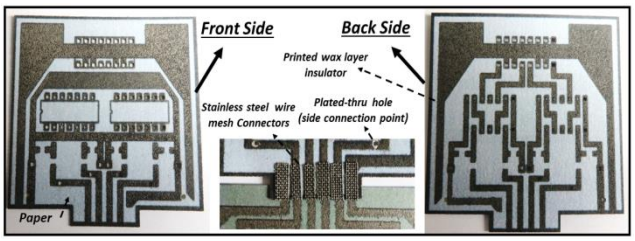

Figure 4: Printed Circuit Board (PCB) on paper substrate.

The proposed papertronic sensing system was fabricated on Whatman#1 filter paper (001-329 Grade 1) by patterning four hydrophilic regions for the anodic reservoirs (Figure 4). These anodic areas were defined by printing wax boundaries with the solid-wax printer (Xerox Phaser8570dn, Xerox Corps ration, Norwalk, CT, USA). The wax patterns were then heat-treated at 130 ºC for 60 seconds to allow complete penetration of the wax through the paper. AutoCAD software was utilized to design the wax patterns on the paper sheet. Then, we constructed two graphite ink electrodes by screen printing method on top of the anodic reservoir. The backside of anodic reservoir was covered by the printed wax to avoid any leakage between the anode and cathode. Our hypothesis is that when we apply DC voltage to the two electrodes, the negative electrode becomes the anode where the bacteria cells attached to it (Figure 3). The produced electrons from the bacterial metabolism are transferred to the anode and moved to the cathode. The electrochemical potential was zero between two equal electrodes before applying the DC power source. When the reservoir was filled with the media or any bacterial inoculum, the cell resistance decreased from the infinite to a few mega ohms, allowing for the bacteria-electrode electron exchange with the DC potential application (Figure 3). Individual bacterial cells have different electrogenic capabilities.

3. Test Setup and Results

The simple sensing platform was obtained by integration of two transistor amplifier (MPQ7093) with four two-electrode microbial sensing wells connected to a four-array LED (Figure 1 & 2). Two resistors (R_1: 200KΩ and R_2: 1MΩ) and metallic wires were patterned on the paper (Figure 4). The sensing well consisted of two graphite electrodes and a microporous/hydrophilic/conductive chamber (Figure 2). The intrinsic capillary force of the paper and the increased capacity from the engineered chamber allowed for rapid adsorption of the bacterial sample and promoted immediate microbial cell attachment to the electrode, leading to instant current generation with even a small amount (microliter range) of liquid (Figure 2). With the increasing concentration of bacterial cells in the sensing well, the resistance of the well decreased due to the more electron transfers from the bacteria to the electrode (Figure 3), resulting in opening of the transistor and consequent powering LEDs (Figure 5a). The LED intensity revealed significant differences between the respective bacterial strains (Figure 5b & 5c). This work (i) created the paper-based approach for biosensing applications, incorporating fluidic and

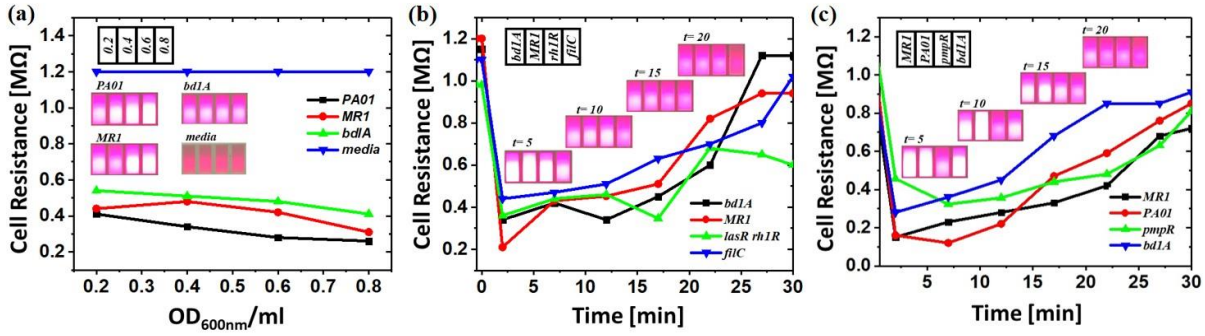

Figure 5: (a) Cell resistivity and optical output of different bacteria concentration, (b) Cell resistivity and optical output of different bacterial strains (bd1A, MR1, rhlR & filC), (c) Cell resistivity and optical output of different bacterial strains (MR1, PA01, pmpR & bd1A)

electronic components, which will augment early work in papertronics and (ii) provided easy, rapid, and sensitive analysis of bacterial electrogenicity.

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