3-D PRINTED REDOX-ACTIVE ORGANIC ELECTRODES TO BRIDGE ACROSS BIOLOGY AND ELECTRONICS

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ABSTRACT

An intimate and direct interface between inorganic electronics and living organisms will revolutionize the next generation of bioelectronics by bridging the signal and material gap between these two different fields. In this work, a redox-active microbial electrode is constructed as the novel interface by simultaneously 3-D printing and electropolymerizing 3,4-ethylenedioxythiophene (EDOT) in a liquid containing electrochemically active bacteria. A custom-made 3-D printer with a concurrent electrochemical control allows a scalable, template-free deposition of electrochemically active organic electrodes in a single printing. Electropolymerized poly(3,4-ethylenedioxythiophene) (PEDOT) acts as redox-active bridges by exploiting extracellularly transferred electrons generated from the bacterial respiration, constructing a seamless contact between the biological processes and the external abiotic systems.

KEYWORDS

3-D printing, redox-active, organic electrodes, bioelectronics, electrochemically active bacteria

INTRODUCTION

Recently, integrating living microbial cells with other building materials has attracted significant attention and has become a rapidly emerging field of study called "engineered living materials (ELMs) [1]." The Defense Advanced Research Projects Agency (DARPA) created the ELM program to significantly improve techniques for the manufacture and maintenance of military systems by exploiting the unique biological functional features. Specifically, electrochemically active bacteria have been considered an excellent biological component for the construction of redox-active electrodes to connect abiotic electronics and biotic living systems. The abiotic electronics exclusively use electrons for the signaling while the living systems rely on ionic molecules for communication, which makes it difficult to bridge the gap between these two fields. By responding to the metabolic ion gradients, the electrochemically active bacteria can generate electrons that can be used to connect the biological system and the inorganic electronics. Despite the vast potential and promise of this technique for the next generation of bioelectronics, there exists an unaddressed need to develop a simple, rapid, and controllable method to combine electrogenic bacteria with electrodes. In 2018, The Ajo-Franklin research group developed a novel technique to embed the bacteria in an electropolymerized polymer [2]. However, the combined material was not able to be patterned in a controllable manner. At Hilton Head 2020 workshop, we demonstrated an innovative proof-of-concept method to fabricate a bacteria-containing living electrode by combining a controllable 3-D printing method and a simple electrochemical polymerization technique [3]. The monomer precursor 3,4-ethylenedioxythiophene (EDOT) was printed into a 3-electrode electrochemical cell that contained microorganisms. The monomer was in-situ electrochemically polymerized to the conducting poly (3,4-ethylenedioxythiophene) (PEDOT) polymer, which entrapped and connected the electrochemically active bacteria to the electrode within 35 min. However, the electroactive materials were limited to the pre-defined microfluidic template with the 3-electrode system. The template could not allow any meaningful functions other than for studying purposes of the materials.

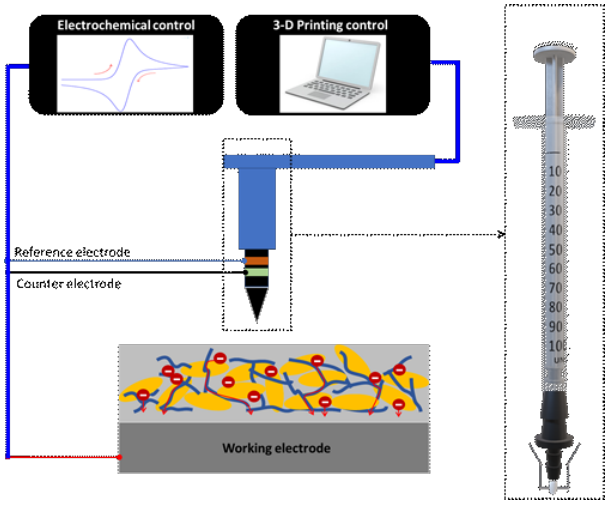

Figure 1: Electrochemical 3-D printing system. Simultaneous 3-D printing and electropolymerizing of a bacteria-containing polymer can construct a seamless biotic-abiotic interface.

TEST SETUPS AND RESULTS

In this work, we created a 3-D, template-free, microbial bioelectrode in a systematic and automated manner by using an electrochemical additive manufacturing technique (Figure 1). Electrochemical additive manufacturing has emerged as a new form of additive manufacturing, but its application has been limited to the deposition of metallic materials through the electrochemical reduction of metal ions. This technique finds the best-fit solution to construct our living bioelectrodes. The living electrode was fabricated by 3-D printing the polymer precursor EDOT in electrogenic bacteria-containing liquid and forming an electrochemically active biomaterial onto an abiotic graphite electrode through the in-situ electropolymerization of the monomer to the redox-active conducting PEDOT. The bioprinting syringe nozzle was modified by integrating an Ag/AgCl reference electrode and a platinum counter electrode which were connected to the external electrochemical control system. The electrodeposition was well-controlled with the potential application to the working electrode of a conductive substrate against the reference and counter electrodes (Figure 1 & Figure 2). When the bacteria containing EDOT liquid contacted the conductive substrate, a stable meniscus formed, and the desired structure was created. The cells were encapsulated within a copolymer matrix of PEDOT and alginate (Figure 2).

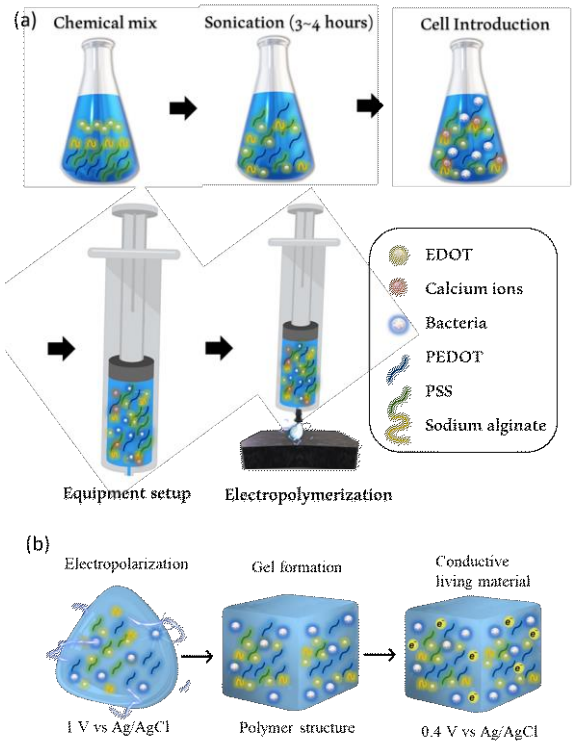

Figure 2: (a) The electropolymerization process to form a conductive microbial electrode. Electrochemical polymerization is followed by alginate crosslinking with $CaCl_2$. (b) Living electrode fabrication stages.

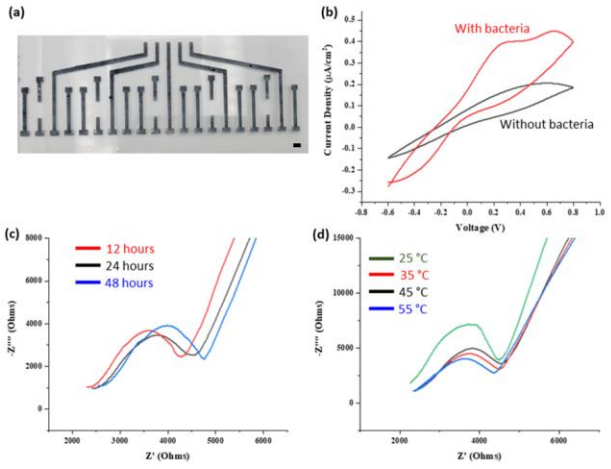

Figure 3: (a) 3-D printed conductive trace (the bar: 2mm), and (b) cyclic voltammetry curves of the electropolymerized PEDOT with and without bacteria. Electrochemical impedance spectroscopic profiles with storage time (c) and temperature (d).

The electropolymerized material was successfully printed as a living electrode in a controllable manner (Figure 3a). The bioelectrode demonstrated higher electrochemical activities and lower charge transfer resistance than that of the PEDOT-only sample (Figure 3b). The polymer maintained its gel structure when used or stored under different conditions. The polymer was storable up to 48 hours maintaining the viability of the cells

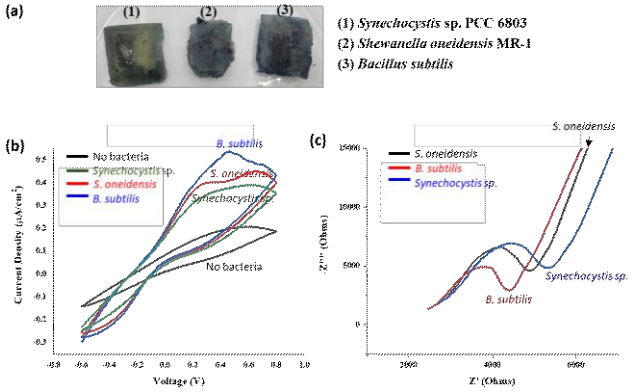

Figure 4: (a) Living electrodes with different types of bacteria ((1) Synechocystis sp. PCC6803, (2) Shewanella oneidensis MR-1, and (3) Bacillus subtilis), (b) their cyclic voltammetry curves and (c) electrochemical impedance spectroscopic profiles.

with the low charge transfer resistance (Figure 3c). Moreover, the gel was functional even when placed for 10 minutes in relatively hot temperatures up to 55°C before breaking (Figure 3d). The living electrodes with different types of electrochemically active bacteria were constructed and their electrochemical activities were characterized along with their electron transfer efficiencies (Figure 4). Our bioelectrodes showed more vigorous electrochemical activities with effective electron transfer capabilities, indicating that they could seamlessly bridge biological systems and abiotic electrodes.

CONCLUSION

This work created a great performing, very electrochemically active, and controllable microbial electrode. The living electrodes can dramatically reduce the physicochemical mismatch between the inorganic electrodes and the biological entities providing an effective coupling at the biotic-abiotic interface through biological electron conduits. Engineering such interfaces will be critical for advancing practical applications in bioelectronics such as *in vitro* biosensors, electronic medical implants, neuroprosthetics, and biofuel cells.

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