3D Bioprinting of Cyanobacteria for Solar-driven Bioelectricity Generation in Resource-limited Environments

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***Abstract*—We demonstrate a hybrid biological photovoltaic device by forming a 3D cooperative biofilm of cyanobacteria and heterotrophic bacteria. 3D bioprinting technique was applied to engineer a cyanobacterial encapsulation in hydrogels over the heterotrophic bacteria. The device continuously generated bioelectricity from the heterotrophic bacterial respiration with the organic biomass supplied by the cyanobacterial photosynthesis. This innovative device platform can be the most suitable power source for unattended sensors, especially for those deployed in remote and resource-limited field locations.**

I. Introduction

Recent advances in wireless sensing blend various functionalities while reducing power consumption using integrated sensors on very small chips [1]. These new unprecedented sensing techniques have led to the rapid evolution of wireless sensor networks (WSNs) for the emerging Internet-of-Things (IoT) [2]. The deployment of larger-scale WSNs has reached a tipping point, even becoming ubiquitous, judging by the increasing number of studies reported in scientific publications and ideas for smart cities, smart energy, security applications, electronic healthcare and intelligent transportation [3, 4]. However, there is a significant challenge in realizing truly stand-alone and self-powered sensing networks that operate without human intervention [5, 6]. The key challenge is the need for a micro- or nano-sized self-sustainable power source for each sensing node in a more effective and efficient way [6]. Power autonomy is critical, allowing miniaturized wireless sensors to work independently and self-sustainably in limited-resource and remote regions, in order to increase lifetimes and reduce maintenance. Standard batteries will not be suitable for these up-to-date applications because of their limited lifetime, relatively high cost, size incompatibility with the sensor, and potential danger to the environment. What is needed is a low-cost, eco-friendly, self-sustainable energy harvesting technique that can be easily integrated or combined with miniaturized wireless sensors.

Among the many micro- and nano-sized energy harvesting technologies with large upside potential [6-10], micro-bio-photovoltaic systems (or micro-BPVs) are arguably the most underdeveloped [11]. Nonetheless, excitement is building for micro-BPVs, as they are the most suitable power source for unattended sensors, especially for those deployed in remote and resource-limited field locations [11-14]. Photosynthetic organisms in the micro-BPV, such as microalgae and cyanobacteria, can self-sustainably harvest the most abundant energy source, solar energy, to produce electrical power even in extreme conditions [5, 11, 14]. Furthermore, micro-BPVs can generate electricity continuously from microbial photosynthetic and respiratory activities under day-night cycles in the absence of an organic feedstock [14-16]. Moreover, this technique resembles the earth's natural ecosystem, where living organisms work with non-living components as a system to self-assemble, self-repair, and self-maintain operational capabilities. Despite the vast potential and promise of micro-BPVs, their performance remains insufficient to realize potential WSN applications. To date, no micro-BPVs exist that can independently power real-world sensor applications. These gaps have relegated today's micro-BPVs to the status of a laboratory curiosity rather than being a viable alternative power source. Their power densities, which are typically a couple of orders of magnitude lower than even the smallest power microbial fuel cells including general heterotrophic bacteria, remain a significant challenge. This is because cyanobacterial electron transfer efficiency is significantly lower than that of those heterotrophic bacteria. To address this issue, we recently created a hybrid micro-BPV that maximized syntrophic interactions between cyanobacteria and heterotrophic bacteria [17]. In this system, heterotrophic bacteria were used for power generation while organic biomass and O_2 required for heterotrophic microbial respiration were supplied by cyanobacterial photosynthesis. In

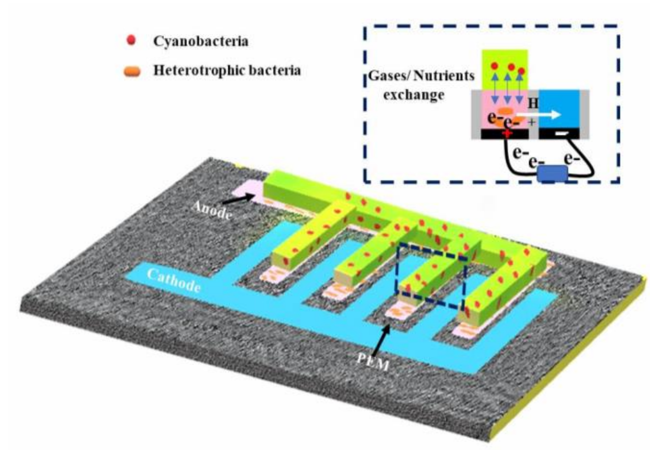

Figure 1. Schematic illustration of the hybrid micro-BPV with the hydrogel encapsulated cyanobacterial biofilm

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Dr. Lee is with Pensées Inc. He is the founder and CEO of Pensées Inc, who newly developed a bio-printer.

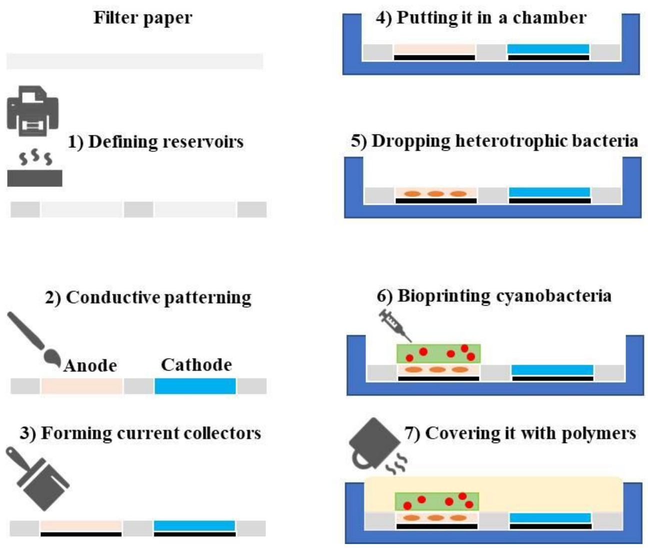

Figure 2. Fabrication processes

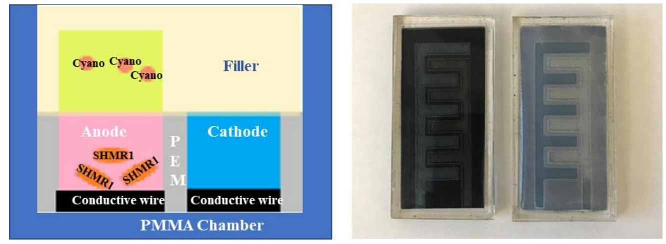

Figure 3. Schematic illustration and photo of the assembled device (SHMR1: Shewanella oneidensis MR-1, Cyano: Cyanobacteria, PEM: Proton exchange memberane, and PMMA: Poly(methyl methacrylate))

the meantime, cyanobacterial reactants, such as CO_2 and H_2O, were produced by the heterotrophic bacterial metabolism. This synergistic cooperation consequently exhibited self-sustainable capabilities with the enhanced power performance. Although this hybrid micro-BPV contributed essential knowledge about the cooperative biofilm formation and syntrophic interactions between photosynthetic co-cultures, their power generation decreased after a few days of operation because of imbalanced bacterial growth/reproduction between the co-cultures. Their intimate co-culturing generated a competitive interaction where only one species benefited and survived. Cyanobacterial population gradually decreased while the heterotrophic bacterial growth increased, degrading the device self-sufficiency.

In this work, we constructed a cyanobacteria-laden hydrogel bio-layer over the heterotrophic bacteria using a 3D bioprinting approach so that there is no physical contact between two bacterial species but still commensalistic or mutualistic relationship can be maintained (Figure 1). Engineered cyanobacterial biofilm can be a potential solution to break through the barriers and limitations hampering conventional hybrid micro-BPV techniques. The heterotrophic bacteria biofilm was formed in the engineered conductive paper substrate and efficiently harvested electrons from the organic biomass delivered through highly porous hydrogel encapsulating cyanobacteria. Cyanobacterial growth was not affected at all by the heterotrophic bacteria, providing an optimal environment for improving the hybrid micro-BPV.

II. Materials and Methods

A. Device Fabrication

The hybrid micro-BPV was constructed on a single sheet of paper substrate (Figure 1). We designed an interdigitated electrode configuration with a hydrophobic wax-based proton exchange membrane (PEM) between the electrodes. First, the hydrophilic anodic/cathodic reservoir regions were defined with hydrophobic wax boundaries on the paper by using a commercial wax printer and spreading the wax vertically through the paper with heat treatment (Figure 2). Then, the anodic ink (200µL PEDOT:PSS with 15% DMSO mixture) and the cathodic ink (200uL 100 mg/mL Ag_2O in PEDOT:PSS mixture) were introduced into the pre-defined anodic and cathodic reservoirs, respectively, followed by screen-printing of carbon pastes on top of the engineered reservoir as a current collector. The PEDOT:PSS conductive polymer allowed the paper reservoirs to be hydrophilic with open pores for subsequent bacterial sample introduction [18-21]. After that, the assembled paper device was placed in a PMMA chamber for biofilm formation. The heterotrophic bacteria, *Shewanella oneidensis* MR-1 (SHMR-1), was first loaded in the anodic reservoir, which acted as a biocatalyst transferring electrons to the anodic current collector. On top of the pre-loaded region, hydrogel-encapsulated cyanobacteria were three-dimensionally printed by using Vitarix^{TM} bioprinter (Pensées Inc, South Korea). Printing parameters are listed as follows: pressure: ~14Mkpa; print speed:25mm/s; infill density: 30%; nozzle size: 0.75mm; shell width: 0.4mm; layer height: 0.15mm. To protect the device from the environmental surroundings, reduce health concerns from microbial cytotoxicity and minimize evaporation, we enclosed the device by filling the chamber with gas permeable PDMS (Figure 3).

B. Bacterial Inoculum

Synechocystis sp. PCC 6803 (cyanobacteria) were cultured by inoculating BG-11 medium with a 24h light cycle (12h light/dark). The BG-11 contained 1.5 g $NaNO_3$, 40 mg K_2HPO_4, 75 mg $MgSO_4$, 36 mg $CaCl_2$, 1 mg of EDTA, and 6 mg of citric acid and of ferric ammonium citrate per 1 L of DI water. The continuous illumination was provided by fluorescent lamps. *Shewanella oneidensis* MR-1 (heterotrophic bacteria) were cultured by inoculating L-broth medium in air for 24h at 35ºC. The L-broth media consisted of 10.0g tryptone, 5.0g yeast extract and 5.0g NaCl per liter. Their growths were monitored by determining the optical density at 600 nm (OD_{600})

C. Operating principle

The heterotrophic bacteria in the anodic reservoir oxidize the organic biomass (supplied by the cyanobacteria) and produce electrons and protons. The electrons flow along the external circuit to reach the cathode while the protons diffuse from the anodic reservoir to the cathode through the PEM. At the cathode, Ag_2O is reduced by the protons and electrons travelled from the anodic reservoir. The voltage generated between the anode and the cathode was measured by using a data acquisition system (National Instrument, USB-6212).

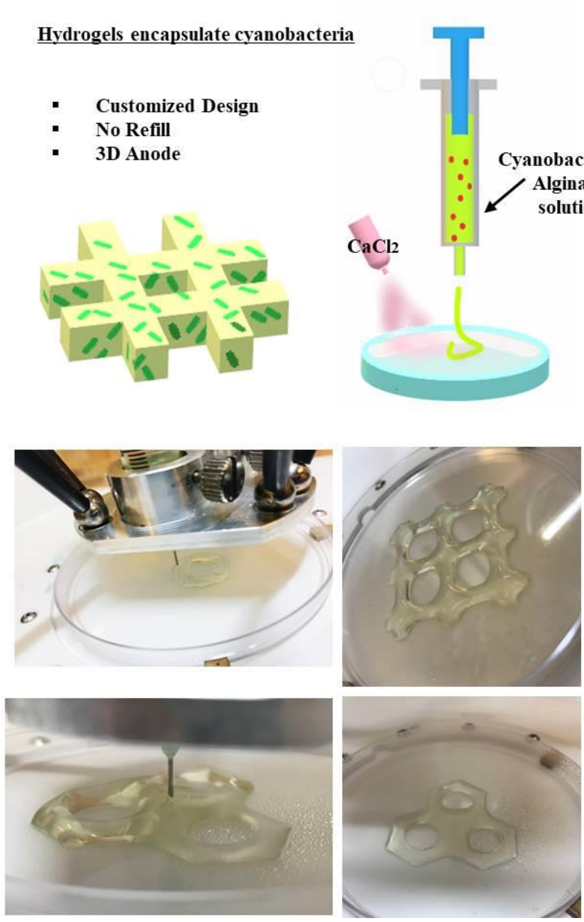

Figure 4. 3D bioprinting of cyanobacteria in alginate gels

III. Results and Discussion

A. 3D Bio-printing and Bio-ink

3D bioprinting is most notably used in diagnostic and medical applications for the purpose of tissue and organ printing [22-24]. Relatively little research has been done into the field of printing three-dimensional microbial biofilms because until recently, little has been known about the specifics of microbial interactions. This bioprinting process requires careful control of sol-gel transition of the bio-ink so that the material can be inviscid sufficient to hold its shape when patterned on a substrate [25]. At the same time, the bio-ink should be readily extruded out of a print nozzle while the cell viability must be preserved by reducing the shear forces on bacterial cells. In this work, alginate hydrogels for cyanobacterial cells were prepared by using ionic crosslinking of alginate with calcium ions. The concentration of alginate and calcium cross-linker is a critical factor to form a sophisticated structure. We chose 6% (w/v) alginate in DI water with 0.5M calcium chloride. With the higher calcium concentration, the diffusion time required for the sol-gel transition increased, leading to higher shear stress on the cells. Various multilayered patterns could be printed with this bio-ink because the reaction rate was slow enough to deposit partial uncrosslinked alginate on the crosslinked layer (Figure 4). Furthermore, the selected condition enabled excellent cell viability.

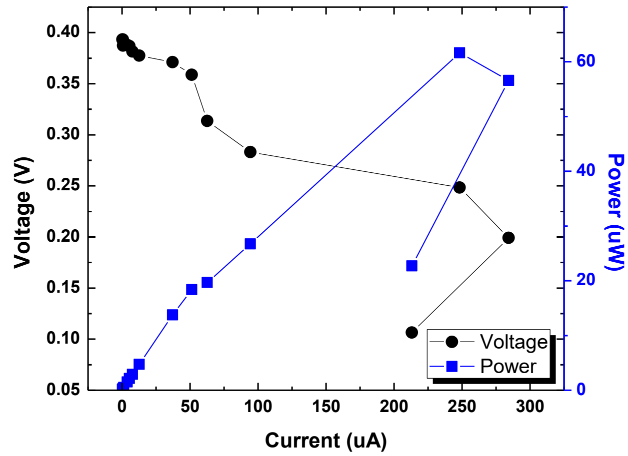

Figure 5. Polarziation curve and output power measured as a function of current with the hybrid micro-BPV using co-culture of heterotrophic bacteria and cyanobacteria.

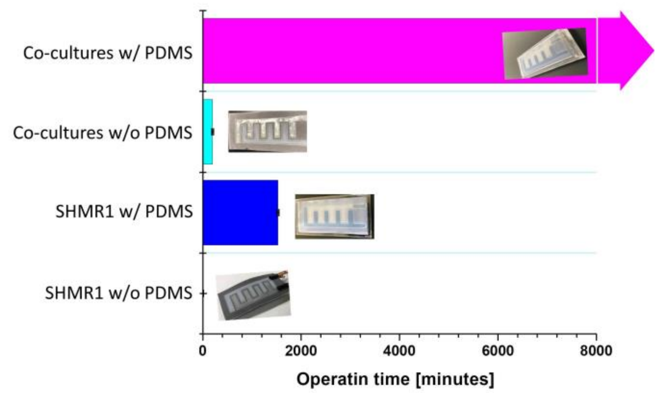

Figure 6. Operation time. Co-cultures of heterotrophic bacteia and cyanobacteria produced sustainging power over a longer-term.

B. Power Generation and Operation Time

The polarization curve and power from the hybrid micro-BPV including co-cultures were measured and calculated based on the current output at a given external load (Figure 5). Very high power and current (~62μW and ~287μA) were obtained, corresponding to ~13μW/cm^2 and ~55μA/cm^2, respectively, which are higher than the latest micro-BPV [26].

Synergistic cooperation of the different bacterial species also provided substantially enhanced sustainability compared to the heterotrophic bacteria only (Figure 6). The heterotrophic bacteria in contact with the anode participated in direct/indirect electron transport with the outer membrane cytochrome, shuttling compounds or pili while the cyanobacteria in hydrogel produced the food necessary for the heterotrophic bacterial metabolism, thus fulfilling the layered community's requirement for self-sufficiency. The co-cultures with the PDMS keep generating power while the device with the heterotrophic bacteria operated only for 13 minutes. As shown in Figure 6, the PDMS sealing prevented liquid depletion through evaporation. Without the PDMS, even the co-culture-based device did not generate a sustained power. The gas permeable PDMS continuously provided necessary gases (e.g., nitrogen) for bacterial metabolism, supporting long-term operation [27-29].

IV. Conclusion

We engineered a 3D, multifunctional polymicrobial biofilm using cyanobacteria-laden hydrogel layer to create a hybrid micro-BPV. This device revolutionarily increased microbial electron transfer efficiency through their syntrophic relationship. A 3D bioprinting technique and cell-laden engineering created a highly functional multi-layered biofilm construct that can optimize the spatial arrangement of community members in the biofilm and maximized metabolic interactions between different bacterial layers. Our hybrid micro-BPV provided sustainable energy production without additional organic fuels for a long-term period.

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