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Plug-and-play modular biobatteries with microbial consortia

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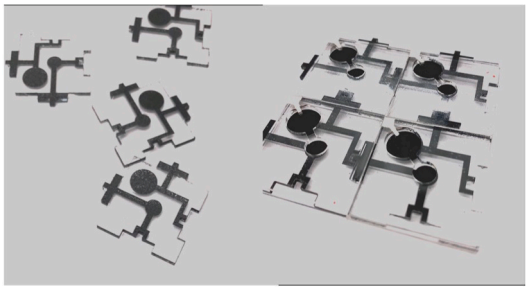
HIGHLIGHTS

- We create a microfabricable and scalable biobattery that includes a microbial consortium.
- A layer-by-layer biofabrication forms a thick, conductive, microbial consortium structure.
- Multiple biobattery modules can be connected with a plug-and-play capability.

GRAPHICAL ABSTRACT



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ABSTRACT

Recently, a bacteria-powered biobattery containing multiple species demonstrated long-lasting and fully self-sustainable power generation through their synergistic interaction. Confining individual species in separate spaces avoids unbalanced competition between neighboring species, maximizing their cooperative interaction to extend battery life. Despite the vast potential and promise, however, a spatially engineered microbial consortium has never been sufficiently scaled in a systematic and controllable manner for immediate power applications. Moreover, the spatial organization of living microorganisms having their seamless and effective electrical coupling with the external electrode remains a significant challenge. In this work, we establish the groundwork for creating a microfabricable and scalable biobattery platform that allows control of a 3D multispecies microbial consortium. A layer-by-layer electropolymerization deposition of microbial-infused polymer solutions creates a vertically multi-layered, conductive, microbial structure where individual species are separately confined in quasi-solid-state polymer layers, ultimately providing effective coupling at the biotic-abiotic interface and efficient ionic environments for cross-feeding interactions between species. An integrated modular "plug-and-play" biobattery platform provides a simple and practical approach for its serial and parallel connections. By connecting multiple biobattery modules, an actual wireless telemetry system was successfully operated, ensuring the practical efficacy of this power supply for real-world wireless sensor network applications.

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1. Introduction

With the rapid evolution of the Internet of Things (IoT) and the electronics industry, ubiquitous interconnected devices promise tremendous improvements in technology, communication, service, and business [1]. Moving into the next decade, the terahertz-enabled sixth-generation (6G) technology combined with artificial intelligence (AI) is expected to be available for massive, ultra-reliable, and low-latency communications [2–4]. Soon, an enormous number of smart, stand-alone, and always-on devices on extremely miniaturized platforms will be deployed in every sphere of human life, collecting real-time information about human health, security, and the environment. The most critical challenge in the buildout of this massive network infrastructure is sustainably and reliably powering individual devices [5,6]. Generally, the 6G-enabled infrastructure will be likely powered by four strategic plans depending on the type of application. (i) Many IoT devices fixed at buildings, homes, and cities will be wired to the electric grid that delivers electricity more reliably and efficiently (ii) Some devices we can readily carry around (e.g., smartphones and laptops). or embedded in large mobile vehicles will rely on batteries or energy storage devices. These devices can access the electric grid while their power supplies can be easily recharged and replaced for sustainable operation. (iii) Wearable and implantable devices for body sensor networks (BSNs) will use batteries and energy storage devices, but the emerging trends and latest innovations are integrating energy harvesting technologies to realize self-sustaining operations with potentially inexhaustible energy supplies [7]. Power can be scavenged from the human body (i.e., human mechanical motion, body heat, and electrochemical energy from body fluids) or ambient environment sources such as the sun and RF waves [8,9]. Because the BSN devices typically interact with nearby mobile or fixed devices, the necessary power will be readily achievable. Moreover, the power sources can be accessed for easy maintenance and replacement. (iv) The most challenging IoT application will be the wireless sensor networks (WSNs) deployed in unattended dangerous, difficult-to-reach and even harsh environments. As the untended WSNs will ultimately allow every corner of the world to be seamlessly connected, power autonomy is the most critical requirement for their self-sustainable function without human intervention and maintenance [5,6]. Because of their immense numbers and pervasive distribution, the devices cannot be powered by being wired to the major electric grid while traditional batteries with finite energy budgets will never provide a long service life. Even the latest advances in energy storage devices, such as supercapacitor and lithium-ion batteries, will not work as a sole power platform due to frequent recharging requirements. Besides, those batteries and energy storage devices deployed in difficult and hostile environments cannot be readily replaced or maintained. Given that the 6G systems will move beyond the millimeter and up into the terahertz wave spectrum [2,3], they will continue to shrink in size making them easy-to-deploy, but consequently, they will require strong, long-lasting power for long-distance communication and even more to accommodate the large data throughput. Although the power efficiency of the electronics and the network systems is significantly improving to prolong their lifetimes, a high-performance, maintenance-free, and fully self-sustaining energy harvesting technique is required. In particular, a miniaturized energy harvester from which power generation can be easily scaled will be the most suitable power solution for the next-generation 6G technology especially to be deployed in untended environments. However, the development of the miniaturized energy harvesters is in its infancy and their integration into the self-powered WSNs remains a challenge because of their short lifetime and low performance [5,10]. Furthermore, power scavenged from their working environments is typically irregular in time, random in location, and inefficiently converted. Even the most advanced photovoltaic cells do not work on cloudy days or at night while the mechanical kinetic or thermal energies are limited to specific locations. Above all, these semiconductor-based energy harvesters are not cost-effective enough for their enormous quantities to be widely distributed.

Microbial energy harvesters (named microbial fuel cells (MFCs) or microbial solar cells (MSCs)) can be the most suitable power source for unattended WSN applications, especially for those deployed in remote and resource-limited environments [11]. The microbial energy harvesters generate electricity continuously from respiratory activities by biodegrading wastewater or other organic materials, or from photosynthesis with solar energy and water in the absence of organic food [12,13]. The microorganisms are self-repairing, self-assembling, and self-sustaining through their metabolisms or photosynthesis using the most abundant renewable energy sources while they are ubiquitous and can adapt to extreme environments. Moreover, miniaturized systems allow significant performance enhancement, and many units can be connected to achieve the required power level, which makes this technique the most suitable energy harvesting technique for the next-generation environmental 6G applications [14–16]. However, many of the previous MFCs for the WSN devices were limited to macro-scale platforms in benthic environments, and even stacking of mid-scale MFCs fail to increase power outputs in the same electrolyte sediment [11]. Moreover, the power generated from the sediment MFCs was not sufficient for signal communication from deep water. Although the miniaturized MSCs can be designed to float on the surface of water for easy signal communication with reduced power consumption [16], they have not been demonstrated as a potential power source because of the extremely low electrogenic capability of the photosynthetic microorganisms. Moreover, previous small MSCs were limited to only a single species to facilitate controlled measurements and reproducible experimental procedures. The MSCs with single species demonstrated a short lifetime because nutrients and gases necessary for bacterial respiration and photosynthesis were not self-supplied in the enclosed small chamber [16,17]. Considering that microorganisms in nature proliferate as individual members of multispecies communities rather than in a single-species group and they maintain their viability even in nutrient-limited environments by using synergistic interactions [18,19], an engineered microbial consortium to mimic microbial interactions under controlled conditions can revolutionize the power and lifetime of the microbial energy harvesters. Previously, several research groups successfully demonstrated how a microbial co-culture platform can improve the power performance and lifespan of the microbial energy harvesters and how the spatial organization of multiple species can be optimized [20,21]. In particular, when individual bacterial species were separately placed, the consortium exhibited significant performance enhancement [21]. Surprisingly, without replenishment of any organic materials, the consortium self-sustainably produced longer and higher electricity than a single species by the synergistic exchange of microbial metabolites. However, the technique requires significant breakthroughs in selective, reliable, and controllable microbial consortium formation and in its seamless integration as a practical microbial energy system. In particular, the device needs an effective coupling between microorganisms and electrodes to improve the efficiency of the microbial electron transfer and thus power performance. Finally, a standardized platform for fabrication of the miniature devices and their easy stacking needs to be created. Stacking multiple small-scale units in an array can be the most practical method to provide the desired power level for the untended WSN applications.

In this work, we build *for the first time* a microfabricable and scalable microbial energy harvester (named biobattery) that can revolutionarily build an artificial microbial consortium through spatial engineering. An electrochemically-driven layer-by-layer biofabrication controllably forms a thick, conductive, microbial consortium structure by vertically stacking individual microcolonies without physical contact between the species. This functional living consortium reduces the physicochemical mismatch between microorganisms and inorganic electrodes and creates an effective coupling at the biotic-abiotic interface through biological electron conduits produced from the cross-feeding microbial

consortium. The engineered consortium is formed in a plug-and-play modular small platform, providing a simple and practical approach to scale-up by assembling and supporting various combinations of serial and parallel connections. This work establishes the groundwork for advances in sustainable energy, with an immediate goal of creating a self-sustainable and scalable microbial energy harvesting system that integrates significantly improved miniature units in an array.

2. Results and discussion

2.1. A plug-and-play modular biobattery platform

Battery-type microbial energy harvester without the need for replenishment of the microbial food simplifies device design, fabrication, and operation because it does not require a complex, energy-intensive fluidic feeding system [11]. Unlike typical batteries that stop generating power upon the depletion of the internally stored chemical fuel, multispecies microbial batteries can serve as a long-lasting, self--sustainable power supply through their cooperative metabolic interactions [21]. One species in a microbial multispecies community can produce specific nutrients and gases that another species requires but cannot generate. However, it has been extremely challenging to integrate well-defined liquid-state microbial multispecies in a microscale spatial structure for the development of a reliable and practical miniature biobattery. Moreover, practical difficulties hamper the design of a fast and simple fabrication system to produce biobatteries that can be easily assembled and reconfigured to achieve various serial and parallel connections. Here, we created a plug-and-play modular biobattery platform that can form a defined microbial consortium systematically, precisely and quickly by electropolymerizing individual microbial layers while the individual modular batteries can be simply connected in series, parallel, and hybrid stacks to achieve the desired power performance (Fig. 1). The biobattery module is based on the typical MFC configuration having a salt bridge chamber between anodic and cathodic compartments (Fig. 1a). With a reference electrode (Ag/AgCl) additionally integrated into the module, the anode (graphite) and the cathode (graphite) served as the working and counter electrodes, respectively, to electrochemically form a conductive biofilm composite in a three-electrode configuration. We mixed 10 mM of 3,4-ethylenedioxythiophene (EDOT) monomer and 1 wt% sodium alginate in 0.2 wt% poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS). The mixture filled in the anodic chamber that contained an M9 medium. Then we electrochemically polymerized the PEDOT. This was followed by alginate crosslinking with introduced 100 mM $CaCl_2$, three-dimensionally entrapping and connecting the bacteria to the anode (Fig. 1b). After electrochemically forming the microbial structure in the anodic chamber, the silver oxide (Ag_2O) in another PEDOT:PSS solution was added as a cathodic catalyst onto the graphite cathode and the cathodic chamber was filled with agar media. The salt bridge chamber was filled with the NaCl-containing agar media. All three chambers provided quasi-solid-phase ionic environments making the biobattery readily operable with other solid-state WSN applications and survivable in dynamic harsh environments [22]. When electricity-producing bacteria break down organic fuels, metabolically produced electrons move from the anode to the cathode through an external circuit while the protons simultaneously generated from the metabolism travel to the cathode through the internal salt bridge. Because the biobattery performance directly relies on the microbe-anode interface, it is very critical to develop a novel method that can increase the number of the cells attached to the anode beyond that of natural biofilms and provide an effective coupling between all cells and the anode. In 2018, Zajdel et al. developed a then-innovative method to form a thick conductive biofilm by embedding electricity-producing bacteria in an electropolymerized PEDOT:PSS [23]. However, the system required additional bulky reference and counter electrodes followed by considerable fabrication steps to transfer and integrate the developed biofilm into a miniaturized device. To address this issue, we recently integrated all three electrodes into a microfluidic chamber so that a thick conductive biofilm can be directly formed in a designated miniaturized device [24]. However, the microscale device containing all three electrodes in one chamber could not provide any meaningful functions other than for analysis purposes of the engineered biofilms. In that sense, this current work is very innovative and transformative because it allows direct integration of bacteria into small biobatteries in a controllable way, enabling immediate powering applications. Given that conventional miniature MFCs or MSCs for a decent power generation require several hours or days to form a biofilm having a high density of cells [14], which even does not ensure electrocatalytic activities from all embedded cells, our technique is simple and rapid with full control of biofilm thickness and cell density. As shown in cyclic voltammetry (CV) and power/polarization curves (Fig. 1c), the electropolymerized electrodes with *Shewanella oneidensis* MR1(blue diamond) generated considerably more vigorous electrochemical activities and better power performance than the control sample with Luria Broth (LB) agar (black square). This indicates that the electropolymerized electrode seamlessly bridges bacteria and inorganic electrodes while the cells in a non-conductive agar chamber cannot effectively transfer electrons to the graphite anode. The electropolymerized electrode without bacteria produced significantly lower performance while no microbial redox peaks were observed (red triangle). Because of the relatively high conductivity of the EDOT monomer and PEDOT:PSS, the liquid sample without going through the electropolymerization process (dark green circle) initially produced a certain level of microbial power, but the toxicity of the EDOT degraded the cell viability over time, decreasing the device performance (data not shown) [23]. Among all electrode samples, the electropolymerized microbial electrode generated the highest power density of 13.65 $\mu W/cm^2$, which is about 13 times greater than the control with the bare graphite electrode. The cell viability even 4 h after the electropolymerization was more than 90%, indicating that our method can preserve cell density and their metabolic activities for reliable and meaningful power generation (Fig. S1).

2.2. Forming multilayered microbial consortium

Our novel platform can be easily leveraged to create a functional microbial consortium by constructing a 3D multilayered construct through vertical layer-by-layer electropolymerization (Fig. 2). Individual reservoir layers contained the reference electrode and three chambers for anodic, cathodic, and salt bridge compartments while an electrode layer included the working and the counter electrodes (Fig. 2a). The electrode layer was repeatedly used to electrochemically form individual species biofilm constructs by combining with the reservoir layer. Each reservoir layer with a specific single species was vertically stacked to form a designed multilayered biofilm construct and the electrode layer was permanently attached to the bottom to ultimately form an MFC configuration. The cathodic and salt bridge chambers were then respectively prepared in the same way as the single-species biobattery. Each device module included a plug-and-play format for easy assembly and stacking (Fig. 2a). To probe the electrochemical activities and the efficiency of the microbial electron transfer of different species and their co-culture combinations, we prepared six different samples by using three electricity-producing microbial species (*Shewanella oneidensis* MR1, *Bacillus subtilis*, and *Synechocystis* sp. PCC 6803); three samples with single species, two with double species, and one with triple species (Fig. 2b). A control without bacterial cells was prepared for comparison. These three bacterial species were selected based on our previous preliminary data-driven [21]. In short, *S. oneidensis* is a well-known Gram-negative electricity-producing bacterial species (or exoelectrogens) that can live in anaerobic and aerobic environments, generating electricity. While many exoelectrogens (e.g., *Geobacter* sp.) require a strict oxygen-free environment for electricity production,

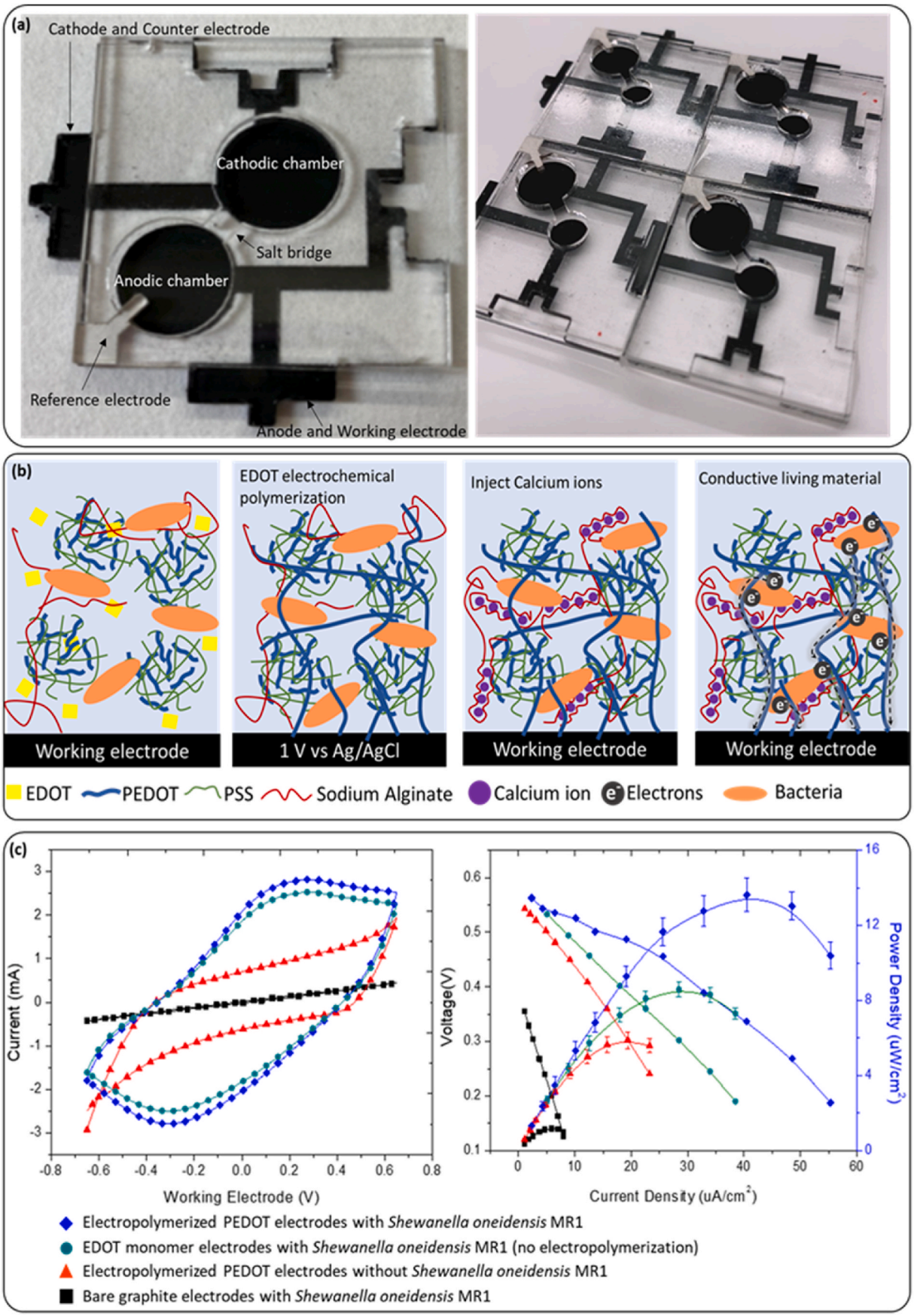

Fig. 1. (a) a plug-and-play biobattery platform and connected multiple biobattery modules, (b) the electropolymerization process to form a conductive microbial construct, and (c) CV profiles and polarization/power curves of various electrode samples with and without bacteria.

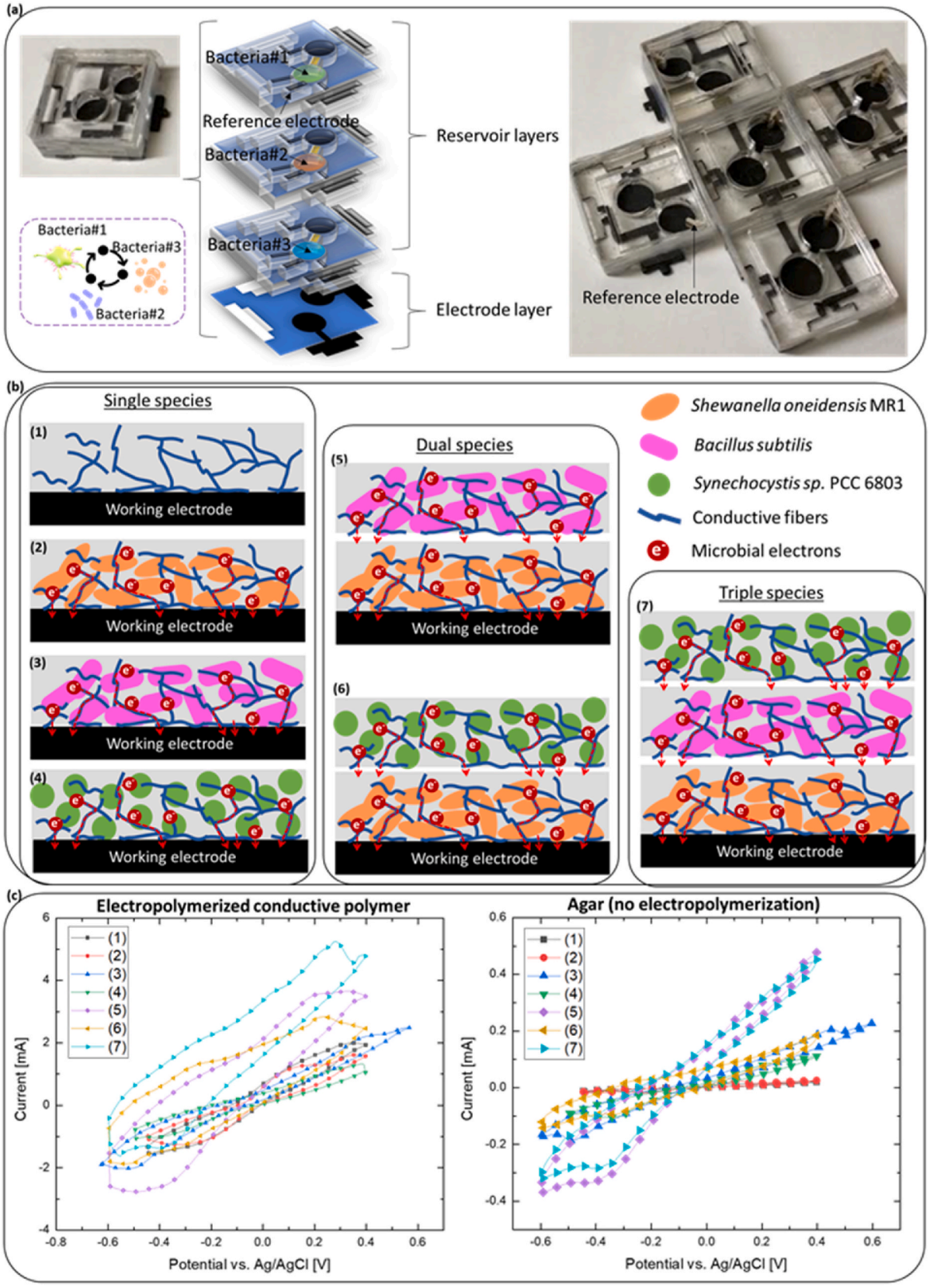

Fig. 2. (a) a plug-and-play biobattery platform having an engineered microbial consortium and connected multiple biobattery modules, (b) different single- and multiple-species samples, (c) CV profiles of the samples in electropolymerized electrode and non-conductive agar (#1: control without bacteria, #2: *S. oneidensis*, #3: *B. subtilis*, #4: *Synechocystis* sp., #5: *B. subtilis* (top) and *S. oneidensis* (bottom), #6: *Synechocystis* sp. (top) and *S. oneidensis* (bottom), and #7: *Synechocystis* sp. (top), *B. subtilis* (middle) and *S. oneidensis* (bottom)).

which makes this technique more challenging for actual application in the environment, *S. oneidensis* provides more flexibility in the device design and fabrication without strict hermetic packaging. While *B. subtilis* is a Gram-positive weak exoelectrogen, riboflavin production can be beneficial when they collaborate with *S. oneidensis* [20,21]. The riboflavin serves as an electron shuttle to indirectly improve the extracellular electron transfer of *S. oneidensis*. Moreover, because *B. subtilis* is strictly aerobic for its fermentation and consumes oxygen, it could generate a very favorable anaerobic environment for better electricity generation from *S. oneidensis* through its anodic respiration. *Synechocystis* sp. PCC 6803 is a photosynthetic bacterial species with weak electrogenicity. Through its photosynthesis, valuable nutrients and gases can be produced [25], which will facilitate the viability of other heterotrophic bacterial species (i.e., *S. oneidensis* and *B. subtilis*), providing a self-sustainable environment for microbial communities. All samples were operated under continuous light illumination. The control without bacteria was first prepared for comparison (Fig. 2b (1)). Individual species in the single-species devices were expected to generate their own electricity from their metabolism or photosynthesis (Fig. 2b (2–4)). For dual and triple species microbial consortium (Fig. 2b (5–7)), *S. oneidensis* was placed on the bottom serving as the main electricity-producing species through its respiratory activities while those above produced valuable molecules, providing more and longer electricity than monocultures. This is because *S. oneidensis* is known to have the stronger electrogenic activity through more diversified pathways for extracellular electron transfer than the other two species [26]. However, because individual layers were designed to have their own seamless biotic-abiotic interfaces in the conductive matrix, additional electron harvestings from the top layers were expected to be obtained. Moreover, individual species were designed to be confined in their own layer without physical contact with other species, preventing competition for limited resources and space. Previously, we confirmed that spatially organized microbial communities demonstrated better electrical performance than well-mixed counterparts [21]. Obviously, the three-species microbial consortium showed significantly higher CV current than two- and single-species ones (Fig. 2c). Two-species microbial consortium samples featured better electrochemical behaviors than single-species samples. The *S. oneidensis* and *B. subtilis* combination showed slightly better performance than the sample with *S. oneidensis* and *Synechocystis* sp. PCC 6803 mainly because *B. subtilis* could facilitate electron transfer of *S. oneidensis* and likely have better electrogenesis than the photosynthetic bacteria, *Synechocystis* sp. Overall, these conductive biofilm constructs show 10 times higher CV currents than the non-conductive agar-based samples without going through the electropolymerization process. The electron transfer efficiency from the cells to the electrode was examined using electrochemical impedance spectroscopy (EIS) measurements. The EIS profiles were fitted with an equivalent circuit consisting of a solution resistance (R_s), a charge transfer resistance (R_{ct}), and a double layer capacitance (C_{dl}). The total ohmic resistance can be calculated by $R_s + R_{ct}$. The electropolymerized conductive biofilm constructs demonstrated much lower R_{ct} values than the non-conductive agar samples (Fig. S2), indicating a better biotic-abiotic interface in the conductive matrix. Surprisingly, the three-species microbial sample showed lower charge transfer resistance than two- and single-species ones. Along with the CV measurement, this indicates that not only overall bacterial electron transfer was enhanced through the vertical stack of multispecies, but the bacterial commensalistic or mutualistic relationship promoted their electrochemical behaviors. *Synechocystis* sp. PCC 6803 placed on the top layer performed photosynthesis to produce carbohydrates, which were used as an organic fuel by other microbial communities below. Facultative *B. subtilis* placed in the middle layer produced riboflavin as an electron shuttle, which considerably enhanced the charge transfer and the electricity generation of *S. oneidensis* located on the bottom layer. This can be explained by the EIS results of the dual-species, showing that the *S. oneidensis* and *B. subtilis* combination has a smaller R_{ct} value than the *S. oneidensis* and *Synechocystis* sp. consortium (Figure S2a-(4) & (5)). Moreover, the fermentative bacteria, *B. subtilis*, could form a more complete oxygen-free environment and facilitate the electron transfer of *S. oneidensis*.

2.3. Output power evaluation of the three-species microbial consortium

The power and polarization curves of the biobattery module were measured and calculated from the voltage responses to different external resistors (Fig. 3a). The biobattery contained the three-species microbial consortium spatially organized in individual quasi-solid-state conductive layers. *S. oneidensis* was placed on the electrode while *B. subtilis* and *Synechocystis* sp. were in the middle and the top layers, respectively. The maximum power density and current density from this consortium were about 42 μW/cm^2 and 220 μA/cm^2. This is about 3 times larger than the single-species biobattery with only *S. oneidensis* (Fig. 1c) and even higher than the three single-species biobatteries connected in series or comparable to the ones connected in parallel (Fig. S3). This implies that the electropolymerized co-culture microbial concept can provide a realistic and practical solution for the microbial energy harvesting techniques that have long been very limited by their low performance. The biobattery polarization curve demonstrated a near-linear drop in potential as current density increased to 220 μA/cm^2 (Fig. 3a). Through the linear fitting of the curve, we estimated the total internal resistance of ~2 kΩ, which is a much lower value than the single-species biobattery (~7 kΩ, Fig. 1c). The reduced internal resistance is attributed to the enhanced electrochemical activity (Fig. 2c) and the decreased charge transfer resistance through synergistic interaction between multispecies (Fig. S2). The plug-and-play multi-species microbial battery module was able to be easily stacked in series or parallel to achieve larger output voltage or power as required (Fig. 3b and c). Although the open-circuit voltage (OCV) of the two-serially connected biobattery pack was not exactly equal to the sum of the two units probably because of the contact resistance between them, their OCV increased to 0.9 V (Fig. 3b), compared to 0.58 V obtained from a single module (Fig. 1c). The two-parallelly connected biobattery pack exhibited almost twice the power and current generation than the single unit, corresponding to 80 μW/cm^2 and 430 μA/cm^2 (Fig. 3c).

Our three-species microbial consortium was designed to enable self-sustaining and long-life power generation even in an unattended wild environment. Because the photosynthetic bacteria placed on the top layer in the biobattery require a constant supply of water and gases (e.g., CO_2, O_2, and N_2) for their photosynthesis which produces metabolic molecules needed for other co-cultures, we passivated the device with a well-explored hygroscopic material, sodium polyacrylate, to keep the device wet and gas-permeable (Fig. 4a) [27]. This material has been well-proven to be great water retention and moisture harvesting material. Moreover, the gas-permeable property of the material was expected to constantly supply necessary gases for bacterial respiration and photosynthesis, supporting the sustained and long-term operation. The biobattery module covered by the hygroscopic material was precisely operated under 12-h light and 12-h dark cycles in a consistent humidity (64%) and temperature (30 °C) environment without replenishing any organic fuels. The device was connected to a 100 kΩ external resistor, and its output power density was continuously monitored with respect to the anode. A very stable and sustaining high-power performance of around 50 μW/cm^2 for more than 22 days was observed with positive light responses where the power increased with light and decreased in the dark (Fig. 4b). This indicates that microbial photosynthesis has a significant influence on the power generation and the self-sustainability of the biobattery by producing organic fuels and other molecules for the other co-cultures below. Slight fluctuations of humidity and temperature with the light-dark cycles (Fig. S4) have nothing to do with the power generation because any meaningful electricity generation is not observed from the control without bacteria (Fig. 1c). The long-term power generation started to gradually decrease after 22 days of

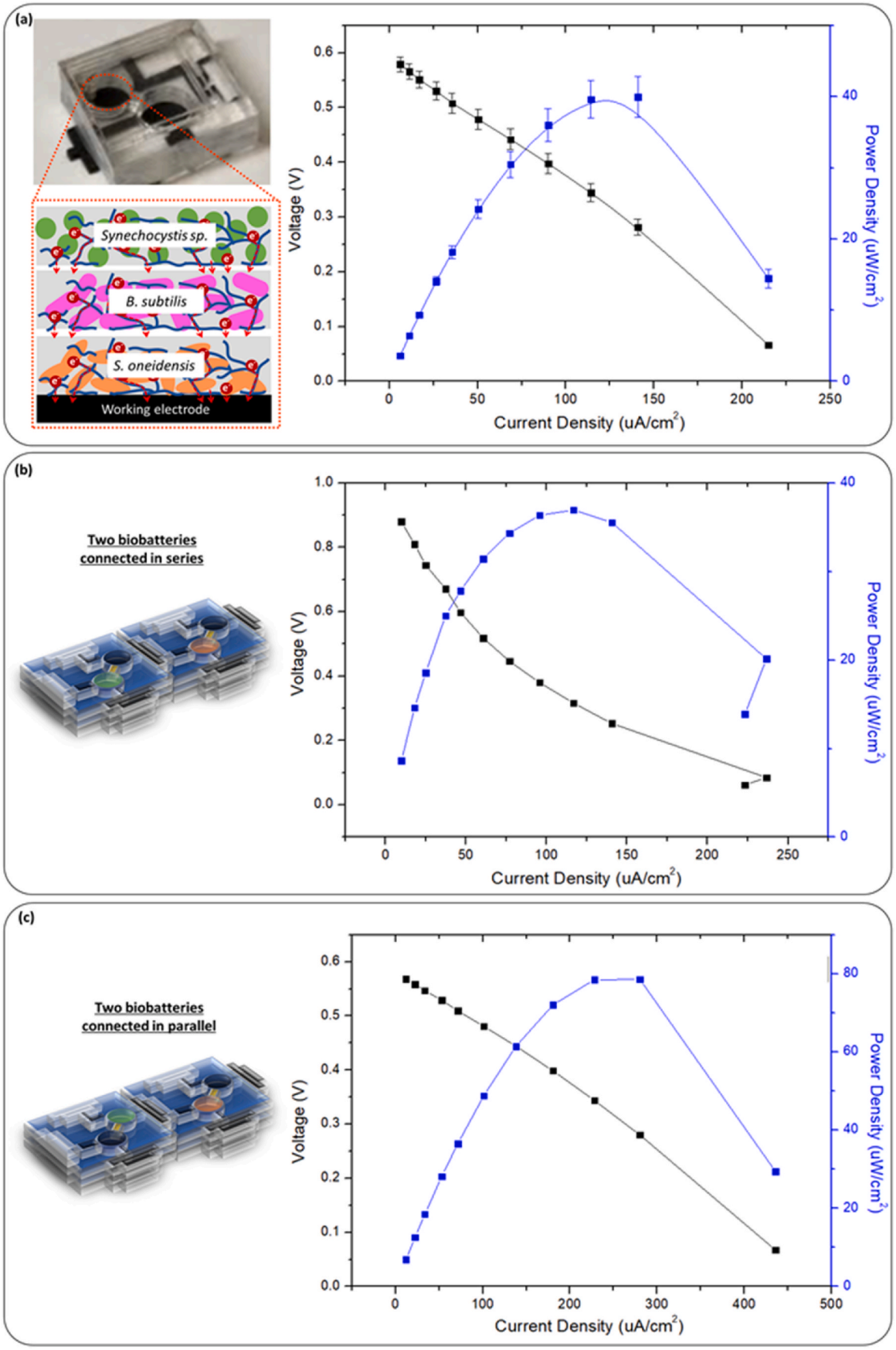

Fig. 3. Electrical performances of the single biobattery with the three-species microbial consortium (a), and the biobatteries connected in series (b) and in parallel (c).

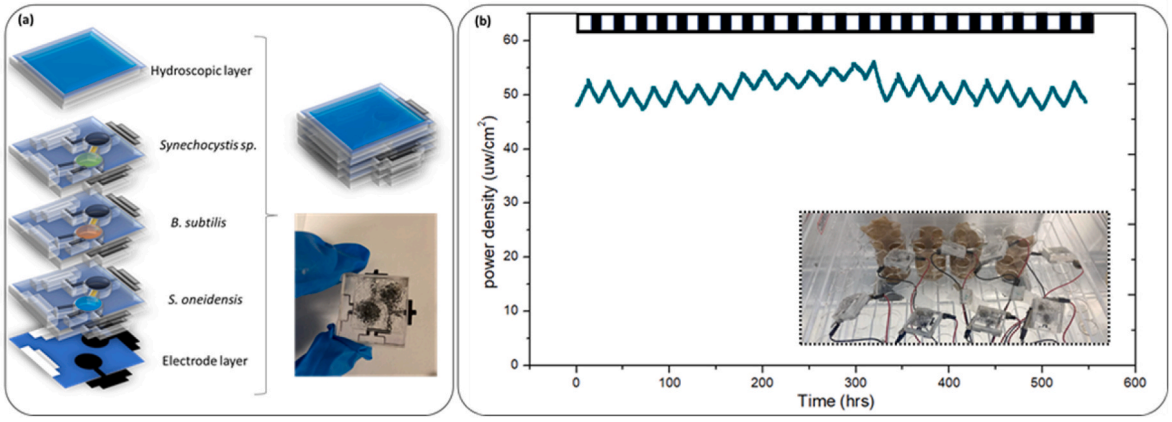

Fig. 4. (a) The three-species microbial biobattery covered by a hygroscopic material, and (b) its power density with 100 kΩ under the light and dark cycles (the white and shadow bars indicate the light and dark period, respectively). Insert: a test setup in an enclosed chamber.

operation. This is mainly because of the gradual increase of CO_2 level and the decreased other gases in the enclosed chamber, which presumably degraded cell activity and viability (Fig. S4) [28]. Further experiments in natural settings are required.

2.4. Powering wireless communication

In this task, we ensure the practical efficacy of the plug-and-play modular biobattery pack as a real power supply to wirelessly monitor the ambient temperature. A low-power Bluetooth temperature monitoring system was successfully powered by 24 biobatteries stacked in series and parallel, wirelessly paired with a smartphone (Fig. 5). A parallel connection of four sets of the serially linked eight biobatteries provided sufficient power for the wireless Bluetooth module (CY8CKIT-042-BLE Bluetooth® Low Energy (BLE)) through a booster converter (BQ25504EVM, Texas Instruments Inc., TX, USA) and a 1000 μF capacitor. The successive-approximation-register (SAR) analog-to-digital converter (ADC) monitored the difference of the reference and the thermistor voltages, and the converted signal was sent to the smartphone through the Bluetooth module (Fig. S5). Although a discharged current from the capacitor fluctuated between 280–400 μA depending on the duty cycle, a full discharged pulse was sufficient to power the whole system for about 4 s. At about 6 s, the system required an additional charge for the operation (Fig. S5). This work confirms that our plug-and-play modular biobattery allows a simple and practical approach to be integrated into the large biobattery pack system for real-world WSN applications.

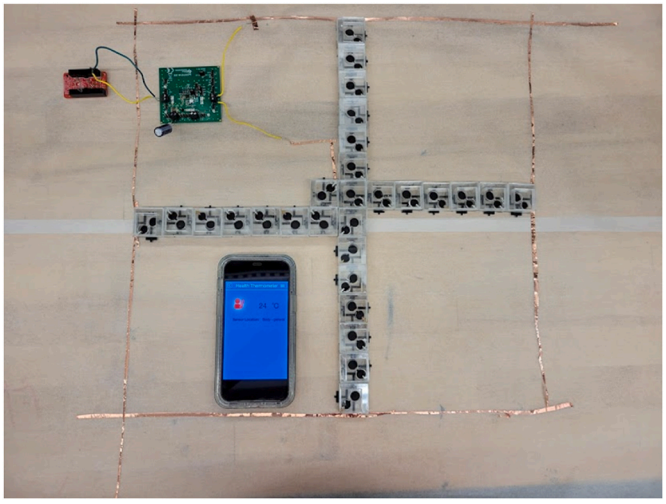

Fig. 5. A test setup to power a wireless communication with a 24-biobattery stack.

3. Conclusion

In this work, we create a microfabricable and scalable multispecies microbial biobattery platform that can be easily connected into a large energy system with "plug-and-play" capability. The biobattery module integrates an innovative technique that can electrochemically construct a thick, conductive, microbial consortium where individual species are spatially organized without physical contacts between species. A 3D, multi-functional polymicrobial structure is built by layer-by-layer electropolymerization of bacteria-polymer solution in a three-electrode microfluidic system, which can be immediately used for power generation. The electropolymerized microbial construct provides a seamless and effective coupling at the bacteria-electrode interface while reducing the physiochemical mismatch. The three-species microbial consortium in the biobattery covered by a hygroscopic material dramatically improves the power performance and lifetime of the system, revolutionizing this microbial energy technique as a superior substitute to conventional batteries, energy storage devices, and energy harvesting technologies for the unattended WSN applications.

4. Materials and methods

4.1. Bacterial inoculum

The photosynthetic bacterial species, *Synechocystis* sp. PCC 6803, was acquired from the American Type Culture Collection (ATCC) and grown in a BG-11 medium at 30 °C in 12 h light and 12 h dark cycles for about two weeks. *Shewanella oneidensis* MR-1 and *Bacillus subtilis* were also obtained from ATCC and grown in LB media with gentle shaking in the aerobic environment for 24 h at 30 °C. Bacterial growth was monitored by using a spectrophotometer with a light source of 600 nm (Optical density at 600 nm, OD_{600}) and all bacterial samples were collected when their OD_{600} reached 2.0.

4.2. Fabrication of a plug-and-play biobattery module

The 30 mm × 30 mm × 3.2 mm single-species biobattery includes three chambers (the anodic, the cathodic, and the salt bridge chambers) and three electrodes (the working, the counter, and the Ag/AgCl reference electrode) formed by two polymethyl methacrylate (PMMA) substrates (Fig. 1 and Fig. S6). Using a laser micromachining technique (Universal Laser Systems VLS 3.5), the PMMA layers were micropatterned to be an electrode layer and a reservoir layer, respectively. The electrode layer having the working (the anode, 0.64 cm^2) and the

counter (the cathode, 0.95 cm^2) electrodes were prepared by screen-printing graphite ink (E3449, Ercon Inc). The reservoir layer included the screen-printed Ag/AgCl reference electrode (0.12 cm^2, E2414, Ercon Inc.) and the window for the chambers. To clean the surface for effective electropolymerization, each electrode was wet-polished by a coffee filter paper and ethanol, followed by oxygen plasma treatment for 45 s. When the individual layers were bonded, 0.38 cm^3 of the anodic chamber, 0.57 cm^3 of the cathodic chamber, and 0.024 cm^3 of the salt bridge chamber were defined. To form a multispecies microbial consortium, individual reservoir layers were separately prepared with specific species, which were vertically stacked with the electrode layer from the bottom.

4.3. Electropolymerization of microbial culture and formation of a microbial consortium

The mixture of 10 mM EDOT (483028, Sigma-Aldrich), 0.2 wt% PEDOT:PSS (Clevios PH1000, Heraeus), and 1 wt% sodium alginate (W201502, Sigma-Aldrich) in M9 medium was first prepared by the processes of mixing/sonication, followed by the introduction of bacterial cultures. The mixture was electropolymerized to the conducting PEDOT polymer with the working electrode set to 1.0 V versus the reference electrode for 25 min. Then, 100 mM $CaCl_2$ was added for the cross-linking of sodium alginate. This process was repeated for individual microbial species which were vertically stacked to build a 3D microbial consortium structure. The silver oxide (Ag_2O) as a cathodic catalyst in a PEDOT:PSS solution was cast onto the graphite cathode and the cathodic chamber was filled with agar media (2% agarose powder in 100 mL LB medium) [29]. The salt bridge chamber was filled with the NaCl-containing agar media. The agar solutions were solidified to form a quasi-solid-state biobattery. Finally, 1 wt % of the hygroscopic material, sodium polyacrylate (432784, Sigma-Aldrich), was used to cover the top layer of the biobattery.

4.4. Electrical measurement setup

The voltage drop across a given external resistor was monitored with a data acquisition system (National Instruments, USB-6212). The power and polarization curves were obtained by calculation with the maximum voltage values at various resistors (470 k, 250 k, 162 k, 100 k, 71 k, 47.5 k, 32 k, 22 k, 15 k, 10 k, 2 k, 1.5 k, 0.45 k, and 0.35 k). Current densities and power densities were normalized to the anode area.

4.5. Electrochemical measurement setup

A potentiostat (Squidstat Plus, Admiral Instruments) was used for CV and EIS measurements. The CV measurements were performed at a scan rate of 10 mV/s FROM -0.6 to +0.6 V voltage window. The EIS tests were operated under an excitation voltage of 0.1 V with a scanning frequency from 0.01 Hz to 100 kHz.

4.6. Powering wireless telemetry system

24 biobatteries with the three-species microbial consortium were stacked with a combination of series and parallel connections to power a standard Bluetooth thermometer. The system measured the ambient temperature using the thermometer and wirelessly transmitted the data in an 8-bit format with a sampling rate of 3000 samples per second. The battery pack was first connected to a power management system including a booster converter (BQ25504EVM, Texas Instruments Inc., TX, USA) and a capacitor (1000 μF). The system successfully converted the low output voltage from the biobattery stack to a practical voltage level (4.2 V) across the capacitor for quick charging and setting a fixed 2 V at the output pins powering the wireless telemetry system (CY8CKIT-042-BLE Bluetooth® Low Energy (BLE)). When the power was generated, the Bluetooth system and a smartphone were successfully paired, wirelessly transmitting temperature data.

CRediT authorship contribution statement

Anwar Elhadad: Investigation, Methodology, Data curation. **Lin Liu:** Investigation, Methodology, Data curation. **Seokheun Choi:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – original draft, Writing – review & editing, Finalization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpowsour.2022.231487.

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