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# Biopower-on-Skin: Electricity generation from sweat-eating bacteria for self-powered E-Skins

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ABSTRACT

Electronic skins or 'e-skins' have recently emerged as a novel platform for electronics, taking on more important roles in health diagnostics, therapeutics, and monitoring. Stand-alone and self-sustained e-skins are essential to providing reliable, effective and sometimes life-saving functions. A stable power supply is the most critical factor in developing practical e-skins because their performance depends significantly on power availability. A realistic and accessible power source is urgently needed for the next generation of smart, stand-alone, always-on e-skin systems. This is by no means a simple challenge because intimately integrating technologically advanced e-skins with human skin requires generating power from an extremely complicated, harsh environment. Skin is cool, dry, acidic and has few potential energy sources. In this work, we create the ability to generate an innovative, practical, and longstanding power from human sweat, which is one of the few available energy resources on skin. We use the metabolisms of sweat-eating bacteria that exist on human skin, *Staphylococcus epidermidis*, *Staphylococcus capitis*, and *Micrococcus luteus*. We also test a microorganism, *Nitrosomonas europaea*, that oxidizes the ammonia in sweat, but is not usually found on the skin. Bioelectrogenesis from all the bacterial species were observed at levels comparable to that of a well-known wild-type electrogenic bacteria, *Shewanella oneidensis* MR1, which inhabits the natural environment. A biobattery, or a microbial fuel cell, that uses bacteria as a biocatalyst, transforms the chemical energy of sweat into electrical power through bacterial metabolism. The skin-mountable biobattery is pre-inoculated with the sweat-eating bacteria and operates with human sweat, delivered by an integrated battery-free skin-interfaced microfluidic system. Given that the total non-human microbial cells inhabiting in and on our bodies outnumber the human cells by at least a factor of 10, the direct use of the microbial cells to produce power is conceivable for e-skins. The significance of this work stems from the demand for self-sustainable and stand-alone e-skin systems for healthcare, security, fitness, and environmental monitoring applications.

## 1. Introduction

Electronic skins, or 'e-skins,' are about to become ubiquitous in healthcare devices, as evidenced by an increasing number of scientific publications, patents and commercial successes [1–3]. With the development of stretchable, biocompatible, and self-healing electronic materials, significant research is dedicated to the seamless and intimate integration of electronics with human skin, which will produce breakthroughs in human-machine interfaces, health monitoring, transdermal drug delivery, and soft robotics [4–6]. As the emerging technologies of artificial intelligence and the internet of things advance at a rapid pace, e-skins will be one of the ultimate forms of next-generation electronics [4,7]. However, current e-skin technology relies on bulky batteries or other energy storage devices to operate, which causes challenges in realizing compact and long-lived advanced functionality because of their size and finite energy budgets [8]. Furthermore, frequent recharging or replacing power devices hinders the practical and sustainable use of e-skins [9]. Power autonomy is a critical requirement, so e-skins can work continuously, independently and self-sustainably. Emerging energy-harvesting technologies that have potentially infinite energy supplies can accomplish the longstanding dream of self-powered e-skin systems [10]. Power can be scavenged from such ambient environment sources as the sun and RF (radiofrequency) waves or directly from human motion, manual touch, body heat, and sweat.

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Among many energy harvesting sources for e-skins, biochemical energy harvesting from human sweat is arguably the most underdeveloped because of immature technologies. Nonetheless, excitement is building for scavenging power from sweat, as it is the most suitable energy source for skin-contacting devices [11]. Sweat is readily and constantly available in sufficient quantities while it can be acquired non-invasively. Furthermore, sweat contains a rich variety of chemical and biological entities that can produce electricity [12]. Despite the vast potential and promise of sweat-driven power generation, the technique is limited to unstable and inefficient enzymatic catalysis, which requires fundamental breakthroughs to enable self-sustaining, long-lived power generation [13]. Even the latest advances in sweat-based energy harvesting are based on enzymatic-based fuel cells, practically limiting work in progress for e-skins [14,15]. Devices that scavenge energy from sweat could be a superior substitute for conventional batteries, energy storage devices, and other energy harvesting devices. However, to achieve this potential an innovative approach is needed to enable high-performing, self-sustaining, and long-functioning sweat-harvesting power generation that will ensure its practical feasibility as a power source.

In this work, we provide a realistic and practical solution for a novel sweat-based power source (Fig. 1a). The proposed sweat-powered batteries are based on microbial fuel cells (MFCs), exploiting sweat-eating bacteria including human skin-inhabiting or non-human ammonia-oxidizing bacteria as a biocatalyst to transform the chemical energy of sweat into electrical power through bacterial metabolism (Fig. 1b). A thin, soft, flexible, and skin-mountable MFC is pre-inoculated with selected electrogenic, or electron-producing, sweat-eating bacteria and operates with human sweat, delivered by an integrated battery-free skin-interfaced microfluidic system (Fig. 1c). The central hypothesis is that some microorganisms are capable of transferring extracellular electrons from sweat and acting as a stable biocatalyst in a MFC to produce reliable electrical power. The MFC will work with ammonia-oxidizing bacteria not typically found on human skin or with typical, sweat-eating bacteria commonly found on the skin. The microbial energy harvesting methods are significantly more resilient than the enzymatic approaches and provide superior self-sustaining features with long-term stability [16,17]. Microorganisms in the MFCs contain complete enzyme pathways and regenerate biocatalytic enzymes as part of their natural metabolism. As a result, they can provide self-assembling, self-repairing, and self-maintaining operational capabilities. Reported work on microbial energy harvesting for wearable electronic applications was either unavailable or quite limited because microbial cytotoxicity may pose health concerns. However, if we consider that humans possess more bacterial cells compared with human cells in their bodies [18], the direct use of bacterial cells as a power resource interdependently with the human body is conceivable for wearable electronics. Furthermore, human skin is home to millions of microorganisms and an ideal place for unobstructed access to those microorganisms [19]. Because the human skin-inhabiting microorganisms for the e-skin power devices ultimately will be extracted from and applied directly to the host, we expect a minimal foreign-body response. Also, ammonia-oxidizing bacteria from outside the host have been shown to improve skin conditions [20], so we again expect minimal harm to the host.

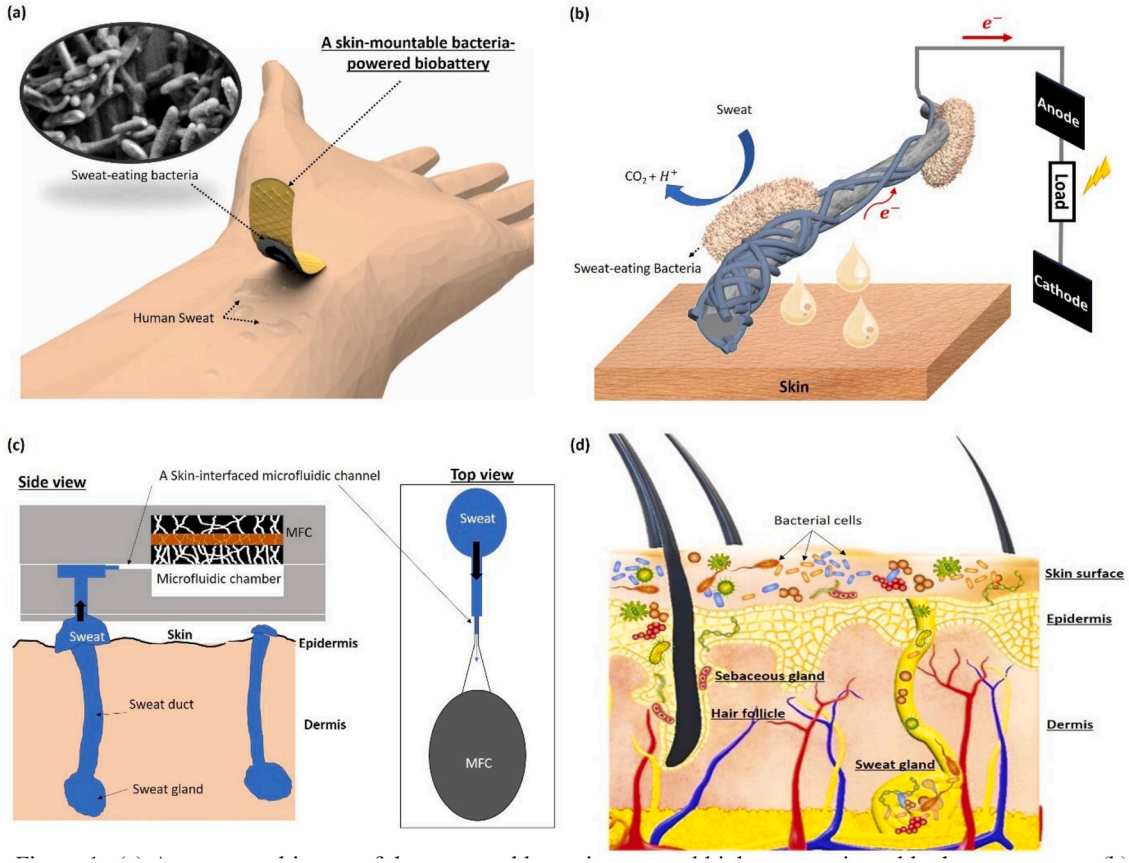


**Fig. 1.** (a) A conceptual image of the proposed bacteria-powered biobattery activated by human sweat, (b) a schematic illustration of the bacterial power harvesting from human sweat, (c) schematic diagram of the skin-interfaced microfluidic system integrated with the MFC demonstrating sweat collection from skin and delivery to the device, and (d) schematic of the human skin with microorganisms.

## 2. Results and discussion

### 2.1. Selecting candidates with the highest potential for e-skin power generation

The human skin is colonized by a wide range of beneficial microorganisms [19,21], which are readily accessible as an energy source if they are capable of extracellular electron transfer. The skin structurally consists of epidermis and dermis layers (Fig. 1d) [22]. The epidermis acts as a physical barrier to prevent invasion by pathogens and potentially harmful substances and contains moisture and necessary nutrients for the body. Structures in the dermis include sweat glands, sebaceous glands, and hair follicles. Specific sites on the layers harbor unique microbial communities. The vast majority of bacteria that inhabit our skin include *Propionibacterium acnes*, *Corynebacterium tuberculostearicum*, *Streptococcus mitis*, *Streptococcus Oralis*, *Streptococcus pseudopneumoniae*, *Streptococcus Sanguinis*, *Micrococcus luteus*, *Staphylococcus epidermidis*, and *Staphylococcus capitis* [19]. Their composition and concentration vary between individuals and depend on the physiology of a particular skin site, which may have moist, dry, or oily conditions. For example, *Propionibacterium acnes* inhabit mainly oily skin sites such as a sebaceous gland, while *Staphylococcus* and *Corynebacterium* species are preferentially abundant in moist skin sites such as the feet and the bends of the elbows [19,21,22]. Interestingly, those bacteria can survive even in the extremely harsh conditions of human skin, which is a cool, acidic and dry environment with insufficient nutrients. Furthermore, although sweat contains antimicrobial substances to prevent the invasion of foreign pathogens, sweat also contains food necessary for bacteria to thrive on the skin. Some non-human bacteria that metabolize ammonia, a major component of sweat, also are suited for the environment of the human skin. Moreover, ammonia-oxidizing bacteria can improve skin health by producing nitrite, and nitric oxide, which are used in physiological skin functions, including wound healing, blood vessel relation, inflammation, and acne treatment [20]. In particular, nitric oxide produced by *Nitrosomonas* species has been found in clinical trials to be critical in basic systemic and cutaneous physiological functions [23]. All those advantageous of sweat-eating bacteria (including skin microbiome and ammonia-oxidizing bacteria) are expected to be part of the best-fit solution for powering skin-worn electronic applications. However, the skin genus *Propionibacterium acnes* and *Corynebacterium*

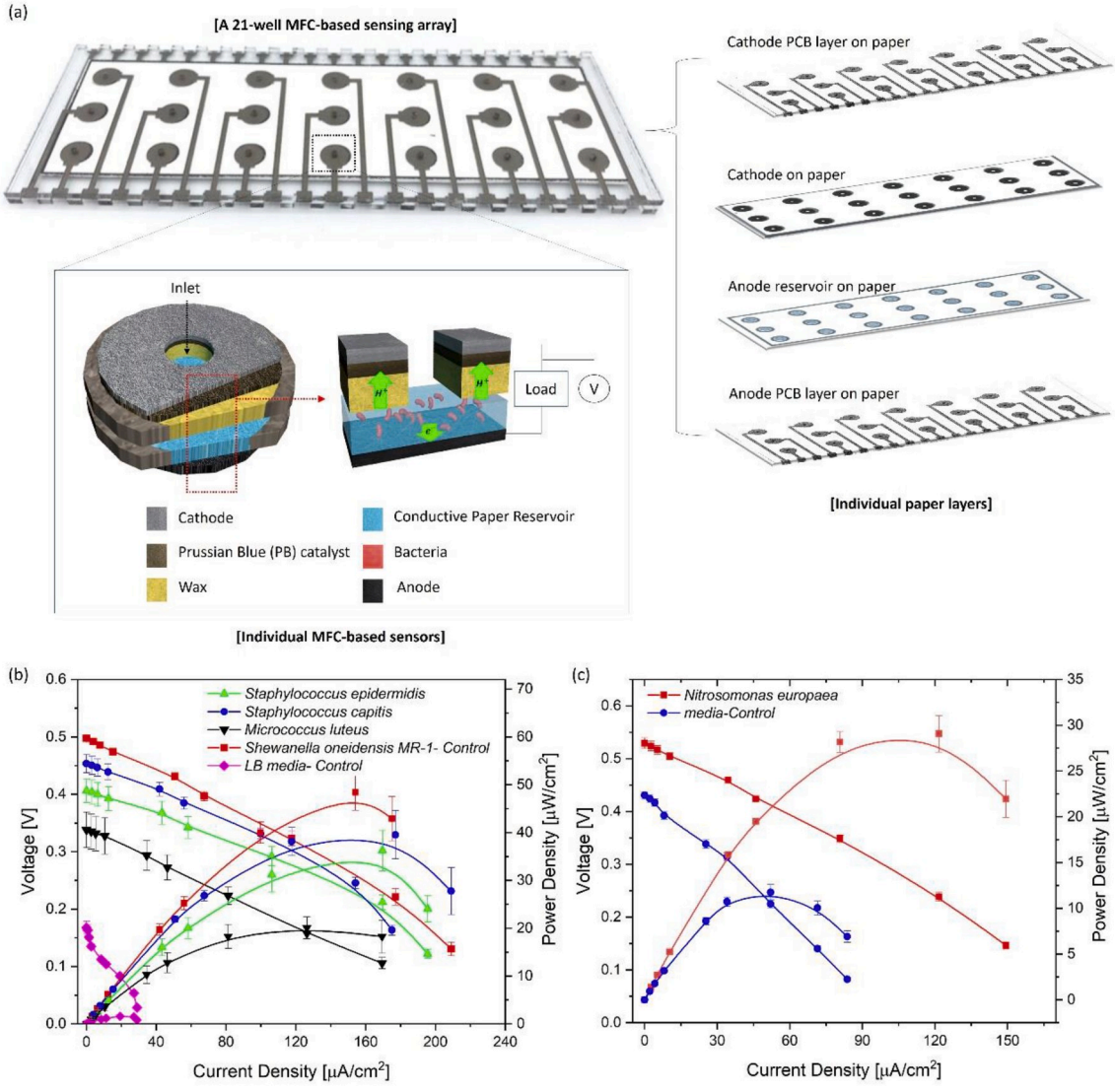


**Fig. 2.** (a) Photos of the assembled 21-well MFC array and its individual layers, and schematic diagram of an individual MFC unit. (b) Power outputs and polarization curves of the biobatteries powered by skin-habitat microorganisms (Staphylococcus epidermidis, Staphylococcus capitis and Micrococcus luteus), Shewanella oneidensis MR-1 as positive control and LB media as negative control. (c) Power output and polarization curve of the biobattery powered by the ammonium oxidizing Nitrosomonas europaea bacteria cells and media as control.

*tuberculostearicum* are more difficult to cultivate than *Staphylococcus* sp. so they are frequently underused in culture-based studies [19]. In this work, *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Micrococcus luteus*, and *Nitrosomonas europaea* are selected for a comprehensive investigation of their electrogenicity.

### 2.2. Discovering sweat-eating exoelectrogens through a paper-based MFC array

We used a high-throughput sensing array system for rapid multiplexed discoveries and characterization of the electrogenicity of three skin bacteria and one ammonia-oxidizing bacteria. Our group's previous technique for a paper-based MFC array was leveraged to develop a 21-well MFC-based sensing array [24–26] (Fig. 2a). A paper-based MFC array was designed on two sheets of Whatman 3MM chromatography paper. The 21 hydrophilic reservoirs were first defined with hydrophobic wax boundaries on the paper simply by using a commercially-available solid-wax printer and letting the wax penetrate the paper with heat treatment. The wax was used as an ion-exchange membrane, as it provided a hydrophobic property to the paper, separating the anodic and cathodic parts and allowing protons to pass through efficiently. The horizontal spread and the vertical penetration of the melted wax was carefully controlled by adjusting the heating time. A Prussian Blue (PB) catalyst was coated onto the membrane and then carbon and nickel were subsequently sprayed as an electrical contact. A mixture of poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) anions (PEDOT:PSS) and dimethyl sulfoxide (DMSO) was pipetted into the wax-defined anodic reservoir to make it conductive and porous for bacterial inoculation and electricity harvesting [27,28]. In an MFC, bacteria in the anodic reservoir oxidize the sweat and produce electrons and protons. The electrons flow through an external circuit to reach the cathode while the protons diffuse from the anodic reservoir to the cathode through a wax membrane to maintain charge neutrality. At the cathode, an electron acceptor is reduced by the protons and electrons that traveled from the anodic reservoir. Our MFC array tested three Gram-positive skin bacterial species (*Staphylococcus epidermidis*, *Staphylococcus capitis* and *Micrococcus luteus*), one Gram-negative ammonia-oxidizing bacteria species (*Nitrosomonas europaea*), one wild-type well-known exoelectrogen (*Shewanella oneidensis* MR1, Gram-negative) for comparison as a positive control, and two bacterial culture media (Luria broth (LB) for skin bacteria and ATCC #2265 for *Nitrosomonas europaea*) as a negative control. Each of those 7 samples was loaded into three units in the 21-MFC array to quantify repeatability and reproducibility. This array allowed rapid and sensitive analysis of bacterial electrogenicity associated with microbial interactions, and electron transport. A National Instrument (NI) data acquisition module (USB-6212) with a customized user interface was used to measure voltages. Device current and power generation were measured and calculated with the connected external resistances and the measured voltage outputs via Ohm's law. Theoretically, exoelectrogens conduct extracellular electron transfer via direct electron transfer, where the cells adhere physically to the anode surface and transfer electrons to the solid anode or where a solid conductive wire is biosynthesized and used for electron transfer, or via shuttle transfer, where the electrons are transferred to the anode by electron mediators [29].

As shown in Fig. 2b, all three Gram-positive skin bacteria (*S. epidermidis, S. capitis* and *M. luteus*) showed distinct electrogenic capabilities ($36.23 \pm 4.24$ μW/cm$^2$, $39.52 \pm 5.05$ μW/cm$^2$, and $20.08 \pm 2.28$ μW/cm$^2$, respectively), which were also comparable to that of the well-known Gram-negative exoelectrogen, *S. oneidensis* ($48.39 \pm 3.74$ μW/cm$^2$) while the LB-media as a negative control generated negligible power output ($1.61 \pm 0.1$ μW/cm$^2$). These results were quite surprising because little attention has been paid to electrogenicity of Gram-positive bacteria while the scientific community has explored electrogenic Gram-negative bacteria mainly found in exotic environments like the deep ocean. It has been thought that Gram-positive bacteria are unlikely to have the electrogenic ability because of their very thick cell membrane. Our results are in good agreement with the recent reports about the discovery of the electrogenicity of the gut microbes, *Listeria monocytogenes* and *Enterococcus faecalis* [30,31]. Although we need to go further to understand extracellular electron transfer pathways of those skin bacteria, this study for the first time reports the discovery of microbial electrogenicity in skin bacteria and provided an opportunity to easily generate power on the skin.

Furthermore, non-human ammonia-oxidizing microorganism, *Nitrosomonas europaea*, demonstrated distinct electrogenic capability ($29.07 \pm 2.00$ μW/cm$^2$) compared to their negative control (ATCC #2265)($11.73 \pm 0.86$ μW/cm$^2$) (Fig. 2c). Ammonia is a major component of human sweat, and can be an excellent energy resource for the MFCs [32].

### 2.3. A skin-mountable MFC integrated with a sweat simulator and a microfluidic interface

After we successfully demonstrated exoelectrogenic activities in human skin-inhabiting bacteria and non-human ammonia-consuming bacteria, and their capacity to produce significant electrical power outputs, we tested their practical feasibility as a power source for potential e-skins by developing a skin-mountable MFC system with a skin-interfaced microfluidic sweat collection device. The single MFC unit in the array we used for high-throughput characterization was modified to be a skin-mountable MFC device by covering it with 3 M Tegaderm medical transparent films (Fig. 3a). The films provide adhesion with the skin and prevent leakage of agents so that the liquid-containing MFC can properly function on a skin. Several holes were cut into the bottom of the Tegaderm layer to introduce sweat samples.

Because a clinical trial for bacterial biocompatibility has not been performed yet, human subject research could not be conducted in this work. Instead, we characterized the MFC by delivering various sweat samples with a syringe pump through an integrated sweat simulator and a microfluidic interface (Fig. 3b). The sweat simulator mimics human perspiration by constructing micro-replicated skin-texture (Fig. S1) while the microfluidic device (Fig. S2) captures the sweat samples released from the sweat simulator and delivers them to the MFC placed on the microfluidic device for power generation. The sweat simulator was developed based on the recent work from the Heikenfeld's group (Fig. S1) [33]. The skin-texture simulator membrane has 2 pores/mm$^2$ over the active area of 100 mm$^2$ with a 50 μm diameter of each pore. The skin-interfaced microfluidic device to be placed on the sweat simulator consists of two well-designed PDMS (Polydimethylsiloxane) layers; the channel layer and capping layer (Fig. S2). The device allows the capture and storage of sweat in the microchannel and delivers the sweat through the five circular openings to the MFC, which will be placed on the microfluidic device. As pressure builds in a narrow exit channel, an arrow-shaped capillary valve releases fluid to fill the five circular reservoirs, preventing overflow. Once the reservoirs are filled with fluid, it is pushed through the holes in the capping layer by new fluid entering these reservoirs. Flow progression through the microfluidic channel was determined by the Young-Laplace equation, which gave a burst pressure defined in a shaped channel [34,35] (Fig. S3).

The bacteria were pre-loaded and freeze-dried in the anodic chambers to generate on-demand power from the sweat. Freeze-drying is one of the most commonly used methods for the long-term, stable storage of microorganisms. Microbial cells are frozen by lowering the pressure to allow the frozen water in the cells to transition directly from a solid to a gas [36,37]. This dehydration results in minimal shrinkage and leads to a completely soluble product that is easily rehydrated for use. Previously, we successfully freeze-dried exoelectrogenic bacterial cells on a paper-based device platform for the long-term maintenance of viability and activity of the cells and the on-demand power generation with the rehydration process [28].

To characterize the skin-mountable MFC system with the sweat

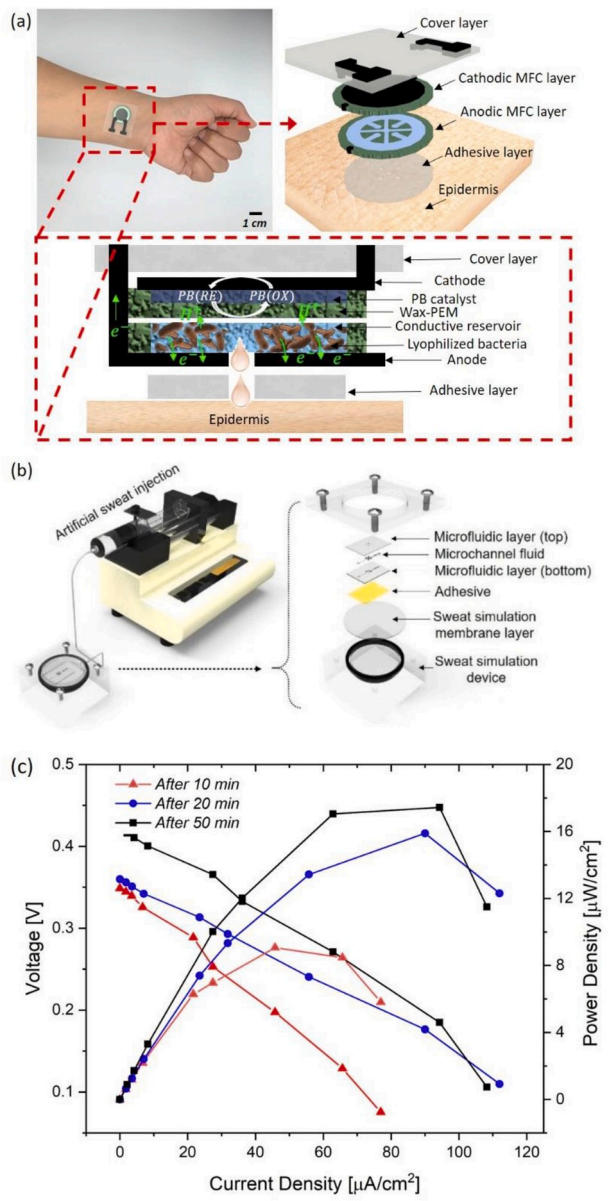


**Fig. 3.** Photo images and schematic diagrams of (a) the wearable bacteria powered biobattery and (b) the skin-interfaced microfluidic device and the artificial sweat simulator. (c) Power generation at different times after injection of artificial sweat at the rate of 50 μL $h^{-1}$.

simulator and the skin-interfaced microfluidic device, the MFC was first pre-inoculated with an electron-producing freeze-dried skin bacteria, *S. epidermidis*. A bacterial concentration with an $OD_{600}$ (optical density at 600 nm) of 2.0 was used to saturate the 10μL volume of the anodic MFC reservoir (Fig. S4). A flow-based syringe pump (KDS 100 digital syringe pump, KDS scientific system) was connected to the sweat simulator with a constant flow rate of 50 μL $h^{-1}$. We chose this rate so that the sweat simulator can reach the closest real sweat flow rate associate with human exercise (0.75 L $h^{-1}$ $m^{-2}$) while the rate should be high enough for ensuring the sweat delivery [33]. With the flow injection for 10 min, the amount of the sweat sample delivered to the MFC was not enough to rehydrate the freeze-dried cells, generating only 8.1 μW/$cm^2$(Fig. 3c). In our system, the highest maximum power (17.5 μW/$cm^2$) was obtained 50 min after the injection started.

### 2.4. On-demand power generation with various samples

After we obtained the optimized operating parameters (e.g. bacterial concentration ($OD_{600}$ of 2), flow rate (50 μL $h^{-1}$), and loading time (50 min)), three skin bacterial species (*S. epidermidis*, *S. capitis* and *M. luteus*) and one ammonia-oxidizing bacteria species (*N. europaea*) were pre-inoculated and freeze-dried in the skin-mountable MFC devices, respectively. For their on-demand power generation, three activation samples (human sweat, artificial sweat, and bacterial media) were introduced to the device through the sweat simulator and the microfluidic interface at a constant flow rate of 50 μL $h^{-1}$. Each sample was to rehydrate the freeze-dried bacteria and produce electricity. All bacterial species generated the most power and current density from human sweat because it contains a variety of nutrition, such as glucose, lactate, urea, ammonia, and electrolytes. As shown in Fig. S5a, the biobattery's performance mainly depended on the lactate and glucose concentrations in the artificial sweat sample. This indicates that the biodegradable organic substrates such as lactate and glucose contain chemical energy convertible to electrical energy by the biobattery. Preferably, the skin-worn biobatteries can be attached directly close to the location of sweat generation, enabling the fast access for energy harvesting. Without exercising or increasing body temperature, however, it may be hard to obtain an adequate sweat source for constant power generation because of the irregular perspiration of individuals [38]. Although this work is limited to naturally collectible sweat, well-established sweat-inducing techniques (e.g. wearable iontophoresis devices) can be readily applied for the continuous, reliable power generation [39]. Alternatively, sweat-based energy harvesting devices can be integrated with energy-storage devices such as lithium-ion batteries and supercapacitors, so that the harvested energy from the sweat can be charged and discharged by the integrated energy-storage device for constant power generation [15]. However, this work mainly focuses on ensuring the practical efficacy of the sweat-eating bacteria as a novel and stand-alone energy harvesting device for e-skin applications. With the freeze-dried *S. epidermidis*, the device activated with human sweat generated an average maximum power density of 41.74 ± 5.35 μW/$cm^2$, while *S. epidermidis* activated by the artificial sweat and LB media generated the average maximum power densities of 36.23 ± 4.24 μW/$cm^2$ and 32.26 ± 5.26 μW/$cm^2$, respectively (Fig. 4). Similarly, the freeze-dried *S. capitis* activated with human sweat generated the average maximum power density of 35.13 ± 1.54 μW/$cm^2$, while the *S. capitis* activated by the artificial sweat and LB media generated the average maximum power densities of 19.13 ± 0.73 μW/$cm^2$ and 12.00 ± 0.52 μW/$cm^2$, respectively. The freeze-dried *M. luteus* activated by human sweat, artificial sweat and LB media generated power densities of 20.62 ± 0.47 μW/$cm^2$, 14.44 ± 0.69 μW/$cm^2$ and 15.78 ± 0.75 μW/$cm^2$, respectively. Finally, the ammonia-oxidizing *N. europaea* generated power densities of 31.14 ± 1.93 μW/$cm^2$, 20.92 ± 1.98 μW/$cm^2$ and 29.07 ± 2.00 μW/$cm^2$, upon activation by human sweat, artificial sweat, and media, respectively (Fig. 4).

To demonstrate the long shelf-life of the device, the devices with *S. epidermidis* were stored in our lab set at 22.8 °C and 45% of relative humidity for up to 200 days (~7 months). To characterize their survival and electrogenicity after a certain shelf-life, the freeze-dried samples were rehydrated with one drop of artificial sweat and the power generated from the devices was measured. Figs. S5–b shows that after rehydration our pre-loaded and freeze-dried exoelectrogens generated electrical current even after the seven-month storage. However, the power decreased by about 45% from Day 1 as the storage shelf life increased to 200 days. For optimizing the shelf-life, the effects of cryoprotectants and storage conditions on the stability of each bacterial strain need to be examined. A variety of cryoprotectants, such as trehalose, glycerol, betaine, adonitol, sucrose, glucose, lactose and polymers, need to be tested to find that additive produces the greatest survival rate of bacterial cells after freeze-drying. Also, various storage conditions with different temperatures, humidity, and light need to be

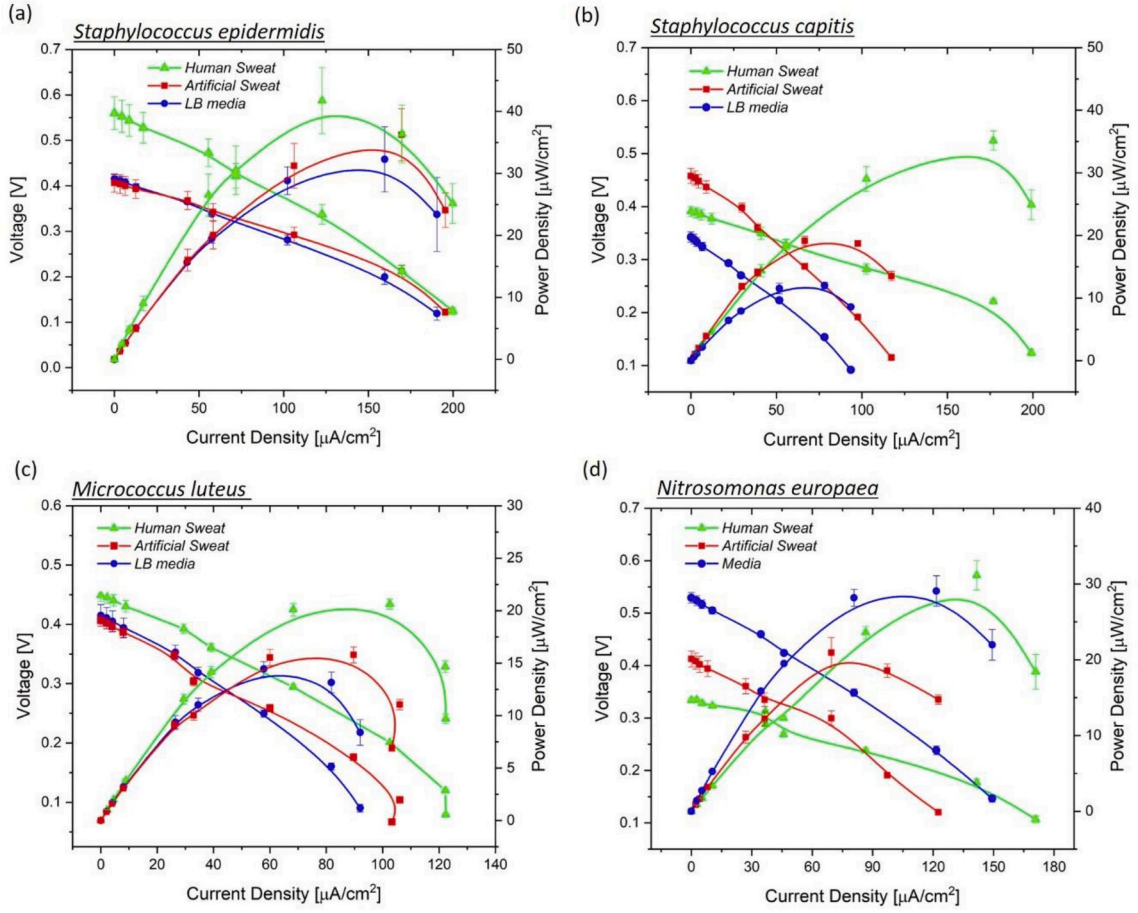


**Fig. 4.** Power outputs & polarization curves of the biobatteries with lyophilized (a) *S. epidermidis*, (b) *S.capitis*, (c) *M.luteus* and (d) *N. europaea* activated by human sweat, artificial sweat & media.

examined to provide a practical strategy for skin biobatteries.

### 2.5. A battery system with a DC-DC booster circuit

The low power density of existing state-of-the-art miniaturized MFCs may limit their practical ability to power useful e-skin applications. The typical sustainable voltage and power outputs from a single miniature MFC are on the order of 0.4–0.6 V and 10–100 μW, respectively [40]. Most conventional e-skins require voltage and power on the order of >3.0 V and >1 mW for far-field wireless transmission of collected information or for other energy-consuming functions. To produce sufficient voltage and power (to reside within the operating range of electronics), we connected the biobattery to a low input voltage booster, BQ255504 (Texas instruments), that is well-suited to operate within the miniaturized biobatteries (Fig. 5). The voltage boost converter can start operation with an input voltage of 0.3–0.6 V. Once started, it can

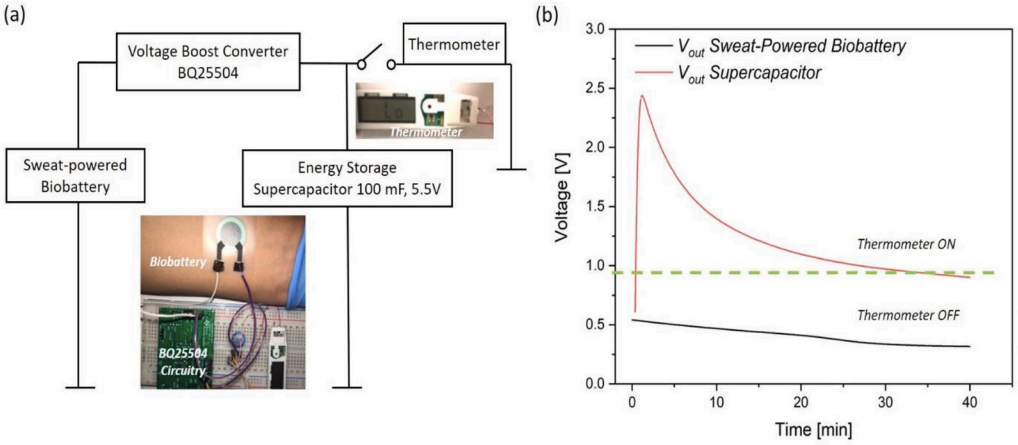


**Fig. 5.** (a) Overview of the experimental setup to power a thermometer. The biobattery is connected to a low input voltage booster, BQ255504 (Texas instruments). The circuit converts the relatively low DC voltage of the biobattery to a more practical range for a thermometer of >3.0 V. (b) Plot of time versus voltage of the sweat-powered biobattery and DC-DC booster circuit.

continuously generate energy with only 0.15 V of input voltage and ultra-low quiescent current of 0.33 μA. As shown in Fig. 5, The DC-DC booster circuit was capable of converting the relatively low DC voltage of the biobattery (0.5 V) to a more practical range of >3.0 V to operate a thermometer.

## 3. Conclusion

In this work, we created a new sweat-based energy harvesting technique, delivering on-chip power to the next generation of e-skin applications. We envision that the developed innovation provides a novel strategy that revolutionizes MFC applications by exploring bio-electrogenicity of unknown electrogenic sweat-eating bacteria and that enables energy scavenging from human sweat. The work connected advanced skin-mountable microfluidics to electromicrobiology through innovative research that is expected to generate a wealth of new scientific and technological results with significant and transformative potential. First, we comprehensively studied the capabilities of extracellular electron transfer in sweat-eating bacteria and thus accelerated the discovery of electrogenically active microbes from skin microbiome and non-human ammonia-oxidizing microorganisms. Then, an innovative, soft, skin-mountable MFC was created, made operable with sweat-eating microorganisms, and potentially integrated with e-skins. Finally, we developed a skin-interfaced microfluidic device to capture and deliver sweat, and integrate it with the MFC as a power generating system.

## 4. Experimental sections

### 4.1. Materials

Tryptone, yeast extract, sodium chloride (NaCl), dimethyl sulfoxide (DMSO), glutaraldehyde solution, L-Lactic acid (L-6402, 98%) and D-(+)-Glucose were purchased from Sigma-Aldrich. Poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS) (Clevios PH1000) was purchased from Heraeus. Conductive graphite ink (#E34561000G) was purchased from Fisher Scientific Company, LLC. Whatman™ Grade 3MM chromatography paper was obtained from VWR International, LLC. Prussian Blue (PB) was obtained from Spectrum Chemical. Finally, artificial sweat was purchased from Quantimetrix and used as received.

### 4.2. Bacterial inoculum

*Staphylococcus epidermidis, Staphylococcus capitis, Micrococcus luteus* and *Shewanella oneidensis MR-1* were grown from −80 °C glycerol stock cultures by inoculating in a 20 mL of L-broth (LB) media 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. Broth cultures were then centrifuged at 5000 rpm for 5 min to remove the supernatant. The bacterial cells were re-suspended in a new medium and used as power source for the device. To monitor bacterial growth, we measured the optical density at 600 nm ($OD_{600}$) of the culture.

*Nitrosomonas europaea* were grown from −80 °C glycerol stock cultures by inoculating in a 10 mL of ATCC broth #2265 medium with gentle shaking in air for 3 days at 25 °C. The ATCC broth #2265 media consisted of Solution 1 (4.95 g of $(NH_4)_2SO_4$ (for 50 mM $NH_4^+$), 0.2 g of $KH_2PO_4$, 0.27 g of $MgSO_4{\cdot}7H_2O$, 0.04 g of CaCl, 0.5 mL of $FeSO_4$ (30 mM in 50 mM EDTA at pH 7.0), 0.2 mg of $CuSO_4{\cdot}5H_2O$ in 1.2 L of distilled water), Solution 2 (8.2 g of $KH_2PO_4$, 0.7 of $NaH_2PO_4$ in 0.3 L of distilled water (pH 8.0 with NaOH 10 N)), and Solution 3 (0.6 g of $Na_2CO_3$ in 12 mL of distilled water). The three solutions were sterilized at 121 °C for 20 min and mixed at room temperature.

### 4.3. A 21-well MFC array

A 21-well MFC array consisted of four functional layers (i) a top polymethyl methacrylate (PMMA) cathodic wiring layer, (ii) a paper cathodic layer with a wax-based proton exchange membrane (PEM), (iii) a paper anodic layer, and (iv) a bottom PMMA anodic wiring layer (Fig. 2). The middle paper layers defined the individual MFCs. The paper cathodic layer was constructed on a region of patterned wax by brushing on Prussian blue (PB) catalysts and then screen-printing graphite ink to collect current. The wax region was used as a proton exchange membrane (PEM). The paper anodic layer was prepared by forming a conductive anodic reservoir with a 20 μL mixture of PEDOT:PSS and dimethyl sulfoxide (DMSO), followed by graphite ink screen-printing. Then, 3-glycidoxypropy-trimethoxysilane was added to the reservoir and air-dried to maintain hydrophilicity of the region.

### 4.4. A skin-mountable biobattery

The biobattery consisted of the engineered conductive paper anodic layer, the paper cathodic layer with the wax-based membrane, the adhesive skin-mountable layer and the cover layer (Fig. 3a & Fig. S6). The conductive anodic reservoir was prepared by introducing a 20 μL mixture of 1 wt% PEDOT:PSS and 5 wt% DMSO into paper reservoirs and air-dried for 8 h. To further increase anode reservoir's hydrophilicity, 20 μL of 2 wt% 3-glycidoxypropy-trimethoxysilane was added to the reservoir and air-dried for 2 h [19]. The PEDOT:PSS polymers provided a conductive 3D paper matrix. Adding graphene removed the need for multiple PEDOT:PSS injection steps to reach the desired sheet resistance range (>300 Ω/sq.).

The cathode was prepared with 8 mg of PB and 3 mg of graphene with a conductive binder solution, followed by ultrasonication for 10 min. The conductive binder solution was prepared with (i) 30 μL of PEDOT:PSS solution, (ii) 5 μL of 5 wt% Nafion, (iii) 100 μL of isopropanol. The mixture was brush coated on the predefined paper-reservoir on the cathodic side of the paper. The cathode electrode was prepared by screen-printing of graphite ink on top of the previously brushed catalyst.

### 4.5. Freeze-drying bacterial cells

After the MFC devices were inoculated with bacterial cells, they were placed in a freeze drier (FreeZone Plus 2.5 L Cascade Benchtop Freeze Dry System, Labconco, MO, USA). The drying operation was performed at a pressure of 0.06 atm for 12 h with freezing and sublimation processes. During the freezing, the chamber temperature dropped to −50 °C and then progressively increased back to room temperature. The device was not affected by the lyophilization processes.

### 4.6. A sweat simulator

The building block of a bi-layer in-vitro sweat simulator was produced by mechanical milling of an acrylic block (Mcmaster-Carr). Bi-layer Polyethyleneterephthalate (PET) film of 0.2 mm thickness served as the outer layer, and etched polycarbonate membrane (0.2 μm, Sterlitech) served as the inner layer [33]. The pores of the outer PET layer were micromachined using a laser engraver system (VLS3.50, Universal Laser Systems) with the optimized setting at 50 μm diameter with a pore density of 2 pores $mm^2$ over the active area of 100 $mm^2$. The bi-layer is held between the rubber holder and the acrylic cap with tightened screws. A flow-based syringe pump (KDS 100 digital syringe pump, KDS scientific system) was connected to the sweat simulator with a constant flow rate of 50 μL h-1 through the active area of the sweat simulator.

### 4.7. A microfluidic interface

The fabrication process of the microfluidic device is presented in

Fig. S7. The stereolithography (SLA) based 3D printer (Form 2 3D printer, Formlabs) was used to prepare a mold through polymerization of photocurable resin (FLGPCL04 clear resin, Formlabs). Computer aid design (CAD) software from Autocad 2020 (Autodesk, Inc.) was used to outline mold for 3D printing. The 3D printed mold was used for standard soft lithography of the PDMS based microfluidic device. PDMS based microfluidic device was fabricated by pouring PDMS (10:1 base: curing agent; Sylgard 184, Dow Corning, MI, USA) on the mold and fully cured (60 °C oven for 12 h). A 22-gage needle was used to punch defined a hole at the inlet of the channel layer of the microfluidic device. Exposure of the channel layer and capping layer at high power at 500 mTorr (Plasma Cleaner PDC-32G, Harrick Plasma, NY, USA) for 60 s facilitated bonding of the two layers after alignment. The joined PDMS device was allowed to rest for a day to recover its hydrophobicity [34]. Skin-adhesive (PC2723U, Scapa Healthcare) with a 6 mm diameter hole was aligned to the center of the inlet hole of the channel layer for conformal contact between the channel layer and the sweat simulator layer.

### 4.8. Electrical measurement setup

The voltage difference between the anodes and the cathodes were measured with a data acquisition system (National Instruments, USB-6212), and were recorded every 30s via a customized LabView interface. The current flow through an external resistor was calculated by Ohm's law.

## 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.

## CRediT authorship contribution statement

**Maedeh Mohammadifar:** Writing - original draft, Data curation, Methodology, Investigation. **Mehdi Tahernia:** Writing - original draft, Formal analysis, Investigation. **Ji Hyun Yang:** Writing - original draft, Formal analysis, Investigation. **Ahyeon Koh:** Writing - review & editing, Funding acquisition, Investigation. **Seokheun Choi:** Writing - review & editing, Writing - original draft, Funding acquisition, Project administration, Supervision, Conceptualization.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nanoen.2020.104994.

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