BIOPOWER-IN-GUT: AN INGESTIBLE BACTERIA-POWERED BATTERY CAPSULE

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ABSTRACT

We report an ingestible, millimeter-sized microbial fuel cell (MFC) capsule that can provide a realistic and practical power solution for ingestible electronics. The capsule integrates a pH-sensitive enteric membrane, a germinant-containing layer, and a microfluidic hydrogel-based anodic channel pre-inoculated with *Bacillus subtilis* spores as dormant biocatalysts, which are directly connected to an integrated MFC. When the pH-sensitive membrane dissolves in a designated gut location with a specific pH, the hydrophilic hydrogel in the anodic channel absorb the gut fluids washing the germinant to trigger the spore germination and generate microbial metabolic electricity in our world's smallest MFC. When the capsule is designed to work in the human intestine, it generates electricity only in the neutral pH solution achieving maximum power and current densities of 64μW/cm^2 and 435 μA/cm^2, respectively, which are substantially higher than the other energy harvesting techniques.

KEYWORDS

Ingestible biobatteries, microbial fuel cells, bacteria-powered batteries, *Bacillus subtilis*, spore germination

INTRODUCTION

Ingestible electronic devices are on the verge of being useful for many healthcare procedures, as evidenced by the commercial success of the capsule endoscope [1]. During the passage of the swallowable devices through the gastrointestinal (GI) tract, the capsules can enable direct visualization of the GI tract and monitor its environment for *in vivo* diagnostics and accurate therapy [2]. However, current technology relies on primary batteries to operate, which causes challenges in realizing compact and long-lived advanced functionality because of their bulky size, and finite energy budgets [3]. Furthermore, toxic battery materials or potential mucosal injury hinders the practical and sustained use of ingestible electronics. Power autonomy is a critical requirement for prolonged monitoring systems, so they can work continuously, independently, and self-sustainably. Alternatively, many energy harvesting methods have been proposed to take advantage of body-produced thermal, mechanical, and acidic energies [4]. However, these body energies are not always available or sometimes not enough for practical applications because of the lack of temperature gradients and slow mechanical movements in the GI tract. Acidic energy harvesting is also limited to the stomach with its low pH.

In this work, we create an ingestible bacteria-powered battery capsule functionalized with spore-forming *Bacillus subtilis* which can generate electricity in the human body intestine. The system has a standard size "0" with 0.68 mL volume and 21.7 mm length integrating a conductive anodic chamber with a commercially available neutral pH-sensitive enteric membrane. The neutral pH-sensitive membrane resists the acidic stomach environment but dissolves in the neutral pH environment of the intestines. The smart capsule generates power after dissolving enteric-coating and dispersing germinant powder through adsorption of gut fluid in the bacteria-containing hydrogel (Figure 1). This work demonstrates the promising potential of the spore-forming MFC as an innovative power source for next-generation ingestible applications.

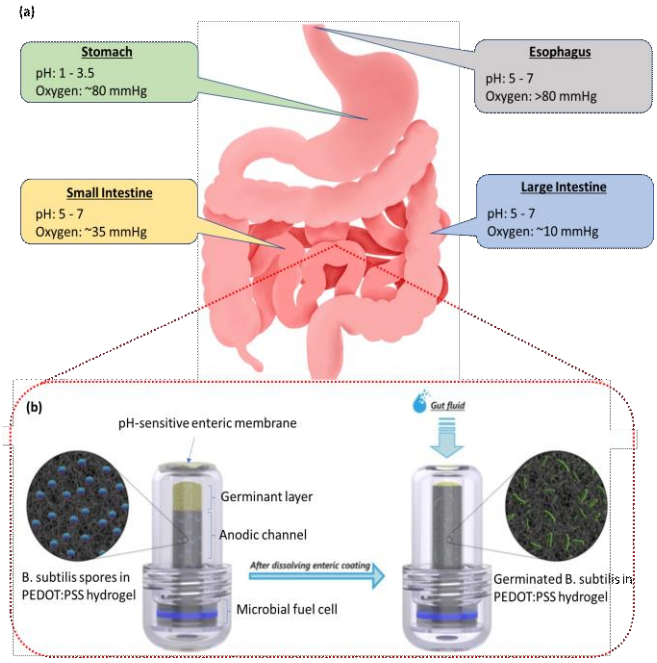

Figure 1: (a) Physicochemical characteristics of the GI tract, and (b) the ingestible MFC system and its working principle.

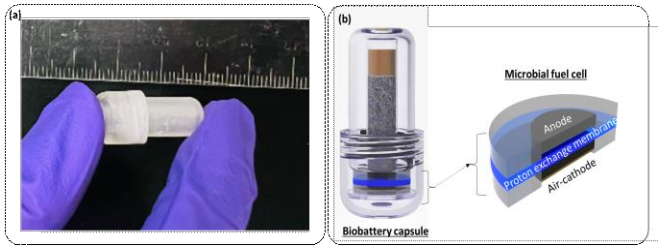

Figure 2: (a) Images of the assembled capsules and (b) the MFC configuration integrated in the capsule.

EXPERIMENTAL PROCEDURE

Capsule design and fabrication

The capsule was fabricated by stereo-lithography-based 3-D printing (Formlabs Form 3B) with high-temperature resin (Figures 1 & 2) [5]. The printed capsule was rinsed in 99% pure isopropyl alcohol (IPA) for 15 minutes to remove remnants and then post-cured with an UV cure station for 60 minutes to complete the

polymerization. The top capsule part including the anodic channel and the germinant layer was screwed into the bottom part having the MFC system.

MFC fabrication

For the MFC structure, an anode and a cathode sandwiched a proton exchange membrane (Nafion 17 membrane), which were all thermally bonded between two poly (methyl methacrylate) (PMMA) layers (Figure 2) [6]. The PMMA layers were designed by AutoCAD and patterned by a laser micromachine (Universal Laser Systems VLS 3.5). The anode was prepared by introducing a mixture of poly (3,4-ethylene dioxythiophene): poly (styrene sulfonate) (PEDOT: PSS) and dimethyl sulfoxide (DMSO) into an untreated carbon cloth. The cathode was constructed on wet-proofed carbon cloth with four layers of polytetrafluoroethylene (PTFE) coating. The cathode was loaded with 10% Pt catalysts for the oxygen reduction reaction. This air-cathode is optimal for the reduction process in the oxygen-dissolved gut environment (Figure 1). The anode and the cathode were pierced with a thin conductive wire as a current collector.

Cultivation and sporulation of *Bacillus subtilis*

Bacillus subtilis strain 168 was acquired from the American Type Culture Collection (ATCC) and was cultivated in Luria Broth (LB) medium at 37°C. The bacterial sporulation was induced by nutrient exhaustion on LB agar plates [7, 8]. The formed endospores were collected from the plate and pelleted by centrifugation at 4000 rpm for 4 minutes. The harvested spores were resuspended in distilled water and stored at 4°C

Anodic channel preparation

The anodic channel was filled with a conductive PEDOT:PSS hydrogel including the bacterial spores. The PEDOT: PSS solution was vigorously stirred for 4 hours, and then the DMSO was added to improve the conductivity of the PEDOT:PSS. The suspension was stirred for 3 hours at room temperature and then the solution was placed in a Teflon autoclave at 180°C for 24 hours. After cooling down to normal temperature, the synthetic material became a porous scaffold structure. *B. subtilis* spores were introduced into the structure and the lyophilization process was conducted in a freeze-drying system (FreeZone Plus 2.5 Liter Cascade Benchtop Freeze Dry System, Labconco, USA).

Germinant layer preparation

The germinant solution was composed of L-Valine (10 mM) and AGFK (10 mM L-Asparagine, 33.6 mM D-Glucose, 33.6 mM D-Fructose, 60 mM KCl) in the LB medium. The solution was freeze-dried in the lyophilization system, which was introduced as the germinant layer on top of the anodic channel. GerA, GerB, and GerK are well-known germinant receptors of B. *subtilis*, that can trigger their spore gemination [7, 8]. GerA interacts with L-Valine while GerB and GerK recognize AGFK.

pH-sensitive polymer membrane

Eudragit® L100 (neutral pH-dependent enteric membrane) was generously donated by Evonik (NJ, USA). 4 g of Eudragit® L100 powder was dissolved in a mixture of 8.8 g of Isopropyl alcohol (IPA) and 6.62 g of acetone. The suspension was vortexed for 15 minutes and sonicated for 60 minutes. Then, it was used in film coating of the capsule to prevent any release of germinant powders/spores and any introduction of the gut fluids in the upper GI tract. A pH 7.0 solution was prepared with a mixture of 100 mL of 0.1 M potassium phosphate and 60 mL of 0.1 M sodium hydroxide in deionized (DI) water. By mixing 46.2 mL of 0.1 M hydrochloric acid, 100 mL of 0.1 M potassium hydrogen phthalate, and 51 mL of DI water, the lowest pH solution was prepared. A pH 6.8 solution was prepared with 6.8 g potassium phosphate and 0.88 g sodium hydroxide in 1000 mL DI water. Its pH was adjusted with 1M NaOH.

Electrical measurement setup

We measured the voltage drops across external resistors by using a data acquisition system (DATAQ Instruments). The current and power outputs were calculated with the connected resistors (470 kΩ, 240 kΩ, 160 kΩ, 100 kΩ, 75 kΩ, 47 kΩ, 33 kΩ, 22 kΩ, 15 kΩ, 10 kΩ, 2 kΩ, 1.5 kΩ, 470 Ω, and 360 Ω). Output densities were normalized to the anode area of the MFC.

RESULTS AND DISCUSSION

Innovation and operating principle

This work is part of a global effort to enable a new generation of smart, stand-alone, and long-lived ingestible electronics designed to serve as practical clinical tools for diagnostics and therapy in the GI tract [9, 10]. While there are many ambient energy resources available in the human body to provide various implantable electronics with sustainable power, the GI tract contains extremely harsh conditions, lacking potential energy sources [11, 12]. Even the latest wireless power transfer and mechanical or thermal energy harvesting techniques are not well suited to ingestible systems [3]. The performance of the wireless power transfer significantly depends on the uncontrollable position and orientation of the devices while it is challenging to securing reliable and practical energy resources from thermal gradients and mechanical movement in the GI tract. On the other hand, the microbial energy harvesting methods in the GI tract are significantly more feasible and can provide superior self-sustaining features with long-term stability because they contain complete enzyme pathways and continuously regenerate biocatalytic enzymes as part of their natural metabolism [13]. Moreover, researchers reported some human gut bacteria can transfer electrons to the exterior of their cells in the nutrient-rich and anaerobic gut environment [13-19]. This intriguing discovery and our additional preliminary data on the electrogenicity of five gut bacterial species inspired us to examine opportunities for a novel microbial power supply in the GI tract [20]. However, it is very challenging to use the human gut-inhabiting microorganisms directly extracted from the host because those incorporated in the capsule cannot maintain their viability and balance their populations throughout the GI tract having different environmental conditions.

In this work, we developed a long-lasting ingestible MFC by using a spore-forming gut bacterium, *Bacillus subtilis*, which can use its survival strategy in harsh gut environmental conditions. *B. subtilis* belongs to a group generally recognized as safe (GRAS) and are usually found in the human gut while their endospores can survive even in the extremely acidic environment of the stomach and tolerates a variety of harsh fabrication conditions with hot/cold temperature, desiccation, high pressure, and other chemical processes, offering flexibility in manufacturing, and long-term operation and stable storage of the capsules [6, 7]. The hydrophilic hydrogel pulls the gut fluid into the anodic channel to trigger the spore germination and provides a promising electrical interface with the germinated bacterial cells to active the MFC.

pH-dependent voltage outputs

The capsules were exposed into several pH solutions which mimicked the different GI environments. Three measurements were conducted along the GI tract, esophagus (pH 7.0 for 1 minutes), stomach (pH 2.6 for 3 hours), and small intestine (pH 6.8 for 3

hours). The neutral pH-sensitive membrane resisted the acidic stomach environment but dissolved in the neutral pH environment of the intestines (Figure 3). Because the ingestible system will have the lowest residence time of tens of seconds in the esophagus with the neutral pH environment, it will not work in the esophagus. The type and thickness of the pH-sensitive membrane can control the operation selectively in different gut areas.

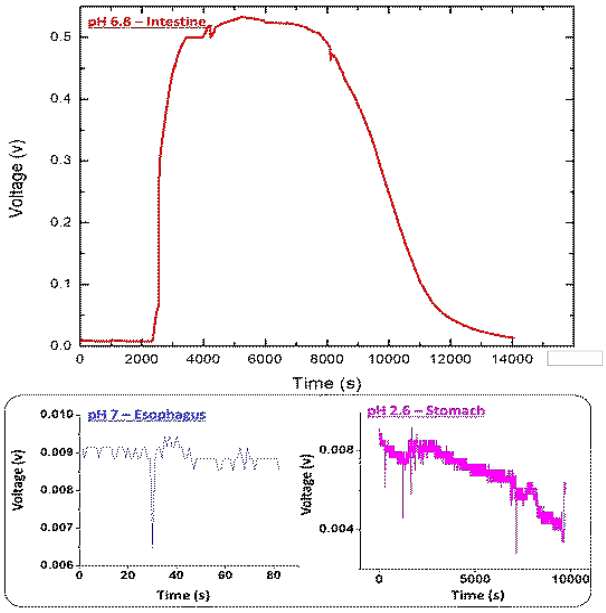

Figure 3: Continuous measurements of the voltages generated from the capsule in different pH solutions.

Power and current generation

A consistent rise was observed in electrical performance as the germination time was increased from 15 minutes to 60 minutes while the control without the spores generated negligible outputs (Figure 4). This demonstrates that the power generation originates from the metabolism of the germinated *B. subtilis* cells. As the spores continued their gemination process with the time, the open circuit voltage (OCV), the current density and the power density improved considerably and reached the maximum in 60 minutes, producing 0.54 V of OCV, 64μW/cm^2 of power density, and 435 μA/cm^2 of current density. This can be attributed to the progressive metabolic improvement in germinated *B. subtilis* cells over time. When the potential gut fluids with germinants triggered the germination of the spores and returned to their vegetative state, the cellular respiration began, generating bioelectricity [6, 7, 22]. Our great performance can be attributed to the revolutionized anodic compartment. Usually, the anode and the anodic chamber play a critical role in affecting the performance of the MFC because its performance depends mainly on the metabolic activity of bacterial cells in the anodic part [16]. The anode component requires a microporous structure large enough to support bacterial growth and a conductive scaffold for stable, consistent bacterial electron transfer. Constructing anodes that are simultaneously biocompatible, conductive, porous, and microfluidic remains challenging especially for miniaturized biological fuel cells. In this work, a highly porous and conductive hydrogel scaffold was innovatively developed by a simple one-step hydrothermal synthetic method and successfully integrated into an extremely small capsule. This work creates a standardized design for the microfabrication of a compact MFC for ingestible applications.

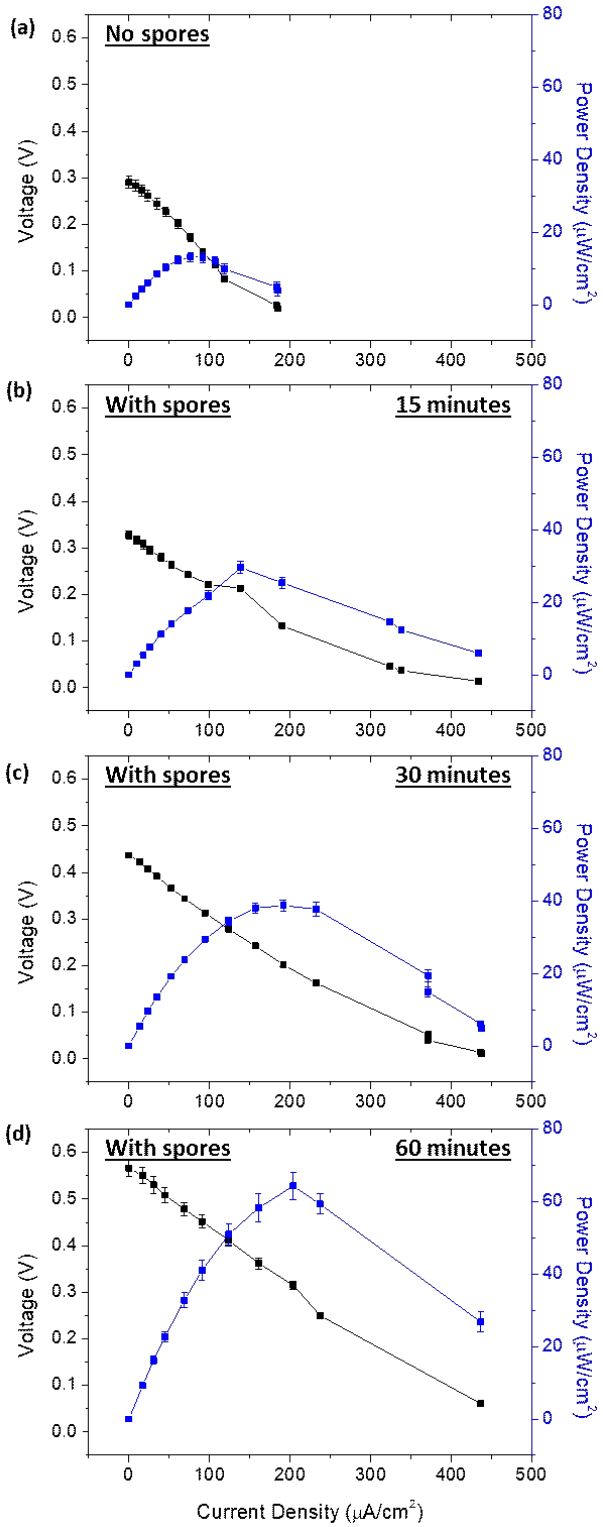

Figure 4: Polarization curves and power outputs of the MFC capsules (a) without and with B. subtilis spores measured at (b) 15 minutes, (c) 30 minutes, and (d) 60 minutes after the enteric membrane dissolves. A consistent rise is observed in open circuit voltage (OCV), power density, and current density as the germination time is increased from 15 minutes to 60 minutes. This can be contributed to the progressive increase in successfully germinated cells over time.

CONCLUSION

The work developed, *for the first time*, a self-contained, self-sustained ingestible MFC battery for devices that can diagnose diseases or deliver drugs where they are needed. The MFC generated reliable and long-lasting power from microbial metabolism in the potential gut environment, delivering on-board energy to the next generation of ingestible devices. This work provided a novel approach to produce electrical power in the gut and improve MFC performance for practical ingestible applications. Reported work on microbial energy harvesting for ingestible electronic applications was unavailable or quite limited because microbial cytotoxicity may pose health concerns. However, if we consider that humans possess more bacterial cells than human cells in their bodies, the direct use of bacterial cells as a power resource interdependently with the human body is conceivable for ingestible electronics. Furthermore, the human gut is home to millions of microorganisms and an ideal place for their cultivation. Because we used the bacterial strain belonging to a group generally recognized as safe, we expect a minimal foreign-body response. Moreover, the GI tract that takes care of various foreign materials for digestion represents one of the human organ systems that cause the least foreign body immune response and consequent malfunction of the ingestible devices. In this work, our MFCs were limited to *in vitro* testing in simulated gut fluids with appropriate pH but we will validate whether the MFC can generate power with *in vivo* animal testing.

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