PROBIOTIC-POWERED INGESTIBLE CAPSULES: A NOVEL APPROACH TO VIBRATIONAL THERAPY

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ABSTRACT

This study presents a pioneering self-sustaining mechanism that exploits metabolic electron production from pre-loaded probiotics to power a vibrating capsule at a specific location in the gut. It is the first research to demonstrate the electrogenic properties of commercially available probiotics in a standard bacterial culture medium, Luria Broth (LB), and its application in generating vibration in a human stomach. The capsule is engineered with a miniature microbial fuel cell containing probiotics, an energy storage component (capacitor), a diode, and a vibrating motor. This assembly is enveloped in a Genipin-crosslinked mucoadhesive polymer to enhance adherence to the stomach lining and is further encapsulated within an acid-sensitive enteric coating to ensure selective dissolution in the stomach. This innovative approach heralds new possibilities for advanced gastrointestinal treatments by merging bio-electricity and biomechanics in a distinctive, patient-centric delivery system.

KEYWORDS

Self-powered vibrating capsules, ingestible biobatteries, electricity-producing probiotics, microbial fuel cells

INTRODUCTION

The advancement of an ingestible vibrating capsule marks a revolutionary, safe, and non-pharmacological method for tackling obesity [1] and effectively managing chronic constipation [2]. Conventional vibrating capsules rely on primary batteries, which pose significant challenges, including toxicity, limited lifespan, and difficulties in sustaining prolonged operations [3, 4]. At the 2022 Hilton Head workshop, our research group unveiled an innovative ingestible capsule that employs a microbial fuel cell technique to generate electricity using electrogenic bacteria [5, 6]. Although this innovation was recognized, concerns were raised regarding the potential health implications associated with the cytotoxicity of the bacterial strains initially utilized. Moreover, the capability of the capsule to serve as a reliable power source for practical ingestible devices was not conclusively established. In response to these concerns, our subsequent research has focused on employing safe probiotics as biocatalysts in the ingestible capsule, thereby mitigating health risks while leveraging the advantageous attributes of probiotics [7]. These freeze-dried live bacteria are instrumental in altering the composition of gut microbiota and promoting a symbiotic interaction with the host's physiology, thereby enhancing the capsule's safety and functional efficacy.

In this investigation, we address the safety concerns highlighted in prior studies by incorporating electricity generation from safe biocatalysts and using them for viable applications within the human gastrointestinal environment (Figure 1). We employed probiotic bacteria [8], integrating these microorganisms into a biocompatible and edible composite made of activated carbon and zinc. This composite was strategically placed within the anodic chamber of a miniaturized microbial fuel cell (MFC), leveraging the metabolic activity of the probiotics for electricity generation. The ingestible vibrating capsule was encased in a flexible, gelatin-based shell. Subsequently, the capsule's exterior was coated with a Genipin-crosslinked mucoadhesive polymer, followed by the application of a pH-sensitive membrane. This design ensures targeted activation at the specific intended location within the gastrointestinal tract, enhancing the capsule's precision and effectiveness. This study elucidates the considerable promise of harnessing commercial probiotics as a renewable energy source within the gastrointestinal environment, showcasing an innovative approach to medical device operation that prioritizes safety and efficiency.

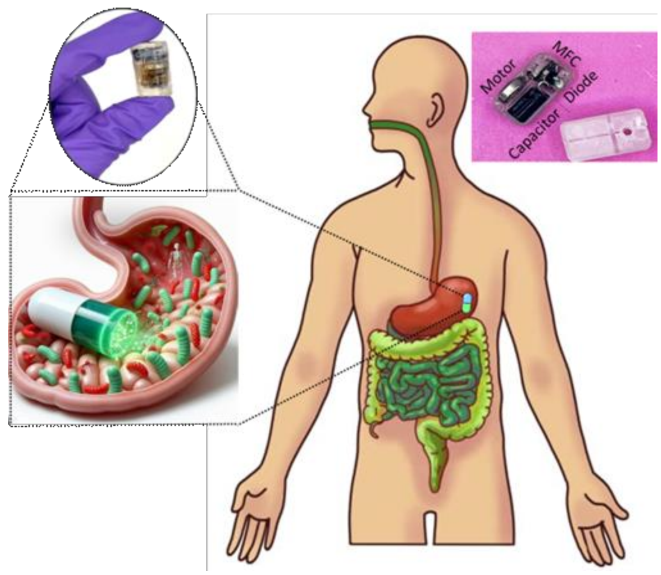

Figure 1: Schematic diagram illustrating the vibrating capsule powered by a probiotic microbial fuel cell and its integrated components.

EXPERIMENTAL PROCEDURE

Capsule design and fabrication

To fabricate our gelatin-based ingestible capsule, we initiated the process by assembling the requisite electronic circuitry (i.e., an MFC, a capacitor, a diode, and a vibrating motor) that facilitates its operation (Figure 1). Subsequently, we employed a specialized 3D printer to construct a mold precisely tailored to the capsule's dimensions. Into this mold, we introduced a gelatin mixture, ensuring the circuit was meticulously positioned within. Following the solidification of the gelatin, we extracted the mold to unveil a robust capsule with the electronic components seamlessly integrated. This meticulous fabrication process guarantees the capsule's functionality and biocompatibility for bodily application. The capsule is characterized by specific metrics: it measures 22 mm in length, has a diameter of 9.2 mm, and encapsulates a volume of 5.847 cm³. It boasts a weight of 2.585 g and a density of 0.442 g/cm³, confirming its suitability for gastrointestinal deployment.

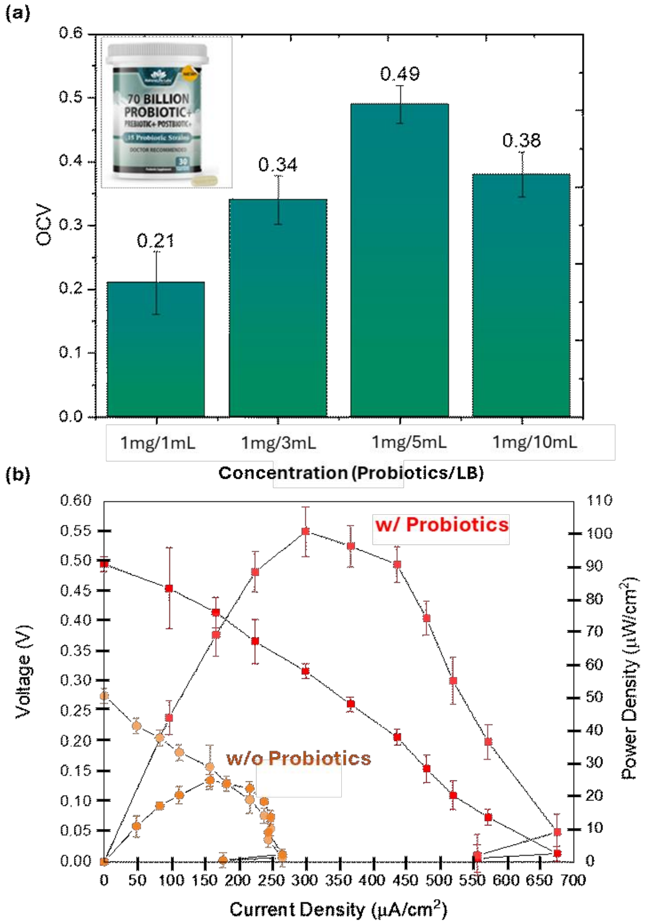

Figure 2: (a) Open circuit voltages (OCV) of the MFC with varying probiotic concentrations in LB. (b) Polarization and power outputs of the capsule containing 1 mg of probiotics in 5 ml of LB.

Probiotics

We sourced commercial probiotics, specifically "Probiotics 70 billion CFU – 15 Probiotic Strains + Organic Prebiotic + Postbiotic 3-in-1 Complete," via Amazon for our experiment. To activate the probiotics, we dissolved the powder into Luria Broth (LB), a standard bacterial culture medium, at different concentrations. We then incubated these solutions at 37°C for 20 minutes. The goal of this procedure was to identify the optimal probiotic concentration that would yield the maximum electrical power output. As depicted in Figure 2a, the analysis of open circuit voltage across various concentrations indicated that the most effective concentration was achieved by dissolving 1 mg of probiotics in 5 ml of LB, highlighting the efficiency of this concentration in maximizing power generation.

Anode

To develop a biocompatible, edible, and conductive anodic compartment for the MFC, we synthesized a composite of activated carbon and zinc. Initially, 100 mg of activated carbon was dissolved in 20 ml of deionized water (DIW). We then added 20 mg of zinc oxide powder to the solution, stirring it continuously for 2 hours to ensure even distribution. Afterward, the mixture was transferred to an autoclave and heated to 180°C for 16 hours to facilitate the synthesis. Once the process was completed, the autoclave was allowed to cool to room temperature. The resulting hydrogel was thoroughly washed with DIW three times to eliminate any impurities, and subsequently freeze-dried to achieve the necessary consistency and stability for its application in the MFC.

Mucoadhesive polymer coating

For the preparation of the mucoadhesive polymer coating for the capsule, we initiated the process by dissolving 50 mg of Genipin powder in dimethyl sulfoxide (DMSO) to achieve a 5% (w/v) concentration. The solution was stirred continuously for 1 hour to ensure complete dissolution of the Genipin in the solvent. Subsequently, 20 mg of chitosan was added to the Genipin solution, followed by additional stirring to ensure a homogeneous mixture. The pH of the solution was adjusted to neutral using a suitable buffer. The solution was then uniformly applied to the capsule's surface through a dipping process, followed by an extensive drying period. The resulting capsule, coated with a Genipin-crosslinked mucoadhesive polymer, exhibits improved adhesion to the stomach lining, thereby enhancing its efficacy for targeted delivery within the gastrointestinal tract [9].

Acid-sensitive enteric membrane

In the preparation of the acidic pH-dependent enteric membrane, 4 grams of Eudragit® EPO powder, generously provided by Evonik (NJ, USA), was dissolved in a solvent mixture of deionized water (DIW) and ethanol with a ratio of 1:0.5. This mixture was agitated continuously for 2 hours to achieve complete dissolution of the Eudragit® EPO powder, a step critical for ensuring the uniformity of the solution. The selection of this specific solvent ratio and the duration of stirring were strategically optimized to enhance the solubility of the Eudragit® EPO powder, as evidenced by previous studies [10]. To prepare a solution with a pH of 3.0, 150 mL of 0.1 M hydrochloric acid (HCl) was combined with 50 mL of DIW. This engineered enteric membrane is designed to dissolve selectively in the stomach's acidic environment, thus permitting the intestinal fluids to initiate the metabolic activity of the encapsulated probiotics, leading to the generation of bioelectricity. This innovative approach underlines the membrane's critical role in facilitating targeted activation and the subsequent bioelectrical output within the gastrointestinal tract.

Electrical measurement

We used a data acquisition system from DATAQ Instruments to accurately measure the voltage drops across external resistors, facilitating the determination of current and power outputs. This measurement process involved using a comprehensive series of resistors with values ranging from 470 kΩ to 360 Ω, specifically including 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. These resistors were connected in series to map out the polarization and power curves across a broad spectrum of loads, ensuring a thorough evaluation of the MFC performance under various electrical conditions. The electrical output densities were then normalized to the anode area of the MFC (Figure 2b), allowing for a consistent and comparable analysis of the MFC's efficiency across different operational parameters.

Electrochemical measurement

Electrochemical analyses were meticulously conducted using the Squidstat Plus potentiostat from Admiral Instruments to assess the anodic performance, both in the absence and presence of probiotics. Cyclic voltammetry (CV) experiments were executed at a scan rate of 100 mV/s, spanning a potential range from -0.8 V to 1.3 V. This protocol facilitated a detailed examination of the biocatalyst behavior, highlighting the electrochemical dynamics under varying conditions. Additionally, electrochemical impedance spectroscopy (EIS) measurements were carried out across a frequency spectrum ranging from 0.1 Hz to 100 kHz. These measurements were instrumental in delineating the charge transfer processes and electrochemical kinetics at the interface between the biocatalyst and anode.

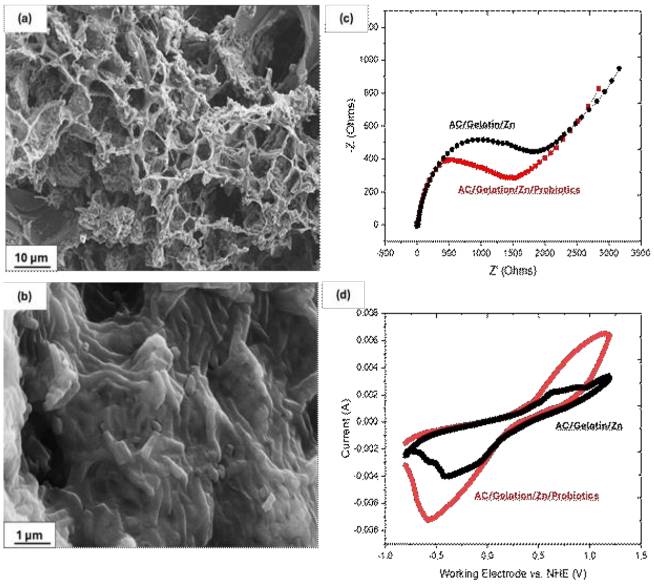

Figure 3: Scanning Electron Microscope (SEM) images of the anode with probiotics: (a) general view, and (b) high magnification view. (c) Electrical Impedance Spectroscopy (EIS) and (d) Cyclic Voltammetry (CV) profiles of the anode. (AC: Activated Carbon; Zn: Zinc).

RESULTS AND DISCUSSION

Initially, we established the optimal concentration of probiotics in LB by evaluating the open circuit voltages (Figure 2a). The peak performance was identified at a concentration of 1 mg of probiotics dissolved in 5 ml of LB. We observed that lower concentrations of bacteria in a larger volume of LB resulted in diminished output voltage, predominantly due to a scarcity of electrogenic bacteria. Conversely, higher concentrations of bacteria in lesser volumes of LB also led to reduced performance, attributed to the inadequate LB relative to the cell count, which in turn limits metabolic reactions. Figure 2b displays the polarization curves of an MFC both with and without the integration of probiotics into the anodic compartment. The inclusion of bacteria significantly bolstered electron transfer and power output, achieving a maximum power density of 100 $\mu W/cm^2$ and current density of 675 $\mu A/cm^2$. Activated carbon is recognized for its extensive surface area and porous structure, providing an abundance of sites for bacterial adhesion and colonization [11-13]. Its electrical conductivity facilitates the efficient transfer of electrons from bacterial cells to the electrode. Zinc, noted for its favorable electrochemical attributes, further augments electron transfer kinetics by encouraging redox reactions at the electrode's surface [14]. In synergy with activated carbon and zinc, probiotic bacteria engage with the electrode interface, harnessing their metabolic pathways to generate electrons. These electrons are then conveyed to the electrode via the conductive matrix established by the activated carbon and zinc, culminating in electrical current production. The bacteria's ability to directly transfer electrons to an electrode surface, acting as biocatalysts within the MFC, enhances power generation and voltage output in comparison to configurations lacking biological elements. As illustrated in Figures 3a and 3b, the anode composed of activated carbon and zinc forms a highly porous network that attracts a significant number of bacterial cells. EIS and CV analyses, presented in Figures 3c and 3d, reveal the probiotics' augmented electrocatalytic properties, underscoring the synergistic effect of the microbial and material components in enhancing the MFC's electrical output.

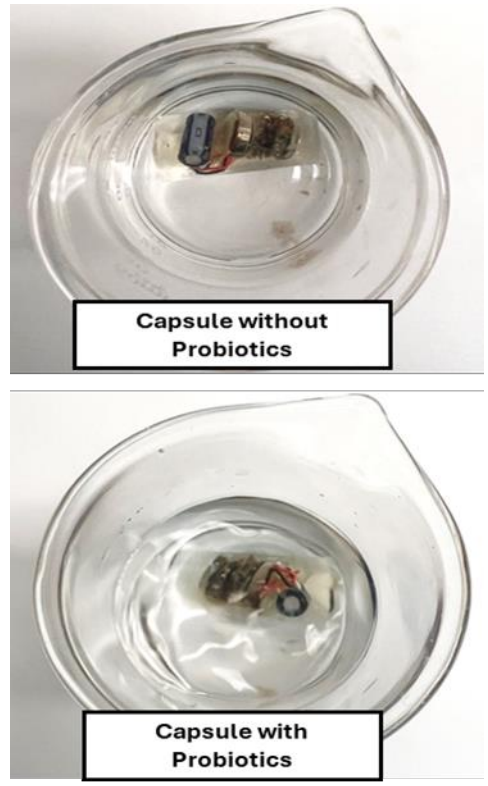

Figure 4: Depiction of the capsule's vibration patterns within simulated stomach fluid waveforms.

Our investigation carefully evaluated the capsule's functionality as an innovative and practical power source [15], particularly for generating mechanical vibrations that hold potential therapeutic benefits [1]. This endeavor significantly advances the field of ingestible medical devices by ensuring safety, practicality, and opening avenues for novel therapeutic applications. The system's design integrates two serially connected MFCs, a 400 µF capacitor, a 0.65 V diode, and a vibrating coin motor, all encapsulated within a gelatin-based shell. To optimize the capsule's performance for gastrointestinal application, it was coated with Genipin-crosslinked mucoadhesive polymer, enhancing its adhesion to the gastric lining. The MFCs were strategically placed on the capsule's upper side, with the uncoated portion designed to absorb gastrointestinal fluid. An outer membrane, dissolvable at the acidic pH of the stomach, encases the device, ensuring activation upon contact with gastric fluids.

For *in vitro* evaluation, capsules were immersed in a simulated gastric solution, with one capsule incorporating probiotics and the other serving as a control without bacteria. Observations conducted 30 minutes post-immersion indicated that the probiotic-incorporated capsule initiated vibrations, thereby inducing turbulence within the solution, in contrast to the stationary control capsule. This phenomenon demonstrates the probiotic-activated, power-demanding vibration mechanism. Upon dissolution of the enteric coating in gastric fluid, the probiotics are activated, generating electricity that charges the capacitor and subsequently powers the vibration motor. Notably, the probiotic-generated power output was significantly high, sufficient to energize the motor efficiently. This activation mechanism was visually validated through the discernible vibration patterns in the simulated gastric fluid's waveforms, underscoring the capsule's operational efficacy (Figure 4).

CONCLUSION

This research marks a significant leap forward in the development of ingestible medical devices, utilizing the metabolic processes of commercial probiotics as a renewable source of energy. Previous investigations into microbial energy harvesting for ingestible electronics have been scarce or hindered by concerns over microbial cytotoxicity and its potential health risks. Unlike other microbes, probiotics are inherently safe, forming an integral component of the natural gut flora. They undergo rigorous safety assessments and are subject to stringent regulatory standards to guarantee their safety, efficacy, and minimal risk of adverse effects. Probiotics act as a biological catalyst for electron production, fueling MFCs that harness these electrons to generate electricity. This bioelectricity is then used to charge a capacitor, which in turn powers a vibration motor.

The focus of this innovation is the stomach, where it is hypothesized to modulate the nervous system as a novel therapeutic strategy for obesity management. Although the current phase of research emphasizes in vitro experiments within simulated gut environments that mimic physiological pH levels, future studies are poised to undertake in vivo trials on animal models. Such endeavors will evaluate the MFC's efficacy in real biological systems, potentially revolutionizing the landscape of ingestible electronic medical devices.

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REFERENCES

[1] Srinivasan, S.S., Alshareef, A., Hwang, A., Byrne, C., Kuosmanen, J., Ishida, K., Jenkins, J., Liu, S., Madani, W.A.M., Hayward, A.M. and Fabian, N., A vibrating ingestible bioelectronic stimulator modulates gastric stretch receptors for illusory satiety. *Science Advances*, 2023. 9(51): p. eadj3003.

[2] Thwaites, P.A., Yao, C.K., Halmos, E.P., Muir, J.G., Burgell, R.E., Berean, K.J., Kalantar-Zadeh, K. and Gibson, P.R., Current status and future directions of ingestible electronic devices in gastroenterology. *Alimentary Pharmacology & Therapeutics*, 2024. 59(4): p. 459-474.

[3] Nadeau, P., El-Damak, D., Glettig, D., Kong, Y.L., Mo, S., Cleveland, C., Booth, L., Roxhed, N., Langer, R., Chandrakasan, A.P. and Traverso, G., Prolonged energy harvesting for ingestible devices. *Nature Biomedical Engineering*, 2017. 1(3): p. 0022.

[4] Van Helleputte, N., Even, A.J., Leonardi, F., Stanzione, S., Song, M., Garripoli, C., Sijbers, W., Liu, Y.H. and Van Hoof, C., Miniaturized electronic circuit design challenges for ingestible devices. *Journal of Microelectromechanical Systems*, 2020. 29(5): p. 645-652.

[5] Rezaie, M., Rafiee, Z., and Choi, S., A Biobattery Capsule for Ingestible Electronics in the Small Intestine: Biopower Production from Intestinal Fluids Activated Germination of Exoelectrogenic Bacterial Endospores. *Advanced Energy Materials*, 2023. 13(1): p. 2202581.

[6] Rezaie, M., Rafiee, Z., and Choi. S., Biopower-in-Gut: An Ingestible Bacteria-Powered Battery Capsule. in *Technical digest of Hilton Head Workshop 2022: A Solid-State Sensors, Actuators, and Microsystems*. 2022. p. 85-88.

[7] Guimarães, J.T., Balthazar, C.F., Scudino, H., Pimentel, T.C., Esmerino, E.A., Ashokkumar, M., Freitas, M.Q. and Cruz, A.G.., High-intensity ultrasound: A novel technology for the development of probiotic and prebiotic dairy products. *Ultrasonics Sonochemistry*, 2019. 57: p. 12-21.

[8] Didari, T., Solki, S., Mozaffari, S., Nikfar, S. and Abdollahi, M., A systematic review of the safety of probiotics. *Expert opinion on drug safety*, 2014. 13(2): p. 227-239.

[9] Xu, J., Strandman, S., Zhu, J.X., Barralet, J. and Cerruti, M., Genipin-crosslinked catechol-chitosan mucoadhesive hydrogels for buccal drug delivery. *Biomaterials*, 2015. 37: p. 395-404.

[10] Moustafine, R.I., Bukhovets, A.V., Sitenkov, A.Y., Kemenova, V.A., Rombaut, P. and Van den Mooter, G., Eudragit E PO as a complementary material for designing oral drug delivery systems with controlled release properties: comparative evaluation of new interpolyelectrolyte complexes with countercharged eudragit L100 copolymers. *Molecular pharmaceutics*, 2013. 10(7): p. 2630-2641.

[11] Gajda, I., Greenman, J., and Ieropoulos, I., Microbial Fuel Cell stack performance enhancement through carbon veil anode modification with activated carbon powder. *Applied Energy*, 2020. 262: p. 114475.

[12] Liu, F., Rotaru, A.E., Shrestha, P.M., Malvankar, N.S., Nevin, K.P. and Lovley, D.R., Promoting direct interspecies electron transfer with activated carbon. *Energy & Environmental Science*, 2012. 5(10): p. 8982-8989.

[13] Tyagi, A., Mishra, K., Sharma, S.K. and Shukla, V.K., Performance studies of an electric double-layer capacitor (EDLC) fabricated using edible oil-derived activated carbon. *Journal of Materials Science: Materials in Electronics*, 2022. 33(11): p. 8920-8934.

[14] Kim, C.H. and Kim, B.-H., Zinc oxide/activated carbon nanofiber composites for high-performance supercapacitor electrodes. *Journal of Power Sources*, 2015. 274: p. 512-520.

[15] Yang, S.Y., Sencadas, V., You, S.S., Jia, N.Z.X., Srinivasan, S.S., Huang, H.W., Ahmed, A.E., Liang, J.Y. and Traverso, G., Powering implantable and ingestible electronics. *Advanced Functional Materials*, 2021. 31(44): p. 2009289.

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