CHARACTERIZING ELECTROGENIC CAPABILITIES OF HUMAN GUT MICROBES

Mehdi Tahernia[1], Yang Gao[1], Maedeh Mohammadifar[1], Melissa R Oefelein[2], Laura C Cook[2], and Seokheun Choi[1]*

[1]Department of Electrical & Computer Engineering, SUNY Binghamton, USA

[2]Department of Biological Sciences, SUNY Binghamton, USA

ABSTRACT

We report a low cost, disposable, paper-based sensing platform for rapid identification and characterization of electroactive microorganisms in human gut. 21 spatially distinct wells of a sensor array parallely characterize microbial extracellular electron transfers of five gut microbes (*Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus agalactiae*, *Lactobacillus reuteri* and *Lactobacillus rhamnosus*) in a defined, microliter culture volume by directly measuring microbial electricity generation in a novel microbial fuel cell configuration. S. aureus, E. faecalis and S. agalactiae showed distinct electrogenic capabilities. The electrogenic potential of gut microbes will be harnessed to act as a biocatalyst and produce a sufficient electrical current for practical applications.

KEYWORDS

Gut microbes; electromicrobiology; electrogenicity; microbial fuel cells

INTRODUCTION

There is a growing appreciation of the role of the gut microbiota in all aspects of human health and disease including metabolism, immunity, and brain functions [1, 2]. Ingestible electronics are fast emerging as a critical technology that can revolutionize gut microbiome research, enabling real-time *in vivo* monitoring from within the body [3-5]. However, even the latest ingestible bacteria-electronic systems for monitoring gastrointestinal health suffer from finite energy budgets available from batteries, hampering long-term operational capabilities [3, 6].

While the scientific community has explored electrogenic bacteria mainly found in exotic environments like the deep ocean [7], they have not paid close attention to the probability of gut microbial electrogenicity residing in a similarly anoxic environment [8]. In its October 4, 2018 issue, Nature published an article about the discovery of the electrogenicity by the microbe *Listeria monocytogenes* and their electron-transfer pathways [9]. This study demonstrated that the food-borne pathogen, *L. monocytogenes*, which colonizes and infects the human gastrointestinal tract, can generate an electric current. Another article published in 2018 also demonstrated the ability of the gut commensal and pathogen, *E. faecalis*, to undergo extracellular electron transfer using biofilm matrix associated iron [10]. Those recent studies suggest that other Gram-positive bacteria likely have the ability to transfer electrons to the exterior of their cells in the oxygen-poor but nutrient-rich gut environment for the same reason we breathe oxygen [9, 11]. The overall goal of this project is to provide an in-depth understanding of gut microbial electrogenic potential for energy harvesting and its capability for a real-time electrical biosensing platform.

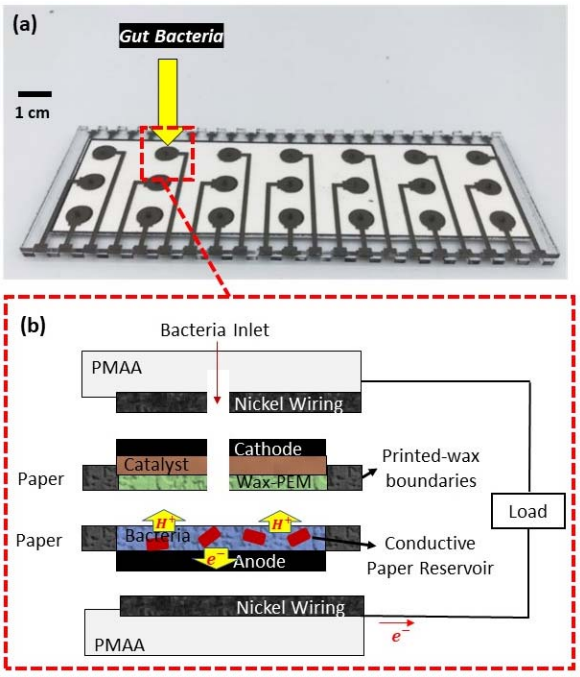

Figure 1: (a) Photo image of the MFC array and (b) schematic diagram of an individual sensing unit.

In this work, we created a unique experimental platform to achieve rapid, highly sensitive electricity generation from five gut microbes, *S. aureus*, *E. faecalis*, *S. agalactiae*, *L. reuteri* and *L. rhamnosus*. We used a high-throughput (21-well) platform to characterize microbial electron transfer in a defined, sub-microliter culture volume by directly measuring microbial electricity generation in a novel MFC device configuration (Fig. 1). We used paper as the MFC substratum that inherently produces favorable conditions for easy and rapid control of a sub-microliter microbial sample through the capillary-driven flow without tubes and fluidic feeding systems. A conductive redox polymer conformally and tightly coated on paper fibers increased the current generation from the 3-D porous electrode surface on which the bacteria are attached. This facilitated microbial electron transfer, resulting in a significant increase in sensitivity. The porous, conductive paper structure ensured a large surface area and efficient mass transfer to and from the paper while the biocompatible redox polymer led to rapid and dense bacterial adhesion. A solid electron acceptor, silver oxide (Ag_2O), was constructed on a paper substrate with carbon or nickel spray providing structural support and functioning as a current collector. The high-throughput

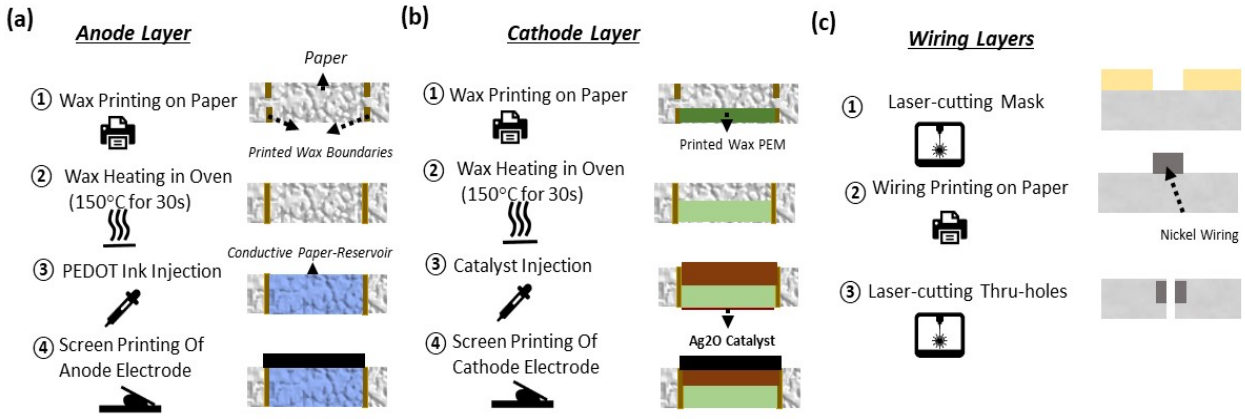

Figure 2: Schematic illustrations of the process used to create the MFC array integrating (a) anodic MFC layer, (b) cathodic MFC layer and (c) PMMA-based wiring layers.

MFCs were batch-fabricated into an array by printing and microfluidic injection processes to build the sensing units on paper.

MATERIALS AND METHODS

Materials

Dimethyl sulfoxide (DMSO), and 3-glycidoxypropy-trimethoxysilane (GLYMO) 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 and Ag_2O (AA11407-14) were obtained from VWR International, LLC. A nickel conductive spray was purchased from MG Chemicals.

Bacterial Inoculum

We selected five species of bacteria commonly found to colonize the human gastrointestinal and genitourinary track: *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus agalactiae*, *Lactobacillus reuteri*, and *Lactobacillus rhamnosus*. We tested the electrogenic abilities of these organisms after growing them in liquid cultures (planktonically) using the micro-sized paper array. *S. aureus*, *E. faecalis* and *S. agalactiae* were cultivated in THY medium (Todd-Hewitt Broth w/ 2% Yeast Extract) while *L. reuteri* and *L. rhamnosus* were cultured in MRS medium (Man Rogosa Sharpe). The well-known exoelectrogen, *Shewanella oneidensis* MR1 was also tested for comparison. *S. oneidensis* cells were cultivated in L-broth (LB) medium. All microbial samples were centrifuged and re-suspended in their medium with the cell titers controlled by the optical density at 600nm. The THY, MRS, and LB medium without bacteria were also tested as the negative controls. All the experiments were performed in a candle jar to provide anaerobic conditions for bacterial respiration.

Preparation of paper-based sensing array

The high-throughput paper-based sensing array is illustrated in Fig. 1 and contains spatially distinct 21 MFC units on 9 cm × 5 cm paper substrates. The array was assembled by sandwiching four functional layers of paper and polymethyl methacrylate (PMMA): (i) PMMA cathodic wiring layer, (ii) cathodic paper layer, (iii) anodic paper layer, and (iv) PMMA anodic wiring layer (Fig 2). All functional layers were carefully assembled. The two paper layers defined the MFC units consisting of a cathode, a wax-based ion exchange membrane, and an anode (Fig. 1 & 2). The conductive paper reservoir in the anodic layer was prepared by injecting 20 µL mixture of 1 wt% PEDOT:PSS and 5 wt% DMSO on paper. A 2wt% of GLYMO solution was introduced to the PEDOT:PSS treated reservoirs to increase the hydrophilicity of the region. The PEDOT:PSS-coated paper improved its conductivity without blocking the paper pores. This technique creates a conductive, biocompatible and porous scaffold for the exoelectrogens placed in each paper reservoir to efficiently transfer electrons to the anode [12].

For the cathodic layer, a silver-based cathode was prepared by mixing and sonicating of 500 mg of Ag_2O and 10 mL of PEDOT:PSS ink [13]. The mixture was injected on the pre-defined paper-reservoir on the cathodic paper layer. The cathode was finally formed by screen-printing of graphite ink on top of the previously brushed mixture. The silver-based cathode allowed high energy recovery efficiencies and stable/reliable performances because of its intrinsically-formed open-framework chemical structure with wide channels. Furthermore, the silver-based cathode was more applicable to paper-based technologies because of its simple fabrication process and low-cost. Finally, nickel metallic wires were deposited on two PMMA layers, which were connected to a voltage/current reader.

All fluidic boundaries and electrical insulators on paper were formed with hydrophobic wax by using a Xerox ColorQube wax printer. The wax patterns were designed using AutoCAD software. The paper with wax patterns was placed in an oven at 150°C for 30s to let the wax penetrate through the paper. The wax was also used as the ion exchange membrane because it provides the hydrophobic property of the paper, separating the anode from the cathode and allowing for proton transfer [14]. The vertical and horizontal penetration depth of the melted wax was carefully controlled by adjusting the temperature and

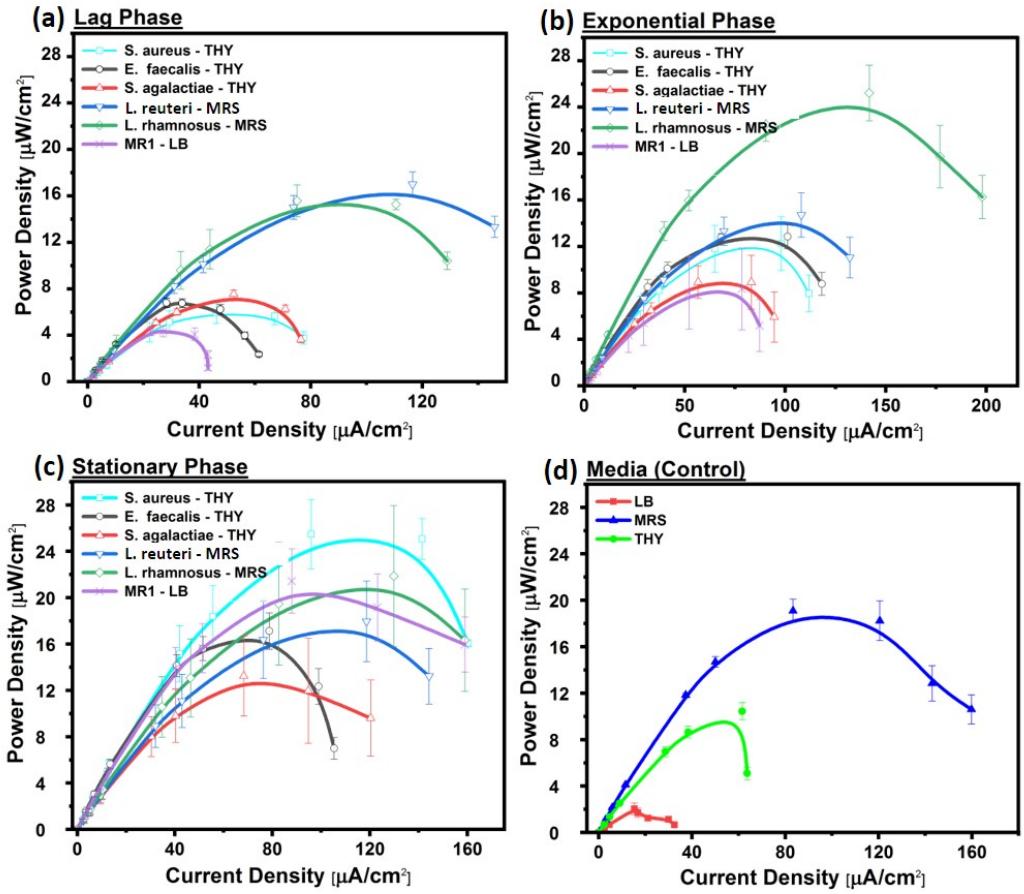

Figure 3: Power outputs of the five gut bacteria and the well-known exoelectrogen, S. oneidensis in three different growth phases: (a) lag phase, (b) exponential phase, and (c) stationary phase. (d) Negative controls.

heating time.

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.

RESULTS AND DISCUSSION

Operating principle

Gut microbes inoculated into the anodic paper reservoirs use respiration to convert chemical energy stored in medium into biological energy. This process involves a cascade of reactions through a system of electron-carrier biomolecules in which electrons are transferred to the terminal electron acceptor. Most forms of respiration use a soluble compound as an electron acceptor but some microbes (named exoelectrogens) are able to respire solid electron acceptors to obtain biological energy. These microbes can transfer electrons produced via metabolism across the cell membrane to an external electrode. The exoelectrogens generate electrons and protons for an MFC operation in which the electrical current is collected through the anodic and cathodic PMMA wiring layers and measured through the external load. The electrons and protons at the cathode finally reduce Ag_2O to Ag, following

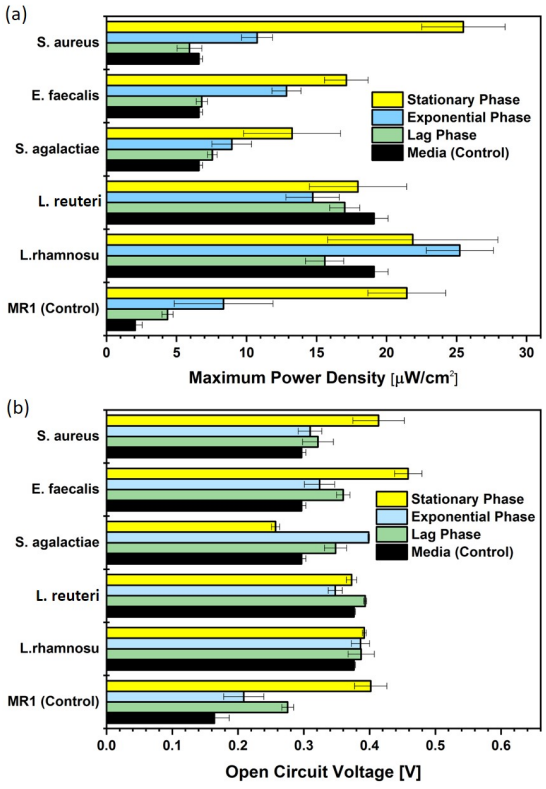

Figure 4: (a) Maximum power densities and (b) open circuit voltages generated from the five gut bacteria and the well-known exoelectrogen, S. oneidensis.

the corresponding reaction:

$$Ag_2O + 2H^+ + 2e^- \rightarrow 2Ag + H_2O$$

Current and power generation

We tested the electrogenic abilities of the five Gram-positive bacteria found in the human gut in three different growth phases: (i) lag phase, (ii) exponential phase, and (iii) stationary phase (Fig. 3). The model exoelectrogens *Shewanella oneidensis* MR1 and three bacterial culture media (THY, LB, and MRS) were used as positive and negative controls. *S. aureus*, *E. faecalis* and *S. agalactiae* showed distinct electrogenic capabilities compared to the negative controls (25.48 $\mu W/cm^2$, 17.12 $\mu W/cm^2$ and 13.25 $\mu W/cm^2$, respectively) while *L. reuteri* and *L. rhamnosus* showed negligible electrogenicity compared to high background seen with MRS medium (Fig. 3 & 4). In particular, *S. aureus* were able to produce significant amount of electricity, which is comparable to that of well-known wild-type exoelectrogens, *S. oneidensis* MR1.

CONCLUSION

In this work, we successfully determined the electricity generation capacity of five gut microorganism by using a novel paper-based sensing platform. The paper-based sensing platform featured (i) the conductive and porous reservoirs for bacterial electron transfer and (ii) the solid-state Ag_2O cathode for high-performance cathodic reaction. High-throughput measurement of microbial electrogenicity was performed by defining 21 spatially distinct individual reservoirs on paper. Although Gram-positive gut microbes generally exhibit very weak electrogenicity due to their thick non-conducting cell membrane, *S. aureus*, *E. faecalis* and *S. agalactiae* showed distinct electrogenic capabilities.

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation (ECCS #1703394 & #1920979), Office of Naval Research (#N00014-81-1-2422), Integrated Electronics Engineering Center (IEEC), and the SUNY Binghamton Research Foundation (SE-TAE).

REFERENCES

[1] J.W. Arnold, J. Roach, M.A. Azcarate-Peril, "Emerging Technologies for Gut Microbiome Research", *Trends in Microbiology*, vol. 24, pp. 887-901, 2016.

[2] A. Kurilshikov, C. Wijmenga, J. Fu, A. Zhernakova, "Host Genetics and Gut Microbiome: Challenges and Perspective", *Trends in Immunology*, vol. 38, pp. 633-647, 2017.

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

[4] M. Mimee, P. Nadeau, A. Hayward, S. Carim, S. Flanagan, L. Jerger, J. Collins, S. McDonnell, R. Swartwout, R.J. Citorik, V. Bulovic, R. Langer, G. Traverso, A.P. Chandrakasan, T.K. Lu, "An ingestible bacterial-electronic system to monitor gastrointestinal health", *Science*, vol. 360, pp. 915-918, 2018.

[5] K. Kalantar-zadeh, N. Ha, J.Z. Ou, K.J. Berean, "Ingestible sensors", *ACS Sensors*, vol. 2, pp. 468-483, 2017.

[6] X. Huang, D. Wang, Z. Yuan, W. Xie, Y. Wu, R. Li, Y. Zhao, D. Luo, L. Cen, B. Chen, H. Wu, H. Xu, X. Sheng, M. Zhang, L. Zhao, L. Yin, "A fully biodegradable battery for self-powered transient implants", *Small*, vol. 14, pp. 1800994, 2018.

[7] D.R. Lovley, "Electromicrobiology", *Annu. Rev. Microbiol*, vol. 66, pp. 391-409, 2012.

[8] L.A. Cahoon & N.E. Freitag, "The electrifying energy of gut microbes", *Nature*, vol. 562, pp. 43-44, 2018.

[9] S.H. Light, L. Su, R. Rivera-Lugo, J. A. Cornejo, A. Louie, A.T. Iavarone, C.M. Ajo-Franklin & D. A. Portnoy, "A flavin-based extracellular electron transfer mechanism in diverse Gram-positive bacteria", *Nature*, vol. 562, pp. 140-144, 2018.

[10] D.Keogh, L.N. Lam, L. E. Doyle, A. Matysik, S. Pavagadhi, S. Umashankar, P.M. Low, J.L. Dale, Y. Song, S.P. Ng, C.B. Boothroyd, G.M. Dunny, S. Swarup, R.B.H. Williams, E. Marsili, K.A. Kline, "Extracellular Electron Transfer Powers Enterococcus faecalis Biofilm Metabolism", *mBio*, vol. 9, pp. e00626-17, 2018.

[11] G. Pankratova L. Hederstedt, L. Gorton, "Extracellular electron transfer features of Gram-positive bacteria", *Analytica Chimica Acta*, vol. 1076, pp. 32-47, 2019.

[12] Y. Gao, M. Mohammadifar, and S. Choi, "From microbial fuel cells to Biobatteries: Moving toward on-demand micro-power generation for Small-scale Single-Use Applications", *Advanced Materials Technologies*, vol. 4, pp. 1970039, 2019.

[13] Y. Gao, and S. Choi, "Merging Electric Bacteria with Paper", *Advanced Materials Technologies*, vol. 3, pp. 1800118, 2018.

[14] M. Mohammadifar, J. Zhang, I. Yazgan, O. Sadik and S. Choi, "Power-on-paper: Origami-inspired Fabrication of 3-D Microbial Fuel Cells", *Renewable Energy*, vol. 118, pp. 695-700, 2018.

CONTACT

*S. Choi, tel: +1-607-777-5913; sechoi@binghamton.edu