BACTERIAL ENDOSPORE BASED WEARABLE BIOSENSORS FOR SELECTIVE AND SENSITIVE GLUCOSE MONITORING

Yang Gao[1], Anwar Elhadad[1], and Seokheun Choi[1,2]*
[1]Bioelectronics & Microsystems Laboratory, Department of Electrical & Computer Engineering, State University of New York at Binghamton, Binghamton, New York, 13902, USA and
[2]Center for Research in Advanced Sensing Technologies & Environmental Sustainability, State University of New York at Binghamton, Binghamton, New York, USA

2024 IEEE 37th International Conference on Micro Electro Mechanical Systems (MEMS) | 979-8-3503-5792-9/24/$31.00 ©2024 IEEE | DOI: 10.1109/MEMS58180.2024.10439331

ABSTRACT

We present a groundbreaking non-invasive, wearable glucose biosensor offering long-term stability, high selectivity, and biocompatibility at low cost. Utilizing bacterial endospores as novel bioreceptors, our sensor exhibits unprecedented robustness, maintaining viability for extended periods without optimized storage conditions. Upon contact with glucose on human skin, these endospores germinate, initiating a microbial process that converts glucose into electrical signals directly correlating with glucose levels. This biosensor discriminates glucose effectively amidst other skin-emitted substances, ensuring precise monitoring. The electrical signals are converted by an integrated circuit, providing a visual LED alert at specific glucose thresholds.

KEYWORDS

Glucose monitoring; wearable; biosensors; bacterial endospores; microbial fuel cells

INTRODUCTION

The landscape of non-invasive glucose monitoring is evolving with the integration of bioreceptors such as enzymes and tailored microorganisms that provide selective glucose detection [1, 2]. Enzyme-based sensors capitalize on the specific catalysis of glucose oxidation, whereas genetically modified microorganisms are designed to respond exclusively to glucose presence. Despite the sophistication of these methods, they are compromised by inherent drawbacks: enzymatic sensors suffer rapid degradation under variable environmental conditions, leading to low stability, and microorganisms necessitate continuous cultivation, imposing constraints on long-term application and electrochemical efficiency [3].

Furthermore, while recent advances have introduced various nanomaterials as non-enzymatic alternatives, these are marred by limitations including diminished glucose sensitivity and specificity, potential biotoxicity, and complex, costly manufacturing processes [4]. Additionally, the surface properties and the interaction of these nanomaterials with glucose molecules are not yet fully optimized, posing challenges in consistent signal transduction and accurate glucose quantification [5].

The push for an innovative paradigm in glucose monitoring is evident, one that marries high performance and stability with biocompatibility. Such an approach would not only transcend the limitations of current methodologies but also enhance the practical deployment of wearable devices. The development of a robust, selective, and sensitive sensor that can endure fluctuating environmental conditions without a compromise in functionality is imperative. Achieving this would revolutionize personal health monitoring by providing reliable, real-time glucose data, thereby facilitating better diabetes management and promoting overall health and well-being. The integration of advanced materials science, microfabrication techniques, and biotechnology holds the key to realizing this goal, paving the way for wearable sensors that can adapt to the dynamic conditions of the human epidermis while maintaining high accuracy and low power consumption [6].

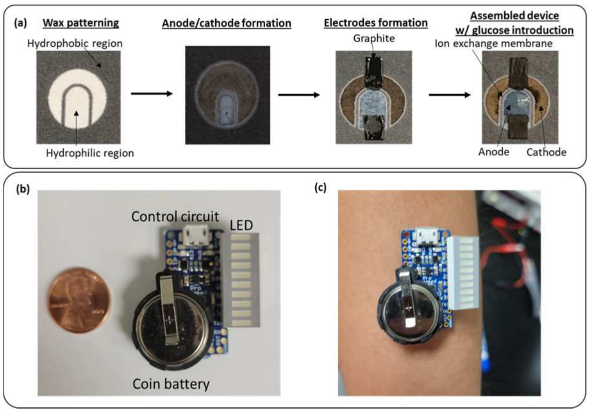

Figure 1: (a) Fabrication process of the proposed paper-based biosensor, (b) a fabricated biosensing system and (c) its wearable feature on human skin.

In this study, we present the innovative development of a paper-based biosensor utilizing dormant *Bacillus subtilis* endospores as a bioreceptor element, embedded within a wearable diagnostic device for non-invasive glucose monitoring. This biosensor is adeptly paired with a dual-modality readout system, which provides both visual cues and digital data conveyance (refer to Figure 1). The device capitalizes on the robust and highly selective germination mechanism of *Bacillus* endospores [7, 8], which, under natural conditions, can be activated by the presence of certain nutrients, specifically a concoction of L-Asparagine, D-glucose, D-fructose, and K+ ions (AGFK) [9-12]. We hypothesize that the glucose component present in human sweat could act as a germinant, selectively triggering the transition of these dormant spores to metabolically active cells. Upon germination, the spores engage in metabolic processes that generate an electrical signal proportional to the glucose concentration [13-16]. Our sensor architecture allows for precise detection of glucose by measuring this bioelectric activity, offering a powerful alternative to traditional enzyme-based glucose sensors. The sensor's operation involves a systematic sequence: sweat permeates the paper-based matrix, comes into contact with the spore-laden region, and if glucose is present, it initiates spore germination. This bioresponse is then converted into an electrical signal by a bio-electrochemical transducer. The integration of this system within a wearable framework enables continuous monitoring, with the visual readout providing an immediate, qualitative indication of glucose levels, and the digital component offering quantitative analysis, facilitating timely medical intervention. We have engineered the paper matrix to ensure optimal spore viability and immediate response upon stimulus, as well as incorporated novel signal amplification strategies to enhance the sensitivity of the biosensor. This makes the proposed device not only a potential tool for diabetes management but also a model for future development of paper-based biosensors for various biomarkers, bridging the gap between laboratory research and real-world biomedical applications.

MATERIALS AND METHODS

Materials

Fisher Scientific supplied conductive graphite ink (#E34561000G) for sensor conductivity. We selected Whatman™ 3MM chromatography paper from VWR International for its consistency, vital for our biosensor's substrate. Heraeus provided the conductive polymer PEDOT:PSS (Clevios PH1000), essential for the sensor's transduction with assured conductivity and biocompatibility. Reagents like Tryptone, yeast extract, NaCl, and DMSO, obtained from Sigma-Aldrich, guaranteed the highest purity for our growth medium and spore assays. Monosaccharides D-glucose and D-fructose, L-Asparagine, and KCl, also from Sigma-Aldrich, were pivotal for *Bacillus subtilis* strain 168 germination, sourced from ATCC to ensure biological reliability. These components collectively underpin our biosensor's precision and traceability, cornerstones of this investigation.

Cultivation and sporulation of *B. subtilis*

For cultivating bacterial specimens, we initiated growth in Luria Broth (LB) medium, agitated at 50 rpm, and maintained at a constant temperature of 37°C for a full 24-hour cycle. Following this proliferation phase, we facilitated sporulation using nutrient-deficient agar plates, adopting protocols established by preceding studies [13, 14]. Upon reaching maturation, spores were harvested, subjected to a centrifugation process at 4000 rpm lasting four minutes, and then resuspended in sterile distilled water. To ensure the elimination of any residual vegetative cells, the preparation underwent a thermal treatment at 80°C for a half-hour duration. The resultant spore suspension was then apportioned into 15 mL airtight tubes and preserved at a refrigerated temperature of 4°C, ensuring their viability for future analytical applications.

Fabrication of wearable biosensors

In this study, we engineered a microfabricated paper-based microbial fuel cell (MFC) with an optimized cathode-to-anode surface area ratio of 3:1 [6]. The microfluidic design of the cell was realized through a precision wax printing process on chromatography paper, followed by a controlled thermal treatment to embed the wax, which acted as an ion-blocking barrier delineating the anode and cathode regions. The anodic segment, designed for direct skin contact, was rendered electrically conductive by infusing it with a PEDOT: PSS conductive polymer ink, which was further treated with DMSO to enhance its electrical conductivity properties. The cathodic counterpart was modified by incorporating silver oxide (Ag_2O) into the PEDOT: PSS matrix to augment its electrocatalytic efficacy for oxygen reduction reactions. Subsequently, the bioelectrical system was coupled with a coin battery and an external circuitry, which included a signal transduction module for real-time analytical readout.

RESULTS AND DISCUSSION

The development of our innovative paper-based biosensor is comprehensively illustrated in Figure 1a, showcasing the sequential integration of electronic and fluidic components onto a cellulose substrate, culminating in the complete assembly depicted in Figure 1b. Figure 1c highlights the biosensor's application on human skin, showcasing its practicality for non-invasive glucose monitoring in real-world scenarios.

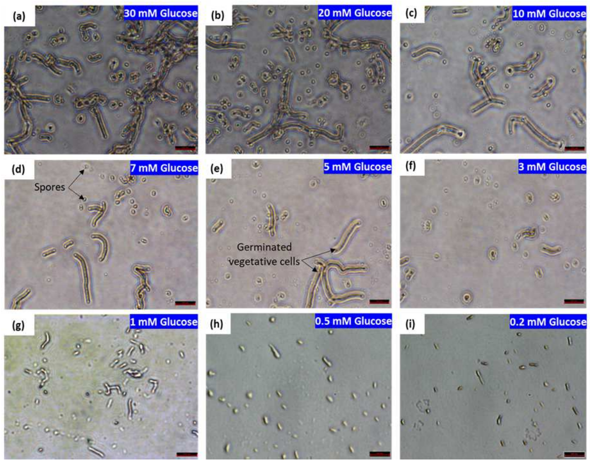

Figure 2: Microscopic images of B. subtilis germination in response to different glucose concentrations.

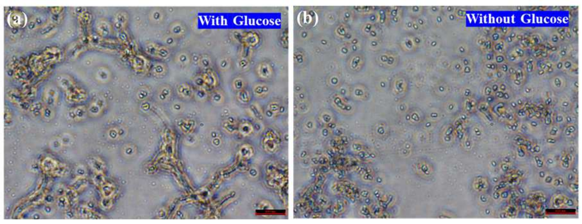

Figure 3: Microscopic image of spore germination (a) with and (b) without glucose

The sensor's anode, in direct dermal contact, captures sweat glucose, leveraging the specificity of spore germination, as illustrated in the microscopic images of Figure 2. These images graphically demonstrate the spores' germination in direct correlation with glucose levels, confirming the biosensor's capacity for quantitative glucose evaluation. Upon introduction of glucose (Figure 3a), the dormant *B. subtilis* spores were observed to germinate, transitioning to metabolically active vegetative cells. This germination was strictly contingent upon the presence of glucose; no germination occurred in its absence, confirming the glucose-specific responsiveness of the spores (Figure 3b). This selective germination is critical for the specificity of the glucose biosensor.

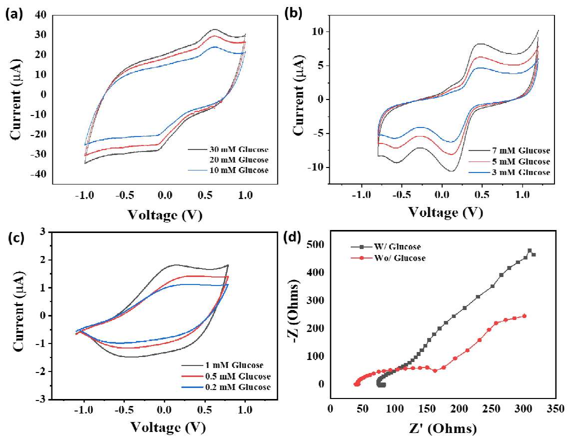

Figure 4: (a)~(c) Cyclic voltammetry curves of B. subtilis spores during its germination with different glucose concentrations. (d) Electrochemical impedance spectroscopy of B subtilis spores with and without glucose (10 mM).

Electrochemical analyses via cyclic voltammetry and impedance spectroscopy elucidate the biosensor's operational dynamics, as shown in Figure 4. The depicted voltammetric and impedance changes upon glucose introduction verify the system's ability to transduce a biological event into an electrical signal.

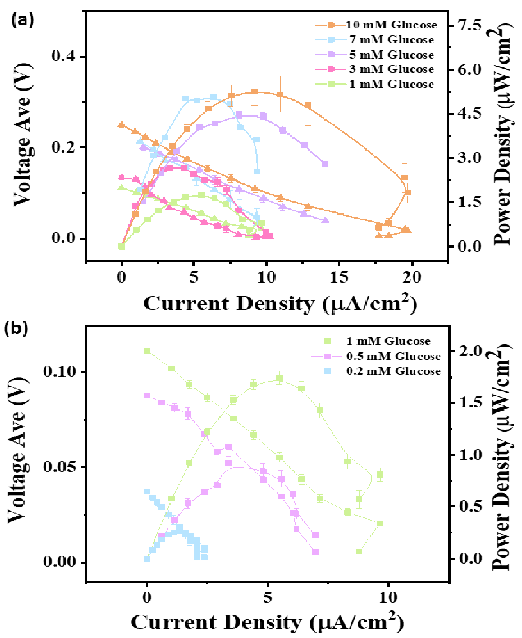

Figure 5: (a) and (b) Voltage and power outputs of the spore-based biosensor as a function of electrical current when a glucose sample at a different concentration is introduced. The electrical output increases with the increasing concentration of glucose.

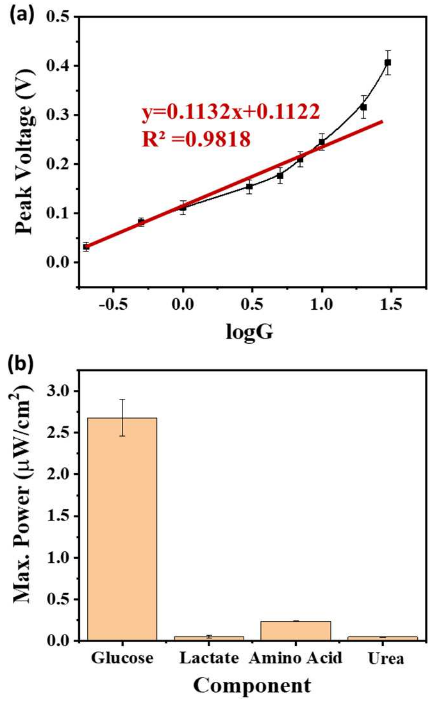

Figure 6: (a) The electrical voltage output versus log scale glucose concentration (0.2 ~ 10 mM). (b) Selectivity test of the biosensor with respect to different biomolecules present in sweat.

The biosensor's electrical output as a function of glucose concentration, portrayed in Figures 5a and 5b, and the log-linear relationship confirmed in Figure 6a (R^2 = 0.98), underscore its accuracy. Figure 6b validates its selectivity, highlighting minimal cross-reactivity with other sweat constituents.

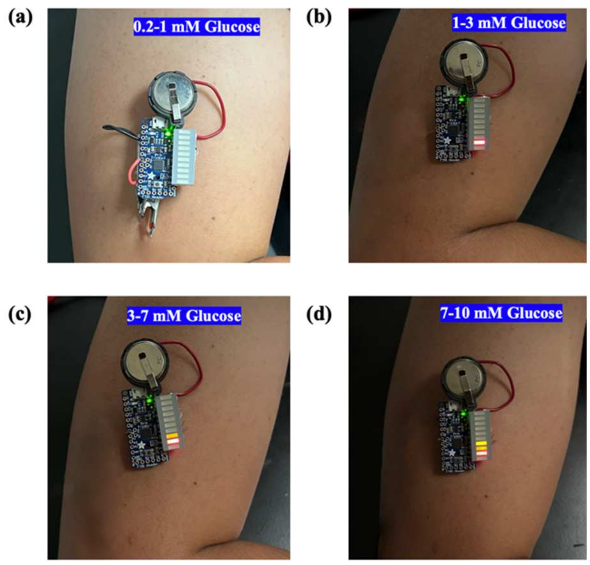

Figure 7: Conceptual in vivo glucose monitoring with different glucose concentration. The output signal was amplified by the readout system and was transduced into LED illumination. The system generated reliable, discrete visual responses to determine glucose levels on skin

Lastly, Figure 7 envisions the biosensor's *in vivo* application, employing LED indicators for glucose concentration levels on skin, offering an innovative and user-friendly glucose monitoring solution. This visual feedback mechanism has the potential to revolutionize self-monitoring for diabetic individuals, simplifying glucose level management through a non-intrusive, instantaneous readout.

CONCLUSION

Our experimental findings substantiate the biosensor's precision in quantifying glucose levels within a 0.2 to 10 mM range. The germination kinetics of *B. subtilis* spores, coupled with the biosensor's electrical signal, display a strong glucose-dependent relationship, yielding a correlation coefficient (R^2) of 0.98. Selectivity assays confirm the biosensor's discriminatory capability, showing minimal cross-reactivity with other common sweat constituents, like lactate and urea. The biosensor's seamless integration with a wearable interface, which translates electrical signals into LED feedback, presents an intuitive method for continuous glucose tracking. This study will explore the ramifications of our findings, juxtaposing our biosensor’s efficacy against current market options and discussing its potential transformative influence on diabetes management. The paper-based endospore biosensor constitutes a substantial innovation in non-invasive glucose monitoring. Utilizing the specific and robust response of *B. subtilis* spores towards glucose, our device addresses significant drawbacks found in existing technologies, emerging as a viable alternative for individuals with diabetes. Its promise for prolonged stability, affordability, and a user-centric interface set the stage for its adoption in wearable healthcare devices. Upcoming research will be directed at refining the biosensor's design for large-scale manufacturing and better incorporation with digital health infrastructures.

ACKNOWLEDGEMENTS

This research was supported by the National Science Foundation under Grant ECCS-2246975. The authors extend their gratitude to the Analytical and Diagnostic Laboratory at SUNY-Binghamton for the use of their facilities.

REFERENCES

[1] L. Johnston, G. Wang, K. Hu, C. Qian, G. Liu, “Advances in Biosensors for Continuous Glucose Monitoring Towards Wearables,” *Front. Bioeng. Biotechnol.*, vol. 19, pp.73381, 2021.

[2] L. Su, W. Jia, C. Hou, Y. Lei, “Microbial biosensors: A review,” *Biosen. Bioelectron.*, vol. 26, pp. 1788, 2011.

[3] M. H. Hassan, C. Vyas, B. Grieve, P. Bartolo, “Recent advances in enzymatic and non-enzymatic electrochemical glucose sensing,” *Sensors*, vol. 21, pp. 4672, 2021.

[4] S. Zhang, W. Zhao, J. Zeng, Z. He, X. Wang, Z. Zhu, R. Hu, C. Liu, Q. Wang, “Wearable non-invasive glucose sensors based on metallic nanomaterials,” *Materials Today Bio*, vol. 20, pp. 100638, 2023.

[5] Y. Liu, S. Zhu, Z. Gu, C. Chen, Y. Zhao, “Toxicity of manufactured nanomaterials,” *Particuology*, vol. 69, pp.31-48, 2022.

[6] Y. Gao, M. Rezaie, S. Choi, “A wearable, disposable paper-based self-charging power system integrating sweat-driven microbial energy harvesting and energy storage devices,” *Nano Energy*, vol. 104, pp.107923, 2022.

[7] X. Zhang, A. Al-Dossary, M. Hussain, P. Setlow, J. Li, “Applications of *Bacillus subtilis* Spores in Biotechnology and Advanced Materials,” *Appl. Environ. Microbiol.*, vol. 86, pp.17, 2020.

[8] J. Errington, “Regulation of endospore formation in *Bacillus subtilis*,” *Nat. Rev. Microbio*., vol. 1, pp.117-126, 2003.

[9] N. Ulrich, K. Nagler, M. Laue, C.S. Cockell, P. Setlow, R. Moeller, “Experimental studies addressing the longevity of *Bacillus subtilis* spores – The first data from a 500-year experiment,” *PloS one*, vol. 13, pp. e0208425, 2018.

[10] K.A.V. Stewart, X. Yi, S. Ghosh, P. Setlow, “Germination protein levels and rates of germination of spores of *Bacillus subtilis* with overexpressed or deleted genes encoding germination proteins,” *Journal of Bacteriology*, vol. 194, pp. 3156, 2012.

[11] C.V. Sayer, B. Barat, D.L. Popham, “Identification of L-Valine-initiated-germination-active genes in *Bacillus subtilis* using Tn-seq,” *PloS one*, vol. 14, pp. e0218220, 2019.

[12] E.P. Riley, C. Schwarz, A.I. Derman, J. Lopez-Garrido, “Milestones in *Bacillus subtilis* sporulation research,” *Microbial Cell*, vol. 8, pp. 1-16, 2021.

[13] J. Ryu, S. Choi, “Bioelectricity production from sweat-activated germination of bacterial endospores,” *Biosens. Bioelectron*., vol. 186, pp.113293, 2021.

[14] J. Ryu, M. Landers, S. Choi, “A sweat-activated, wearable microbial fuel cell for long-term, on-demand power generation,” *Biosens. Bioelectron*. vol. 205, pp.114128,2022.

[15] M. Rezaie, S. Choi, “Moisture-Enabled Germination of Heat-Activated *Bacillus* Endospores for Rapid and Practical Bioelectricity Generation: Toward Portable, Storable Bacteria-Powered Biobatteries,” *Small*, vol. 19, pp. 2301125, 2023.

[16] M. Rezaie, Z. Rafiee, S. Choi, “A biobattery capsule for ingestible electronics in the small intestine: biopower production from intestinal fluids activated germination of exoelectrogenic bacterial endospores,” *Advanced Energy Materials*, vol. 13, pp. 2202581, 2023.

CONTACT

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