MICRONEEDLE-INTEGRATED ELECTROCHEMICAL SENSOR TOWARD DETECTION OF BASOLATERAL SEROTONIN IN THE GI TRACT

Sydney N. Overton, Joshua A. Levy, Michael A. Straker, Jinjing Han, and Reza Ghodssi[*]
University of Maryland, College Park, Maryland, USA

ABSTRACT

Here we present a **microneedle (MN)-carbon fiber microelectrode (CFME) sensor for basolateral gastrointestinal (GI) serotonin (5-hydroxytryptamine, 5-HT) measurements**. This is the first report of an MN-CFME 5-HT sensing platform and is a major step towards realizing real-time GI 5-HT sensing. This technology was developed to measure 5-HT below the epithelium in the GI tract, where the novel use of a 3D printed MN reference electrode enables sub-epithelial detections. In this paper, we present the fabrication and validation of a MN-CFME sensor, demonstrating successful detection of 2.5 µM 5-HT in *ex vivo* GI tissue. Building on the MN-CFME sensor, we aim to develop a non-invasive deployable sensing platform for *in vivo* basolateral 5-HT sensing.

KEYWORDS

Serotonin, Electrochemistry, Biosensing, Gut-Brain Axis

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) has been implicated as a key biomarker regulating the gut-brain axis (GBA) due to its coincident role in brain and gastrointestinal (GI) functions. While 5-HT is responsible for regulating vital functions in the brain, more than 90% of 5-HT is produced in the GI tract by enterochromaffin (EC) cells in the epithelium (Figure 1), primarily on the basolateral side of the epithelium. There, 5-HT regulates critical processes like GI motility, fluid secretion, and immune response [1,2]. However, the specific physiological pathways of 5-HT in the GBA are not well understood. Therefore, much interest exists in measuring *in vivo* 5-HT in real-time to elucidate specific pathways, necessitating the development of quantitative tools to study spatiotemporal 5-HT dynamics below the GI epithelium.

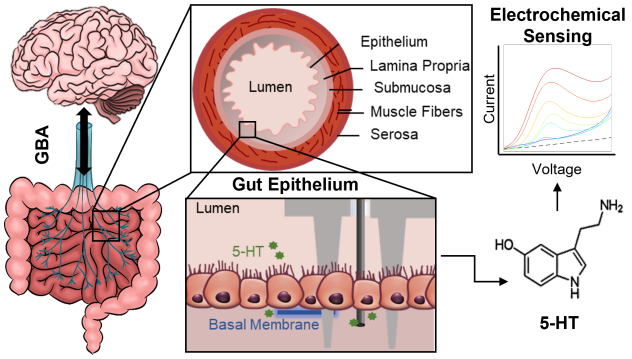

Figure 1: A schematic depicting the GBA and the GI epithelium where EC cells secrete 5-HT from the basal membrane and an illustrative representation of electrochemical detection of 5-HT.

Here, we demonstrate an integrated microneedle (MN)-carbon fiber microelectrode (CFME) electrochemical sensor (⌀ = 2.5 mm) for measuring basolateral GI 5-HT (Figure 1). The sensor leverages a surface modified CFME, which enables excellent electron transfer for sensitive 5-HT detection, and a reference electrode (RE) featuring four Ag/AgCl MNs. By integrating the MNs and CFME into a unified sensing platform, it can be attached to a previously validated cantilever actuator to access and quantify subepithelial 5-HT [3]. In this work, we characterize the MN-CFME sensor and conduct 5-HT measurements in *ex vivo* porcine intestinal tissue.

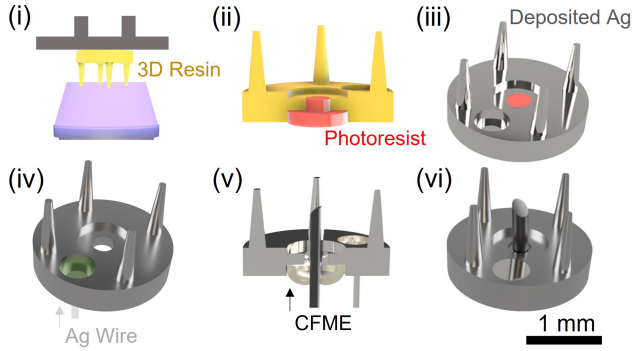

Figure 2: Fabrication of the MN-CFME sensor: (i) 3D print MN part using Surgical Guide Resin (Formlabs Inc), (ii) coat MN part with Parylene C and selectively passivate with S1813, (iii) deposit Ag using E-beam deposition, (iv) remove S1813, connect to wire with Ag epoxy (green circle), and electrochemically convert to AgCl, (v) insert and adhere CFME, and (vi) final MN-CFME sensor.

MATERIALS AND METHODS

The fabrication of the integrated MN-CFME electrochemical sensor is shown in Figure 2. The MN-RE features four Ag/AgCl MN (L = 700 µm, sharpness = 100 µm). MN parts were 3D printed using digital light processing (DLP) (CADworks3D), cleaned for 1 min in isopropanol, and cured for 40 min under UV light. To form the RE, samples were coated with Parylene C (500 nm), selectively passivated with Shipley S1813 photoresist, and metalized with 250 nm Ag using electron beam evaporation (Angstrom Engineering). 36 AWG wire was attached to the MN part using Ag epoxy. The Ag/AgCl MN-RE surface was created by immerging the MN-RE in a 1 M KCl solution and applying 0.15 mA to chloride the surface.

The CFME (⌀ = 50 µm, L = 500 µm) is comprised of T-650 carbon fiber bundles (Solvay) and electrically connected to 30 AWG copper wire using conductive paint (Bare Conductive) and sealed with a layer of Parafilm (Amcor). The CFME was modified following the protocol demonstrated in to improve sensitivity and selectivity via increased surface area and electrostatic interaction, respectively [4]. Finally, the CFME was inserted into the MN-RE and adhered with cyanoacrylate adhesive (Newell Brands).

To determine the sensor's suitability for insertion into the GI epithelium, the MN force profile was evaluated using the 5942 Mechanical Tester (Instron). MNs were deployed into 0.2 w/v% Agar tissue phantom at a crosshead speed of 1 mm/min and evaluated using a 10 N load cell. Additionally, the MN-CFME was characterized with 5-HT concentrations in 1X phosphate buffer saline (PBS). A benchtop potentiostat (VSP-300, BioLogic) was used to record the cyclic voltammetry (CV) response for 5-HT concentrations of 0, 1, 2, 3, 5, 7, and 10 µM. CV was performed from -0.1 to 0.6 V at a scan rate of 100 mV/sec. All data was filtered and analyzed in MATLAB (MathWorks).

Finally, the sensor's performance was evaluated using *ex vivo* porcine intestinal tissue soaked in 10 µM 5-HT for 10 min. The sensor was pressed into the tissue at multiple locations and CV was performed. pH was evaluated with pH indicator strips. Data was post-processed via low pass filtering and analyzed in MATLAB.

978-1-940470-05-4/HH2024/$25©2024TRF
DOI 10.31438/TRF.HH2024.120

RESULTS AND DISCUSSION

The MN-CFME sensor was successfully fabricated (Figure 3) and mechanical tests were performed to characterize the MN penetration force in epithelial tissue (Figure 4a). The force increased until penetration and then dropped rapidly, where the maximum exerted force was 270 µN±59.5 µN. After penetration, the force increased again due to friction between the MNs and tissue to a maximum force of 334 µN±35.6 µN. The maximum force required for penetration is less than the deployment force previously demonstrated with a flexible cantilever (180 mN [3]). Therefore, integrating the MN-CFME sensor onto the cantilever facilitates access to basolateral 5-HT in the GI tract (Figure 3c).

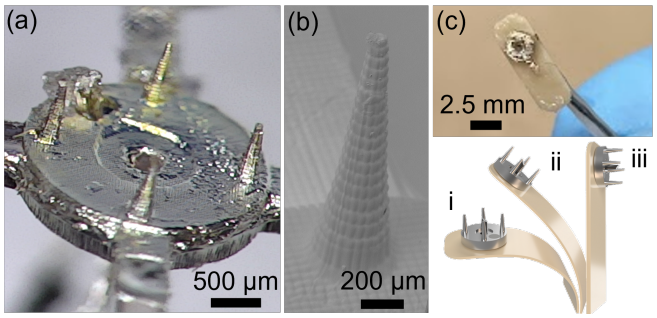

Figure 3: (a) An image of the MN-RE, (b) a scanning electron microscopy image of the assembled MN-CFME sensor, (c) an image and CAD rendering of the sensor on a previously demonstrated cantilever actuator (i) before, (ii) during, and (iii) after deployment.

CV measurements were performed with 5-HT concentrations from 1-10 µM to evaluate the performance of the MN-integrated sensor (Figure 4b). The results show a linear relationship between the oxidation anodic current peak (Ipa) at 0.35 V for all concentrations, with a limit of detection of 16 nA and sensitivity of 38 nA/µM.

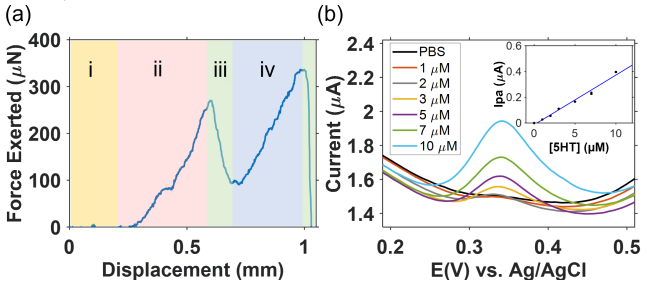

Figure 4: (a) The force versus displacement profile for MN platform in tissue phantom (i) transit of needle to tissue (ii) forces before penetration into the tissue, (iii) penetration of the MNs and rapid decrease of force and (iv) surface tension and friction forces, (b) The CV responses of the sensor in PBS and 0-10 µM 5-HT, depicting Ipas at 0.35 V. (Inset) A calibration curve of the Ipa from the experiment shows the linear response for 0-10 µM (R^2=0.99).

CV measurements were also performed in *ex vivo* porcine intestinal tissue to determine the effectiveness of the sensor in tissue. The CV responses are shown in Figure 5, where the results clearly show an Ipa in response to 5-HT. The oxidation potential shifted approximately 100 mV compared to beaker characterization. This may be due to the change of pH between PBS (7.4) and tissue (6), where pH is known to affect the oxidation potential [5]. The Ipa corresponds to a 5-HT concentration of 2.5 µM, likely due to the incomplete saturation of 5-HT into the tissue. Nonetheless, the clear detection of 5-HT in tissue provides evidence for the successful implementation of this sensor *in vivo*. Repeated CV measurements over 20 min were evaluated to show the effects of sensor fouling

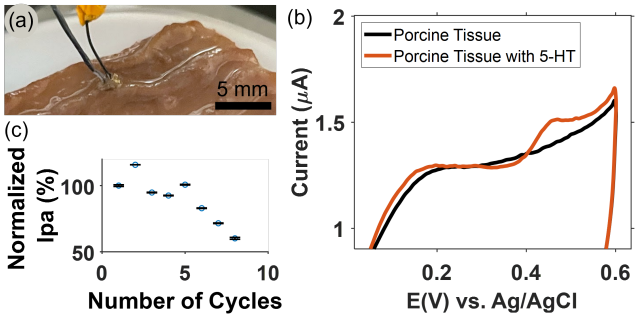

Figure 5: (a) An image of the sensor in contact with the ex vivo porcine tissue, (b) The CV response of the sensor in porcine tissue before and after soaking in 5-HT, (c) The Ipas over 8 consecutive measurements. The Ipas are normalized to the initial cycle's Ipa.

and biofouling. There was no significant decrease in Ipa response in the first five measurements, after which the Ipa decreased significantly (16% per cycle). Minimizing sensor fouling is critical to applying the sensor to long-term repeatable measurements, and future work will address minimizing its effects. Furthermore, it is critical to evaluate the sensor's selectivity against other biological interferents present in the GI tract, such as histamine and uric acid.

CONCLUSION

This paper reports on a novel functionalized 3D printed MN-RE for electrochemical sensing, the first demonstration of an integrated MN-CFME sensor for 5-HT detection and represents an important step towards achieving *in vivo* detection of basolateral 5-HT in the GI tract. Building upon technologies established in our lab, we aim to integrate the MN-CFME sensor with our previously demonstrated PCB and ingestible capsule technologies to achieve wireless measurements of *in vivo* 5-HT dynamics.

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation under NCS Grant #1926793. The authors acknowledge support from the M-Power MSI-pain team and University of Maryland NanoCenter and FabLab.

REFERENCES

[1] J. M. Rojas Cabrera *et al.*, "Techniques for Measurement of Serotonin: Implications in Neuropsychiatric Disorders and Advances in Absolute Value Recording Methods", *ACS Chem Neurosci*, (2023).

[2] N. Liu, S. *et al.*, "The Mechanism of Secretion and Metabolism of Gut-Derived 5-Hydroxytryptamine", *Int J Mol Sci*, 22, 15, (2021).

[3] J. A. Levy, M. A. Straker, L. A. Beardslee, and R. Ghodssi, "Cantilever Actuator Capsule for Magnetically Triggered Drug Delivery in the GI Tract", *2023 IEEE SENSORS*, Vienna, Austria, 10/29-11/1, IEEE, (2023).

[4] J. Han, J. M. Stine, A. A. Chapin, and R. Ghodssi, "A portable electrochemical sensing platform for serotonin detection based on surface-modified carbon fiber microelectrodes", *Analytical Methods*, 15, 9, (2023).

[5] O. E. Fayemi, A. S. Adekunle, and E. E. Ebenso, "Electrochemical determination of serotonin in urine samples based on metal oxide nanoparticles/MWCNT on modified glassy carbon electrode", *Sens Biosensing Res*, 13, (2017).

CONTACT

*R. Ghodssi, (301) 405-8158; ghodssi@umd.edu