INGESTIBLE DEVICE FOR NOISE-RESILIENT BIOIMPEDANCE MONITORING IN GASTROINTESTINAL TRACT

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ABSTRACT

This work demonstrates the first Bluetooth®-enabled ingestible device capable of continuous, noise-resilient monitoring of epithelial bioimpedance, a surrogate for barrier function and inflammatory processes. Existing ingestible devices cannot transmit bioimpedance data beyond the esophagus, limiting understanding of its relationship with tissue histology. We address this technological gap, by minimizing measurement variability associated with hardware limitations of low-power integrated potentiostat circuitry. The flexible bioimpedance sensor features a tetrapolar microelectrode array treated with poly(3,4-ethylene-dioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS) for mitigating interfacial impedances and contact resistance. Sampling at 10kHz, sample-to-sample magnitude variation was reduced by 58.7% and 68.3% on control and treated tissues, respectively. Electrochemical characterization confirms the ability of the device to dynamically differentiate tissues in motion, marking significant progress toward the use of bioimpedance as robust feedback for gastrointestinal (GI) diagnostics.

KEYWORDS

Ingestible device, bioimpedance, Bluetooth, electrochemical impedance spectroscopy

INTRODUCTION

Crohn's disease and ulcerative colitis, known collectively as inflammatory bowel disease (IBD), exhibit progressive patterns of inflammation in the GI tract, sharing overlapping symptoms with other illnesses and complicating diagnosis. Abnormal intestinal permeability, commonly referred to as “leaky gut”, has been well documented as a result of chronic inflammatory disease, diet, or drug abuse; however, there is no standard test to measure intestinal permeability directly in patients. In afflicted intestinal mucosa, an overabundance of pro-inflammatory cytokines manifest, resulting in tight junction dilation and increased tissue conductivity [1]. To quantify intestinal permeability, preclinical research utilizes invasive tissue biopsy and Ussing chamber equipment to measure transepithelial conductivity. However, monitoring mucosal tissue integrity *in vivo* warrants localized sensing of dielectric properties to characterize relative changes in tissue impedance. Tethered impedance sensing capsules and pH-impedance probes have previously been developed for monitoring esophageal mucosa but are unable to measure in the small intestine [2]. To achieve non-invasive, continuous monitoring of tissue impedance throughout the GI tract, several challenges limiting the applicability of bioimpedance in ingestible devices must be addressed.

To accurately monitor the progression of inflammation using continuous bioimpedance sensing in dynamic environments such as the small intestine and colon, interfacial impedances must be minimized. In GI tract, measurement variation is highly undesirable due to shifting electrode position during peristalsis and varying tissue conductivity between GI regions obscuring measurement accuracy. Microelectrodes are well-suited for impedance sensing in the GI tract since their geometry can be optimized for maximizing regional sensitivity to mucosal tissue, but interfacial impedance is inherently greater compounding noise [3]. Measurement accuracy especially suffers at low frequencies due to capacitive impedances inherent in polarizable metals such as Au or Pt [4]. Due to these challenges, existing ingestible device platforms have yet to integrate electronics capable of robust four-wire impedance measurements and wireless data transmission. To enhance electrode conductivity and resolve high interfacial impedances, surface treatments may be applied to metal electrodes [5]. Suitable treatments include Pt black, carbon nanotubes (CNTs), or conducting polymers such as polyaniline or polypyrrole. PEDOT:PSS is among the most extensively used conductive polymer surface treatments in bioelectronic applications due to its excellent stability [6]. PEDOT:PSS is biocompatible and has been previously employed in neural interfaces that feature dense microelectrode arrays [7]. It has been shown to significantly reduce the impedance of pure metals at lower frequencies.

In this work, we present an ingestible device capable of noise-resilient, wireless four-wire impedance measurement (Figure 1). This device expands upon our previously demonstrated bioimpedance sensing capsule prototype [8] through PEDOT:PSS treatment to minimize interfacial impedance and Parylene-C coating for insulating traces, greatly improving measurement reliability and repeatability. A four-probe electrode configuration is used to mitigate electrolyte-interface impedances that arise in bipolar sensing by separating current-injecting and voltage-sense electrode pairs. The flexible bioimpedance sensor is coated with PEDOT:PSS to significantly reduce measurement variability and enhance sensor performance at low interrogation frequencies. The system is capable of differentiating tissues in motion and activates an LED in response to a decrease in impedance magnitude below a dynamically set threshold value, demonstrating the device’s efficacy as a feedback mechanism for ingestible devices.

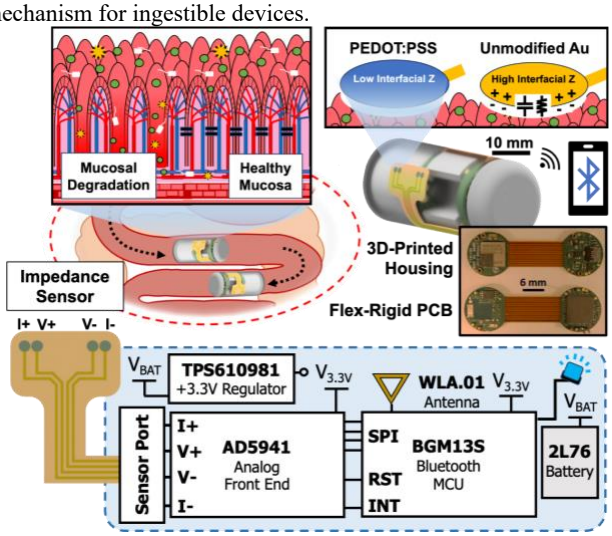

Figure 1. Overview of ingestible device design and operation in GI tract. Mucosal degradation enhances epithelial permeability, resulting in local alterations in tissue impedance. PEDOT:PSS coated electrodes reduce interfacial impedance for noise-resilient sensing. Treated impedance sensor interfaces with low-power potentiostat AFE and Bluetooth-enabled MCU on flex-rigid PCB in a 3D-printed housing shell as shown in schematic.

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MATERIALS AND METHODS

Sensor Fabrication and Assembly

Au microelectrodes spanning 500 µm in diameter were patterned in a tetrapolar configuration onto polyimide film using a standard liftoff process as previously described [8]. Electrode pairs are separated by an inner spacing of 1 mm and individual electrodes are spaced 100 µm apart. As shown in Figure 2-i, 1 mil Kapton® Polyimide film (DuPont, Wilmington, DE) was adhered to a silicon substrate by spincoating a thin layer of Omnicoat (at 3000 rpm) and 16 µm layer of SU-8 2015 photoresist (Kayaku Advanced Materials, Westborough, MA). Electrode traces are patterned using Shipley S1813 photoresist, followed by the deposition of Cr/Au (20/70 nm) using electron-beam evaporation and subsequent lift-off (Figure 2-ii). In Figure 2-iii, the substrate was coated with Parylene-C (1.6µm thick) to insulate metal traces using the Parylene Deposition System 2010 (Specialty Coating Systems, Johnstown, PA). The electrodes and contact pads were exposed by reactive ion-etching (RIE) for 240 seconds at 50 W using 100 sccm O_2 at 100mTorr. (Figure 2-iv) and photoresist is removed using solvent (Figure 2-v). Sensors are cut to shape along the traces to conform to the capsule shell during assembly (Figure 2-vi,vii). To minimize variation, all electrodes are simultaneously electropolyermized in a 200 µL working cell filled with 3-4% high conductivity grade PEDOT:PSS aqueous solution (Sigma Aldrich, St. Louis, MO) using a standard commercial Ag/AgCl reference and Pt wire counter electrode (CH Instruments, Bee Cave, TX). Galvanostatic deposition is performed for current densities ranging from 1µA/mm^2 to 5µA/mm^2 for 600 seconds using a BioLogic VSP300 potentiostat (Biologic, Seyssinet-Pariset, France).

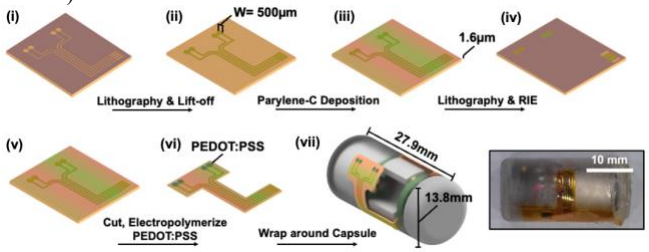

Figure 2. Fabrication steps (i)-(vii) for bioimpedance sensor and integration with capsule electronics. The final device spans 13.75 mm in diameter and 27.9 mm in length.

Evaluation of PEDOT:PSS Electrode Treatment in Solution

Electrochemical characterization of the treated electrodes in solution was conducted to evaluate the quality of the PEDOT:PSS films, determine their frequency dependence, ionic sensitivity, and sensor lifetime for the bioimpedance measurement. The charge transfer capabilities of PEDOT:PSS-coated electrodes were characterized using cyclic voltammetry (CV) in a 10 mL solution of 1x-Phosphate Buffered Saline (PBS) using the VSP300. Voltage was swept from -0.5 V to +0.5 V using a standard Ag/AgCl reference and Pt counter electrode configuration for three cycles. Electrochemical impedance spectroscopy (EIS) was performed using the EVAL-AD5941 electrochemical evaluation board (Analog Devices, Wilmington, MA) to assess the full spectrum impedance magnitude response and ion sensitivity for unmodified and PEDOT:PSS treated sensors (N=3). The interrogation frequency was swept from 100 Hz to 200 kHz in varied concentrations of potassium chloride (KCl) solution (1 µM-100 mM) and recorded using the SensorPal interface software. To determine the operational lifetime of the PEDOT:PSS coating in solution and potential for sensor drift, sensors were stored in PBS (10 mL) for 150 min and EIS was performed at 15 min intervals with the benchtop potentiostat.

Capsule Electronics

The bioimpedance sensor is interfaced with a custom flex-rigid printed circuit board (PCB) previously described in [9]. The device uses a commercially available analog front end (AFE) IC (AD5941, Analog Devices) paired with a low-power Bluetooth 5 microcontroller (BGM13S, Silicon Labs) and 2.45GHz antenna (WLA.01, Taoglas) for wireless transmission (up to +18 dBm) of bioimpedance data. The system is powered from a 3.0V lithium manganese dioxide battery (2L76, Energizer) supplied through a 3.3V voltage regulator (TPS610981, Texas Instruments) and uses a magnetic reed switch (HSR-502RT, Hermetic Switch) to disconnect the power when not in use. The flexible bioimpedance sensors are inserted into a four-pin flat-flex connector (FFC) and sensor port along the LCD 3D-printed capsule shell (Ø=13.8 mm), comprising FormLabs Surgical Guide v2 Resin. Once inserted, the sensor is contoured around the exterior of the shell and sealed with hot-melt adhesive.

Sensor Evaluation on Ex Vivo Porcine Tissues

Unmodified and PEDOT:PSS-treated sensors were validated on *ex vivo* porcine intestinal tissue, first, using the AFE evaluation board and then following integration with the packaged capsule electronics. Porcine tissue was procured (Animal Biotech Industries Doylestown, PA), segmented, and stored at -20°C until experimentation. For benchtop experiments, *ex vivo* porcine small intestinal tissue was segmented into five (1-cm) non-treated (N.T.) and PBS-treated samples, respectively. EIS was performed for each sensor using the EVAL-AD5941 to assess the impedance magnitude and phase response. Next, the impedance sensors were integrated with the ingestible device and attached to a previously developed linear actuator [10]. The capsule was translated across two segments of ex vivo tissue, comprising one N.T. and one PBS-treated segment (dyed blue), at a rate of 3mm/s. Impedance magnitude was wirelessly reported to a mobile device via the EFR Connect app (Silicon Labs, Austin, TX). The interrogation frequency was fixed at 10 kHz based on prior sensor characterization. Finally, to demonstrate the sensor feedback capabilities of the device, an impedance magnitude threshold was wirelessly set using the phone app. This threshold was determined to 'turn on' the onboard LED in response to a drop in magnitude below a threshold value indicative of increased tissue conductivity.

EXPERIMENTAL RESULTS

Electrode Coating and Bioimpedance Results

Chronopotentiometry recordings reveal that lower applied current densities result in longer PEDOT:PSS electrodeposition times but yielded more reliable films (Figure 3a). During electropolymerization, the working electrode voltage response is denoted by a constant value, followed by a sharp rise and plateau. This sharp rising behavior can be attributed to formation of the film due to the rise in voltage needed to sustain current once the electrode surface is fully covered. The subsequent plateau denotes formation of the film. To achieve a low deposition rate and uniform film, sensors were electropolymerized using a current density of 1µA/mm^2 for subsequent experimentation. The resulting cyclic voltammogram, shown in Figure 3b, denotes the enhanced charge injection capacity of the PEDOT:PSS treated sensors compared to unmodified electrodes. The current response of the PEDOT:PSS-coated electrodes is greater than that of the unmodified electrodes by about two orders of magnitude. The frequency dependence of the unmodified Au sensors (Figure 3c) and PEDOT:PSS treated sensors (Figure 3d) was characterized using a full spectrum impedance analysis in ionic potassium chloride (KCl) solutions. PEDOT:PSS treated sensors exhibited distinguishable impedance values, uniform

frequency response, and sensitivity to sub-10µM ion concentrations using the AD5941 AFE, ideal for in vivo application.

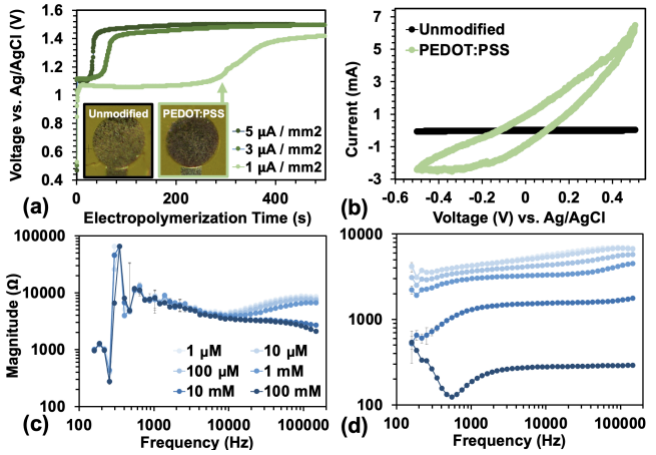

Figure 3. (a) Chronopotentiogram of PEDOT:PSS electropolymerization onto Au electrodes at various current densities. (b) Cyclic voltammogram of unmodified and PEDOT:PSS-coated Au electrodes (1µA mm^{-2}, 600s) in PBS (Scan rate: 100 mV s^{-1}). EIS recording via AD5941 portable potentiostat development kit of (c) unmodified and (d) coated sensor (N=3), exhibiting uniform frequency response and excellent sensitivity to ion content.

Characterization of PEDOT:PSS Coating

Therefore, repeated EIS measurements and exposure to conductive media may affect the integrity of the PEDOT:PSS film over time. Repeated EIS measurements using the PEDOT:PSS-treated sensor were performed at select frequencies in PBS. In Figure 4a, the magnitude response exhibits drift at lower frequencies but shows excellent stability over time at 10 kHz and beyond. Repeated measurement in PBS demonstrates that the impedance magnitude response of the PEDOT:PSS-treated sensor slightly increased, demonstrating the potential for time-dependent degradation of the PEDOT:PSS film (Figure 4a). Low frequencies (100 Hz – 3 kHz) experienced significant measurement variation during measurement, whereas higher frequencies experienced less than 10% time-dependent variation. An interrogation frequency of 10.5 kHz was empirically determined to minimize relative alterations in impedance and was used for measurement during translation across tissue (Figure 4b). At this frequency, impedance magnitude only increased 3.7% from its original value. Future investigation must be conducted to assess the charge transfer capacity of the PEDOT:PSS film after capsule traversal through the GI tract.

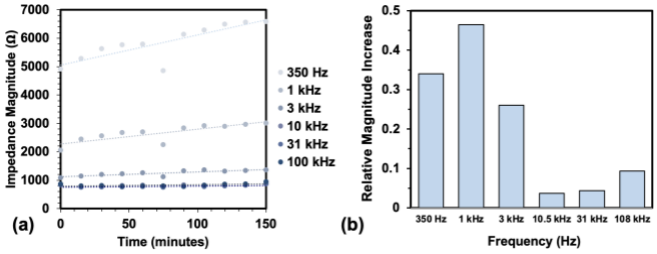

Figure 4. PEDOT:PSS-treated sensor measurement variability after repeated EIS measurements in PBS: (a) impedance magnitude response to time at various frequencies, (b) relative increase in impedance magnitude from initial measurement after 150 minutes.

Validation on Ex Vivo Tissue

The unmodified sensor produced indistinguishable magnitude values for untreated and PBS-soaked porcine tissues below 10kHz (Figure 4a). Additionally, acquired phase values measured with the unmodified sensor are equivalent across the frequency spectrum for all tissue samples. The PEDOT:PSS treated sensor greatly reduced sample-to-sample variation and yielded clearly distinguishable values between the untreated and PBS-soaked group. Sample variation was reduced by 58.7% for unmodified tissue and 68.3% for PBS-treated tissue groups at a sampling frequency of 10kHz. The PEDOT:PSS treated sensor also reported significantly lower tissue impedance magnitude. The magnitude and phase response are depicted in Figure 4b and Figure 4c, respectively.

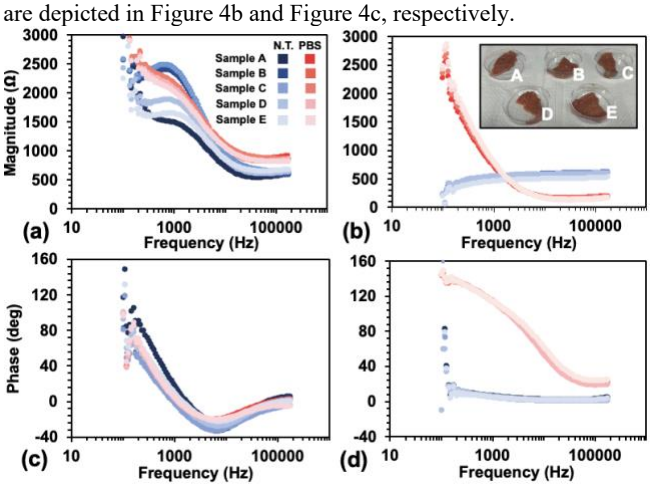

Figure 5. EIS magnitude recording via AD5941 devkit on five intestinal tissue samples using (a) unmodified and (b) PEDOT:PSS coated impedance sensor, phase response for (c) unmodified and (d) coated sensor. An interrogation frequency of 10kHz was chosen for subsequent experimentation to maximize magnitude difference while maintaining contact pressure invariance and minimizing variation due to PEDOT:PSS degradation.

Device Measurement Across Tissue

Following assembly, the sensor integrated capsule was placed onto each segment of the devised tissue model and translated using the developed linear actuator. Based on initial wireless impedance measurements with the device, the threshold to trigger the LED was set to 650 Ω. Wireless measurements received through the EFR Connect app confirm the device's ability to successfully differentiate tissue state during linear translation using the PEDOT:PSS treated sensor. Upon contacting the dyed PBS-treated tissue, the blue LED activated, as shown in Figures 5a-b. Due to tissue ridges caused by the underlying adhesive, the sensor made partial contact at two intervals during translation, resulting in a spike in impedance magnitude. A significant reduction in bioimpedance magnitude (N.T.: 890±107Ω, PBS: 513±45Ω) was observed with low variation during translation. However, capsule translation across the tissue model using the unmodified bioimpedance sensor resulted in a small increase in impedance magnitude upon contacting PBS-treated tissue. Average reported impedance values using the unmodified sensor (N.T.: 1227±23Ω, PBS: 1453±189Ω) were unsuitable for setting an LED activation threshold capable of differentiating N.T. and PBS-treated tissue groups. Average impedance values during translation are compared in Figure 5c. Previous studies comparing gastrointestinal tissue impedance based on permeability or tissue state have been conducted in the esophagus; however comparable datasets from the small or large intestine are lacking [11]. Additional work must be performed to

establish baseline values for various gastrointestinal tissues using the developed sensor for accurate characterization of tissue state in vivo. Future steps to verify device utility as a diagnostic tool entails correlation of measured bioimpedance with Ussing chamber data on inflamed tissue models.

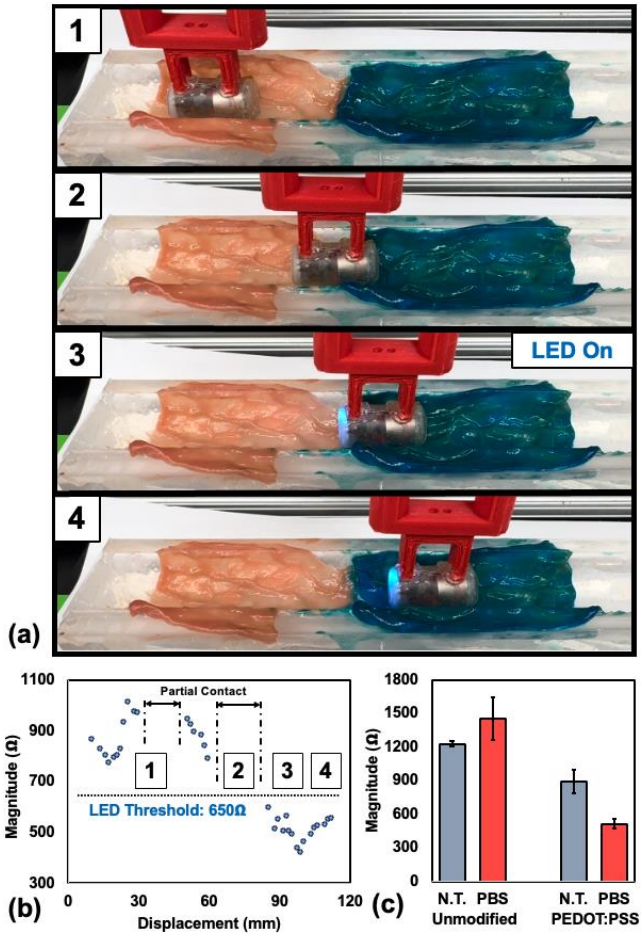

Figure 6. (a) Linear translation of integrated capsule measuring impedance along small intestinal tissue (pink) and PBS-treated tissue (blue) depicting LED activation upon contact with treated tissue, (b) impedance magnitude data transmitted to mobile device via Bluetooth during translation, (c) average impedance magnitude reported for unmodified tissue and PBS-soaked tissue by Au and PEDOT:PSS coated sensor following capsule-integration.

CONCLUSION

This work demonstrates the first untethered ingestible device capable of wireless impedance monitoring of gastrointestinal tissues. The developed capsule is capable of differentiating tissues in motion with low variation and continuously reporting impedance magnitude to mobile device via Bluetooth®. Although PEDOT:PSS degradation may affect measurement variability at the lowest frequencies, the electrodeposited film exhibited excellent stability and greatly enhanced sensor performance. Future work entails investigation into sensor biofouling to validate that the device is capable of continuous readout throughout the GI tract. Additionally, experimentation must be conducted to correlate impedance values with mucosal barrier integrity starting with small animal models. Ultimately, the developed device signifies major progress towards non-invasive bioimpedance sensing as sensor-driven feedback for ingestible capsule systems.

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