

ABSTRACT

Title of Thesis: INGESTIBLE BIOIMPEDANCE SENSING
 DEVICE FOR GASTROINTESTINAL TRACT
 MONITORING

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Gastrointestinal (GI) diseases, such as inflammatory bowel disease (IBD), result in dilated adherens and tight junctions, altering mucosal tissue permeability. Few monitoring techniques have been developed for in situ monitoring of local mucosal barrier integrity, and none are capable of non-invasive measurement beyond the esophagus. In this work, this technology gap is addressed through the development of a noise-resilient, flexible bioimpedance sensor integrated ingestible device containing electronics for low-power, four-wire impedance measurement and Bluetooth-enabled wireless communication. Through electrochemical deposition of a conductive polymeric film, the sensor charge transfer capacity is increased 51.4-fold, enabling low-noise characterization of excised intestinal tissues with integrated potentiostat circuitry for the first time. A rodent animal trial is performed, demonstrating successful differentiation of healthy and permeable mice colonic tissues using the developed device. In accordance with established mucosal barrier evaluation methodologies,

mucosal impedance was reduced between $20.3 \pm 9.0\%$ and $53.6 \pm 10.7\%$ of its baseline value in response to incrementally induced tight junction dilation. Ultimately, this work addresses the fundamental challenges of electrical resistance techniques hindering localized, non-invasive IBD diagnostics. Through the development of a simple and reliable bioimpedance sensing module, the device marks significant progress towards explicit quantification of “leaky gut” patterns in the GI tract.

INGESTIBLE BIOIMPEDANCE SENSING DEVICE FOR
GASTROINTESTINAL TRACT MONITORING

by

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Dedication

This work is dedicated to Jesus Christ of Nazareth, who died on the cross for our sins and rose again in victory over death. I would like to thank my parents, Debra and Michael Holt, for their continued support and love.

Ad maiorem Dei gloriam

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I would like to acknowledge my research advisor Dr. Reza Ghodssi for his continued guidance and mentorship throughout the research presented in this thesis. Additionally, I would like to thank Dr. Younggeon Jin and Hammed Ayansola for their patience and assistance in seeing this work successfully validated on animal models. I also thank my committee members Dr. Ryan Sochol and Dr. Jin for their feedback and support.

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List of Abbreviations

(Listed Alphabetically)

AMI: Acetone, methanol, isopropanol

AC: Alternating Current

AFE: Analog front-end

AI: Artificial intelligence

BLE: Bluetooth Low-Energy

CD: Crohn's Disease

CE: Counter electrode

CNT: Carbon nanotubes

CTC: Charge transfer capability

DI: Deionized

DSS: Dextran sodium sulfate

EDTA: Ethylenediaminetetraacetic acid, a calcium chelator used in cell culturing and medical applications capable of disrupting tight and adherens junctions in mucosal tissue

EGTA: Ethylene glycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid, a chelating agent related to EDTA

EIS: Electrochemical impedance spectroscopy

EoE: Eosinophilic Esophagitis

EPROM: Electrically programmable read-only memory

EVA: Ethylene vinyl acetate

FEM: Finite-element method

FFC: Flat-flex connector

GERD: Gastroesophageal Reflux Disease

GI: Gastrointestinal

H&E: Hematoxylin and Eosin

IBD: Inflammatory Bowel Disease

IBS: Inflammatory Bowel Syndrome

IC: Integrated circuit

IL-4: Interleukin-4

ISM: Industrial, Scientific, and Medical

KRB: Krebs's-Ringer Bicarbonate

LMR: Lactulose and mannitol ratio

LPS: Lipopolysaccharide

MCU: Microcontroller

NT: Non-treated, referring to control tissue groups during animal experiments

PCB: Printed circuit board

PDMS: Polydimethylsiloxane, a transparent, biocompatible, and chemically inert silicone-based polymer.

PEDOT:PSS: Poly(3,4-ethylenedioxythiophene) polystyrene sulfonate

PEEK: Polyether ether ketone

PLA: Polylactic acid, a thermoplastic monomer used as a sustainable bioplastic in 3D printing and medical applications

PPI: Proton pump inhibitor

RE: Reference electrode

RF: Radio frequency

RIE: Reactive ion etching

SEM: Surface electron microscopy

TEER: Transepithelial electrical resistance

UC: Ulcerative Colitis

VPP: Vat photopolymerization

WE: Working electrode

ZO-1: Zonula Occludens-1

Chapter 1. Introduction

1.1. Background and Motivation

Cases of inflammatory bowel diseases (IBD) have surged within recent decades due to a combination of genetic and nongenetic factors such as stress, changes in diet, and antibiotic use, affecting over 1.4 million Americans as of 2009 [1]. IBD, comprising Crohn’s Disease (CD) and Ulcerative Colitis (UC), manifests various patterns of progressive inflammation in the gastrointestinal (GI) tract characterized by degradation of mucosal barrier integrity. IBD shares symptoms with common GI disorders such as inflammatory bowel syndrome (IBS), posing a challenge to diagnosis without continuous monitoring of disease progression [2], [3]. Although common methods for monitoring IBD include traditional endoscopy or colonoscopy, molecular probe testing, and serum or fecal sampling, these techniques cannot locally quantify epithelial barrier integrity.

For localized measurement in the GI tract, ingestible devices offer a non-invasive platform for research and diagnostics. Microscale physiological sensors embedded into these capsules have been employed in medical research to monitor a variety of IBD-related biomarkers *in situ*. Alternatively, video capsule endoscopy, such as the PillCam, has enabled continuous visualization of the GI tract in a clinical setting. However, previously developed ingestible devices share the same limitation as traditional methodologies: they cannot provide localized, selective measurements of mucosal barrier integrity for the continuous characterization of inflammatory patterns.

Electrical impedance measurement can be performed across a spectrum of frequencies to evaluate tissue state, providing information about tissue viability, density, morphology, ion permeability, and extra- and intra- cellular resistivity [4]. In this thesis, the first untethered impedance sensing ingestible device is presented capable of continuous epithelial monitoring beyond the esophagus (Figure 1-1). A noise-resilient bioimpedance module is developed and integrated with compact, Bluetooth-enabled PCB electronics for wireless diagnostics. Through the first animal study of its kind, the sensor-integrated device was capable of reporting impedance alterations in response to induced “leakiness” through tight-junction dilation in colonic epithelial tissue.

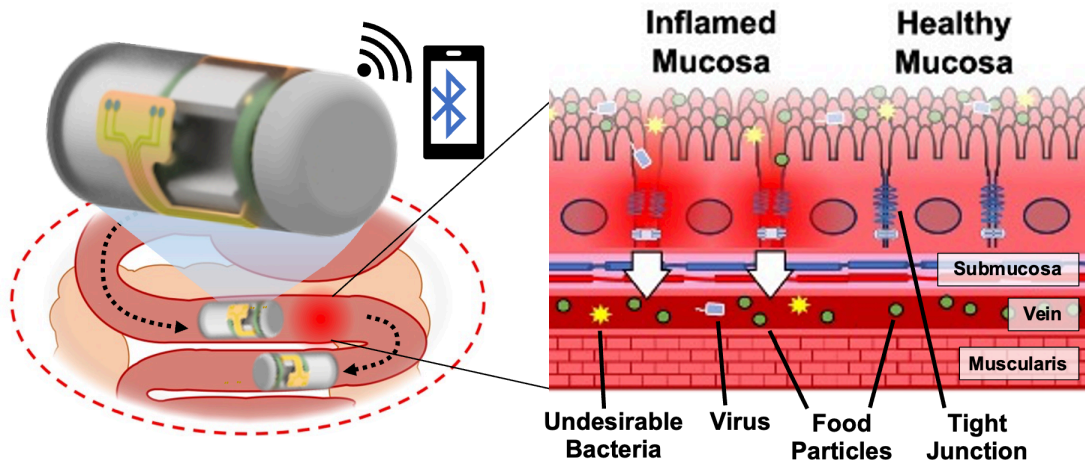


Figure 1-1. Conceptual overview of bioimpedance sensor-integrated ingestible capsule traversing through GI tract. Impedance values reported via Bluetooth to mobile device are used to differentiate inflamed and healthy epithelial tissues. Inflammation is characterized by enhanced mucosal permeability, enabling uptake of viruses and undesirable bacteria through tight junctions.

1.1. Summary of Accomplishments

1.1.1. Address technology gap for four-probe, noise-resilient ingestible impedance sensing module

A flexible bioimpedance sensor was designed to maximize regional sensitivity to mucosal tissue layers, supporting integration with capsule electronics. First, finite element method (FEM) modeling was performed to evaluate how sensor geometry affects electric field propagation through small intestinal tissue. Sensors of varying geometry were initially fabricated, and benchtop measurement results in saline and on agarose tissue phantoms mirrored behavior observed during FEM modeling. Next, electrochemical deposition of conductive polymer films was performed to resolve microelectrode interfacial impedances, enhancing ion sensitivity and electrode performance across a wider frequency range.

1.1.1. Integrate sensor with electronics and validate device

The efficacy of the conductive polymer coating was assessed on porcine intestinal tissues using the fabricated sensor with an evaluation kit designed to perform benchtop measurements using the potentiostat low-power integrated circuit (IC) in the capsule's electronics. Next, the sensor was integrated with custom printed circuit board electronics, consisting of the potentiostat, a Bluetooth Low-Energy (BLE) enabled microcontroller (MCU), high-capacity battery, and supporting electronics, and subsequently packaged within a biocompatible 3D-printed capsule shell. Programming was modified to achieve wireless measurement of impedance magnitude to a mobile device, which was verified through device translation on a segmented tissue model.

1.1.2. Correlate bioimpedance with intestinal tight junction disruption

A small animal trial is conducted using a mice model to demonstrate that the device can differentiate permeable epithelial tight junctions. First, mice colonic tissue samples are extracted, and mucosal permeability is assessed using an established benchtop technique analogous to impedance sensing. A calcium chelating agent is then applied to a subset of samples to validate its efficacy in enhancing epithelial permeability. Next, pilot trials are conducted to initially evaluate the fabricated sensor and optimize the experiment protocol to simulate varying degrees of induced tight junction damage. Through a five-sample trial, full-frequency benchtop impedance measurement using the sensor and device potentiostat is performed on mice colonic tissue to determine optimal impedance measurement parameters. Analysis is performed to verify statistically significant differentiation between degrees of epithelial integrity. Finally, the sensor-integrated ingestible device is placed on tissue, reporting impedance magnitude in accordance with benchtop measurement using the evaluation kit.

1.2. Literature Review

In this section, we explore relevant literature supporting the need for bioimpedance integration with ingestible device electronics towards its use in GI diagnostics. First, we overview the physiological implications of IBD within intestinal mucosal tissue and existing methodologies used for assessing epithelial permeability. Second, we establish bioimpedance as a promising technique for tight junction characterization and note the practical shortcomings of its current implementation in the GI tract. Next, we overview literature addressing sensor design and electrochemical

treatment toward noise-resilient bioimpedance sensing. Finally, we examine pertinent studies to design a robust animal study for device validation.

1.2.1. Existing clinical approaches for monitoring inflammation

Fundamentally, the intestinal epithelium is responsible for uptake of nutrients and acting as a barrier against undesirable bacteria [5]. Enterocytic mucosal cells, comprising the majority of the intestinal epithelium, are adjacently bound together by adherens and tight junctions [6]. Villous tight junctions mediate transcellular transport of small molecules ($< 6\text{\AA}$), and cryptic tight junctions enable uptake of larger molecules ($< 50\text{\AA}$) through the paracellular route, located between villi [7]. Continuous assessment of intestinal permeability is crucial for early detection and treatment of symptoms. During annual check-ups, enhanced mucosal permeability is a known precursor for Crohn's relapse or flare-ups of inflammation [8]. In afflicted intestinal tissue, an overabundance of inflammatory cytokines alters the protein composition of tight junctions resulting in enhanced paracellular permeability [9] (Figure 1-2). For example, treatment of interleukin-4 (IL-4) reduces expression of Occludin [10] and Zonula Occludens-1 (ZO-1) [11] while increasing Claudin-2 presence [12]. Claudins are the primary structural component of tight junctions and are closely linked to paracellular ion conductance [13], [14]. These proteins play a crucial role in regulating epithelial permeability [15] and mucosal barrier repair [16]. A diagram of intestinal tight junction bonds between enterocytic cells is shown in Figure 1-2.

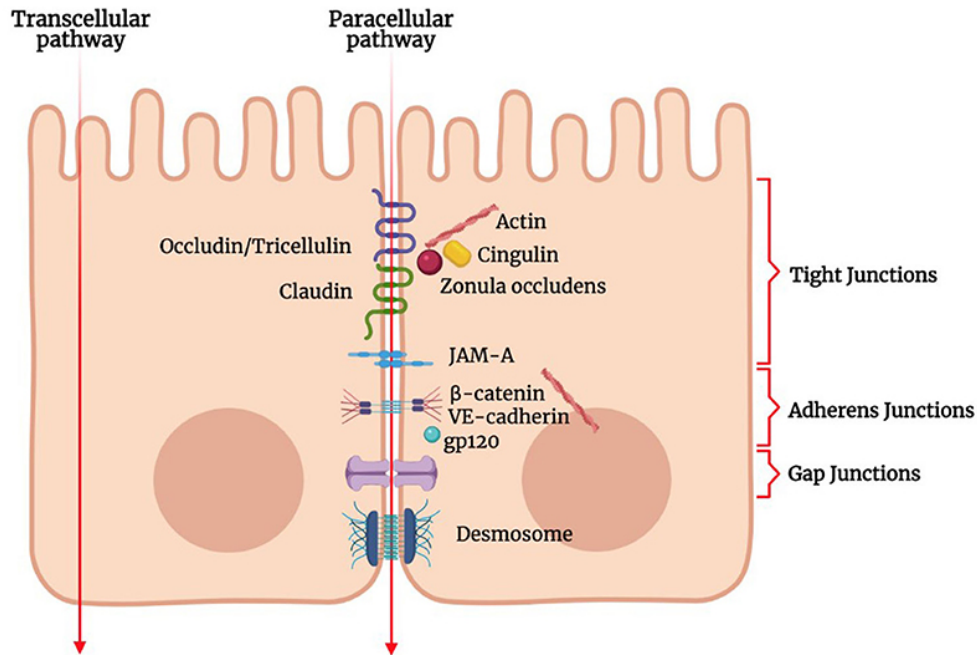


Figure 1-2. Illustration of tight-junction bonding and relevant proteins in enterocytic cells in intestinal epithelium obtained from [17].

Endoscopy is extensively used for visualization of the GI tract, early detection of dysplasia, and guidance for IBD therapy [18]. However, endoscopes are unable to provide continuous access to long sections of the GI tract [19]. A common alternative to traditional endoscopy is video capsule endoscopy such as the PillCam, enabling medical practitioners to continuously monitor the GI tract without risks from sedation [20]. However, collected footage requires review of hours of footage by a trained professional and cannot be used to identify subclinical inflammation, necessitating detection of inflammatory biomarkers through other methods such as serum or fecal sampling [21]. Due to accessibility of ingestible devices like the PillCam, the development of swallowable sensing capsules to measure these biomarkers *in situ* has also become prevalent [22], [23]. Ingestible physiological sensors for the measurement of pH [24], [25], gas [26], temperature [27], and GI motility [28] have been

demonstrated, boasting the ability to wirelessly measure these biomarkers for diagnostics or real-time feedback [29]. Despite their advantages, these sensing modalities are often non-specific and cannot be used to locally quantify epithelial permeability or “leakiness”.

From a clinical perspective, a common technique to measure intestinal permeability non-invasively is through oral delivery of non-metabolizable probe molecules [30]. This method boasts the unique benefit of explicit quantification of mucosal barrier integrity enabling medical practitioners to identify subclinical inflammation in patients. In lactulose-mannitol testing, the ratio of lactulose and mannitol (LMR) is compared following urinary excretion [31]. One large-scale study on 1420 healthy relatives of CD patients demonstrates that increased intestinal permeability as measured through LMR predicted the onset of CD by years [32]. Additional types of molecular probe testing include sucrose [33], [34], polyethylene glycol [35], [36], and Cr-EDTA (ethylenediaminetetraacetic acid) [37], [38]. While these techniques are sufficient for continuous monitoring of systemic changes in GI tract permeability, this method is unable to locally identify patterns of tight junction integrity. Additionally, these techniques only effectively measure paracellular permeability to non-charged molecules but cannot selectively communicate ion permeability of cryptic and villous tight junctions, implicated in electrolyte uptake [39]. Changes to villous tight junctions that manifest during inflammation are less understood, as most literature examines paracellular tight junctions, most closely associated with altered protein composition and IBD [40]. In inflamed tissue, failure of intestinal ion transport channels causes perturbed uptake of ions such as sodium,

calcium, and potassium, causing variations in intra- and extra- cellular ion content and a resultant increase in conductivity [41]. For measuring changes in paracellular ion permeability, alternative methods must be used.

One such method is measuring electrical resistance, where changes in trans- and para- cellular ion transport dynamics are recorded [42]. Common techniques include transepithelial electrical resistance (TEER) for cell culture evaluation [43] and four-electrode Ussing chamber measurement for excised intestinal tissue [44]. In these methodologies, electrodes are placed on opposing sides of a tissue to measure transmural impedance [45], [46], [47]. Principally, inflammation increases mucosal conductivity; in UC patients, it has been shown that colonic epithelial conductivity significantly enhanced ($39.3 \pm 7.1\%$) in cases of mild inflammation, and more than quadrupled ($409.5 \pm 71.8\%$) in moderate to severely inflamed tissue with visible lesions [48]. In healthy epithelia, the electrical conductance of the transcellular pathway far exceeds that of the paracellular pathway since it is normally the most ion permeable route. Hence, if tissue paracellular conductance exceeds transcellular conductance, the epithelial barrier may be described as “leaky” [49]. Due to the proximal location of these channels and the fact that ionic fluid uptake is not exclusively conducted through the transcellular route, it is difficult to exclusively measure transcellular or paracellular conductance, as these transport channels are inherently coupled [50]. For example, although transcellular tight junctions are responsible for uptake of sodium ions and glucose [51], ions may be redirected through paracellular tight junctions to conserve energy as demonstrated in claudin-2 deficient mice [52]. It has been shown that both structural integrity and protein content of tight junctions influence TEER [53]. In

epithelial cells, claudin-2 overexpression in tight junctions demonstrated an increase in paracellular ion permeability and decreased TEER, aligning with mesoscale electrophysiological measurement on UC patients [54], [55]. Despite their efficacy, TEER and Ussing chamber measurements necessitate invasive biopsy or mucosal sampling and thus are limited to a benchtop context.

Bioimpedance, an analog to TEER, may be used to measure the electrical resistance of tissues *in situ* for assessing epithelial integrity in the gastrointestinal tract [56]. In this technique, a small alternating current (AC) excitation signal is used to elicit an electric field, and the potential difference across the tissue is then measured to evaluate its electrical resistance. Unlike TEER or Ussing chamber measurement, bioimpedance sensing may be non-invasively achieved through coplanar electrodes. In this manner, current injection and voltage sensing occurs intraluminally rather than transmurally across tissue, and interrogation frequency may be tailored to isolate extra- and intra- cellular resistivity and phase responses [57], [58]. At low frequencies, bioimpedance can be used to probe intercellular ion content, most affected by alterations in the electro-osmotic gradient caused by changes to tight junction proteins [59], [60], [61]. Due to these advantages, a plethora of bioimpedance sensing technology has been developed for use in the GI tract.

1.2.2. Previous bioimpedance monitoring strategies for monitoring epithelial permeability

Bioimpedance measurement in the GI tract has been used for various applications including monitoring small intestinal ischemia [62], [63], identification of malignant lesions [64], and artificial intelligence (AI) enabled tissue differentiation

[65]. With respect to epithelial barrier dysfunction, many studies have demonstrated bioimpedance as a biomarker of esophageal mucosal permeability for acid-reflux-related conditions [66], [67], [68], [69], [70]. Similarly, esophageal catheters integrating impedance and pH sensors (Fig 1-3a) have been used clinically to identify inflammation associated with gastroesophageal reflux disease (GERD) and quantify proton pump inhibitor (PPI) treatment efficacy [71]. As an alternative to catheters which require sedation, ingestible impedance sensing capsules have also been developed for detection of esophageal permeability, shown in Figure 1-3.

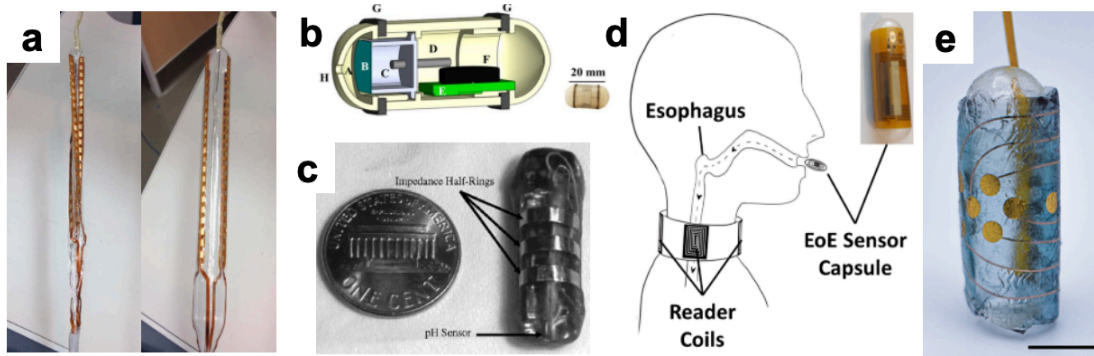


Figure 1-3 (a) Photograph of deflated and inflated mucosal impedance pH catheter [71], (b) Smart capsule for bioimpedance feedback-driven drug delivery in the esophagus [72], combined pH and impedance sensing capsule for GERD detection [73], (d) NFCapsule design for monitoring eosinophilic esophagitis [74], (e) gelatin-based impedance sensing pill for esophageal EIS using benchtop potentiostat [75].

A general-purpose drug delivery capsule was previously demonstrated [72] utilizing impedance sensing for differentiation of healthy and pathological tissues (GERD, IBD, etc.). The capsule uses two Pt ring electrodes shaped around the device Fig (1-3b) to sample impedance twice per second using an interrogation frequency of 30kHz. The device adheres to the IEC60601-1 standard, ensuring that the injected current does not exceed 100 μA in standard operation and 500 μA in fault conditions. Collected impedance data on a GERD patient was stored in electrically programmable

read-only memory (EPROM) for analysis following capsule passage. Though no comparison was made with healthy esophageal tissue, averaged reported impedance magnitude agreed with both benchtop impedance measuring equipment and previously published impedance measurement on GERD patients. Although the system is capable of closed-loop therapy, the device cannot transmit impedance data in real-time and offers little power capacity (6 mAh), not suitable for continuous measurement in the GI tract. Furthermore, it was not verified that the sensor could differentiate changes in epithelial permeability.

Gonzalez-Guillaumin et al. designed an ingestible capsule device [73] toward characterization of GERD featuring three half-ring stainless steel electrodes for two-channel bipolar impedance sensing (Fig 1-3c). The excitation signal used was a 3 V square pulse at 100 Hz, using a large resistor to limit current flow for safety. The capsule was untethered and featured radio-frequency (RF) electronics for wireless communication but required the use of an external magnet to keep the device stationary for esophageal measurement. Though impedance measurement using the capsule was verified using two resistors of known value, no experimentation was conducted to assess whether the device could differentiate healthy and permeable esophageal tissue.

Other capsules lacking integration with onboard electronics have also been proposed. These designs use thin-film metallic electrodes fabricated onto flexible polyimide or Parylene-C-based substrates. One such example, NFCapsule, was developed for monitoring eosinophilic esophagitis (EoE) and used a flexible neck choker for external powering and communication [74]. Although the device successfully differentiated between healthy and damaged porcine esophageal tissue, misalignment

between reader antennas embedded on the choker resulted in a reduction in measurement accuracy. A future iteration of the capsule from the group was demonstrated by Balakrishnan et al. [75]. Here, external benchtop potentiostat equipment is used to perform impedance measurement using a tethered capsule on healthy and inflamed porcine esophageal mucosa verified using hematoxylin and eosin (H&E) staining. Both systems achieve full-frequency electrochemical impedance spectroscopy (EIS) in the esophagus but are unable to measure or transmit impedance in the stomach or beyond, limiting their applicability for IBD and GI disorders associated with enhanced mucosal permeability.

While these bioimpedance sensing capsules have yielded limited success in quantifying esophageal mucosal integrity, no ingestible devices have been developed capable of noise-resilient, wireless bioimpedance measurement in the distal GI tract. In the following section, we discuss the drawbacks of existing bioimpedance sensing capsule devices and noise concerns followed by solutions.

1.2.3. Sensor design challenges in GI bioimpedance sensing

The development of ingestible impedance sensing devices presents multiple technical challenges, limiting their applicability beyond the esophagus. For example, miniaturized potentiostat integrated circuitry must interface with microcontroller and RF communication electronics while balancing size and power constraints, rendering bioimpedance sensing via an ingestible device difficult to achieve. Though Gonzalez-Guillaumin achieved a device capable of measurement beyond the esophagus, no investigation was performed to verify its ability to differentiate healthy and permeable epithelial barriers. Previous sensors perform bipolar impedance measurements using

polarizable electrode materials, contributing to measurement variability at low interrogation frequencies. Additionally, no previous investigation has been performed to optimize capsule-integrated bioimpedance sensor design for measuring mucosal permeability.

Careful design of bioimpedance sensor geometry and electrode configuration may be used to enhance regional sensitivity to mucosal tissues and thus tight junction integrity. Previous impedance sensing capsules use two-electrode or bipolar sensing. In bipolar configurations, measured impedance also comprises the interfacial impedance of the working (WE) and counter electrode (CE), which can be decreased by making them span a large area [76]. This technique is not infallible, however, as electrode polarization cannot be achieved quickly for large electrodes, significantly increasing the time for stable measurement. This is especially unsuitable for non-stationary measurements taken during peristaltic movements in dynamic environments like the GI tract. Although polarizable metals such as Au and Pt are highly conductive and biocompatible, in bipolar configurations, the contact impedance at the tissue interface exceeds actual tissue impedance, leading to high noise and poor sensitivity [77]. These challenges may be overcome through separation of current injecting and voltage sense electrodes using a tetrapolar electrode configuration, greatly reducing measurement contributions of the injecting interfacial impedance [78]. One common example of tetrapolar sensing is a Wenner-Schlumberger topology whereby injecting and measuring electrodes corresponding to outer and inner electrode pairs are arranged linearly [77]. In this arrangement, electrode size and spacing may be optimized using Geselowitz sensitivity theory, where regional sensitivity to tissue is assessed through

the cross-product of current density experienced through separate injecting and measuring electrode pairs. Unlike Ussing chamber measurement which reports transmural resistance, the generated electric field of coplanar Wenner-Schlumberger electrodes may be confined to epithelial layers, maximizing regional sensitivity to the mucosal barrier. This design optimization was previously validated by Kassanos et al. on small intestinal tissue, as reported impedance reflected induced mucosal ischemia through time-series measurement [77]. Here it was shown that compact microelectrode geometries increase the relative contribution of mucosal tissue. However, this can compound interfacial impedances, posing challenges for accurate impedance measurement [79].

Surface modification through electrochemical deposition of highly conductive materials is extensively used to reduce interfacial impedances and enhance electrode properties for low-noise measurement *in vivo* [80]. At the interface of tissues and electrodes, electrical charge must be transferred to aqueous ions through capacitive charge injection across the electrical double layer at the electrode surface or through Faradic redox reactions. The charge injection capability of unmodified polarizable metal electrodes is low and cannot easily overcome double layer capacitances, introducing high measurement variability [81], [82]. This inhibits bioimpedance measurement at low excitation frequencies, obscuring information about paracellular ion permeability. Additionally, without surface treatment, contact pressure has been shown to influence measured bioimpedance at lower frequencies due to high capacitance, tissue compression, and extracellular fluid migration [83]. In Ruiz-Vargas et al., an electrical impedance probe equipped with contact pressure and temperature

sensors was developed to correlate impedance variations with GI conditions *in vivo* [84]. In the GI tract, motion due to peristalsis may compound impedance measurement variability. Since stable electrode positioning and capsule orientation cannot be ensured, it is critical to minimize interfacial impedances.

Numerous electrochemical treatment methods have been reported. The most popular approach for reducing interfacial impedances is through electrodeposition of Pt-black [76], [85], [86], but the resultant film is mechanically rigid and may adhere weakly without additional treatment [87]. Carbon nanotubes (CNT) have also been used to treat metal electrodes, offering reduced interfacial impedances compared to Pt-black modified electrodes [88], [89]. However, as with Pt-black, CNT adhesion to electrodes can be poor [90]. As an alternative, conductive polymers such as polyaniline [91], [92], polypyrrole [93], and poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) [94], [95] are extensively used in bioelectronic sensing applications to enhance electrode performance. PEDOT:PSS is elastic, electrochemically stable, and has been shown to decrease interfacial impedances of various polarizable metal microelectrodes sized between 10 and 200 μm by 39-fold [96]. Composite coatings, such as polypyrrole-CNT [97] or PEDOT:PSS-CNT [98], have also been reported, greatly reducing interfacial impedances while boasting improved adhesion characteristics.

By addressing these technical challenges through sensor design, surface treatment, and electronics integration, it is possible to enhance the performance of ingestible devices for reliable and accurate bioimpedance monitoring beyond the esophagus, enabling quantification of mucosal tight junction integrity.

1.2.4. Device validation and animal models for inflammation

Capsule device verification *in vitro* can be achieved using gastric fluids and mock tissue [99]. For example, placement of ingestible device into biofluids may prove useful for elucidating packaging and noise concerns, such as biofouling. Additionally, *ex vivo* capsule testing using excised intestinal tissue is sufficient for initially evaluating reliability and device performance in simulated conditions [100]. For this reason, it is extensively used in ingestible device-based drug delivery [101]. However, unless freshly excised and adequately preserved under controlled conditions, excised tissue degrades and cannot be used to tie measured impedance to tissue physiology.

Porcine models are popular for preclinical ingestible device verification *in vivo* [102] and have been used to correlate capsule-obtained impedance with acid-induced esophageal damage [75]. However, acid-induced damage is only pertinent to mucosal cell damage caused by GERD, Barrett's Esophagus, EoE, and other esophageal disorders. No reported literature has verified that ingestible device-collected bioimpedance can be used to quantify tight junction damage in the distal GI tract. Toward this end, an animal study must be devised capable of modulating mucosal permeability in small intestinal or colonic tissue in a controlled manner.

Previous animal studies using mice and rats have proven useful for correlation of electrical resistance methods such as TEER or Ussing chamber data with intestinal tissue permeability. Previously, Ussing chamber measurement using inflamed rat colonic tissue demonstrates alteration in paracellular, but not transcellular, permeability, coinciding with protein deficiency in cryptic tight junctions [103]. In

healthy mice colonic tissue, the impedance of the paracellular pathway has been shown to exceed transcellular impedance by 23-fold through impedance analysis conducted between 10 Hz and 10 kHz [104]. Using cultured colonic HT-29/B6 cells, the same study demonstrated that transcellular TEER exceeded paracellular TEER by a factor of ten.

For selective disruption of cryptic tight junction integrity, calcium chelation can be used to disrupt adherens junction binding sites through redistribution of ZO-1 and E-cadherin [105], [106]. This translates to a decrease in tight junction integrity between intestinal epithelial cells. For example, ethylene glycol-bis-(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) has been shown to remove calcium ions from enterocytic Caco-2 epithelial cells, inhibiting tight junction formation [107], [108]. As a result, impedance magnitude and TEER decrease in a time-dependent manner due to enhanced paracellular ion permeability. A microfabricated TEER electrode integrated organ-on-chip has also been previously demonstrated, capable of performing full-frequency EIS on intestinal epithelial cells [109]. Following 5 mM EGTA treatment, the measured transmural impedance rapidly decreased by 50% after twenty minutes of treatment and slowly converged to 35% of its original value, eventually plateauing. The same study demonstrated that low frequency measurement (< 10 kHz) exhibited the largest relative alteration in impedance on lung epithelial cells, confirming that calcium chelation selectively enhances paracellular ion permeability and hence extracellular conductivity.

1.3. Contributions

The work presented in this thesis contributes a reliable solution for quantifying tight junction dilation in intestinal mucosal tissue, addressing shortfalls of existing electrical resistance-based techniques. In Chapter 2, a general bioimpedance sensor design strategy is described, applicable to tailored monitoring of intestinal epithelial tissues. A fabrication process for realizing flexible, insulated sensors is also presented to interface the sensors with mucosal tissue while enabling convenient integration with ingestible device electronics. Optimized parameters for electrochemical surface modification are reported, yielding a considerable increase in electrode charge injection capacity for low noise biosensing. In Chapter 3, a thorough comparison of unmodified and treated sensors is conducted, confirming that the designed sensor can adequately differentiate ion-permeable small intestinal tissue when integrated with low-power ingestible device electronics. Here, wireless feedback is validated in motion using an embedded LED. Finally, in Chapter 4, an animal study is performed to demonstrate that wireless impedance monitoring with the developed device can characterize altered mucosal integrity induced through calcium chelator challenge. No previous study has correlated impedance magnitude as measured via an ingestible device with intestinal tissue physiology, and the results generated by this thesis show promise toward wireless, localized IBD diagnostics.

Chapter 2. Capsule-Conformal Bioimpedance Sensor

In this chapter, we overview the design of the flexible bioimpedance sensor. Initially, sensor topology and electrode dimensions are optimized for selective

monitoring of mucosal tissues. Next, various sensor geometries are fabricated and verified in saline and on agarose tissue phantoms. Finally, electrochemical treatments are investigated to minimize interfacial impedances and noise to support low-frequency impedance measurement via low-powered potentiostat circuitry. **Dr. Justin Stine** extensively assisted with sensor modeling, initial fabrication, and devising an adaptor for benchtop evaluation of the sensor. **Dr. Luke Beardslee** provided guidance for the fabrication process, such as adhering polyimide films to Si and using the Heidelberg MLA150 maskless aligner. Finally, **Mateo Lim** supported by modifying the photomask minimize trace impedances for reduction of measurement noise.

2.1. Optimization of sensor design

Here, modeling and design considerations for bioimpedance sensing via ingestible devices are presented. In the following sections, sensor fabrication and electrode enhancements are to be discussed.

2.1.1. FEM modeling of tetrapolar sensor geometry

The initial goal of the sensor design was to optimize electrode size and spacing. A model was devised using dielectric parameters of small intestinal tissue to evaluate how regional sensitivity is affected by design parameters for a four-probe Wenner-Schlumberger type impedance sensor. Only a tetrapolar design was considered to mitigate current-injecting contact impedances and maximize tissue sensitivity. Dielectric properties including electrical conductivity σ and relative permittivity ϵ_r are obtained from existing literature [77] for small intestinal tissue layers are tabulated below. The thickness of each layer is denoted by t . Compared to tissue layers more

distant from the lumen, mucosal tissues are especially ion-permeable and hence the most electrically conductive.

	σ (S/m)	ϵr	t (μm)
<i>Mucus</i>	1.1	72	50
<i>Mucosa</i>	0.532	$2.64 * 10^5$	900
<i>Submucosa</i>	0.383	$3.03 * 10^5$	800
<i>Circular Muscularis</i>	0.5	$4.35 * 10^5$	450
<i>Longitudinal Muscularis</i>	0.321	$1 * 10^6$	450
<i>Serosa</i>	0.383	$3.03 * 10^5$	50
<i>Surrounding Tissue</i>	0.148	$1.97 * 10^5$	$1 * 10^5$

Table 2-1. Dielectric parameters used in FEM model obtained from [77].

The AC/DC Electrical Currents module featured in COMSOL Multiphysics 5.4 was used to construct a model consisting of four planar gold electrodes interfacing with stacked rectangular small intestinal tissue layers with the properties represented in Table 2-1. Geselowitz sensitivity theory was used to visualize regional impedance contributions through each layer. The essence of this technique is to apply equal current through current-injecting and voltage-sense pairs, resulting in two separate volumetric current densities. The resultant measured impedance should remain identical. However, this is not necessarily true in practice, since there are variations between the two current

densities in tissue regions proximal to each electrode pair. This introduces a positive or negative regional bias, skewing the measurement. The regional sensitivity field S is calculated using the normalized dot-product taken between the two generated current densities.

$$S = \frac{J_1 * J_2}{I^2}$$

When scaled by complex resistivity and integrated over the volume of the target media, the total resultant impedance may be obtained.

$$Z = \rho \int_V S dv$$

In the simulation, injected current was set to 80 μA for both electrode pairs, within safety limits imposed by medical standard IEC60601-1. In tissue regions located between electrodes, the cross-product of each current density is negative since current flow is in an opposite direction. In a real measurement, distance between current-injecting and voltage-sense electrodes introduces error because the measured voltage is not representative of the true potential difference at injecting and ground electrodes. Conversely, a positive dot-product results in positive-sensitive regions in the model, representing targeted tissue regions. The generated model featuring negative-sensitive and positive-sensitive tissue regions is depicted in Figure 2-1. The relative regional impedance contribution with respect to tissue depth is shown in the inset. This was calculated by integrating over a limited vertical distance from the lumen and dividing by the total impedance.

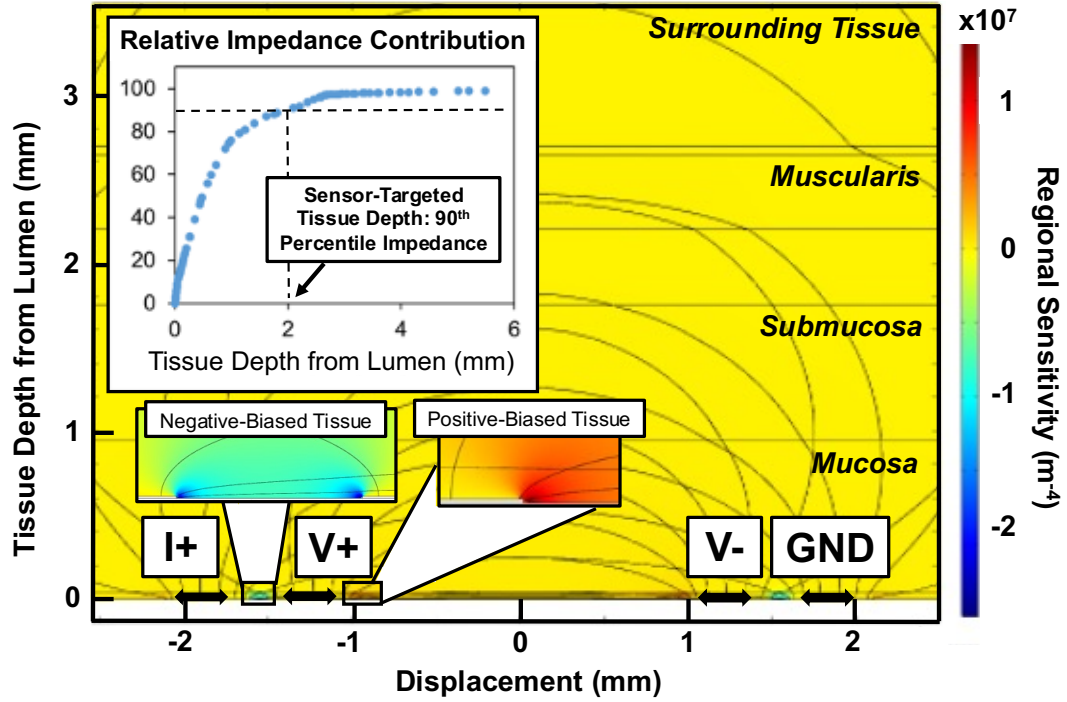


Figure 2-1. Labeled COMSOL model consisting of stacked rectangular small intestinal layers. Electrodes are uniformly sized. The current-injecting electrode pair is located on the outside, with voltage-sense electrodes placed between them separated by a variable spacing parameter.

The regional sensitivity for tissues greatly diminishes when the relative impedance contribution exceeds 90%. Intuitively, tissue adjacent to the coplanar electrodes have the highest contribution to measured impedance. Hence, in the model, the sensor-targeted tissue depth will be obtained using the 90th percentile impedance contribution. The sensor geometry featured above uses an electrode diameter of 500 μm and a sensor width of 2 mm as prescribed by the distance between voltage sensing electrodes. Additionally, current-injecting and voltage-sense electrodes are separated by a spacing of 100 μm to minimize negative-sensitive tissue regions while maintaining ease of fabrication. For optimizing sensor configuration, the sensor-targeted tissue depth of various geometries was compared, with electrode diameter ranging from 500

μm to 2 mm and voltage-sense electrode spacing between 100 μm and 10 mm. The sensor-targeted tissue depth response is plotted below in Figure 2-2.

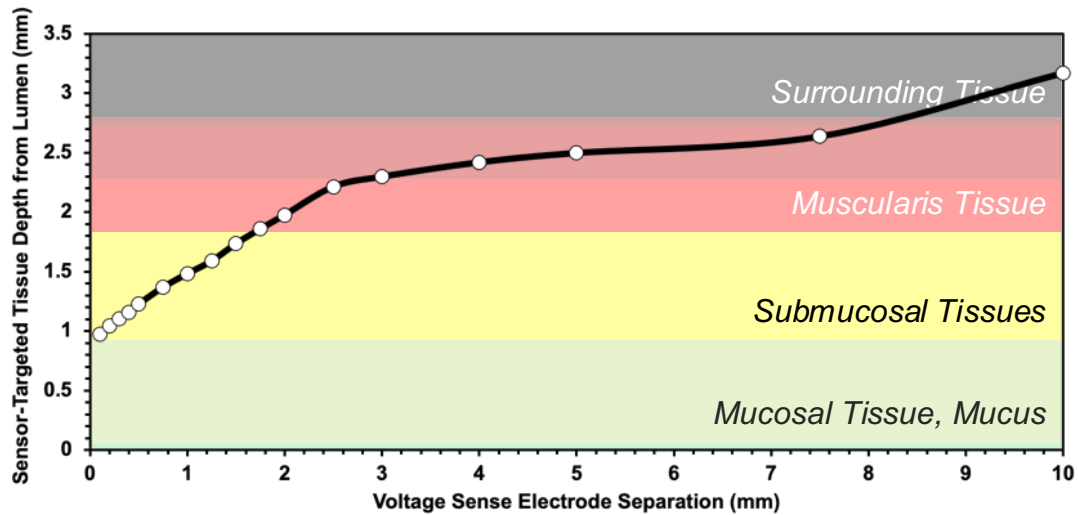


Figure 2-2. Regional bioimpedance sensitivity (i.e., 90th percentile field confinement) is evaluated with respect to sensor width, or inner separation between voltage-sense electrodes. Mucosal tissue contributions are prioritized below a width of 1 mm, and irrelevant tissues contribute to measured impedance beyond an electrode width of 2 mm.

Simulation results demonstrate that the sensor-targeted tissue depth is confined to the mucosal and submucosal tissue layers for an inner electrode distance of 1.6 mm or below. For wider sensor configurations, the targeted tissue depth will factor irrelevant tissues such as the muscularis and serosa into the measured impedance. The sensitivity field was integrated over each respective tissue layer and divided by the total measured impedance to determine the relative contribution of each intestinal layer. During simulation, the relative layer contribution with respect to sensor width and electrode diameter was evaluated. Microelectrodes using a diameter of 500 μm or less and width of 1.5 mm or less prioritized mucosal tissue and mucus secreted by intestinal goblet cells. Conversely, electrode sizes larger than 500 μm tended to equalize relative tissue contributions and sensors that separate voltage-sense electrodes by more than 1.5

mm increasingly targeted tissues beyond the mucosal layer. The relative layer contribution response to voltage-sense spacing using a microelectrode size of 500 μm is shown below in Figure 2-3.

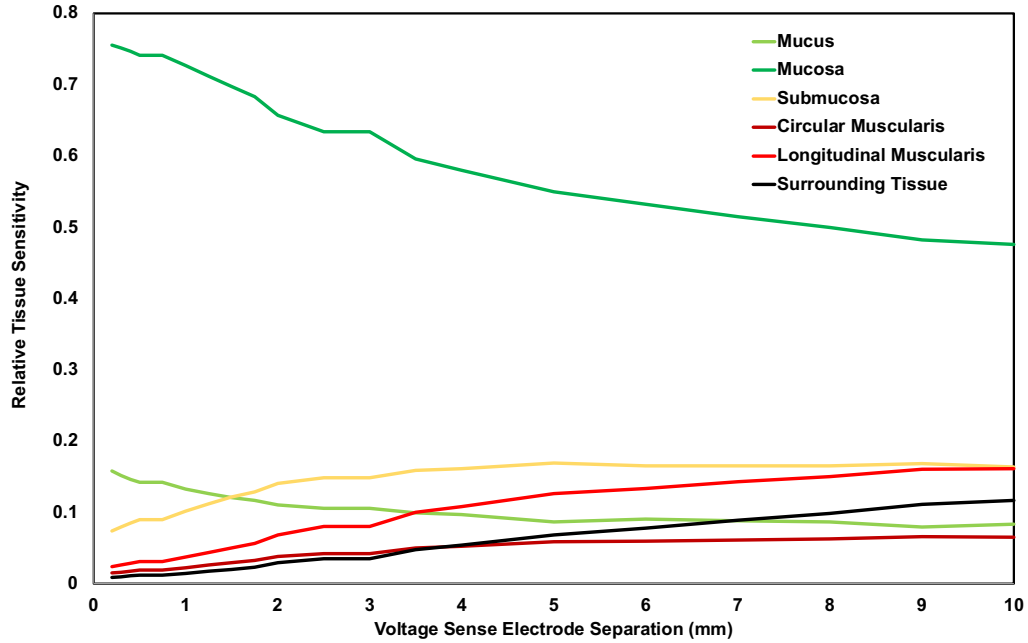


Figure 2-3. Relative bioimpedance sensitivity for each layer plotted against separation between electrode pair as before.

2.1.1. Sensor Design

Previous ingestible devices leverage ring-like electrodes integrated into the device or microfabricated circular electrodes using a flexible substrate such as polyimide film or Parylene-C, a biocompatible dielectric polymer. Flexible substrates facilitate microfabrication of thin-film metallic electrodes because they can contour to the cylindrical shape of a traditional ingestible capsule. Integrated ring-like electrodes were not considered during sensor design, as these hinder the fabrication and comparison of electrode geometries toward maximizing mucosal sensitivity. Additionally, ring-like electrodes are less compatible with highly localized actuation

mechanisms such as drug delivery [110] or biopsy [111]. During fabrication, it became evident that Parylene-C is mechanically fragile when used as a substrate, so polyimide film is used for its thin size, thermal resistance, and durability as a flexible substrate. For biocompatible trace insulation, the developed sensor used Parylene-C, as previously demonstrated in literature [75].

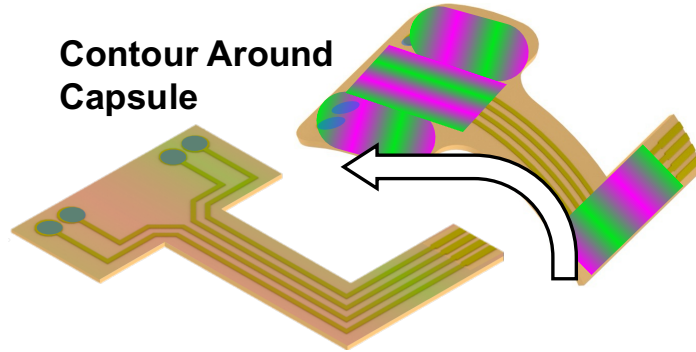


Figure 2-4. Illustration of thin-film metallic electrodes fabricated onto flexible substrate with translucent Parylene-C coating.

Although device electronics and integration are to be discussed later in Chapter 3, the developed sensor must feature contact traces to enable connection with an internal potentiostat for four-wire measurement. A four-pin flat-flex cable (FFC) port is used as an interface between the fabricated sensor and device electronics. The traces form a right angle between the sensor port and planar electrode array so that it is oriented down the length of the capsule (Figure 2-4), mirroring the FEM simulation. As determined through modeling, mucosal contributions cannot be completely isolated, but can be prioritized using an inner electrode separation of 1.5 mm or less. Additionally, compact electrodes of 500 μm or less are optimal for maximizing epithelial tissue. A variety of electrode topologies were fabricated and tested using a benchtop potentiostat to verify trends observed in the FEM model.

2.1.2. Fabrication process of flexible bioimpedance sensor

A fabrication process to create nine bioimpedance sensor geometries was performed. Initially, a bi-layer liftoff process was attempted. Kapton® Polyimide film was cut to the shape and size of a 100 mm silicon wafer. The wafer backing was cleaned with acetone, methanol, and isopropanol (AMI), followed by a deionized (DI) water rinse. A transparency mask was designed using the Autodesk AutoCAD software (see Appendix A). Kapton tape was initially used to adhere the polyimide film to the silicon backing. Once the polyimide film was adhered to the silicon backing, the wafer was covered in LOR 10B lift-off resist. A spinning process consisting of a warm-up step (100 RPM for five seconds), a spreading step (500 RPM for five seconds), and final step (3500 RPM for forty-five seconds) was used to achieve a desired film thickness of 0.9 μm . Next, the wafer was placed on a hotplate set to 180 C for five minutes to ensure good undercut resolution. A pipette was then used to coat the substrate in Shipley 1813 (S1813) positive photoresist, and a spinning process was performed with the spreading step modified to be 3000 RPM for thirty seconds. The substrate was soft baked at 115 C for 110 seconds and exposed using the transparency mask at a dosage of 180 mJ/cm^2 as to not expose the underlying lift-off resist. Two development steps were performed using slight agitation in Microposit 352 Developer, followed by a DI water rinse and hard-bake step at 125 C for five minutes. Using an Angstrom Electron-Beam Evaporator PLC Driven -B, 20 nanometers of Ti and 100 nanometers of Pt were deposited in sequential order (20/100nm) to promote adhesion with the polyimide substrate. Finally, liftoff was performed in Kayaku Remover PG. Upon close inspection, it became evident that the high-heat deposition process caused damage to the S1813

and that adhesion was insufficient between the sensor and substrate. The results are shown below in Figure 2-5. Additionally, the use of tape tended to trap moisture and gases beneath the polyimide film, hindering the vacuum step during cleanroom processes.

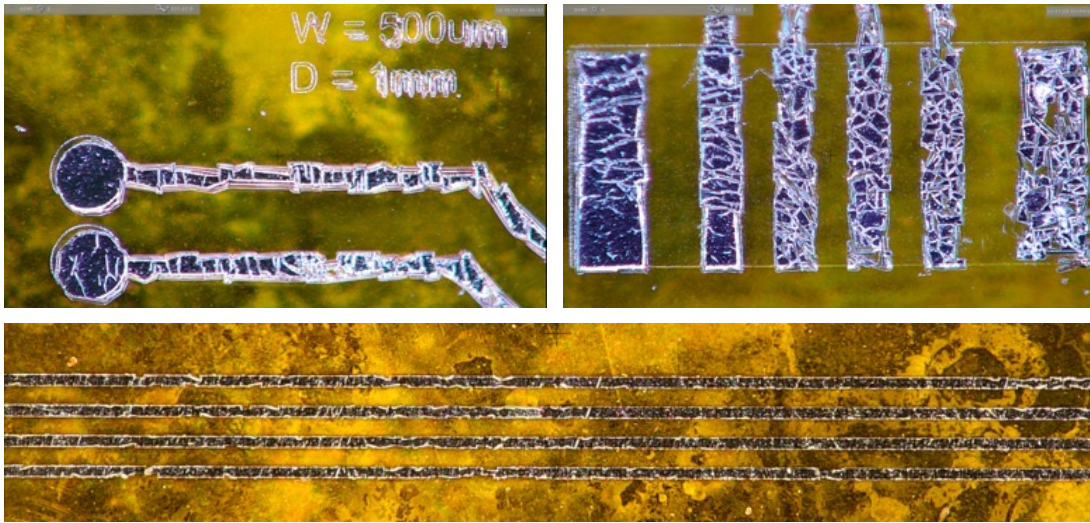


Figure 2-5. Poor adhesion between platinum and substrate resulted in “shattered” sensor appearance and irregularities in electrical traces following liftoff.

A second streamlined fabrication process was devised without the underlying liftoff resist layer to resolve these issues. First, adhesion between the polyimide film and silicon backing was simplified through application of Kayaku Omnicoat spun at 3000 RPM followed by a bake step at 200 C in accordance with the datasheet. Subsequently, Kayaku SU-8 negative photoresist was poured onto the Omnicoat layer and spun at 2000 RPM. A circular polyimide film was then placed onto the silicon backing and flattened via rolling. Next, a bake step at 115 C was performed. This process eliminated the need for tape, reducing trapped moisture and air. Following this, application of the lift-off resist was skipped and an identical S1813 process was used. Trace insulation was not performed for initial sensor verification of the nine geometries.

Hence, for initial sensor verification, the S1813 was exposed using a standard aligner, but for later fabrication processes was exposed with a Heidelberg MLA150 maskless aligner. Following photolithography, a layer of 20/100nm Cr/Au was deposited in the same manner using the e-beam evaporator. In this process, adhesion improved between the sensor traces and polyimide and the sensor remained intact following lift-off, which was performed in an ultrasonic bath using acetone. Liftoff resulted in the removal of the underlying Omnicoat, causing the polyimide film to detach from the silicon backing. The fabricated sensors are depicted in Figure 2-6.

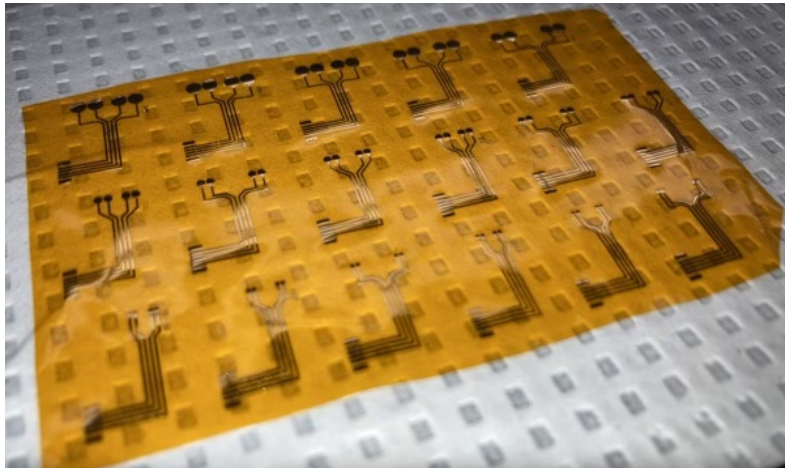


Figure 2-6. Successful fabrication process of various Cr/Au sensor configurations.

The sensor fabrication process was later altered for trace insulation using Parylene-C. Using the optimal sensor geometry determined through verification, two sensor types were devised for capsule integration and benchtop tissue testing. In earlier trials, sensor thickness was lowered to 20/70 nm resulting in high trace impedance, introducing higher measurement variability. This was solved by widening the trace width and increasing the thickness to 20/200nm (Appendix A). Covering the traces in a second layer necessitated development of a second photolithography step and

photomask to selectively expose the Parylene-C covered contact pads and electrodes for etching. First, following lift-off, chemical vapor deposition of 1.6 μm Parylene-C was performed using a DEP-06 Parylene Deposition System Model 2010. Subsequently, the substrate is re-adhered to the silicon wafer using the aforementioned Omnicoat and SU-8 step. S1813 is deposited at 2000 RPM to achieve a taller film thickness of 1.8 μm to exceed the Parylene-C layer, with pre-bake and post-bake steps as described. Reactive ion etching (RIE) was used to expose the electrodes and contact pads. A Trion Phantom RIE machine was set to a pressure of 100mTorr, and the substrate was exposed for 240 seconds at 50W with 100 sccm of O_2 . For each process, the etch step time was adjusted until a trace-to-electrode connection could be established using an ohmmeter. The optimized fabrication process is shown below in Figure 2-7.

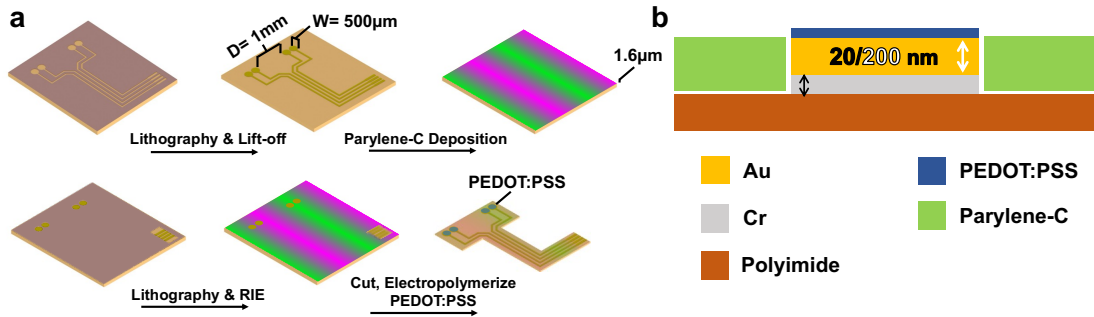


Figure 2-7 (a) Successful fabrication process of various Cr/Au sensor configurations, (b) cross-sectional view of sensor following electrodeposition of PEDOT:PSS (see 2.4).

2.2. Initial verification of depth-targeted design *in vitro*

Before development of the insulation step, sensors were evaluated using a benchtop potentiostat to assess whether they can isolate impedance contributions on ionic agar tissue phantoms of varying thickness and saline content. For initial sensor

verification, traces were insulated with Kapton tape. A four-pin FFC connector port was soldered to an adapter for connection with a Gamry Interface 1010E potentiostat and future experimentation. EIS was first performed across frequencies ranging from 10 Hz to 1 MHz in NaCl solutions of varying ion content to characterize ion sensitivity, as shown in Figure 2-8 (a). Next, agarose powder obtained from Sigma Aldrich was dissolved in 100 mL solutions of NaCl to control phantom conductivity and 8.4g sucrose to enhance permittivity. The solution was poured into 3D-printed polylactic acid (PLA) molds of varying thickness and set to cool overnight. Next, EIS was performed for each fabricated geometry. Full-frequency impedance magnitude is depicted in Figure 2-8 (b), (c), and (d) for electrode diameters of 500 μm , 1 mm, and 2 mm, respectively.

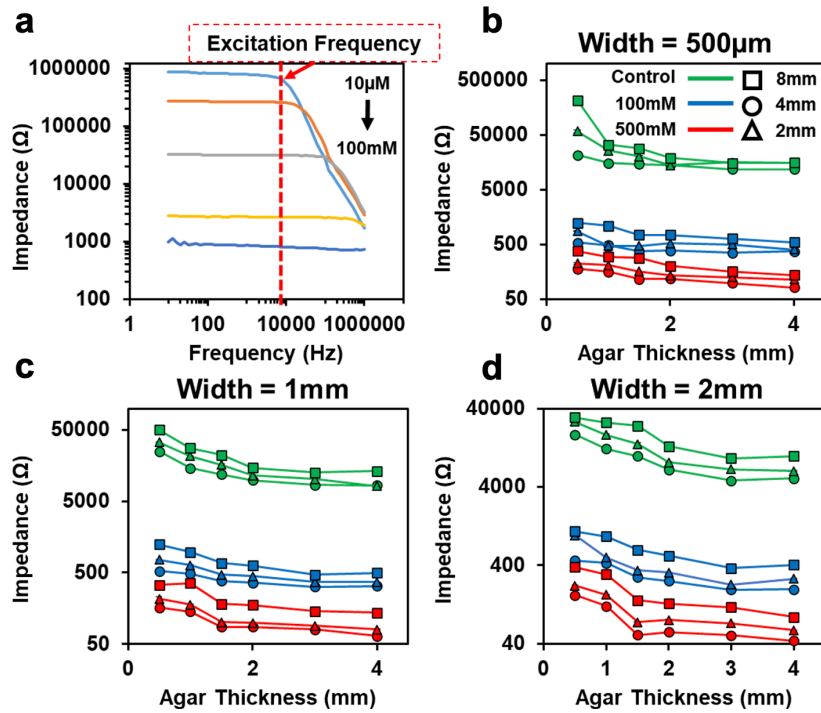


Figure 2-8 (a) EIS characterization in various NaCl saline solutions using an unmodified sensor with a diameter of 500 μm and voltage-sense spacing of 4 mm, fixed-frequency impedance magnitude response to agar thickness for electrode spacings denoted by shapes and ion concentrations denoted by color using an electrode width of (b) 500 μm , (c) 1 mm, and (d) 2 mm.

Results indicate that impedance magnitude decreased with increasing saline content. For NaCl concentrations below 10 mM, capacitive contributions became negligible in the 10 kHz – 100 kHz range and the measured impedance was reduced. In 100mM saline, the sensor reported a magnitude nearly 1 k Ω , indicative of high interfacial impedances. Since intercellular impedance is isolated at low frequencies in mucosal tissue, capacitive contributions were included for low ion concentrations by performing impedance recordings at 10kHz on the prepared agar phantoms. A wider sensor spacing of 4 to 8 mm resulted in higher impedance magnitude, confirming that increasing the separation between voltage-sense electrodes enlarges the volume of tissue that the generated excitation field traverses. Larger electrodes (1-2 mm) produced significantly lower impedance values due to reduced interfacial impedances. Smaller electrodes (500 μ m), despite exhibiting higher impedance magnitudes, showed low variation for compact spacing ($\leq$ 4 mm), indicating minimal impedance contributions beyond 500 μ m of ionic target media. Ultimately, these results echo behavior observed during initial modeling. An electrode width of 500 μ m and a sensor spacing of 1 mm were selected for the final design to maximize mucosal contributions.

2.3. PEDOT:PSS electropolymerization and film assessment

Benchtop characterization confirmed that the developed microelectrodes suffered from high interfacial impedances as demonstrated through high magnitude in concentrated saline. One common method to resolve interfacial impedances is through the electropolymerization of a conductive polymer, such as PEDOT:PSS. In this work, PEDOT:PSS was chosen for its biocompatibility, favorable mechanical properties, and electrochemical stability compared to its counterparts [96]. A composite PEDOT:PSS-

CNT treatment was also considered to assess its efficacy in further mitigating measurement variability [98]. First, high-conductivity grade 3-4% aqueous PEDOT:PSS was obtained from Sigma Aldrich and placed into a 200 μ L working cell. Galvanostatic deposition has been reported to yield superior films over potentiostatic deposition, so a fixed current density was used during electropolymerization [76]. A standard Ag/AgCl RE and Pt CE are inserted into the working cell, along with the fabricated sensor featuring the insulation layer. The electrodes are connected to the working lead using the adapter described in 2.3, and chronopotentiometry is performed for a variable amount of time. The electrodeposition setup is illustrated below in Figure 2-9.

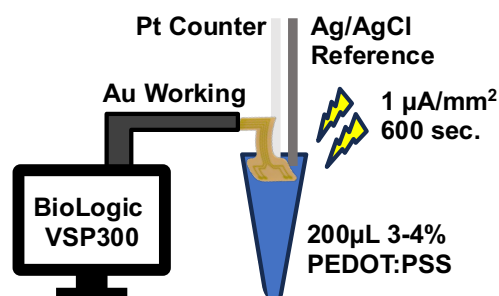


Figure 2-9: Electrode setup for PEDOT:PSS electropolymerization.

Chronopotentiometry recordings shown in Figure 2-10 (a) relate the working electrode voltage to electropolymerization of PEDOT:PSS during applied current. The potential on the sensor must rise after successful film formation to sustain constant current, denoted by a rise and subsequent plateau in the chronopotentiograph. It became evident that lower applied current densities extended the time needed to coat the electrode, and that these films were far more stable and effective in reducing interfacial impedances. A cyclic voltammetry sweep was performed in 1x phosphate-buffered saline from -0.5V to +0.5V using the unmodified sensor and optimal PEDOT:PSS

treated sensor (density of $1 \mu\text{A}/\text{mm}^2$) to characterize charge transfer capability (CTC). A standard Ag/AgCl RE and Pt CE is used. The result, shown below in Figure 2-10 (b), demonstrates a 51.4x fold improvement in CTC, as calculated using integration over the cyclic voltammogram. Chronopotentiometry and cyclic voltammetry are performed using a Biologic VSP300 potentiostat.

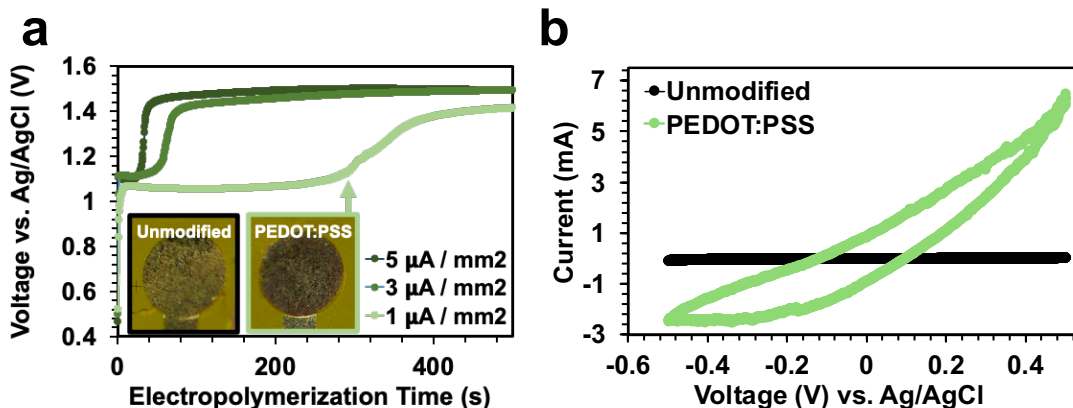


Figure 2-10 (a) Chronopotentiometry recording for various applied current densities featuring photograph of unmodified and electrode coated at $1 \mu\text{A}/\text{mm}^2$, (b) cyclic voltammetry response of pure Au and PEDOT:PSS coated electrode at $1 \mu\text{A}/\text{mm}^2$.

A PEDOT:PSS-CNT working cell was also compared for electropolymerization to evaluate whether a composite treatment further enhances CTC. In this iteration, a $5 \mu\text{A}/\text{mm}^2$ current density was used in the application of both the pure PEDOT:PSS and composite films. In the PEDOT:PSS-CNT working cell, 1 mg SWCNTs are added and PEDOT:PSS is used to fill the remaining $200 \mu\text{L}$ cell. When coated with a high current density, no electrode discoloration was observed. Scanning electron microscopy (SEM) revealed little to no alteration in surface morphology, but exhibited lower surface charging compared to unmodified Au, confirming successful coating (Figure 2-11).

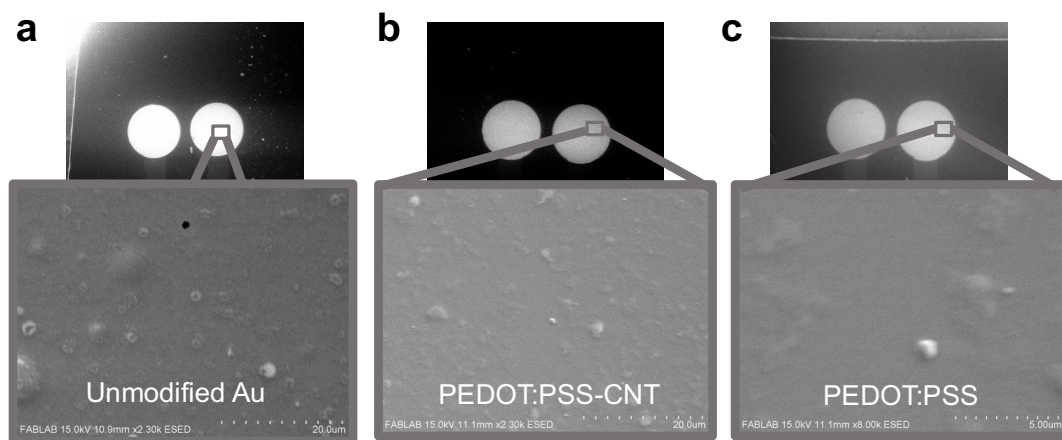


Figure 2-11 (a) SEM imaging of pure Au sensor, (b) PEDOT:PSS-CNT composite treated sensor, and (c) PEDOT:PSS sensor.

As before, cyclic voltammetry is used to compare the CTC for each sensor (Figure 2-12 (a)). The $5 \mu\text{A}/\text{mm}^2$ offered poor performance compared to the low current density coating, and the CTC of the pure PEDOT:PSS treatment exceeded that of the PEDOT:PSS-CNT. Next, EIS was used to evaluate ion sensitivity for each sensor type. Impedance measurement at an excitation frequency of 1 kHz has been determined to be a good metric for monitoring electrochemical electrode performance [112]. Thus, EIS measurements were performed using an Analog Devices electrochemical evaluation PCB (EVAL-AD5941) and SensorPal GUI software to record the impedance magnitude and phase response using a PC. The excitation signal consisted of a 100-mV p-p sinusoidal waveform with a frequency sweep ranging from 100 Hz to 146.6 kHz at 50 points per decade. The EVAL-AD5941 evaluation kit is the counterpart to the AD5941 low-power potentiostat IC featured in ingestible device electronics [113] and is capable of four-wire impedance sensing. The EIS impedance magnitude response to KCl solutions of varying concentration is shown in Figure 2-12 (b), (c), (d) for the unmodified, composite, and pure PEDOT:PSS sensor, respectively. Here, the

sensors coated with PEDOT:PSS demonstrated distinct impedance values, a consistent frequency response, and were sensitive to ion concentrations below 10 μM .

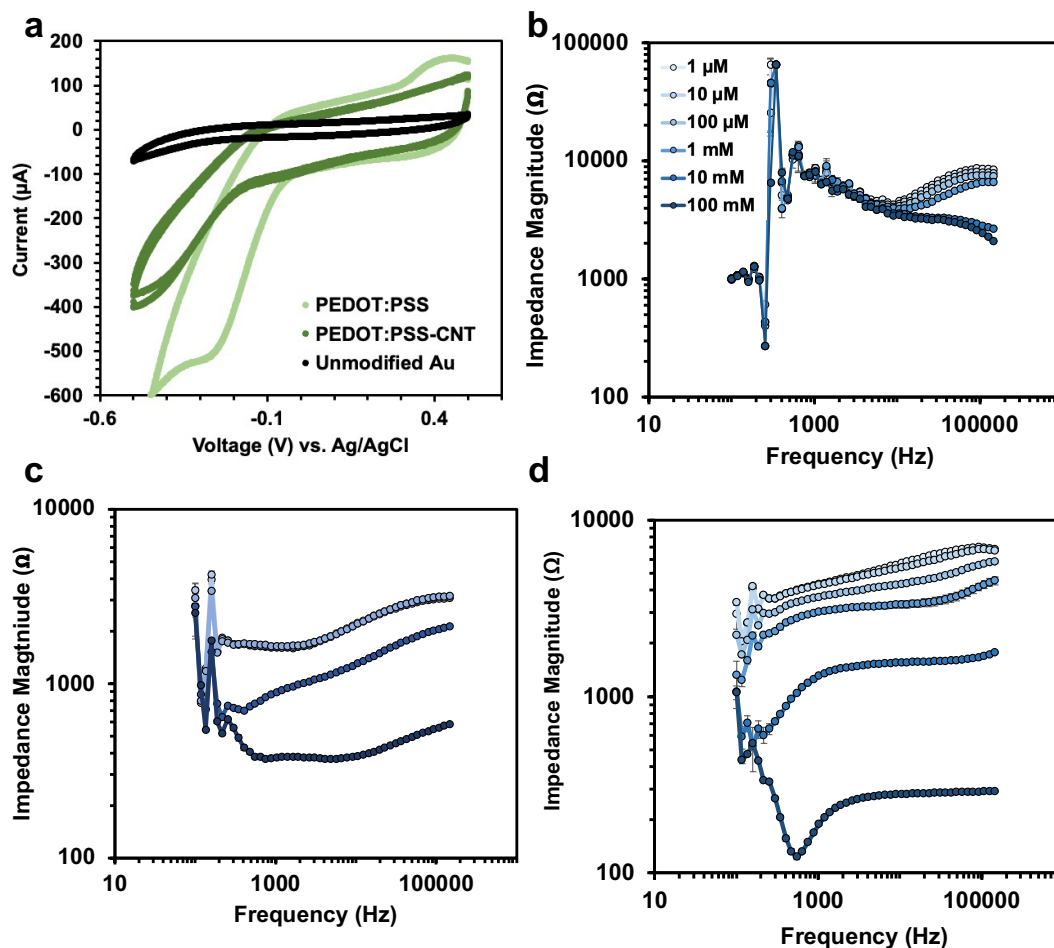


Figure 2-12 (a) Cyclic voltammogram of unmodified sensor, PEDOT:PSS sensor, and PEDOT:PSS-CNT composite treated sensor coated using current density of $5 \mu\text{A}/\text{mm}^2$, (b) EIS performed using unmodified Au impedance sensor between 100 Hz and 146 kHz using EVAL-AD5941 kit, (c) EIS using PEDOT:PSS-CNT composite treated sensor, (d) EIS with PEDOT:PSS treated sensor.

During this trial, the poor performance of the composite film was surprising. A second attempt was performed using a 1.5 mL tube to determine whether low CTC was caused by limited volume in the working cell. In this experiment, 1g SWCNTs were ultrasonically dispersed in 1 mL ethanol and 500 μL PEDOT:PSS was used to fill the cell. A lower electrodeposition current of $3 \mu\text{A}/\text{mm}^2$ was used. During this attempt, the

working voltage of the electrode during chronopotentiometry was much lower than in the PEDOT:PSS cell, and the subsequent plateau value was also lower. This indicates that the composite solution was more conductive and required a lower voltage to coat the sensor. EIS was used to evaluate the new composite treatment using the EVAL-AD5941, which exhibited superior ion sensitivity as observed with the PEDOT:PSS sensor treated in a 200 μ L working cell. The magnitude response is depicted in Figure 2-13 below.

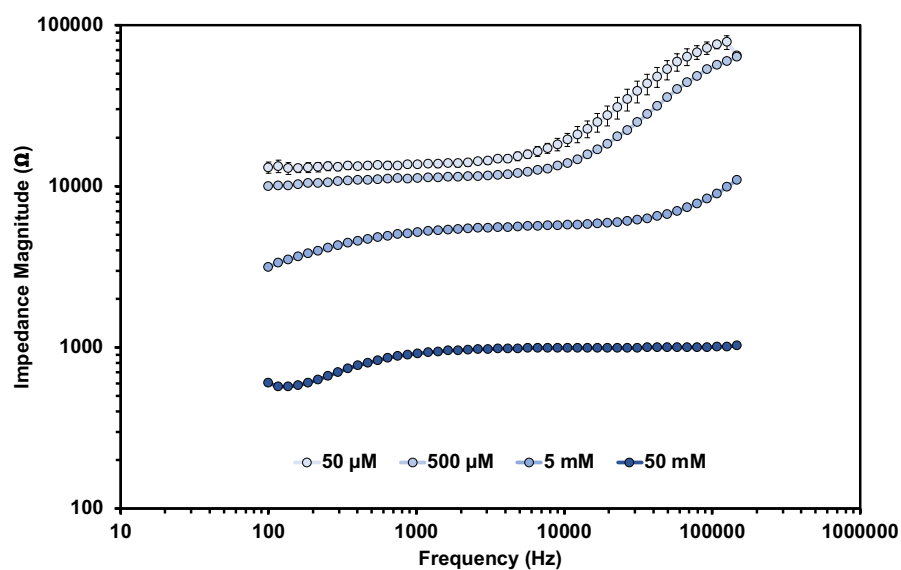


Figure 2-13: Impedance magnitude response obtained during EIS using PEDOT:PSS-CNT composite sensor treated in larger working cell.

Using a fixed current density of 1 μ A/mm², a coating of 5 minutes in duration resulted in a uniform, dark blue discoloration of the electrode, confirming successful film formation [114]. However, subsequent coatings using the same working cell yielded non-homogenous electrode coverage, as shown in Figure 2-14. Using a fresh working cell, a deposition time of 20 minutes resulted in film overexpansion and sensor shorting.

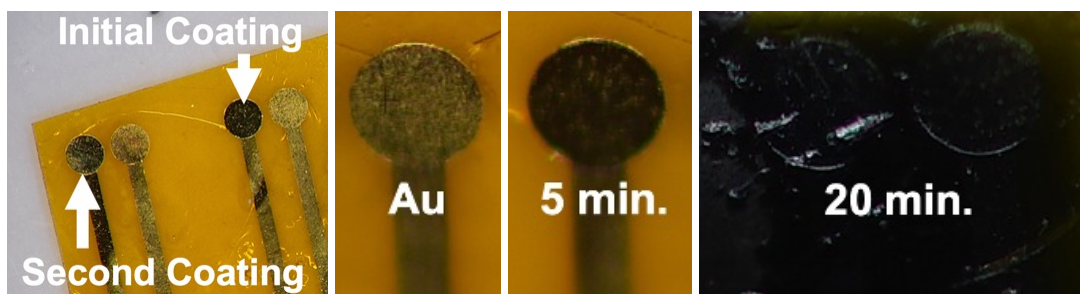


Figure 2-14: Variation in electrode discoloration of electrodes observed under different PEDOT:PSS treatment conditions using a current density of $1 \mu\text{A}/\text{mm}^2$.

Electrodes were then coated using a current density of $1 \mu\text{A}/\text{mm}^2$ without swapping the working cell ($200 \mu\text{L}$ PEDOT:PSS) to assess cell re-usage. Chronopotentiometry recordings indicate a slightly longer deposition time following initial coating as shown below in Figure 2-15 (a). Despite longer deposition times, exhibited electrode discoloration decreases for subsequent coatings. The resultant CTC generated by these treatments is nearly equivalent to unmodified Au, as shown in Figure 2-15 (b).

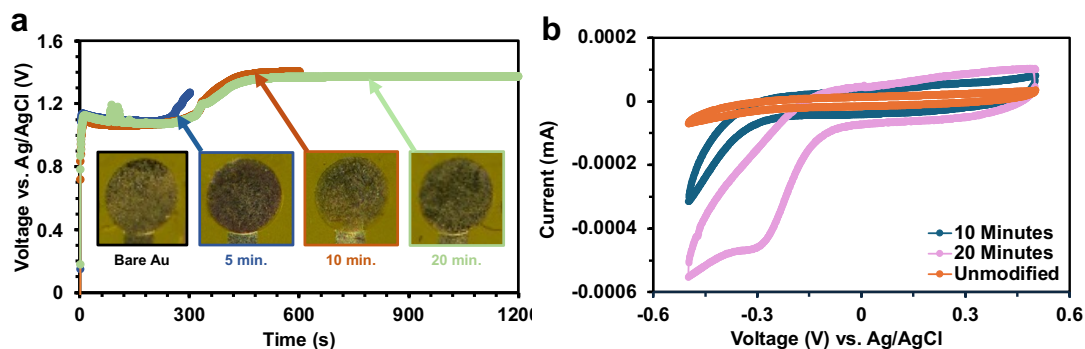


Figure 2-15 (a) Chronopotentiogram of electrodes during electropolymerization of PEDOT:PSS without swapping working cell, (b) cyclic voltammogram of electrodes coated in re-used cell exhibit little improvement in overall CTC.

Through this experimentation, it was empirically determined that the pure PEDOT:PSS treatment was more effective than the composite treatment at resolving interfacial impedances in smaller working cells. With the pure PEDOT:PSS treatment,

the optimal applied current density of $1 \mu\text{A}/\text{mm}^2$ for 600 seconds results in the highest CTC values as determined through cyclic voltammetry. Although the composite treatment may yield higher CTC when used in a larger working cell, differences in EIS performance are negligible between the optimal PEDOT:PSS treatment and the composite treatment. Variation in CTC between each electrode was minimized by simultaneously coating all four electrodes and replacing the working cell between each sensor.

Using the optimized sensor, experimentation was needed to assess time-dependent film degradation through repeated EIS measurements in aqueous ionic media. Since PSS is a hydrophilic counter-anion, PEDOT tends to absorb moisture and gradually de-dope the film over time [115], [116]. Hence, PEDOT:PSS lacks long-term chemical stability in ionic conditions like the GI tract. Since EIS is a good metric for assessing performance of interfacial impedance, EIS was conducted on the PEDOT-PSS-treated sensor at specific frequencies in 1x-PBS for 2.5 hours. Low-frequency measurement ($< 3 \text{ kHz}$) exhibited a relative magnitude increase between 26.1 – 46.5% during measurement, and high-frequency measurement was resistant to time-dependent variation. An excitation frequency of 10.5 kHz was empirically determined to minimize relative alterations for which magnitude increased 3.7% from its original value. The EIS response is shown in Figure 2-16. Overall, the increase of impedance magnitude indicates a rise in interfacial impedance due to progressive de-doping of the PEDOT:PSS treatment, but careful selection of impedance measurement parameters such as frequency can be used to mitigate these effects.

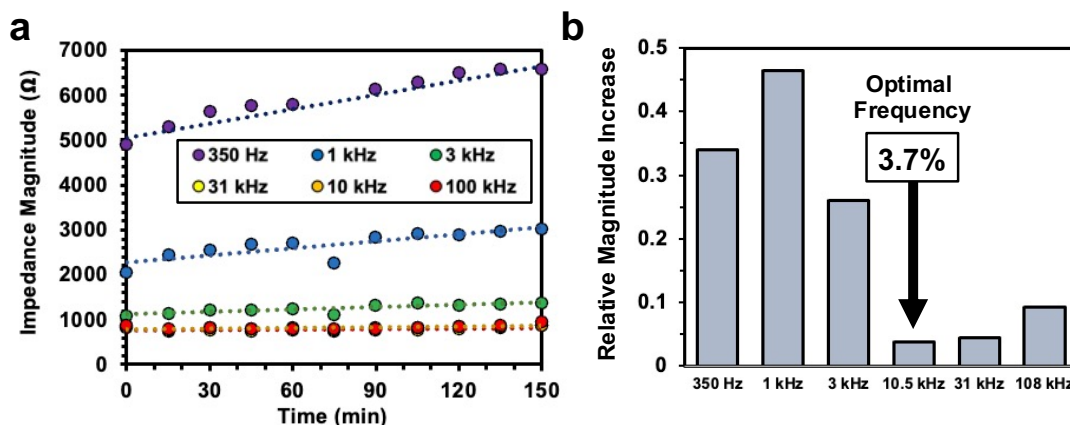


Figure 2-16 (a) Impedance magnitude increases over time in PBS due to time-dependent degradation of PEDOT:PSS film, (b) relative magnitude increase over 150 minute trial for select frequencies shows that low frequency measurement is especially susceptible to relative magnitude increase.

Future research should focus on evaluating alterations in the CTC of the PEDOT:PSS treated sensor following traversal through regions of the GI tract. It has been shown that pH variations can impact the conductivity of PEDOT:PSS films [117]. However, previous studies have demonstrated excellent film stability for extended electrical measurement on various gastric tissues [118], [119]. Further investigation into the stability of the PEDOT:PSS film under realistic GI tract conditions can be achieved through CV or EIS measurement simulated intestinal fluid. If future experimentation reveals that this is a serious issue, pH-dependent effects on PEDOT:PSS film conductivity may be mitigated through patient pre-treatment with proton pump inhibitors or protective pH-dependent eudragit-based packaging schemes [120].

Chapter 3. Evaluation of Ingestible Bioimpedance Device

3.1. Sensor integration with ingestible device

In this section, an overview of the ingestible device electronics and capsule packaging are presented to support *in situ* measurement using the sensor fabricated in the previous chapter. I would like to credit **Dr. Justin Stine**, who designed the capsule electronics and supported initial assembly and impedance measurement on porcine tissues.

3.1.1. Capsule electronics

Circuitry capable of four-wire impedance spectroscopy, data processing, and wireless transmission is needed to successfully address drawbacks of existing ingestible devices and extend access to the distal GI tract. Additionally, unlike previous impedance sensing capsules which are externally powered, the designed ingestible device must include an onboard battery and an antenna for communication to enable full remote operation.

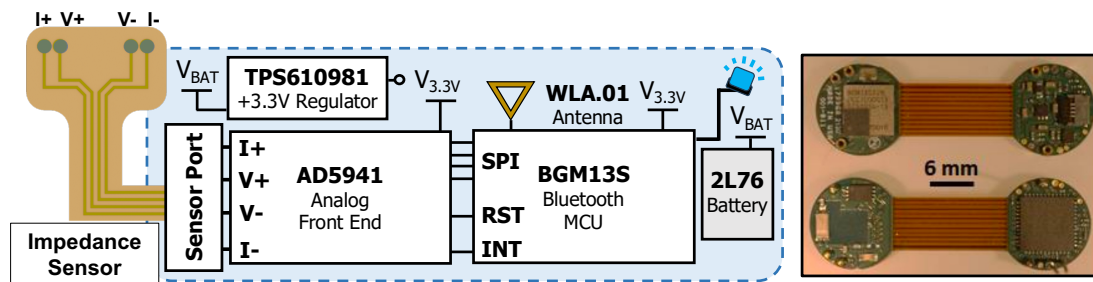


Figure 3-1. Simplified electronics schematic illustrating impedance sensor interfacing with AD5941 potentiostat which forwards measured four-wire impedance to BGM13S BLE-enabled MCU for wireless transmission to mobile device. Supporting electronics include 2L76 high-capacity LiMnO₂ battery, WLA.01 antenna, and voltage regulator. Additionally, an MCU-programmable LED is integrated into the flex-rigid PCB (pictured right) for visual feedback purposes.

The developed device electronics incorporate a miniaturized potentiostat IC, a BLE-enabled microcontroller, and passive components onto a custom circular flex-rigid PCB [113], as shown in Figure 3-1. An Analog Devices AD5941 low-power electrochemical analog front end is employed to generate the excitation signal and perform four-wire impedance measurements. This low-power component can perform EIS between 100 Hz and 200 kHz with an error margin within 2.8% of a standard benchtop impedance analyzer [121]. Impedance magnitude values are transmitted via Bluetooth Low-Energy (BLE) using the Silicon Labs BGM13S MCU and Taoglas 2.45 GHz WLA.01 antenna. Wireless biomedical devices such as wearables and ingestible devices often operate in the high-frequency industrial, scientific, and medical (ISM) RF communication spectrum at frequencies such as 405 MHz, 915 MHz, or 2.45 GHz [122]. Bluetooth, operating at 2.45GHz, is highly regarded for its capability of continuous data streaming and compatibility with PC or smartphone communication [123]. BLE is a protocol adapted for low-power devices which conserves energy through limiting transmission rates with infrequent, short data bursts [124]. It has been extensively used in the biomedical domain, such as for glucose monitoring [125], but is yet to be integrated with ingestible impedance sensing electronics for communication.

Data is received from the device through the Silicon Labs EFR Connect phone application using a customized GATT profile that updates fixed-frequency magnitude twice per second. Additionally, GATT fields are programmed for user data entry allowing for a dynamically set excitation frequency and LED activation threshold impedance from the mobile device. This functionality enables a user to wirelessly control impedance measurement parameters during transit through the GI tract.

Although silver-oxide batteries have been federally approved for clinical use in video capsule endoscopy [126], a LiMnO_2 battery was selected for its high capacity of 160 mAh and compact form factor. This battery can continuously power the onboard electronics for 29 hours [113].

3.1.2. Packaging and flat-flex connector

The battery, reed switch, and printed circuit board (PCB) are enclosed within a 3D-printed capsule shell spanning 13.8 mm in diameter and 27.9 mm in length. The shell is printed using LCD vat photo-polymerization (VPP) of FormLabs Surgical Guide v2 Resin, a biocompatible material. For dynamic activation, a magnetic reed switch is soldered between power and ground terminals on the PCB. A 30 AWG wire is used for electrical connection between the battery and PCB using spot-welding to minimize the capsule diameter, as this wire must pass across the battery. The PCB only features 24 AWG power terminals, so the 30 AWG wire is soldered to its 24 AWG counterpart for insertion. A four-pin flat-flex cable (FFC) port is located on the side of the PCB, accompanied by a rectangular intrusion in the capsule shell for sensor insertion. Additionally, a rounded cap was attached, and the entire assembly was sealed using hot-melt adhesive for testing.

3.2. Ex vivo validation of preliminary integrated capsule

An unmodified Au sensor using a voltage-sense electrode spacing of 4 mm and electrode diameter of 500 μm were connected to device electronics housed in a 3D-printed PLA shell for initial validation of capsule electronics. The impedance magnitude response of three target media were initially compared at a fixed frequency

of 10 kHz, comprising an ionic agar mold of 100mM NaCl, untreated porcine small intestinal tissue without mesentery, and tissue soaked in 1x PBS for 24 hours prior to measurement. Impedance was also measured on PBS tissue placed onto the agar tissue phantom, representing “surrounding tissues.” This experiment was performed to confirm that the sensor’s targeted probing depth was mostly confined to the intestinal tissue. The measurement setup and resultant magnitude response are shown below in Figure 3-2.

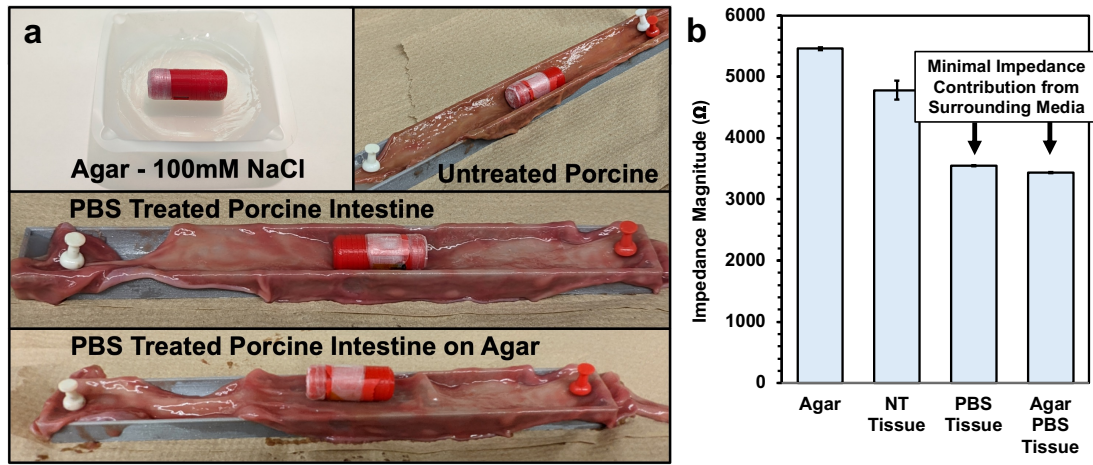


Figure 3-2 (a) Photograph of untreated sensor integrated capsule performing wireless measurement on ionic agar mold, untreated and PBS-treated porcine small intestinal tissue (mesentery removed), and PBS-soaked tissue backed by agar to verify targeted impedance measurement. (b) Impedance values obtained on each target media.

When backed with the tissue phantom, minimal impedance alteration was observed on the PBS-treated tissue, confirming that impedance contributions from proximal tissue was isolated. However, it must be noted that the reported magnitude obtained using the pure Au sensor exceeded 3 kΩ, denoting high interfacial impedances. Following capsule integration, impedance magnitude measured using the sensor was very sensitive to orientation, movement, and applied pressure due to high measurement variability. The performance of unmodified and PEDOT:PSS-treated bioimpedance

sensors were compared using device electronics to evaluate whether the enhanced CTC of the PEDOT:PSS treatment reduces noise during translation.

3.3. Ex vivo evaluation of PEDOT:PSS treated impedance sensor

Previously the efficacy of the PEDOT:PSS treatment has been demonstrated in saline solutions. In this section, EIS was performed on porcine intestinal tissue *ex vivo* using the EVAL-AD5941 to determine whether the surface modification reduces measurement variability on GI tissues. Next, the PEDOT:PSS treated sensor is integrated with the capsule and programmed to wirelessly report impedance magnitude as in the previous section, and also indicate tissue state via the onboard LED. I would like to thank **Michael Straker, Joshua Levy, and Vivian Borbash**, who designed the linear actuator used to characterize capsule-obtained impedance during translation across tissue.

3.3.1. Measurement parameter optimization via stationary measurement

Before evaluation with the capsule, a preliminary trial is performed on excised small intestinal porcine tissue samples using the EVAL-AD5941. Ten small (~1cm) samples were segmented. A subset of five were soaked in 1x-PBS for 24 hours prior to measurement. An EIS sweep was conducted between 100 Hz and 200 kHz. Plotted in Figure 3-3 are the impedance magnitude and phase response obtained with the unmodified sensor and PEDOT:PSS-treated sensor. Non-treated (N.T.) samples are denoted by a blue color, and PBS-soaked impedances are shown in red.

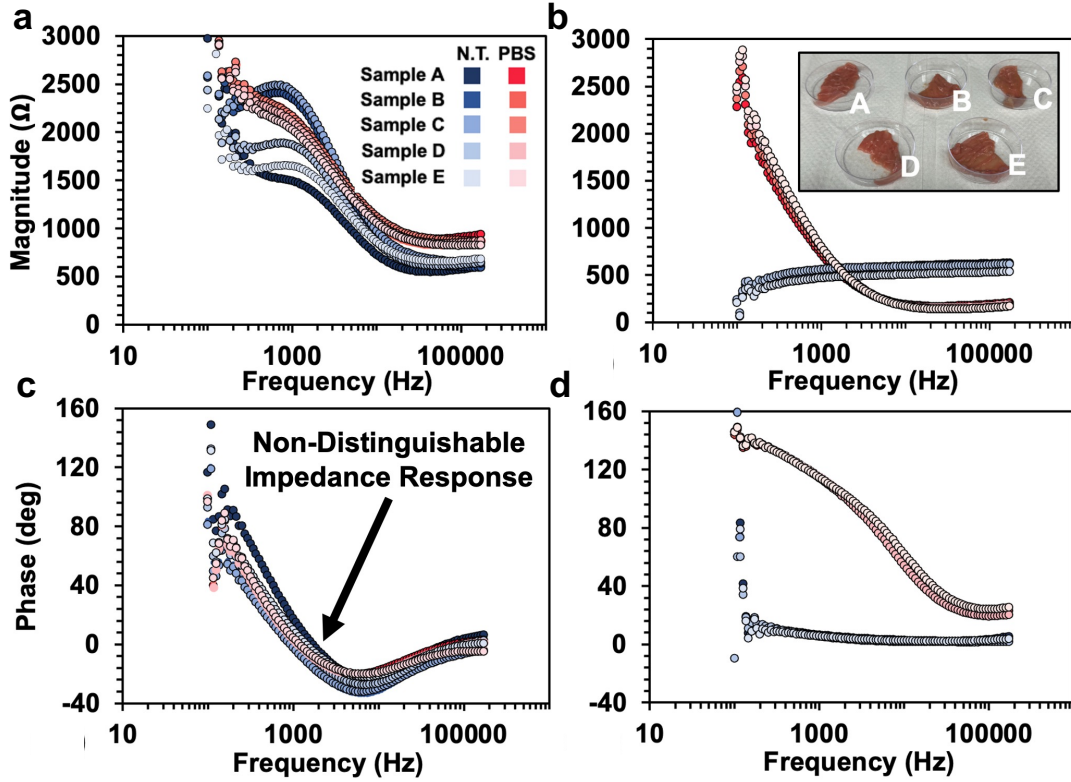


Figure 3-3 (a) Impedance magnitude response to frequency using untreated Au sensor. (b) Magnitude response of PEDOT:PSS-treated sensor and (inset) photograph of excised porcine small intestinal tissue samples. (c) Phase response of unmodified and (d) PEDOT:PSS-treated impedance sensor.

From this experiment, it became evident that the unmodified sensor was incapable of differentiating each tissue group, supporting the low decrease in impedance magnitude observed in Chapter 3.2. At a sampling frequency of 10.5 kHz, the PEDOT:PSS sensor reduced sample-to-sample variation by 58.7% on untreated tissue and 68.3% on PBS-soaked intestinal tissues. Furthermore, magnitude between each group were clearly distinguishable, stabilizing at 10 kHz. Hence, the performance of the PEDOT:PSS sensor far exceeds the unmodified Au sensor, supporting its integration with ingestible impedance sensing electronics.

3.3.2. Evaluation of device performance during translation

Following initial verification using the EVAL-AD5941, experimentation was conducted to determine whether the PEDOT:PSS-treated sensor was capable of low-noise characterization of tissues using device electronics. First, the sensor was inserted into the FFC port of the device and sealed using Kapton tape. The sensor was contoured around the shell and secured using hot-melt adhesive. The resultant capsule is shown in Figure 3-4 (a). Next, a tissue model consisting of untreated and PBS-soaked intestinal tissue was devised to determine whether the capsule could continuously report variations in impedance magnitude during translation. The tissue was adhered to a half-pipe silicone tissue holder featuring a diameter of 25 mm, mimicking human lumen. For initial measurement, the device was placed on both tissue groups, easily identifiable by washing the PBS-soaked tissue a blue dye (Figure 3-4 (b)). These measurements were used to configure the LED activation threshold via the EFR Connect mobile application. It should be noted that at the wirelessly programmed sampling frequency of 10 kHz, the measurement obtained with the PEDOT:PSS-treated sensor was invariant to contact pressure and capsule orientation as long as contact was made with tissue. When placed onto the PBS-soaked tissue, the measured impedance magnitude decreased below 650 Ω , causing the onboard blue LED to shine as shown in Figure 3-4 (c).

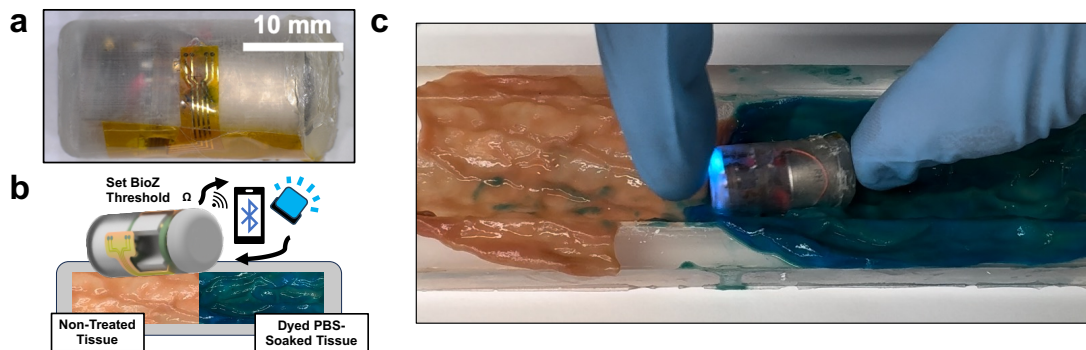


Figure 3-4 (a) Sensor-integrated capsule with Kapton tape covering the sensor port for insulation during initial measurement, (b) illustration of tissue model used for tissue differentiation using the feedback-driven LED. (c) Photograph of device placement on PBS-treated porcine intestinal tissue and resultant LED activation.

The capsule was actuated across the tissue model in a linear fashion using a previously demonstrated apparatus [127] to simulate GI transit. The LED activated upon contacting PBS-soaked tissue and deactivated upon placement onto untreated tissue, confirming that the capsule could differentiate between each tissue group (Figure 3-5 (a)). Wirelessly reported impedance magnitude spiked due to partial contact with tissue at two intervals as denoted by arrows in Figure 3-5 (b). Faulty contact was most likely caused by mechanically rigid extrusions formed by the underlying adhesive. A second translation trial was performed using the unmodified sensor, resulting in minimal impedance variation (N.T.: $1227 \pm 23\Omega$, PBS: $1453 \pm 189\Omega$) as observed using the EVAL-AD5941. Average impedance magnitude values are shown in Figure 3-5(c).

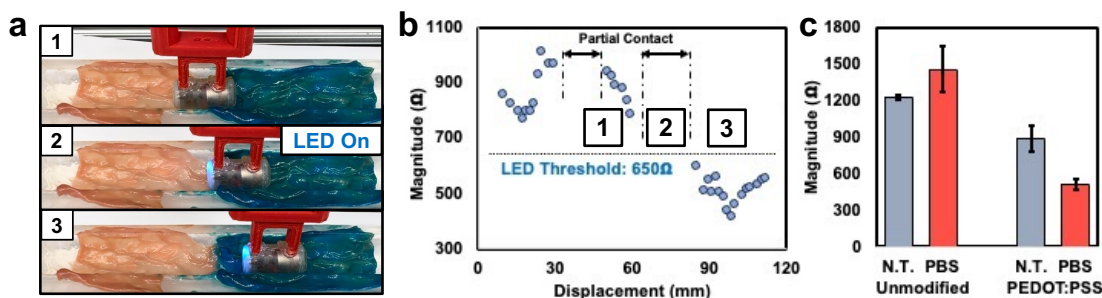


Figure 3-5 (a) Controlled translation of integrated capsule device across tissue model to simulate peristaltic motion. LED activates in response to measured drop in impedance. (b) Corresponding impedance magnitude transmitted wirelessly to mobile device in relation to bioimpedance threshold, (c) average values obtained during translation using unmodified sensor (left) and PEDOT:PSS-treated sensor (right).

Measurement with the PEDOT:PSS-treated sensor yielded a considerable reduction in impedance magnitude (N.T.: $890 \pm 107 \Omega$, PBS: $513 \pm 45 \Omega$). This result confirms that stable impedance magnitude values can be wirelessly achieved through four-probe sensing using the PEDOT:PSS-treated sensor and integrated AD5941 potentiostat. In contrast, the untreated sensor proved incompatible with the capsule device, as no significant difference in impedance was observed during translation across saline-soaked tissue, limiting its applicability in feedback-driven applications.

Chapter 4. *Ex vivo* Validation of Bioimpedance Sensing on Rodent Animal Model

In this chapter, we validate that the fabricated bioimpedance sensor and capsule electronics can quantify paracellular permeability on small animal tissues. An animal study is designed with incremental enhancement of colonic mouse tissue permeability via tight junction dilation. Experimental protocols are validated with TEER using an Ussing chamber. Then, alterations in mucosal permeability are recorded with the

bioimpedance sensor, first using the **wired** EVAL-AD5941 to establish statistical significance, followed by the ingestible capsule device to demonstrate feasibility for **wireless** acquisition of bioimpedance data. **Dr. Younggeon Jin** and **Hammed Ayansola** significantly contributed to the experiments, assisting with planning, tissue extraction, Ussing chamber measurement, and data analysis.

4.1. Overview of animal model

For correlating impedance measured from the sensor-integrated capsule device with mucosal permeability on intestinal tissue, an animal trial was devised using C57BL/6 mice. Experimentation with living animal tissues is required to relate physiological changes in tight junction integrity with measured bioimpedance to eventually quantify mucosal permeability *in vivo*. Although porcine tissues are more representative of human tissue, rodent tissue models have also been used due to convenient animal care and tissue extraction. According to a recent study by Merrero et al., exposure of intestinal enterocytic Caco-2 epithelial cells to 4 mM ethylenediamine-tetra-acetic acid (EDTA), a calcium chelating agent related to the more calcium ion selective EGTA [128], resulted in a 50% drop in TEER within minutes due to enhanced paracellular permeability [129]. After 30 minutes, EDTA was removed, and TEER increased to baseline levels due to recovery. EDTA is often employed to detach cell cultures for passage by binding calcium and magnesium, weakening cell adhesion, leading to disrupted tight junction integrity.

4.2. EDTA challenge for *ex vivo* mouse tissues

4.2.1. Extraction and preservation of tissue samples

Mice are first euthanatized via CO₂ suffocation and subsequently opened to extract the colonic tissue. Colonic tissue is immediately washed in 1x-PBS and luminal contents are removed. The samples are then opened luminally and segmented into 0.1 cm² sizes for Ussing chamber verification and longer sections (~1 cm) for bioimpedance measurement. For preserving the tissue, the samples are pinned to a foam biopsy sponge and placed into a beaker of Kreb's-Ringer Bicarbonate (KRB) buffer supplemented with glucose and carbogen gas of 95% O₂ and 5% CO₂. Paracellular permeability is increased by placing the tissue into a separate KRB beaker containing EDTA. During experimentation, increasing concentrations of EDTA ranging from 1 mM to 10 mM are applied into the separate beaker to incrementally decrease tight junction integrity.

4.2.2. TEER measurement of tight junction damage induced via EDTA

Initially, colonic tissue samples were placed into a Physiologic Instruments P2300 Ussing chamber to verify that the EDTA challenge could be used to increase paracellular permeability [103]. Concentrations of 1, 3, and 5 mM were applied into the mucosal hemi-chamber of two samples. Transepithelial resistivity (TEER) values shown in Figure 4-1 remained relatively unchanged for control samples, colored in shades of orange. Conversely, EDTA treated tissue impedance was increasingly lowered with each treatment over the forty-minute measurement interval.

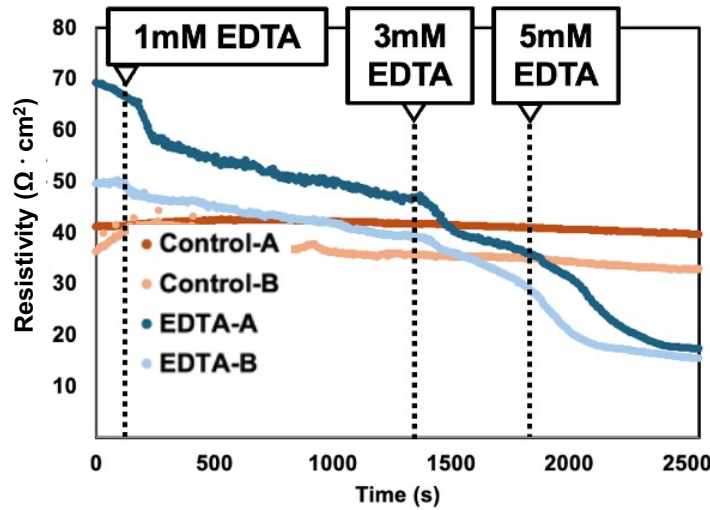


Figure 4-1. Ussing chamber TEER measurement on four samples. Two samples denoted EDTA-A and EDTA-B undergo identical EDTA treatment at various times denoted by labels, disrupting adherens junctions and reducing tissue resistivity. After 5min. of 5mM EDTA treatment, a plateau in tissue impedance is observed, denoting saturation of tissue permeability.

Through this experiment, it became evident that EDTA increases paracellular permeability in a time- and dosage- dependent manner. Additionally, following application of 5 mM EDTA, measured electrical resistance sharply declines and plateaus at about 20 Ω . From this, it may be deduced that once tight junctions have fully dilated, the permeability saturates and measured impedance cannot be further reduced. This pattern, evident in previous studies [129], will also be observed during later benchtop experimentation using the bioimpedance sensor and EVAL-AD5941.

4.2.3. Measurement protocol

First, colonic tissues samples extracted using the aforementioned technique are placed into a beaker of KRB-glucose solution. Figure 4-2 (a) outlines the experimental procedure for tissue extraction and treatment. For each measurement, baseline measurements are first performed without EDTA challenge to establish the impedance

of healthy epithelial tissue. This is performed using the EVAL-AD5941 evaluation kit. For all measurements, to determine the optimal interrogation frequency for quantifying paracellular permeability, a frequency sweep is performed between 100 Hz and 200 kHz and excitation voltage of 600 mV p-p. Next, the tissue is moved to a beaker containing EDTA for a variable time parameter, denoted “T”. The tissue is rinsed with agitation in a beaker of fresh KBR to remove e. Removal of EDTA from the tissue is necessitated to inhibit further enhancement of epithelial permeability during measurement. Initially, EIS (N=3) was performed in KRB to ensure that impedance magnitude was unchanged by increasing EDTA concentration. The result, as shown in Figure 4-2 (b), confirms that measured impedance is not affected by EDTA.

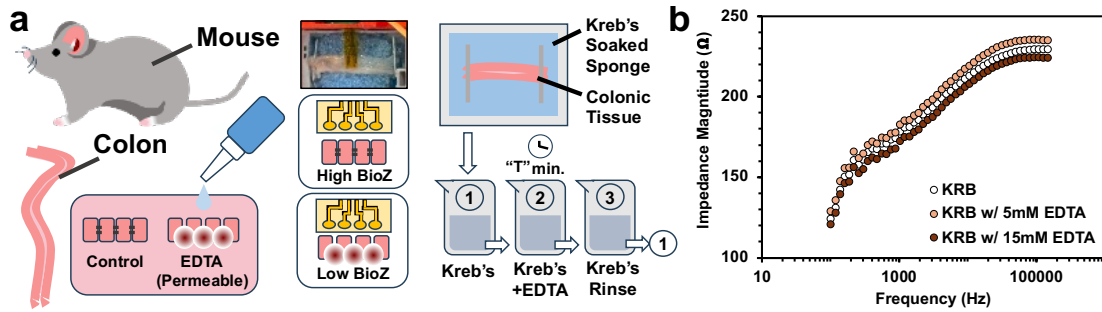


Figure 4-2 (a) Conceptual overview of tissue extraction and EDTA treatment protocol, enhancing paracellular permeability and reducing bioimpedance (BioZ). Tissue is placed in KBR solution to stabilize before EDTA challenge (1, 3, 5, 10 mM) induced for a time “T” and rinsing to remove EDTA. (b) EIS measurement in KRB solution (N=3) using fabricated PEDOT:PSS treated sensor and EVAL-AD5941 verifies that added EDTA has little effect on the impedance of the solution itself.

Following the EDTA rinse, the tissue sample is returned to the original oxygenated KBR-glucose beaker for thirty seconds and removed for each measurement. The measurement took place outside of the beaker to better replicate how the sensor interfaces with tissue in the GI tract. Initial measurements were performed on tissue submerged in KBR solution, which caused inconsistencies in impedance trends due to

its high ion content. Between each measurement (N=4), the tissue was placed back into the oxygenated KBR-glucose to preserve the tissue and ensure that the measured impedance could only be attributed to epithelial permeability and not tissue KBR content.

4.2.4. Optimization of experimental conditions

In early trials, tissue was treated with 5- and 10-mM concentrations of EDTA. For example, during the experiment protocol featured in Figure 4-3, the tissue was placed into the (5mM EDTA)-KBR beaker for three minutes before bioimpedance measurement. Next, concentration was increased to 10 mM EDTA in the treatment beaker, and the tissue was submerged for five minutes before removal and measurement. The KBR-soaked sponge impedance magnitude, colored in pink, ranged between 200 and 300 Ω consistently between trials in the targeted frequency range of 3 kHz to 100 kHz. This value, acting as a series resistance beneath the tissue, represents complete ion permeability. In practice, measured impedance plateaus before this due to the presence of non-epithelial tissues.

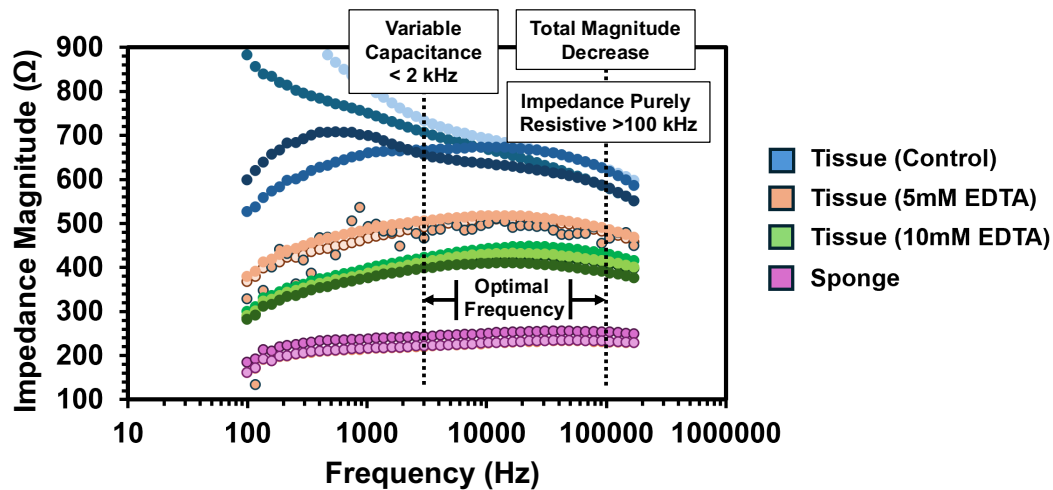


Figure 4-3. Bioimpedance magnitude measurement ($N=4$) on tissue treated with 5 mM EDTA for three minutes followed by 10 mM EDTA for five minutes. This early trial reflects that the optimal frequency for measurement lies between 3 kHz and 100 kHz. The KRB-soaked sponge pinned beneath the tissue represents the minimum attainable impedance (colored pink) if tissue was completely ion-permeable.

The measured impedance magnitude is stable in the optimal frequency range labeled above. Measurement at frequencies greater than 100 kHz exhibit a sharp decline in magnitude, as impedance becomes purely resistive. Conversely, below 3 kHz, dominant capacitive effects induce measurement variation. This ultimately confirms the need for the PEDOT:PSS treatment to reduce the effect of interfacial and electrode-electrolyte impedances, extending the viable frequency range for targeted measurement of paracellular ion permeability at low frequencies. This general trend was also observed during other trials, shown in Figure 4-4. In these two trials, tissue was submerged in 2.5, 5, and 10 mM EDTA uniformly for five minutes each. During experimentation, it became evident that impedance magnitude was most effective at communicating alterations in tight junction integrity, since no clear trend could be established using phase measurements. In Figure 4-4, the impedance magnitude

observed following each treatment plateaued within 100 Ω of one another, indicating saturated tight junction permeability.

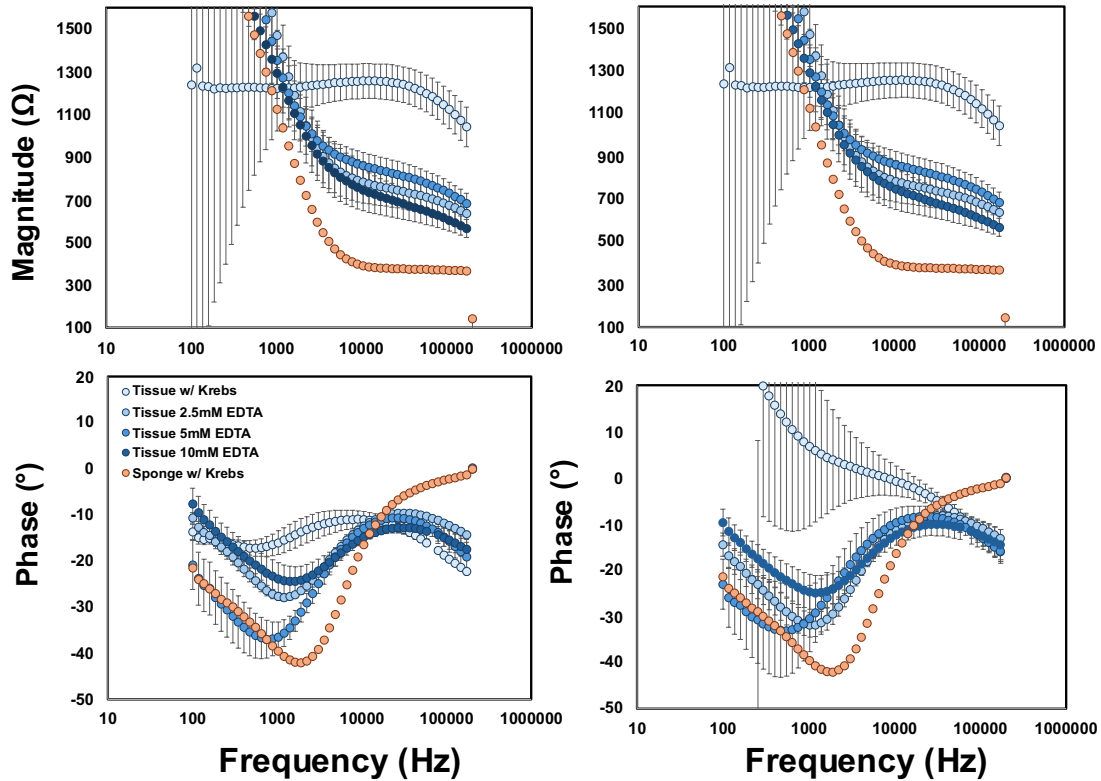


Figure 4-4. Comparison of full-frequency EIS magnitude and phase response using five minutes of 2.5mM, 5mM, 10mM EDTA treatment. Unlike impedance magnitude, although variations in measured phase are evident between each tissue group, a definitive trend cannot be established.

Between the trials depicted in Figure 4-3 and Figure 4-4, a general rise in impedance was observed. This is most likely caused by use of a different sensor as well as variations in tissue thickness between mice. During experimentation, sensors were swapped between mice to ensure that PEDOT:PSS film or sensor-to-sensor variation did not obscure the observed trend. The impedance magnitude was plotted relative to the healthy baseline values sampling at 10.5 kHz for each mouse to account for this variability. Although a clear magnitude trend was evident for Mouse I, this could not be established between each treatment group for Mouse II-III. This is likely due to the

prolonged exposure to EDTA, diminishing variation in tight junction disruption between treatments. Hence, for the final mice experiment, treatment durations were reduced from five minutes to one minute and EDTA concentration was adjusted to incrementally enhance mucosal permeability.

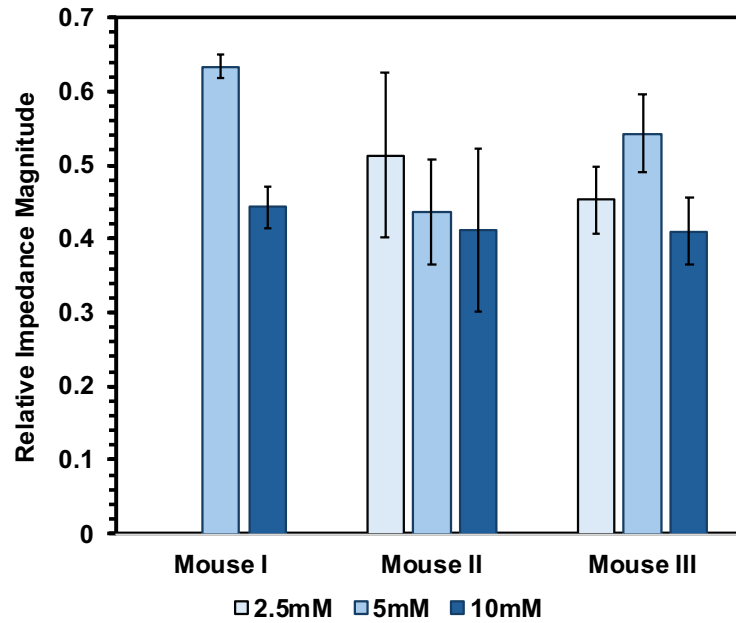


Figure 4-5. Comparison of relative impedance magnitude at 10kHz results in little magnitude difference in early trials using five minutes of increasing EDTA treatment in small increments.

4.3. Small animal trial using optimized treatment protocol

4.3.1. Measurement with wired eval kit

Colonic tissue samples are extracted from five mice and treated with 1 mM, 3 mM, 5 mM, and 10 mM EDTA for one-minute intervals to establish a general trend in impedance response. Analysis was only performed on bioimpedance magnitude. The results for full-frequency EIS using the developed sensor and EVAL-AD5941 are shown in Figure 4-6. Among the sensors, the 1-kHz impedance magnitude [112]

consistently increases, confirming de-doping of the PEDOT:PSS film and a general increase in interfacial impedance over time. However, in agreement with longevity experimentation in Figure 2-16 (a), the impedance magnitude at 10.5 kHz is the lowest excitation frequency for which the measurement remains stable with time.

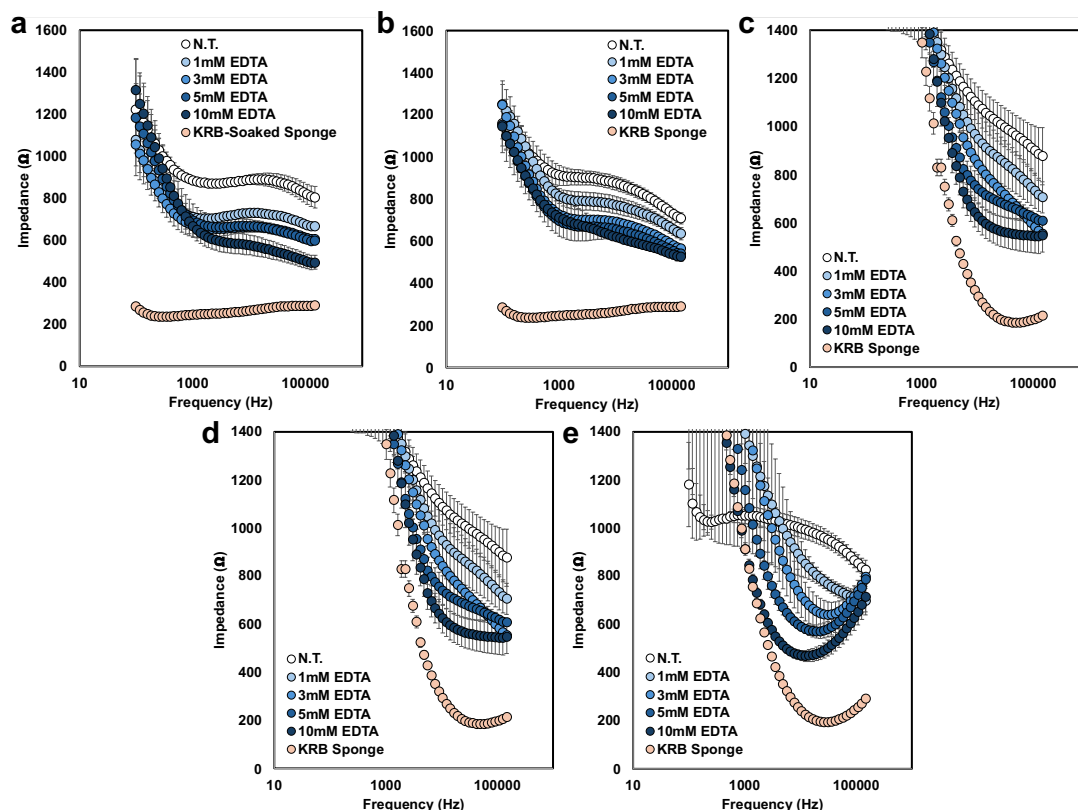


Figure 4-6. EIS magnitude response for five mice using EVAL-AD5941 and PEDOT:PSS treated sensor. EDTA challenge time is reduced to one minute for each concentration, resulting in generally distinguishable impedance magnitude values.

The impedance magnitude sampled at 10.5 kHz is plotted in Figure 4-7. An unpaired T-test was employed between each tissue group to assess whether a statistically significant difference exists between measured bioimpedance magnitude on untreated and EDTA-treated colonic tissue samples. An unpaired T-test is useful for comparing mean values between two groups using independent observations, whereas a paired T-test assesses differences on an individual basis. In accordance with standard

scientific practices, the baseline significance threshold for determining significance was set at $P=0.05$. With exception to the 1 mM treatment on Mouse IV, a significant difference could be established between untreated colonic tissue and all permeability-enhanced EDTA tissue groups. The impedance of the KBR sponge ($270.0 \pm 18.7 \Omega$) remained relatively unchanged between each mouse, confirming the measurement validity of all sensors.

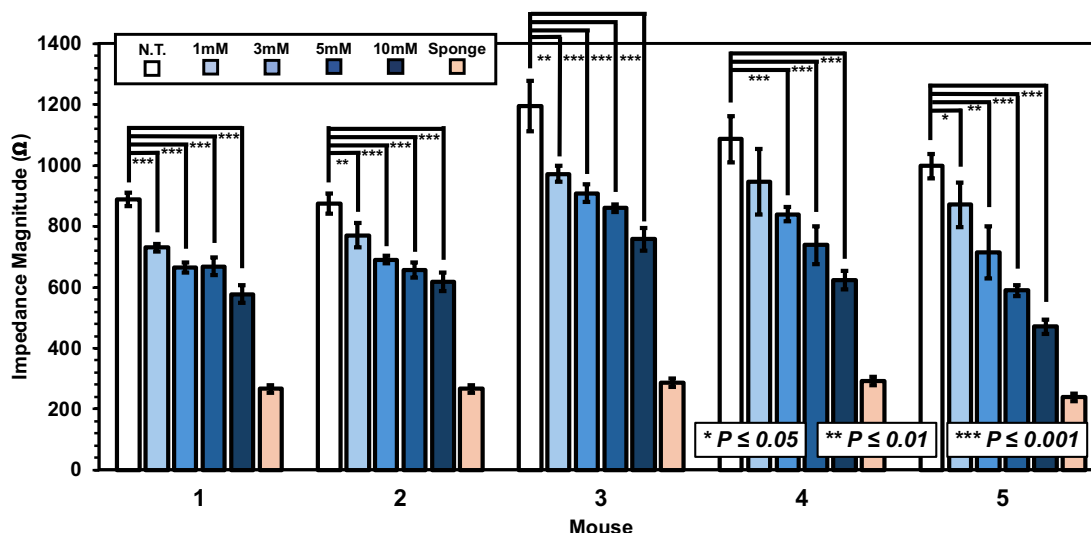


Figure 4-7. EIS magnitude response for five mice using EVAL-AD5941 and PEDOT:PSS treated sensor. EDTA challenge time is reduced to one minute for each concentration, resulting in generally distinguishable impedance magnitude values.

All impedance values were averaged and plotted relative to the baseline magnitude value collected on untreated colonic tissues to account for variability. In contrast to the unpaired T-test, a paired T-test ($N=5$) was used to compare unmodified impedance magnitude under each treatment condition to ensure that variations in tissue thickness and fabricated sensors did not bias the results. Ultimately, this design enabled unbiased comparison of impedance magnitude value, shown in Figure 4-8 (a). Under each challenge, magnitude was reduced to 79.7 ± 9.0 , 66.8 ± 6.3 , 58.8 ± 8.4 , and $46.4 \pm 10.7\%$ of its baseline value. Statistical significance was established between most

treatment groups, demonstrating the ability of the PEDOT:PSS treated sensor to quantify incremental alterations in paracellular permeability.

4.3.2. Wireless capsule device measurement

Finally, a PEDOT:PSS-treated sensor was integrated with a capsule device and placed on the colonic tissue extracted from a sixth mouse. Magnitude values with and without EDTA challenge were wirelessly transmitted to a mobile device through the EFR Connect application, plotted in Figure 4-8 (b).

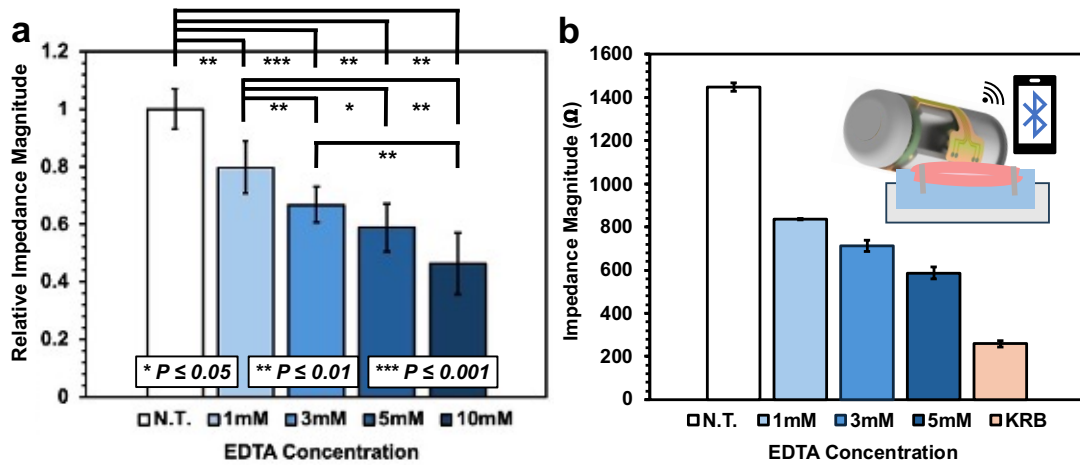


Figure 4-8. (a) Impedance values averaged among five mice plotted relative to their original values as obtained using the EVAL-AD5941 and (b) impedance magnitude values wirelessly obtained using integrated capsule device.

Unlike the behavior exhibited by the capsule in 3.3.2, the device was relatively sensitive to changes in the capsule's orientation, but this is to be expected given the small size of mice colonic tissue. Average impedance magnitude values reported from the capsule for 1-, 3-, and 5-mM EDTA challenge ($836.0 \pm 4.3 \Omega$, 713.3 ± 26.7 , and $586.3 \pm 27.4 \Omega$) mirrored the averaged magnitude among all five mice (858.5 ± 113.5 , 764.1 ± 103.6 , and $702.6 \pm 98.1 \Omega$). A large discrepancy was observed between unmodified tissues measured via the device ($1447 \pm 19.5 \Omega$) and EVAL-AD5941

($1008.9 \pm 133.5 \Omega$) during the five-mice trial. However, capsule-transmitted impedance did align with magnitude values observed in Figure 4-4 also obtained with the evaluation kit. These results confirm that the developed bioimpedance sensing capsule can effectively characterize intestinal mucosal permeability through incremental reductions in magnitude in accordance with benchtop results obtained using the EVAL-AD5941.

Chapter 5. Conclusion and Future Work

5.1. Summary

This thesis presents the design and fabrication of a noise-resilient flexible bioimpedance sensor compatible for integration with low-power ingestible device electronics. Through validation on excised porcine small intestinal tissue and systems integration with other capsule-embedded microsystems, the efficacy of the developed sensor as a tool for feedback and wireless diagnostics is demonstrated. A small animal trial is performed on mice colonic tissue using the EVAL-AD5941 evaluation kit and sensor-integrated capsule to ensure that the capsule device is capable of reporting alterations in intestinal paracellular permeability. Results demonstrate a significant reduction in impedance magnitude in response to incremental tight junction damage induced using a calcium chelating agent.

Chapter 2 details the comprehensive design of targeted bioimpedance sensors for characterizing intestinal epithelial tissues. A methodology for resolving sensor interfacial impedances and increasing CTC is presented through PEDOT:PSS electropolymerization. In Chapter 3, the PEDOT:PSS-treated sensor is integrated with

ingestible device electronics to verify low-noise characterization of intestinal tissues compared to polarizable metallic electrodes, employed in previous literature. In Chapter 4, an animal experiment is performed using a rodent model to correlate various degrees of induced intestinal tight junction dilation to bioimpedance magnitude. In the first study of its kind, a significant reduction in mucosal impedance is wirelessly reported by the developed ingestible device, confirming that the challenges presented in Chapter 1 may be addressed using the techniques contributed through this work.

5.2. Future work

5.2.1. Path towards employing bioimpedance device *in vivo*

A simple initial trial for *in vivo* validation of the developed device may entail embedding the capsule in the caecum of a large, anesthetized rat. Through dextran sodium sulfate (DSS) challenge, GI inflammation may be induced, enhancing mucosal permeability. Wireless impedance measurement will reveal the efficacy of the device in time-series impedance monitoring of inflammation progression. Additionally, device verification is needed in larger animal models (i.e., pigs) before translation into a clinical context. This will prove useful for establishing baseline impedance values of various GI tissues [130] under healthy conditions. An integrated pH sensor may prove useful to further aid in differentiating tissue *in vivo*, since the GI tract features a distinguished pH gradient from the esophagus through the large intestine. This would also elucidate pH-induced variations in PEDOT:PSS conductivity [117] during traversal. Finally, a study should also be performed to assess how passage of bolus or luminal fluids affect impedance measurement. PEDOT:PSS post-treatment in HCl- or

HCOOH- acids has been shown to enhance mechanical properties and chemical stability while mitigating sensitivity against passage of H₂O [131], [132]. This added step could serve to further enhance the sensor's resistance to environmental conditions *in vivo*.

5.2.2. Bioimpedance for closed-loop intervention

The design of the ingestible device electronics enables internal capsule connections and programming to be modified to support integration with other capsule-embedded microsystems. In this section, a proof-of-concept ingestible device is developed to demonstrate that the PEDOT:PSS-treated bioimpedance sensor can be used as a feedback mechanism for highly localized drug delivery. The device presented in Chapter 3 is modified to support a previously demonstrated thermomechanically actuated drug delivery module [110]. In response to an acute reduction in impedance magnitude, rapid closed-loop drug delivery is demonstrated on porcine intestinal tissues. In this section, **Joshua Levy** and **Mateo Lim** were extensively involved in the integration of the drug delivery module and characterization of the resultant device.

In the gastrointestinal tract, bioimpedance feedback-driven drug delivery may be used for autonomous therapeutic intervention in response to variations in tissue properties such as paracellular permeability, tissue type (i.e., lesions), extracellular fluid. Toward this end, a general-purpose ingestible device utilizing an electrolytic pump has been reported in the literature [72]. Here, a previously demonstrated cantilever actuator module was integrated with the bioimpedance sensing device to improve response time and drug retention in tissue. An illustration of bioimpedance-driven drug delivery is depicted in Figure 3-6 (a).

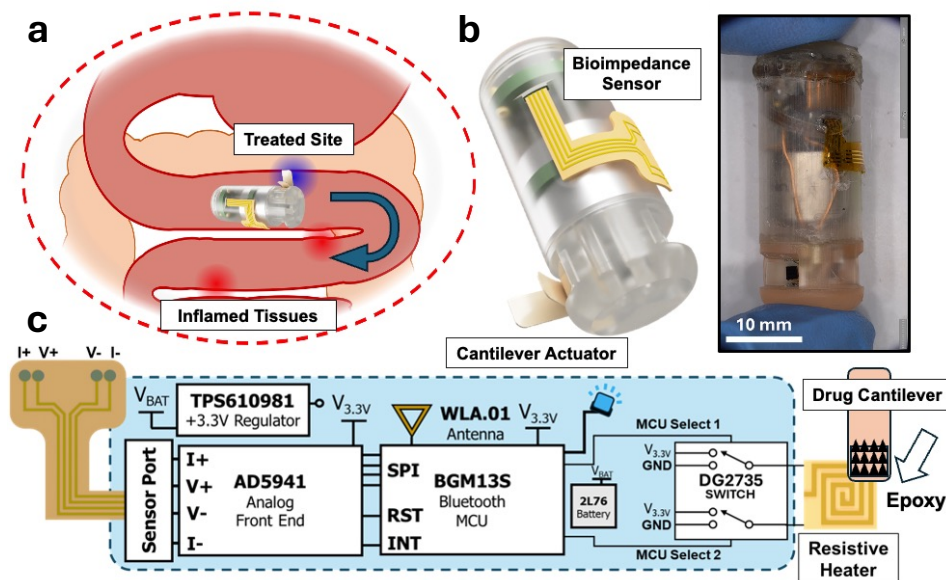


Figure 3-6 (a) Conceptual illustration of bioimpedance module applied as feedback tool for targeted drug delivery via ingestible device. (b) Rendering and photograph of bioimpedance sensing capsule integrated with thermomechanical actuated cantilever module, (c) electronics modification used to achieve rapid actuation in response to acute alterations in impedance magnitude.

A resistive heater was fabricated using lift-off of 20/70 nm Cr/Au on polyimide film. The geometry and resistance (70 Ω) of the resistive heater were optimized to efficiently dissipate heat into a low-melt ethylene vinyl acetate (EVA), facilitating the release of the cantilever. The cantilevers are laser cut from 254 μm thick sheets of low-creep polyether ether ketone (PEEK). Next, the cantilevers are topped with a drug-loaded microneedle array for delivery into intestinal tissue, as previously described. The heater was electrically connected to the PCB using conductive silver epoxy and 28-gauge insulated wires. An image of the capsule is shown in Figure 3-6 (b). Internally, a DG2735 analog switch is used to interface two MCU select ports with each heating element to enable controlled application of 3.3 V regulator output. The module can support two drug-loaded actuators, located adjacent to the impedance sensor and on the reverse side. The updated electronics schematic is depicted in Figure 3-6 (c). In the

code, the GATT profile was customized to support a second threshold for high-current output to a resistive heater as an alternative to LED activation. Additionally, a GATT field for manual deployment of the actuator was included to enable characterization of response time.

Using a mobile device, the cantilever was deployed (N=4) through the EFR Connect application. On average, thermomechanical release occurred within 1.59s, suitable for instantaneous delivery into afflicted tissue sites. Results are shown below in Figure 3-7.

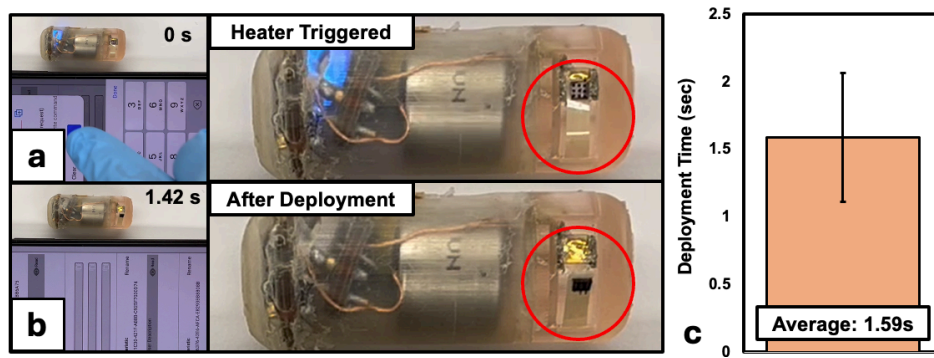


Figure 3-7 (a) Image of controlled cantilever deployment before and (b) after activation via mobile device, (c) average deployment time (N=4).

Next, the linear actuator and model consisting of N.T. and PBS-soaked tissue was used to characterize wirelessly obtained bioimpedance magnitude during translation as shown in Figure 3-8 (a). During each trial (N=3), tissue ridges caused by the underlying adhesive resulted in high measurement variability for the N.T. tissue, but the reported magnitude for PBS tissue was consistent (Figure 3-8 (b)). An activation threshold of 1500 Ω was programmed via the EFR Connect application for triggering of the drug delivery module. Average values are plotted in Figure 3-8 (c).

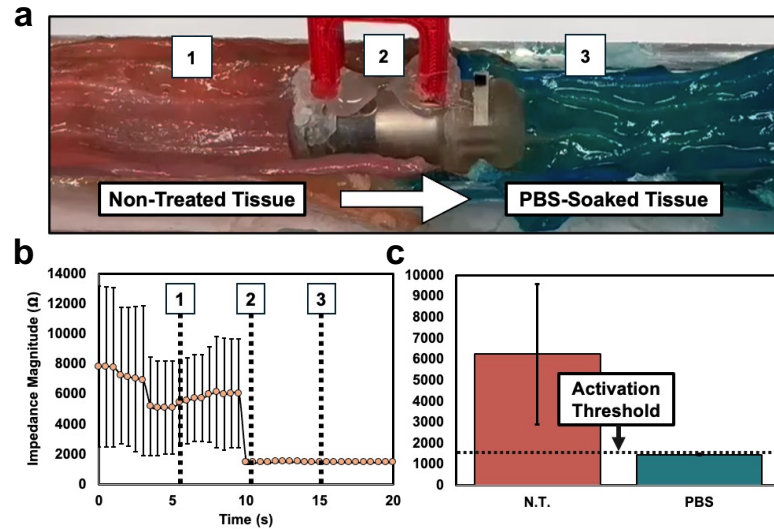


Figure 3-8 (a) Translation of capsule device across tissue model devised in Chapter 3.1.1., (b) impedance magnitude characterization wirelessly reported to mobile device among three trials, (c) averaged values among non-treated and PBS-soaked tissue relative to cantilever activation threshold.

Finally, the device was translated across tissue and successfully deployed the cantilever actuator upon contacting the PBS segment. For visualization, the deployed cantilever was assembled to the opposite side of the device's impedance sensor. This behavior, confirming the device's capability for closed-loop localized actuation, is depicted in Figure 3-9. In the GI tract, the cantilever actuator would separate from the device and pass naturally.

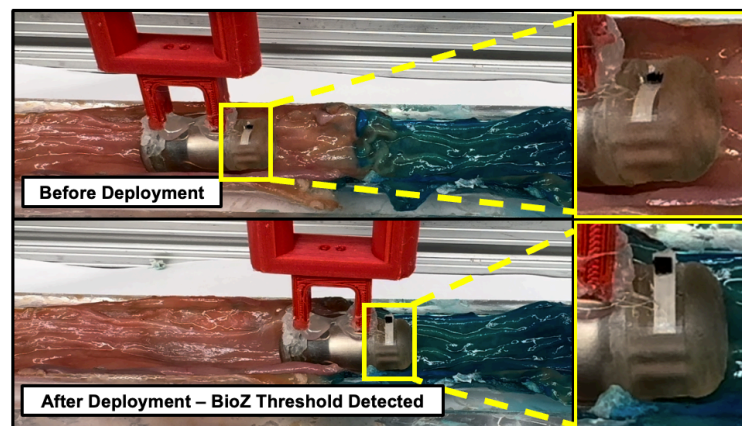


Figure 3-9. Exploded view of cantilever actuator automatically deploying in response to an acute drop in measured impedance when contacting PBS-soaked tissue.

Though a proof-of-concept, this modified device is among the first examples of combined wireless diagnostics and localized drug delivery for the GI tract. Rapid-response sensing tools such as the developed bioimpedance module are needed to accurately characterize tissue physiology and inform autonomous intervention. Oral treatment is the preferred route for drug delivery due to high patient compliance. In the GI tract, targeted drug delivery eliminates side-effects of systemic or traditional oral delivery while increasing drug absorption in afflicted sites. Using bioimpedance as a feedback tool, the device cantilever can be equipped with corticosteroids for tailored treatment of IBD. Currently, there are few examples of ingestible drug delivery devices that are capable of both wireless measurement and closed-loop delivery. This is due to the multifaceted issue of (1) few suitable sensing tools for explicit measurement of tissue properties and (2) sizing and power constraints hindering systems integration. Through closed-loop control of ingestible sensors and actuators, the array of available patient treatment options may be broadened, enabling combined monitoring and drug delivery with minimal side effects.

While this example applies bioimpedance as a feedback tool for drug delivery, the developed module also supports integration with other capsule-embedded microsystems. For example, the highly localized nature of the sensor is useful for targeted mucosal biopsy [111], where deployment can be autonomously or manually triggered in response to alterations in epithelial impedance. Through the modular systems integration techniques presented in Chapter 3, minimally invasive feedback-driven intervention can be achieved. Preliminary work conducted towards this end is depicted in Figure 5-1.

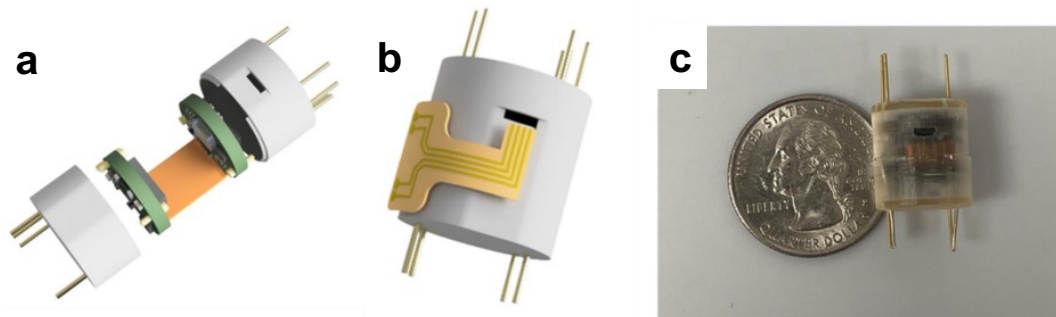


Figure 5-1. Pin-out concept for convenient attachment of PCB electronics and bioimpedance sensor with other modular capsule systems in plug-and-play fashion.

5.2.3. Application of machine learning in tissue differentiation

Beyond mucosal permeability, machine learning techniques may prove useful in the identification of complex relationships between tissue physiology and measured bioimpedance for an array of diagnostic applications. Before development of the PEDOT:PSS treatment, an early iteration of the bioimpedance sensor (voltage-sense electrode spacing of 8 mm, electrode diameter 2 mm) was used to demonstrate this concept. Benchtop measurements on various excised GI tissues were obtained using a Gamry 1010E Interface. Two small Yorkshire pigs were euthanized, with one having received lipopolysaccharide (LPS) treatment for an extended duration prior to measurement. LPS has been shown to induce intestinal inflammation, resulting in enhanced epithelial permeability. Plotted in Figure 5-1 are normalized impedance magnitude and phase values of various GI tissues extracted at 10 kHz. Support vector machines (SVM) classification [133] was performed using a radial technique available in MathWorks MATLAB R2023b. Initial data obtained immediately after tissue extraction are plotted in Figure 5-1 (a), and a larger dataset containing subsequent measurements is featured in Figure 5-1 (b).

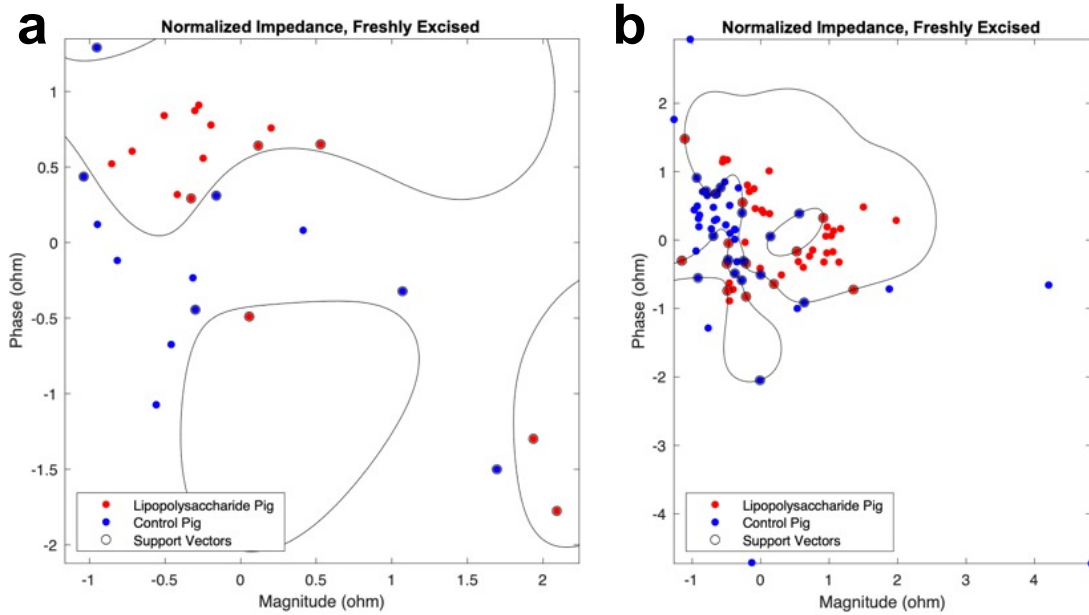


Figure 5-2. Trained SVM classifier differentiating impedance between LPS-treated and untreated excised porcine GI tissues. A radial SVM technique is demonstrated using (a) an initial measurement subset and (b) full dataset featuring two additional rounds of impedance measurement after two and four hours (right).

Classifier variation between initial and later measurements indicate poor generalizability of the model. Only two pigs were assessed, warranting a larger scale study. Additionally, this work was performed before PEDOT:PSS treatment on large electrodes not optimized for epithelial monitoring. However, this small experiment demonstrates the feasibility of simple machine learning techniques in tissue differentiation, compatible with embedded processors such as the BGM13S.

5.3. Conclusion

Few existing clinical diagnostic tools can characterize intestinal permeability, and none are capable of highly localized monitoring beyond the esophagus. This poses a challenge to medical practitioners, unable to non-invasively quantify patterns of tight junction dilation in the distal intestine toward diagnosis of inflammation and other GI diseases. This work addresses these issues through the development of an ingestible

bioimpedance sensing device capable of differentiating tissue based on paracellular permeability modulated during inflammatory processes. Through future validation *in vivo* using pilot studies and large-scale animal trials, this device may prove useful for a variety of autonomous diagnostics using artificial intelligence or machine learning.

Appendix A – Photomasks for developed sensors

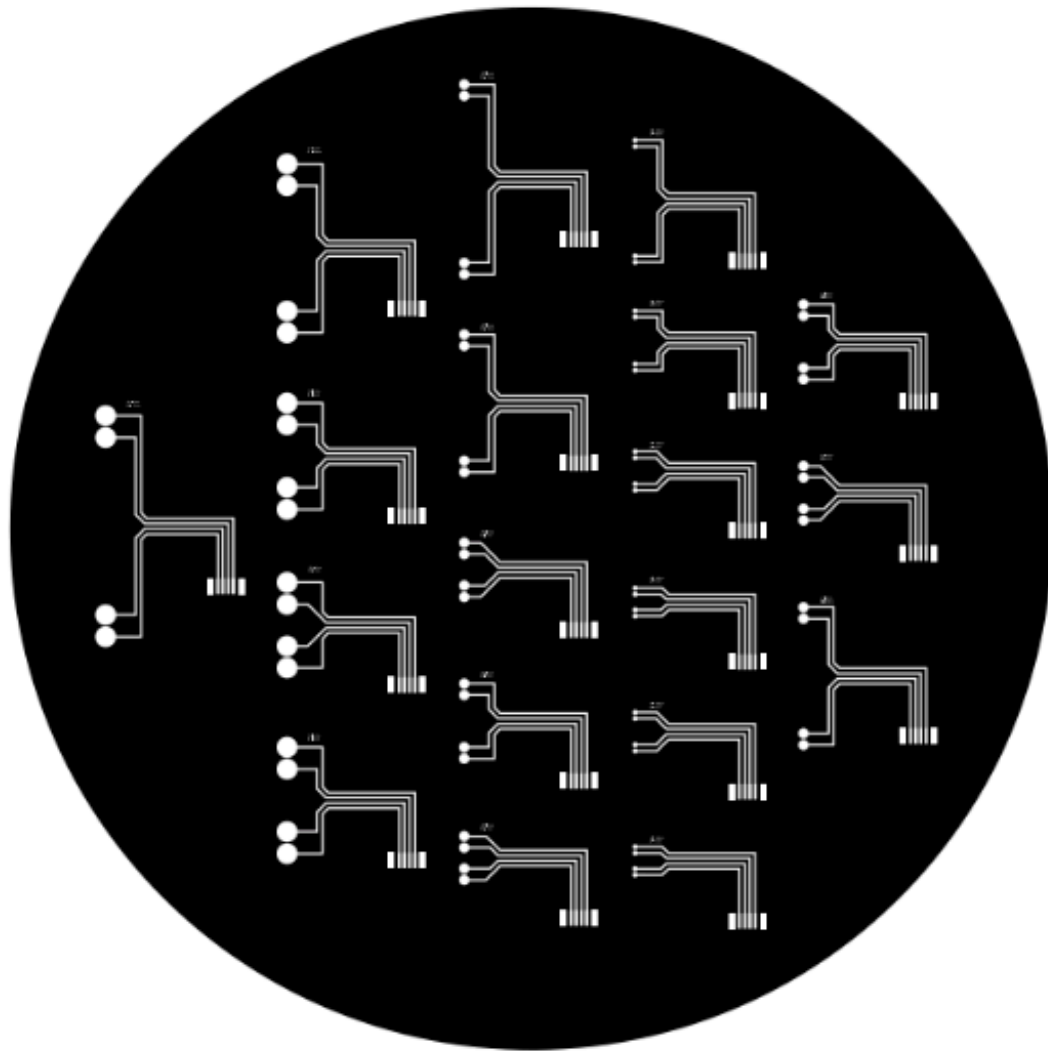


Figure A-1. Initial photomask containing various bioimpedance sensor geometries.

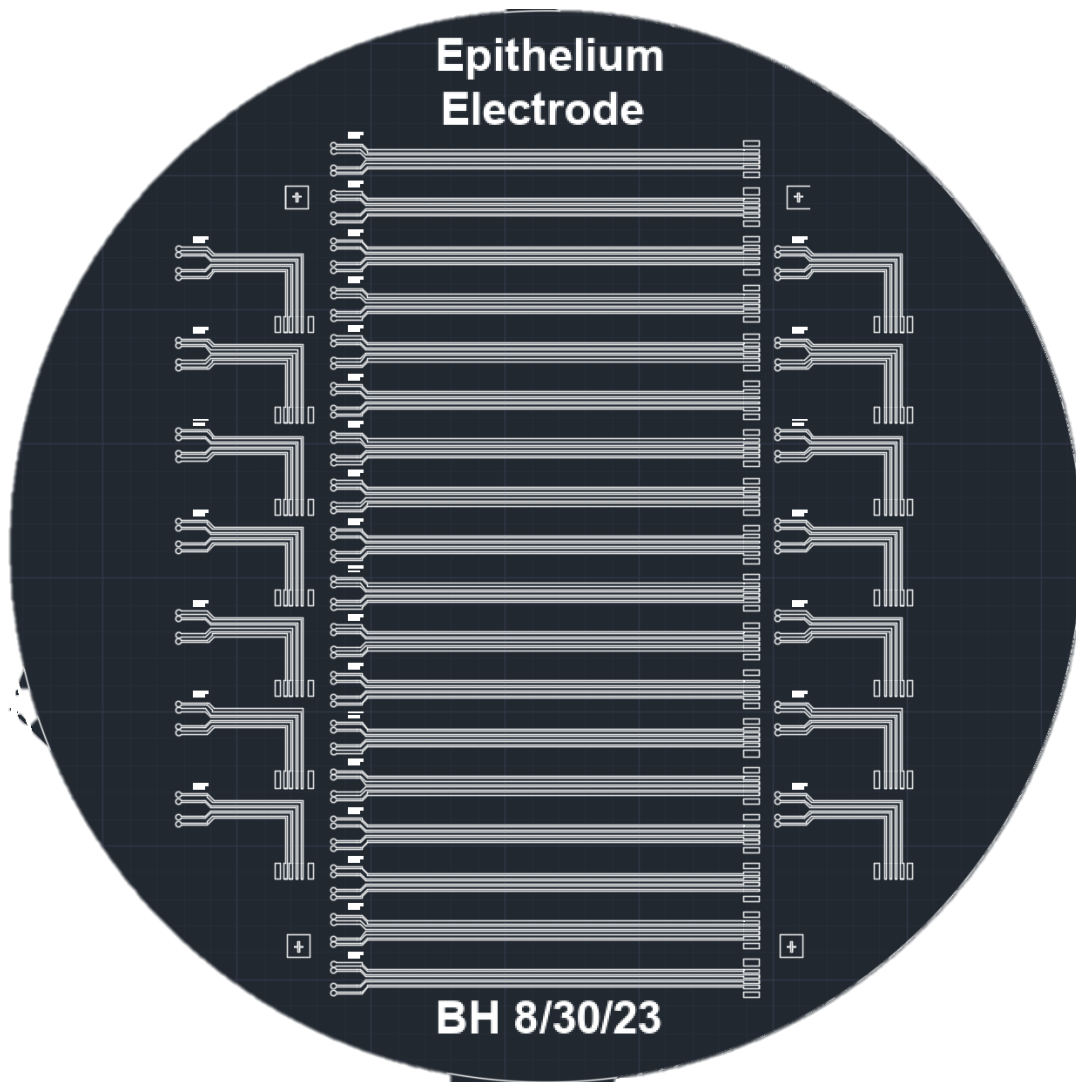


Figure A-2. Initial photomask containing various two sensor geometries. The compact geometry featuring a right angle is used for integration with the capsule device. Here, square alignment markers are featured in each corner to perform alignment using the Heidelberg MLA150. For this mask, a thickness of 20/70 nm Cr/Au is used during fabrication. During testing, it became evident that trace impedance was very high, causing measurement variability.

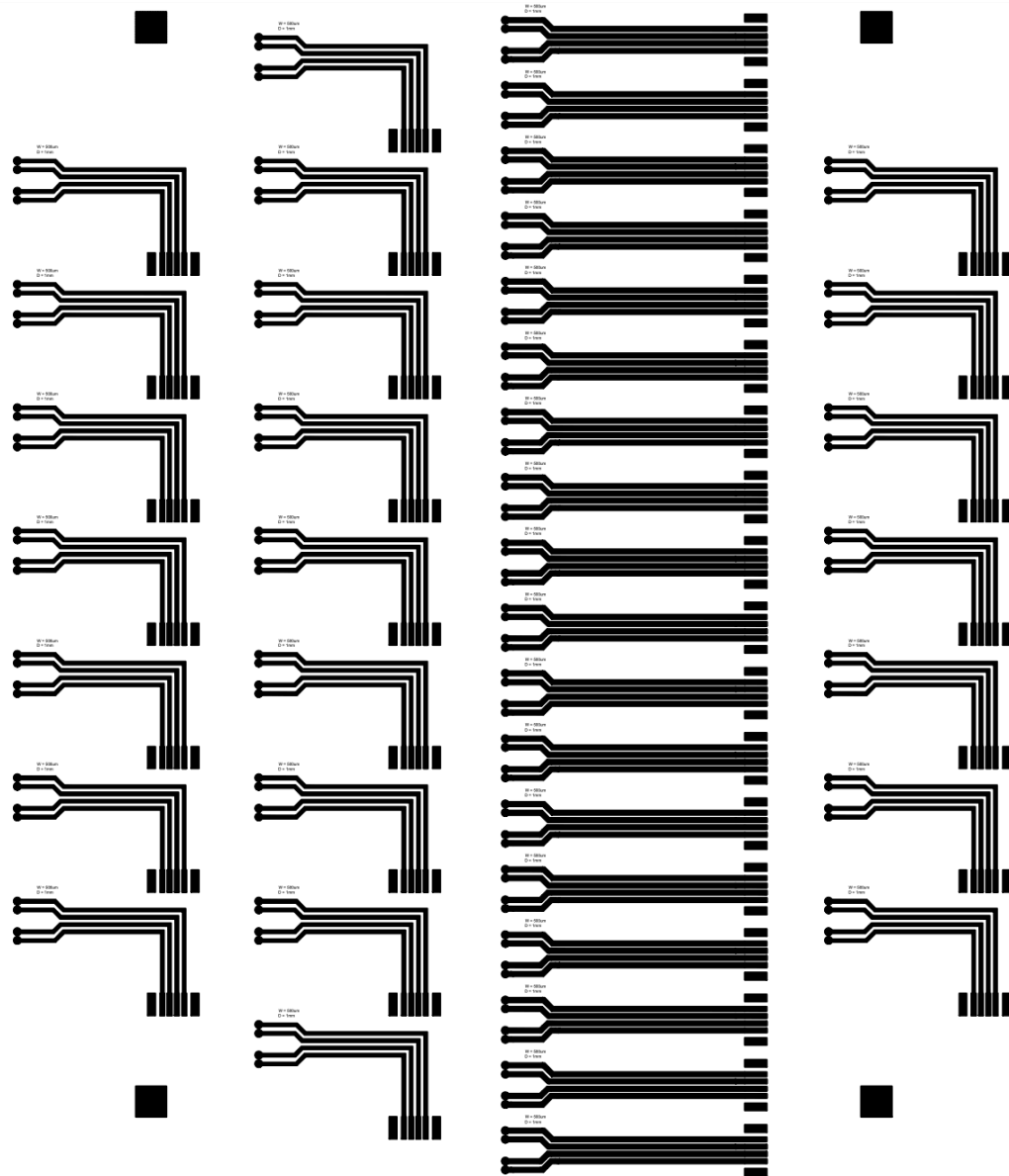


Figure A-3. Final photomask used for angular sensor geometry for integration with capsule electronics and long geometry for benchtop validation during animal trial. Note that the “long” geometry features thicker traces for minimizing trace impedance. Additionally, a thickness of 20/200 nm Cr/Au is used during fabrication to further minimize impedance.

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