In-Line Microelectrode Arrays for Impedance Mapping of Microphysiological Systems

Ashlyn T. Young[1], Vladimir A. Pozdin[3], Michael Daniele[1,2*]
[1] Joint Department of Biomedical Engineering, NC State University and UNC at Chapel Hill, Raleigh, NC, USA
[2]Department of Electrical and Computer Engineering, NC State University, Raleigh, NC, USA
[3]Department of Electrical and Computer Engineering, Florida International University, Miami, FL, USA
*mdaniel6@ncsu.edu

***Abstract*—Herein, a 60-electrode array is fabricated down the length of a microchamber for analysis of a microphysiological system. The electrode array is fabricated by standard photolithographic, metallization, and etching techniques. Permutations of 2-wire impedance measurements (10 Hz to 1 MHz) are made along the length of the microchannel using a multiplexer, Gamry potentiostat, and custom Labview code. An impedance "heat map" is created via custom algorithms. Spatial resolution and mapping capabilities are exhibited using conductive NaCl solutions and 2D cell culture.**

Keywords—microphysiological system; impedance mapping; transendothelial electrical resistance; cell culture

I. INTRODUCTION

Microphysiological systems (MPS) are miniature tissue constructs that recapitulate biological function. MPS can be used for drug screening, developmental biology research, and pathology studies [1-3]. Important MPS include the vascular-tissue barriers, such as the blood brain barrier [4-7], which consist of vascular and tissue units within a single device. Typical analysis of MPS require post-operative histology or immunohistochemical staining; consequently, the device must be sacrificed at a single time point to collect quantitative data. As an alternative to immunohistochemistry, in-line electrical impedance sensors can be used to collect electrical impedance measurements to determine barrier permeability.

Available MPS with in-line sensing capabilities measure the transendothelial electrical resistance (TEER) of vascular-tissue interfaces [8, 9]. TEER is used to determine ionic permeability between cells across a barrier and is defined as the resistance across the barrier multiplied by the area of the barrier. The resistance of the barrier is determined using Ohm's law, where the voltage measured across the barrier is divided by the driving current to calculate the resistance. These measurements are collected using electrodes placed on either side of a vascular-tissue barrier. When using TEER to define cell growth in a barrier system, a single value is reported. TEER measurement do not provide spatial information, failing to capture heterogeneity in monolayer formation within a single MPS. TEER exclusively provides information regarding gaps between cells, valuable in determining proliferation rates, though not informative on paracellular or junctional protein formation. Regardless, TEER remains a primary reported metric defining development and permeability of an MPS.

As an alternative to TEER, impedance measurements can be used to analyze MPS development and function. Rather than using direct current (DC) to quantify resistance, alternating current (AC) can be employed to characterize both the resistive and capacitive characteristics of the reside cell population. This provides information on the ionic permeability between cells, *i.e.* resistive component, as well as cell-cell interactions or membrane dynamics, *i.e.* capacitive components [10]. Impedance values measured by sweeping an AC signal across multiple frequencies offers information on the capacitance of the cell layer, cytoplasm conductivity, and other important transcellular and paracellular characteristics. Capacitive measurements have been used to determine cell viability, differentiation, and endocytosis, and potentially the uptake and movement of particles through the blood-brain barrier (BBB)

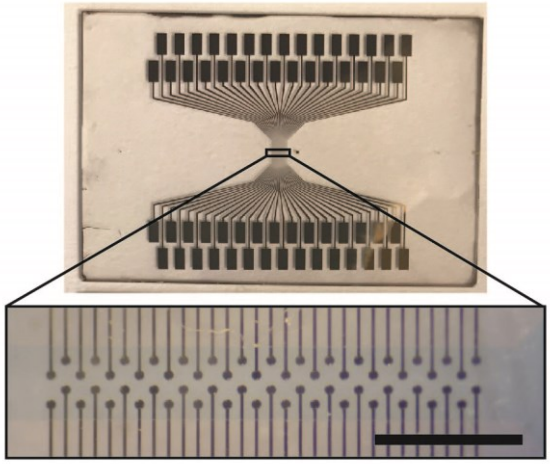

Fig 1. The microelectrode array consists of 60 planar electrodes for measuring a microchannel width of 400 μm and a length of 2.9 mm. The scale bar represents 1mm.

This work was supported by The National Science Foundation Grant CCSS-1846911. A.T.Y was supported by the Pre-doctoral Training Program in Integrative Vascular Biology at the University of North Carolina at Chapel Hill (NIH 2T32HL069768-16). This work was performed in part at the Chapel Hill Analytical and Nanofabrication Laboratory, CHANL, a member of the North Carolina Research Triangle Nanotechnology Network, RTNN, which is supported by the National Science Foundation, Grant ECCS-1542015, as part of the National Nanotechnology Coordinated Infrastructure.

978-1-7281-6801-2/20/$31.00 ©2020 IEEE

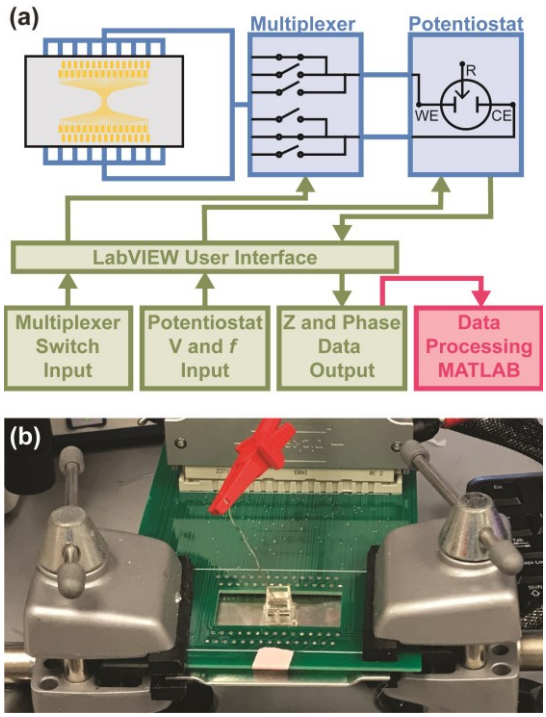

Fig 2. (a) Impedance mapping hardware consists of a PCB for connecting a multiplexer to a potentiostat, controlled by custom LabVIEW. MATLAB code is used to process data post measurement. (b) The custom PCB consists of spring-loaded pins for connection to the electrode array. A well is used to hold solution for preliminary testing.

via active transport [11, 12]. Spatiotemporal information can be collected using a microelectrode array, where impedance measurements are collected between paired electrodes along the length and width of the channel. This allows confirmation of uniform MPS development, as well as the extraction of key features from both the tissue and vascular portion of an MPS.

Herein, we demonstrated the design and operation of a microelectrode array that can be integrated within an MPS to achieve spatiotemporal resolution for MPS characterization and monitoring.

II. ELECTRODE AND HARDWARE DESIGN

A microelectrode array with 60 electrodes of 50 µm diameter spaced 141 µm from the nearest neighboring electrode was designed in AutoCAD and fabricated using photolithography and wet etching. In short, 30 nm Cr and 100 nm Au were deposited on a 2 by 3 inch glass slide using a Kurt Lesker physical vapor deposition 75 magnetron sputtering system. Shipley Microposit S1813 photoresist and a Karl Suss MA6/BA6 Mask Aligner is used to pattern electrodes. All photolithography masks are designed in AutoCAD and printed on transparency film by Fineline Imaging. Photoresist is developed in MF- CD-26 developer. An additional 100 nm of Au is electroplated onto the gold layer using Elevate Gold 7990 solution (Technic) and a power source. Photolithography was used as previously described to cover electrode area. The slide was then covered in Au etchant, rinsed, and then covered in Cr etchant. Final electrode patterns are then present, and photoresist is washed off with acetone.

Electrodes were insulated in parylene C. Slides were coated in a self-assembled silane monolayer, 3-(Trimethoxysilyl)propyl methacrylate. A Labcoater 2 Parylene Deposition System (Specialty Coating Systems) is used to insulate the slide in 4.1 µm of parylene C. First, photolithography is used to coat areas in photoresist that are not to be etched. Thick AZ 9260 photoresist is spun onto the slide and pattern using a Karl Suss MA6/BA6 Mask Aligner. Photoresist is then developed in AZ Developer 1:3. An Alcatel AMS 100 Deep Reactive Ion Etcher is used to etch through parylene and complete the device.

The custom hardware set-up consists of a NI PXIe 2524 in NI PXIe-1073 chassis Multiplexer and a Reference 600+ Gamry Potentiostat. The flow diagram for data collection is shown in **Figure 2a.** The PXIe-2524 multiplexer switch module 64 channel bank configuration is used, allowing all 60 electrodes to be connected both banks. This set-up permits two-point impedance measurements by connecting the working electrode to one bank and the counter electrode to the other bank, allowing any electrode pair to be selected. Connections are made between the multiplexer and the electrode array using a custom printed circuit board (PCB). The PCB was designed using EagleCAD and printed by ALLPCB. PCBs are populated with spring loaded gold pins, a 160-pin heads, and banana plugs using solder paste and a reflow oven. Spring loaded pins, on the bottom side of the PCB, are aligned to the contact pads on the glass slide, and clamps are used to compress pins onto pads for proper connection. A 160-pin cable assembly (Pickering) connects the header on the PCB to the multiplexer. Banana plug connecters connect the potentiostat to the selected working and counter electrodes.

III. EXPERIMENTAL PROTOCOL

Using the multiplexer, potentiostat, and custom LabVIEW code, impedances of 5M NaCl, 100 mM NaCl, and 1 mM NaCl solutions were measured. A well from an 8-well chamber slide was glued to the electrode array using PDMS at a 10:1 ratio. NaCl solution was added to the well at a 500µL volume, with an Ag/AgCl reference electrode, shown in **Figure 2b**. Data was collected from all 60 electrodes with nearest neighbor pairing at a driving voltage of 600 mV, at frequencies from 10 Hz to 1 MHz, with 5 points measured per decade using custom LabVIEW code.

Impedance of media and adherent cells was also measured in a chamber slide well. Human brain microvascular endothelial cells (hBMVEC) (Angioproteomie) were cultured in microvascular endothelial growth media 2 (EGM-2 MV) (Lonza) using a 37°C and 5% CO_2 incubator at 100% humidity. The electrode array was coated in 50 µg mL^{-1} fibronectin in PBS for 30 minutes at 37°C. HBMVECs were seeded at a 100,000 cells mL^{-1} density and allowed to adhere for 30 minutes. The cell monolayer was then washed twice with EGM-2 MV and 500 µL of EGM-2 MV was introduced for impedance measurements. Impedance measurements were collected with a 600 mV driving voltage at frequencies ranging from 1 Hz to 1 MHz with 5 points per decade. Data was collected for all electrode nearest neighbor pair. Impedance values between

each nearest neighbor pair were visualized on a heatmap using single pixels in a grid to represent a measurement, processed using custom MATLAB code.

IV. RESULTS AND DISCUSSION

To achieve spatial resolution down the length of the channel, as well as across both the vascular and tissue portions of the MPS, a 60-electrode array is incorporated within the microchannel. The electrode array, shown in **Figure 1**, is equidistant in the width of the channel and covers 2.9 mm down the length of the central channel. By incorporating many electrodes along the length of the channel, homogeneity of MPS growth down the channel can be determined during development. Electrodes of equal size and space from the nearest neighbor are used, allowing interpolation of measured two-point impedance values between combinations. This design enables multiple measurements in the 500 µm channel width without covering a large percentage of the growth area, therefore visual monitoring of MPS formation is enabled.

Using the complete hardware system including the multiplexer, potentiostat, custom PCB, and custom LabVIEW code, impedances of conductive solutions were measured. Solution conductivity is proportional to NaCl molarity. Impedance magnitude decreased with increasing solution conductivity at frequencies lower than 1 kHz, shown in **Figure 3a**. Impedance phase peak also shifted toward lower frequencies with decreasing conductivity. Impedance magnitudes were collected between all neighboring electrode pairs and spatial resolution at 998 Hz was demonstrated using a heat map, shown in **Figure 3b.** An increase in impedance is observed with decreasing conductivity for most electrode pairs. Some electrode pairs failed to detect impedance magnitude differences depending on solution conductivity. These

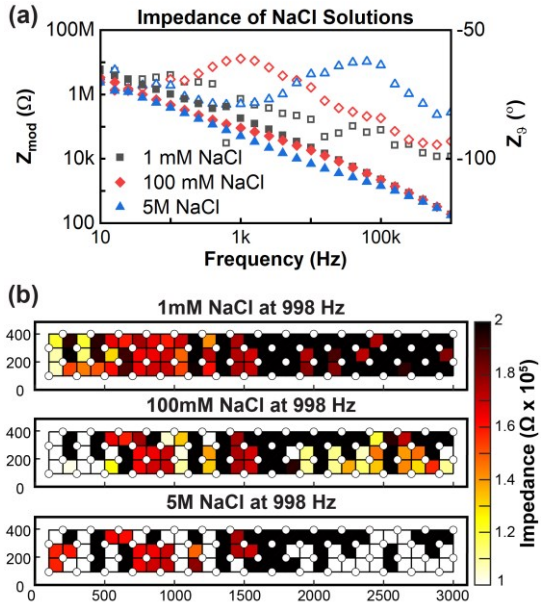

Fig 3. (a) Impedance (filled) and Phase (open) measurements are collected across a 10 Hz to 1 MHz frequency range for NaCl solutions with varying conductivity. (b) Two-point impedance magnitude measurements are mapped along the length and the width of the electrode array.

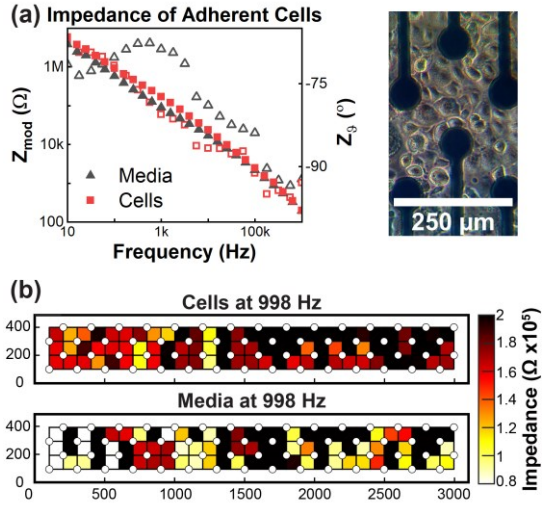

Fig 4. (a) Impedance (filled) and Phase (open) was measured across a 10 Hz to 1MHz frequency range with and without adhered microvascular cells. (b) Two-point impedance magnitude measurements are mapped along the length and width of the electrode array, normalized to 5M NaCl measurements.

measurements can be attributed to electrode fouling, fabrication defects, or defects in PCB construction.

The same protocol was used to measure the impedance of an adherent cell monolayer. An increase in impedance at frequencies less than 10 kHz was observed when cells were adhered to the electrode surface, shown in **Figure 4a**. Peak phase values also shift to lower frequencies with the addition of cells. Heatmaps of the impedance magnitude were also created comparing adherent cells to media alone within on the electrode array at 998 Hz frequency, shown in **Figure 4b**. As a general trend observed in the heatmap values, impedance values were higher when cells were adherent on the electrode surface compared to media alone. These experiments confirm the microelectrode hardware can be used to detect biological changes in cell culture.

We will employ lock-in amplifiers to improve the sensitivity and accuracy of TEER mapping throughout the device at very low signal-to-noise ratios. Optimization of electrode performance is also necessary to reduce "dead pixels." Validation of BBB model formation will be carried out by constructing a 3D scaffold and juxtaposed vascular channel, using human-derived brain microvascular endothelial cells and astrocytes. Other cells can also be utilized to evaluate system performance. Future effort may support the integration of electrochemical sensor arrays integrated in the microdevices.

V. CONCLUSIONS

In this study, we describe the design and operation of a microelectrode array for impedance mapping applications in MPS. A custom hardware and software configuration were used to measure impedance between neighboring electrodes. Heatmaps were used to visualize and compare impedance magnitude measurements between conductive solutions and adherent cells, demonstrating feasibility of microelectrode use in collecting data with spatial resolution.

REFERENCES

[1] L. P. Lee, "Microphysiological Analytic Platforms (MAPs): Precision Organs on Chip," *Advanced Healthcare Materials,* vol. 7, Jan 24 2018.

[2] Y. I. Wang, C. Carmona, J. J. Hickman, and M. L. Shuler, "Multiorgan Microphysiological Systems for Drug Development: Strategies, Advances, and Challenges," *Adv Healthc Mater,* vol. 7, Jan 2018.

[3] A. T. Young, K. R. Rivera, P. D. Erb, and M. A. Daniele, "Monitoring of microphysiological systems: integrating sensors and real-time data analysis toward autonomous decision-making," *ACS sensors,* 2019.

[4] A. Oddo, B. Peng, Z. Q. Tong, Y. K. Wei, W. Y. Tong, H. Thissen*, et al.*, "Advances in Microfluidic Blood-Brain Barrier (BBB) Models," *Trends in Biotechnology,* vol. 37, pp. 1295-1314, Dec 2019.

[5] A. Bhalerao, F. Sivandzade, S. R. Archie, E. A. Chowdhury, B. Noorani, and L. Cucullo, "In vitro modeling of the neurovascular unit: advances in the field," *Fluids and Barriers of the Cns,* vol. 17, Mar 16 2020.

[6] M. P. Ferro, S. C. Heilshorn, and R. M. Owens, "Materials for blood brain barrier modeling in vitro," *Materials Science & Engineering R-Reports,* vol. 140, Apr 2020.

[7] B. M. Maoz, A. Herland, E. A. FitzGerald, T. Grevesse, C. Vidoudez, A. R. Pacheco*, et al.*, "A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells," *Nature Biotechnology,* vol. 36, pp. 865-+, Sep 2018.

[8] B. Srinivasan, A. R. Kolli, M. B. Esch, H. E. Abaci, M. L. Shuler, and J. J. Hickman, "TEER measurement techniques for in vitro barrier model systems," *J Lab Autom,* vol. 20, pp. 107-26, Apr 2015.

[9] O. Y. F. Henry, R. Villenave, M. J. Cronce, W. D. Leineweber, M. A. Benz, and D. E. Ingber, "Organs-on-chips with integrated electrodes for trans-epithelial electrical resistance (TEER) measurements of human epithelial barrier function," *Lab Chip,* vol. 17, pp. 2264-2271, Jun 27 2017.

[10] K. Benson, S. Cramer, and H. J. Galla, "Impedance-based cell monitoring: barrier properties and beyond," *Fluids Barriers CNS,* vol. 10, p. 5, Jan 10 2013.

[11] D. Hyun Jo, R. Lee, J. Hyoung Kim, H. Oh Jun, T. Geol Lee, and J. Hun Kim, "Real-time estimation of paracellular permeability of cerebral endothelial cells by capacitance sensor array," *Sci Rep,* vol. 5, p. 11014, Jun 5 2015.

[12] Y. Xu, X. Xie, Y. Duan, L. Wang, Z. Cheng, and J. Cheng, "A review of impedance measurements of whole cells," *Biosens Bioelectron,* vol. 77, pp. 824-36, Mar 15 2016.