A Detailed Model for High-Frequency Impedance Characterization of Ovarian Cancer Epithelial Cell Layer Using ECIS Electrodes

Abdur Rub Abdur Rahman∗, Chun-Min Lo, and Shekhar Bhansali, Member, IEEE

Abstract—We report on the electrical impedance spectroscopy characterization of OVCA429 ovarian cancer cells. A commercially available eight-well cell culture impedance array (ECIS-8W1E), commonly used in electrical cell–substrate impedance sensing (ECIS), was used for OVCA429 characterization. Impedances of OVCA429 cell layer were recorded with cell culture medium (without cells) and with OVCA429 cell layer in the culture medium between 100 Hz and 10 MHz frequency. Physiological and interfacial components of experimental impedance data were modeled by equivalent circuit fitting, using a newly developed model. Impedance measurements with cell culture medium show two semicircles in the admittance plane, which are identified as 1) low-frequency semicircle due to impedance of gold electrode in contact with the electrolyte and 2) high-frequency semicircle due to impedance of polymer-coated region of the gold electrode in contact with the electrolyte. In the presence of OVCA429 cell layer, three convolved semicircles are observed in the Cole–Cole plane, which are identified as the interfacial impedance in series with the cell layer impedance. The average resistance and capacitance of the OVCA429 cell layer was found to be 152 ± 59 Ω·cm² and 8.5 ± 2.4 μF/cm², respectively.

Index Terms—Cell adhesion, cell impedance model, electrical cell–substrate impedance sensing (ECIS), impedance spectroscopy.

I. INTRODUCTION

T HE measurement of complex impedance as a function of frequency has long been used in bioimpedance investigations to study the frequency dispersions of biological tissue [1]. Impedimetric sensing has evolved as a powerful alternative to traditional methods in a variety of biosensing applications such as immunosensing, DNA hybridization studies, and cytotoxicity assessment [2]. Among the various types of impedimetric biosensors, cell-based biosensors utilize the living cells for monitoring environmental variables, cell–drug interactions, and infections [3]. Majority of the impedimetric-cell-based biosensors, including electrical cell–substrate impedance sensing (ECIS), rely on the alterations in the impedance of electrode–cell interface due to adhesion and motility of anchorage-dependent cells.

The ECIS technique was originally introduced by Giaever and Keese [4]. By measuring the change in impedance of gold electrodes in the presence and absence of WI-38 fibroblast cells, they successfully demonstrated the use of impedance in monitoring cell adhesion and locomotion. The overall impedance of cell-covered electrodes comprised impedance contributions from cells, medium, and electrode. Hence, the measured impedance needed to be parameterized to deconvolve the complex impedance data. Giaever addressed the need for parameterization of changes in impedance due to cell adhesion by developing a simple and elegant model [5], which allowed for the determination of cell–substrate and cell–cell separation from measured impedance data. Over the years, the primary use of ECIS has been in monitoring the changes in real and imaginary components of overall impedance of the electrode–cell system at a single frequency (typically 4 kHz). The parameterization of frequency-dependent cell–substrate impedance has been utilized to a lesser degree.

Since the introduction of ECIS, researchers have continued to develop models that advanced the understanding of physicochemical aspects of cell–electrode interface. Lo and Ferrier [6] developed an ECIS model that approximates the cell shape to a rectangle with two semicircles attached to its end. Cell–substrate and cell–cell separation parameters were calculated based on this model. Using this model, Lo and Ferrier demonstrated that the fibroblast cell–substrate separation reversibly increases with temperature. Recently, Goda et al. [7] developed a detailed mathematical model for ECIS measurements, which circumvented the need to make certain assumptions regarding cell size and geometry, which are necessary in employing the original ECIS model developed by Giaever and Keese [4]. The model developed by Goda et al. [7] comprised two parallel combinations of a resistor (R) and a capacitor (C) in parallel with each other, with one parallel RC combination representing the resistance and capacitance of the cell membrane, and the other parallel RC combination representing the cell to solution resistance and capacitance, which quantifies the cell–cell separation. The aforementioned circuit arrangement is then placed in series with the electrode polarization impedance, which quantifies the cell–substrate gap. The model developed by Goda et al. was not fit to the measured impedance. It was, however, shown that the model and measured impedance follow similar graphical profiles (visual correlation). Goda et al. extended their analysis of theoretical ECIS impedance up to 1 MHz.
Xiao et al. [8] developed a model for electrode–electrolyte impedance of ECIS system that accounted and externally compensated for each of the eight individual working electrodes (WEs) of the ECIS 8W1E cell culture well by using an external RC combination for each of the measuring channels. The complex impedance was, however, not parameterized in this study. Wegener et al. [9] developed an electrode system that was slightly different from the original ECIS electrodes. A model was proposed comprising a series resistor–constant phase element (CPE) combination representing electrode impedance and a parallel RC combination representing cell layer impedance.

The impedance data, however, were not fit to this model. Although the electrode configuration developed by Wegener et al. was different from ECIS-8W1E, the authors noted the changes as being insignificant and that the basic ECIS theory and data analysis procedures, including the ECIS model, would still apply to their electrode system. Hence, they resorted to the Giaever model [5] for the modified electrode system [10].

The ECIS as well as the electrode system developed by Arndt et al. [10] were manufactured by photolithography and metal deposition processes. A thin layer of gold is deposited on an insulating substrate. The deposited metal is then patterned using standard lithography and metal etching to form the electrode patterns on the substrate. The metal patterns thus formed include the WE and the counter electrode (CE), along with their connections to measurement electronics. It is desirable to isolate all metalized areas on the substrate from the electrolyte except for the two electrodes (WE and CE). This is achieved by coating the entire substrate with a thin polymer or passivation film, and lithographically [11]–[14] patterning the substrate to reveal only the WE and CE. In the case of ECIS-8W1E, the desired diameter (250 µm) of WE is achieved by lithographically patterning a larger gold area so as to isolate all but a 250-µm-diameter circle of gold from the electrolyte. Thus, the bipolar impedance of the WE and CE arrangement of ECIS will reflect the interactions at the electrode–electrolyte interface, impedance of cell layers (physiological), and the artifacts due to measurement fixture (leads and polymer-covered area of WE). Careful data modeling and analysis is therefore necessary to deconvolute the interfacial, physiological, and measurement fixture impedance components.

The total area of gold deposited to serve as the WE (TAWE) in the case of modified electrode system used by Wegener et al. [9] is 3 mm²; all of this area is directly in contact with the electrolyte, whereas in the original ECIS-8W1E construction, the total area of gold deposited is ≈14 mm² (TAWE) of which 0.049 mm² (WE) of gold is photolithographically exposed to be in direct contact with the electrolyte and the rest of the electrode is buried under the polymer resin. In the case of the CE used by Wegener et al. [9], the area of CE is ≈3 cm², whereas in ECIS, the CE area exposed to the electrolyte in each well is 18 mm² [8]. The WE:CE ratio in the electrode array used by Wegener [9] is ≈1:100, whereas in the case of ECIS, the ratio of TAVE:CE is almost 1:1 and the ratio of WE:CE is ≈1:300.

For reasons discussed before, an ECIS model that accounts for the overall WE area (TAWE ≈ 14 mm²) is more representative of the observed impedance behavior than the one which considers only the WE area that is in direct contact with the electrolyte (0.049 mm²). In this paper, we developed a polymer-coated electrode model to parameterize the ECIS impedance from 100 Hz to 10 MHz. In this model, the overall WE area (TAWE) acts as a polymer-coated gold electrode with a macroscopic pore of dimension equal to the effective WE area (0.049 mm²). The effective WE area is in direct contact with the electrolyte, whereas the polymer-covered region has access to the electrolyte through microscopic pores in the polymer.

The implication of this study is that it will result in: 1) a more physically realistic parameterization of cell–substrate and cell–cell impedance by accounting for effects such as electrode coating and lead track inductance; 2) provide insights for electrode design to reduce the contribution of TAWE to the overall impedance; and hence, 3) allow for high-frequency characterization of biological cell cultures using ECIS-8W1E. This will expand the realm of ECIS-8W1E applications from the existing cell adhesion and micromotion applications to high-frequency applications, possibly in the study of biomolecular interactions, where cell-culture-based impedimetric sensing is making inroads [15]–[17]. The model presented in this paper is also applicable to ECIS electrode arrays used with non-ECIS measurement systems such as commercially available two-port impedance analyzers [18], [19], which have been increasingly used in cell-based impedimetric sensing [20]–[22].

II. THEORY

The ECIS-8W1E culture well array consists of eight separate culture wells equipped with individual WEs and a CE, which is common to all eight wells. The eight devices are arranged in two columns with four devices in each column. The measurement points are located at the edge of the substrate, with metal trace lines connecting the WEs and CE to the measurement pads. During impedance monitoring of cell cultures, cell suspension is inoculated into the culture well and impedance is recorded as a function of time and frequency. The electrolyte, which is the cell culture medium, electrically couples the WE and the CE. The polymer-covered region of the WE blocks the ionic pathway and acts as a capacitive barrier; this capacitance is known as the coating capacitance.

The capacitance of a coated metal–electrolyte interface is $2 \pi \varepsilon_r \varepsilon_0 / \text{cm}^2$ for a 2-µm-thick polymer coating (relative permittivity $\varepsilon_r = 5$), when the coating is dry [23]. This value is a few orders of magnitude less than the double-layer capacitance, which ranges from 10 to $50 \mu\text{F/cm}^2$ [24]. This implies that at low frequencies, current will tend to travel through the exposed regions of the electrode surface. However, due to water (ε_r = 80) uptake in polymers as well as the large area ratio between coated and exposed metal, the capacitive reactance of the polymer-coated region becomes comparable to the capacitance of the exposed regions. This is particularly true in the case of microelectrodes, where long lead lines often coated with thin polymer or other insulation layers are used to connect the microelectrode to measurement pads. The lead line area could be several times larger than the area of the microelectrode. In the case of an ECIS-8W1E culture device, a gold pattern of total...
at Harvard Medical School. These cells were grown in M199 and MCD 105 (1:1) (Sigma, St. Louis, MO) supplemented with 10% fetal calf serum (Sigma), 2 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin under 5% CO2 and 37 °C high-humidity atmosphere. Cell culture medium was changed every two to three days, depending on the rate of cell growth, and cells were subcultured at 80% confluence using 5× trypsin solution. Forty-eight hours after inoculating cells into electrode wells at 8 × 10^4/cm², confluent cell layers were formed and the normal medium was replaced by Hanks’ balanced salt solution (HBSS; Mediatech, Inc., Herndon, VA) without phenol red. Impedance data were obtained at room temperature.

B. Impedance Measurements

An Agilent 4294 A impedance analyzer was used to record impedance of the ECIS array. The ECIS-8W1E was mounted on the Cascade Summit 11000 probe station equipped with microprobes with probe tip diameter 5 µm [29]. The measurement setup calibration was carried out by performing open, short, and load compensation according the procedure outlined in the instrument application note [30]. The amplitude of ac voltage signal was 10 mV peak-to-peak, which satisfies the linearity criteria of impedance spectroscopy [31]. Impedance was recorded in the frequency range between 100 Hz and 10 MHz. Device 4 was found to be defective and its parameterization led to poor results; hence, its impedance data have been omitted from the results and analysis. Recorded impedance data were then modeled using the equivalent circuit modeling. The LEVM, complex nonlinear least square (CNSL) simulation and fitting software [32], which uses the Levenberg–Marquardt algorithm [33] for fitting and optimization, was used for impedance data parameterization in this paper. The average normalized parameters estimated using the equivalent circuit modeling. The LEVM, complex nonlinear least square (CNSL) simulation and fitting software [32], which uses the Levenberg–Marquardt algorithm [33] for fitting and optimization, was used for impedance data parameterization in this paper. The average normalized parameters were calculated by normalizing the average of the resistances and capacitances determined for all devices to the area of the WE, which is 0.049 mm².

IV. RESULTS

A. Measurements With HBSS

The culture wells of ECIS-8W1E were filled with HBSS and allowed to equilibrate overnight before recording impedance between the CE and each of the eight WEs. These measurements serve as control and establish the baseline impedance of the individual WEs in the absence of OVCA429 cells. For a gold–electrolyte system, the expected impedance is that of a combination of an interfacial CPE and solution resistance [9]. The expected Bode diagram of such a system is a slope followed by a plateau, representing a transition from capacitive to resistive behavior from low to high frequency, respectively, which has been widely observed in experiments [9], [13], [34], [35].

Fig. 2 is the overlaid Bode magnitude and phase diagram of impedances of the ECIS-8W1E devices with HBSS only. The Bode diagram of HBSS reveals a dispersion due to the R–CPE series combination until approximately 100 kHz, beyond which another dispersion is observed. Dispersion is indicated by a
transition of the phase angle from capacitive to resistive. Two such transitions are observed in the case of HBSS in ECIS-8W1E. The two dispersions are due to the effects of coating (CPE coat) and double layer (CPE dl) in combination with solution resistance \( R_{\text{sol}} \) and the spreading resistance of the exposed region of gold WE \( R_{\text{exp}} \). This model is illustrated in Fig. 1 and used in parameterizing the HBSS impedance data in the next section. Fig. 3(a) is the complex plane admittance plot of device 3 of the ECIS-8W1E containing HBSS. The complex admittance plot indicates two well-separated time constants contributing to the admittance spectrum. In general, a parallel combination of conductance and capacitance gives rise to a semicircle in the admittance plane [36]. The circuit in Fig. 3(b) (elements connected by solid lines) describes the observed behavior very well, as indicated by the agreement between fit and measured impedance in Fig. 3(a). This agreement is quantified by the fit quality factor, Sigma \( \Sigma \), in Table I, which summarizes the estimated parameters of various devices of the ECIS-8W1E. The circuit of Fig. 3(b) (elements connected by solid lines) is used in corrosion measurements for evaluation of coating effectiveness [37]. Pores in the coating allow restricted access to the metal, which is analogous to the present situation with the 250-µm-diameter exposed metal area representing a macroscopic pore. In addition to the macroscopic pore, microscopic pores are also present throughout the coating, but their effect is masked by the dominant effect of the macroscopic 250-µm-diameter pore forming the electrode. The polymer-covered model for ECIS is also supported by the data presented by We-gener et al. [9] and Arndt et al. [10]. They used an electrode arrangement similar to ECIS but with the entire WE (Tawe) area in direct contact with the electrolyte. In this case, the second dispersion is not observed.

The expression for the overall impedance of the electrode system with HBSS modeled by the equivalent circuit in Fig. 3(b) (elements connected by solid lines) is given by the following expression:

\[
Z_{\text{HBSS}} = R_{\text{sol}} + R_{\text{lead}} + j\omega L + \frac{1}{R_{\text{exp}} + 1 + (j\omega)^{\text{coat}} A_{\text{coat}}}
\]

where \( R_{\text{sol}} \) is the solution resistance, \( R_{\text{lead}} \) is the resistance of the metal trace connecting the WE to the measurement pad, \( L \) is the lead inductance, \( R_{\text{exp}} \) is the spreading resistance of the exposed (250 µm diameter) WE, \( A_{\text{all}} \) and \( n_{\text{all}} \) are the double-layer CPE parameters, and \( A_{\text{coat}} \) and \( n_{\text{coat}} \) are coating CPE parameters.

Table I summarizes the model parameters of the equivalent circuit of Fig. 3(b) (elements connected by solid lines). With the exception of some parameters of device 8, all parameters show device-to-device repeatability. This is expected because of batch fabrication of the devices and due to the use of same electrolyte (HBSS) in all wells. The effect of lead resistance has been assimilated into the solution resistance parameter, as evidenced by the increase in the solution resistance with the device number. Assigning two separate resistors, one for solution resistance and another for lead resistance led to poor fitting results, indicates the inability of the solver to resolve these components. The pore resistance is \( \sim 1.23 \) kΩ for all devices, as expected. The two
The high-frequency region, i.e., the frequency system, there is a substantial low-frequency slope variation in the length of the WE gold track. This quantity varies with device number, due to the systematic variation in the length of the WE gold track length. This quantity is well determined (small standard deviation) in all the datasets, which indicates that it is a necessary parameter in the fitting circuit. The parameters of device 8 are inconsistent with the rest of the devices. We believe that this is due to a defect in the device such as polymer delamination or photolithographic defect. This result reiterates the utility of the modeling presented in our paper as a quality control measure of the 8W1E devices before they could be employed for biophysical investigation.

### B. Measurements With OVCA429 Ovarian Cancer Cells

OVCA429 cells form a confluent layer upon culturing that covers a broad region of the substrate including the WE (coated and exposed) and the CE. The cell layer can be conceptualized as a blanket covering throughout the surface area of the electrodes. Consequently, it is expected that the impedance of the cell layer should be in series with that of the electrode–electrolyte interface. Since the cells form close contact with the electrode, the interfacial impedance parameters will also be altered. The cell layers are usually represented by a parallel resistor–CPE combination [9]. The CPE of the cell layer represents the averaged effect of the membrane capacitance over the area of the electrodes, whereas the resistance is due to cell–cell junction constraining the flow of ions and the intracellular resistance.

Fig. 4 is the Bode diagram of cultured OVCA429 cancer cell layer in ECIS-8W1E devices. Unlike the HBSS–gold electrode system, there is a substantial low-frequency slope variation in the case of OVCA429. This is due to the formation of intimate contact between the substrate and the cells. From Fig. 4, it is observed that the high-frequency region, i.e., the frequency range where the impedance of polymer coating on the electrode dominates (approximately >100 kHz), is relatively less modified between HBSS data and OVCA429 data. The double-layer slope is substantially shifted upward in relation to the HBSS-only impedance. Further insight can be gained by examining the complex permittivity diagram (Cole–Cole plot) of the OVCA429 dataset.

Fig. 5 is the complex permittivity plot of OVCA429 cell cultures. The complex permittivity is obtained from complex impedance using the relationship

$$\varepsilon_r = 1/\omega C (\varepsilon_0),$$

where $Z$ is the complex impedance, $CC$ is the cell constant, $\varepsilon_0$ is the permittivity of free space, and $\omega = 2\pi f$, where $f$ is the frequency in hertz. Data points in the descending order of real axis represent increasing frequency. Three time constants are observed in the complex permittivity plane (“Cole–Cole” plot) of Fig. 5. Fig. 3(b) (all circuit elements) is the fitting circuit used to model the OVCA429–coated electrode data. As indicated earlier, the cell layers contribute an additional $R$–CPE element of Fig. 5. Fig. 3(b) (all circuit elements) is the fitting circuit used to model the OVCA429–coated electrode data. As indicated earlier, the cell layers contribute an additional $R$–CPE element of Fig. 5.
TABLE II
SUMMARY OF EQUIVALENT ELECTRICAL MODEL PARAMETERS OF THE CIRCUIT OF FIG. 3(b) (ALL CIRCUIT ELEMENTS), WHICH REPRESENTS THE IMPEDANCE OF ECIS-8W1E CULTURE DEVICE WITH CULTURED OVCA429 CELL LAYER ON THE ELECTRODES

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>DEVICE-1</th>
<th>DEVICE-2</th>
<th>DEVICE-3</th>
<th>DEVICE-5</th>
<th>DEVICE-6</th>
<th>DEVICE-7</th>
<th>DEVICE-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rsol*E2</td>
<td>1.93</td>
<td>0.56</td>
<td>2.19</td>
<td>0.90</td>
<td>2.55</td>
<td>1.04</td>
<td>1.88</td>
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<td>Rcell*E5</td>
<td>2.04</td>
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<td>3.04</td>
<td>4.18</td>
<td>5.24</td>
<td>4.69</td>
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<td>R pore*E3</td>
<td>1.54</td>
<td>3.78</td>
<td>1.48</td>
<td>7.00</td>
<td>1.59</td>
<td>10.12</td>
<td>1.70</td>
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<tr>
<td>CPE(cell)*E-9</td>
<td>5.11</td>
<td>3.31</td>
<td>3.94</td>
<td>3.46</td>
<td>2.66</td>
<td>3.60</td>
<td>6.51</td>
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<tr>
<td>n(cell)*E-1</td>
<td>8.98</td>
<td>0.75</td>
<td>8.98</td>
<td>0.74</td>
<td>9.43</td>
<td>0.77</td>
<td>8.89</td>
</tr>
<tr>
<td>CPE(dl)*E-1</td>
<td>9.54</td>
<td>8.78</td>
<td>7.08</td>
<td>17.25</td>
<td>12.28</td>
<td>11.99</td>
<td>11.00</td>
</tr>
<tr>
<td>n(dl)*E-1</td>
<td>8.36</td>
<td>1.51</td>
<td>8.42</td>
<td>2.16</td>
<td>7.62</td>
<td>1.28</td>
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<tr>
<td>CPE(coat)*E-9</td>
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<td>11.48</td>
<td>4.06</td>
<td>21.42</td>
<td>3.00</td>
<td>29.81</td>
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<td>n(coat)*E-1</td>
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<td>0.69</td>
<td>8.56</td>
<td>1.44</td>
<td>8.86</td>
<td>1.98</td>
<td>9.01</td>
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<tr>
<td>L*E-7</td>
<td>1.87</td>
<td>8.29</td>
<td>2.65</td>
<td>9.58</td>
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<tr>
<td>Sigma f</td>
<td>0.0068</td>
<td>0.0085</td>
<td>0.0093</td>
<td>0.0035</td>
<td>0.0092</td>
<td>0.0072</td>
<td>0.0086</td>
</tr>
</tbody>
</table>

The notation $A/B$ is the determined parameter value $A$ with a standard deviation (SD) of $B \times 10^2$. Resistances are in ohms, the parameter $A$ of CPE is in siemens-second$^A$, and inductance $L$ is in henry. The parameter $n$ is dimensionless.

Fig. 5. Complex plane permittivity (“Cole-Cole”) diagram of cultured OVCA429 cells and equivalent circuit fit from 100 Hz to 10 MHz for a single device. Measured data (dots) and fitted data (line). The three sections of the plot divided by the solid line represent impedance contributions from exposed metal area of the WE and the overall area of gold (TAWE) and the impedance contribution of the cell layer. Fitting was carried out with the equivalent circuit of Fig. 3(b) (all circuit components). The parallel combination of $R_{cell}$ and CPE cells accounts for the contribution of cells.

Circuit in Fig. 3(b) (all circuit elements) is given by the following expression:

$$Z_{cell} = R_{sol} + R_{pore} + j\omega L + \frac{1}{\frac{1}{R_{pore}} + \frac{1}{j\omega \rho_{cell} A_{cell}} + \frac{1}{j\omega \rho_{coa} A_{coa}}}$$

where $R_{cell}$ is the resistance due to the cell layers, and $A_{cell}$ and $n_{cell}$ are the CPE parameters due to the cell layers.

Table II summarizes the extracted parameters from the circuit model of Fig. 3(b) (all circuit elements). The addition of cell layers has not only contributed two additional impedance elements to the circuit, but also modified the other parameters such as double-layer capacitance and pore resistance. The presence of cells has considerably altered the interfacial space charge distribution of the electrode–HBSS system. This is because the cells form an intimate and adherent layer to the substrate, thereby altering the equilibrium charge density distribution that extends a few micrometers into the bulk of the electrolyte.

C. Comparison Between Control and OVCA429 Impedance

Fig. 6 is the Bode diagram of device 3 (D3) of ECIS-8W1E with OVCA429 cells and with medium only. As indicated earlier, the high-frequency region displays similar characteristics in HBSS and OVCA429 systems, as is evident from the impedance magnitude and phase angle beyond approximately 1 MHz. This implies that this section of the impedance spectra is dominated by the coating impedance. Hence, it is attributed to the polymer-covered portions of the gold WE. The impedance magnitudes and phase angle of cells and medium are considerably different at frequencies below 1 MHz. The cell layer impedance appears as a single slope. This characteristic is due to the overlap of time constants of the RC elements in the equivalent circuit. In such situations, visual analysis of impedance in the Bode plane alone would be inaccurate.
Fig. 7. Complex permittivity plot of device 3 of ECIS-8W1E with and without OVCA429 cell layer. (Inset) Enlarged view of the high-frequency portion of the permittivity plot.

V. DISCUSSION

The generally accepted value of cell membrane capacitance is 1 \( \mu \text{F/cm}^2 \) [28]. Cell membranes are composed of phospholipid bilayers that act as insulators, except for ion channel openings that conduct transcellular currents. Unlike the membrane capacitance, cell layer capacitance indicates the capacitance of a layer of interconnected cells. Cell layers consist of trans-cellular junctions, folded cell walls, and in some cases, multiple cell layers that lead to a capacitance value other than that of a flat sheet cell wall capacitance of 1 \( \mu \text{F/cm}^2 \). Since the cell wall capacitance is in series with the coating and double-layer capacitance in the coated regions, the smallest of the capacitances, namely the coating capacitance, dominates the impedance. Consequently, the effect of the membrane capacitance will be visible in the frequencies higher than those at which the double-layer capacitance appears.

In systems with multiple CPEs, it is important to identify and associate the CPEs with the corresponding physical quantity they represent. Some indicators that help in this differentiation are the CPE parameters, namely, magnitude (\( A_{\text{coat}} \)) and power factor (\( n_{\text{coat}} \)). Due to the thickness of the polymer and its relative permittivity, \( A_{\text{coat}} \) is small compared to the double-layer CPE. The power factor (\( n_{\text{coat}} \)) of the coating CPE is closer to unity in the case of good (void-free) polymer coatings. The power factor (\( n_{\text{dl}} \)) of metal-deposited gold electrodes exposed to simple electrolytes is closer to unity for a smooth deposition of metal. We recall that the power factor of the CPE is an indicator of its fractal nature [38]. For very smooth metal surfaces, the CPE approaches the regime of perfect capacitance with a power factor approaching 1 [39].

VI. CONCLUSION

OVCA429 ovarian cancer epithelial cell layer was characterized in terms of electrical equivalent circuit elements. The trans-layer resistance and capacitance were determined to be 152 ± 59 \( \Omega \cdot \text{cm}^2 \) and 8.5 ± 2.4 \( \mu \text{F/cm}^2 \), respectively. Equivalent circuit models developed for device containing HBSS and OVCA429 cells indicate that the polymer-coated regions contribute to the impedance spectrum above \( \sim 1 \text{MHz} \). The model presented in this paper approximates the experimental data very well over a broad frequency range of 100 Hz to 10 MHz. The coated electrode model developed in this paper is comprehensive, taking into account the effects of polymer-coated WE area as well as the electrode-contact pad lead effects. This paper paves the way for future high-frequency studies of cell cultures using the ECIS-8W1E in combination with commercially available impedance analyzers.

REFERENCES


Q1: Author: Please provide the IEEE membership details (membership grades and years in which these were obtained), if any, for A. R. A. Rahman and C.-M. Lo.
Q2: Author: First author has been set as the corresponding author. Is it OK?
Q3: Author: Ref. [34] has been deleted as it was exactly identical to Ref. [13], and subsequent references have been renumbered. Please check.
Q4: Author: Is the edit OK?
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Index Terms—Cell adhesion, cell impedance model, electrical cell–substrate impedance sensing (ECIS), impedance spectroscopy.

I. INTRODUCTION

THE measurement of complex impedance as a function of frequency has long been used in bioimpedance investigations to study the frequency dispersions of biological tissue [1]. Impedimetric sensing has evolved as a powerful alternative to traditional methods in a variety of biosensing applications such as immunosensing, DNA hybridization studies, and cytotoxicity assessment [2]. Among the various types of impedance-based biosensors, cell-based biosensors utilize the living cells for monitoring environmental variables, cell–drug interactions, and infections [3]. Majority of the impedimetric-cell-based biosensors, including electrical cell–substrate impedance sensing (ECIS), rely on the alterations in the impedance of electrode–cell interface due to adhesion and motility of anchoragedependent cells.

The ECIS technique was originally introduced by Giaever and Keese [4]. By measuring the change in impedance of gold electrodes in the presence and absence of WI-38 fibroblast cells, they successfully demonstrated the use of impedance in monitoring cell adhesion and locomotion. The overall impedance of cell-covered electrodes comprised impedance contributions from cells, medium, and electrode. Hence, the measured impedance needed to be parameterized to deconvolve the complex impedance data. Giaever addressed the need for parameterization of the changes in impedance due to cell adhesion by developing a simple and elegant model [5], which allowed for the determination of cell–substrate and cell–cell separation from measured impedance data. Over the years, the primary use of ECIS has been in monitoring the changes in real and imaginary components of overall impedance of the electrode–cell system at a single frequency (typically 4 kHz). The parameterization of frequency-dependent cell–substrate impedance has been utilized to a lesser degree.

Since the introduction of ECIS, researchers have continued to develop models that advanced the understanding of physicochemical aspects of cell–electrode interface. Lo and Ferrier [6] developed an ECIS model that approximates the cell shape to a rectangle with two semicircles attached to its end. Cell–substrate and cell–cell separation parameters were calculated based on this model. Using this model, Lo and Ferrier demonstrated that the fibroblast cell–substrate separation reversibly increases with temperature. Recently, Goda et al. [7] developed a detailed mathematical model for ECIS measurements, which circumvented the need to make certain assumptions regarding cell size and geometry, which are necessary in employing the original ECIS model developed by Giaever and Keese [4]. The model developed by Goda et al. [7] comprised two parallel combinations of a resistor (R) and a capacitor (C) in parallel with each other, with one parallel RC combination representing the resistance and capacitance of the cell membrane, and the other parallel RC combination representing the cell to solution resistance and capacitance, which quantifies the cell–cell separation.

The aforementioned circuit arrangement is then placed in series with the electrode polarization impedance, which quantifies the cell–substrate gap. The model developed by Goda et al. was not fit to the measured impedance. It was, however, shown that the model and measured impedance follow similar graphical profiles (visual correlation). Goda et al. extended their analysis of theoretical ECIS impedance up to 1 MHz.
Xiao et al. [8] developed a model for electrode–electrolyte impedance of ECIS system that accounted and externally compensated for each of the eight individual working electrodes (WEs) of the ECIS 8W1E cell culture well by using an external RC combination for each of the measuring channels. The complex impedance was, however, not parameterized in this study. Wegener et al. [9] developed an electrode system that was slightly different from the original ECIS electrodes. A model was proposed comprising a series resistor–constant phase element (CPE) combination representing electrode impedance and a parallel RC combination representing cell layer impedance.

The impedance data, however, were not fit to this model. Although the electrode configuration developed by Wegener et al. was different from ECIS-8W1E, the authors noted the changes as being insignificant and that the basic ECIS theory and data analysis procedures, including the ECIS model, would still apply to their electrode system. Hence, they resorted to the Giaever model [5] for the modified electrode system [10].

The ECIS as well as the electrode system developed by Arndt et al. [10] were manufactured by photolithography and metal deposition processes. A thin layer of gold is deposited on an insulating substrate. The deposited metal is then patterned using standard lithography and metal etching to form the electrode patterns on the substrate. The metal patterns thus formed include the WE and the counter electrode (CE), along with their connections to measurement electronics. It is desirable to isolate all metalized areas on the substrate from the electrolyte except for the two electrodes (WE and CE). This is achieved by coating the entire substrate with a thin polymer or passivation film, and lithographically [11]–[14] patterning the substrate to reveal only the WE and CE. In the case of ECIS-8W1E, the desired diameter (250 µm) of WE is achieved by lithographically patterning a larger gold area so as to isolate all but a 250-µm-diameter circle of gold from the electrolyte. Thus, the bipolar impedance of the WE and CE arrangement of ECIS will reflect the interactions at the electrode–electrolyte interface, impedance of cell layers (physiological), and the artifacts due to measurement fixture (leads and polymer-covered area of WE). Careful data modeling and analysis is therefore necessary to deconvolve the interfacial, physiological, and measurement fixture impedance components.

The total area of gold deposited to serve as the WE (TAWE) in the case of modified electrode system used by Wegener et al. [9] is 3 mm²; all of this area is directly in contact with the electrolyte, whereas in the original ECIS-8W1E construction, the total area of gold deposited is \( \approx 14 \text{ mm}^2 \) (TAWE) of which 0.049 mm² (WE) of gold is photolithographically exposed to be in direct contact with the electrolyte and the rest of the electrode is buried under the polymer resin. In the case of the CE used by Wegener et al. [9], the area of CE is \( \approx 3 \text{ cm}^2 \), whereas in ECIS, the CE area exposed to the electrolyte in each well is 18 mm² [8]. The WE:CE ratio in the electrode array used by Wegener [9] is \( \approx 1:100 \), whereas in the case of ECIS, the ratio of TAWE:CE is almost 1:1 and the ratio of WE:CE is \( \approx 1:300 \).

For reasons discussed before, an ECIS model that accounts for the overall WE area (TAWE \( \approx 14 \text{ mm}^2 \)) is more representative of the observed impedance behavior than the one which considers only the WE area that is in direct contact with the electrolyte (0.049 mm²). In this paper, we developed a polymer-coated electrode model to parameterize the ECIS impedance from 100 Hz to 10 MHz. In this model, the overall WE area (TAWE) acts as a polymer-coated gold electrode with a macroscopic pore of dimension equal to the effective WE area (0.049 mm²).

The effective WE area is in direct contact with the electrolyte, whereas the polymer-covered region has access to the electrolyte through microscopic pores in the polymer.

The implication of this study is that it will result in: 1) a more physically realistic parameterization of cell–substrate and cell–cell impedance by accounting for effects such as electrode coating and lead track inductance; 2) provide insights for electrode design to reduce the contribution of TAWE to the overall impedance; and hence, 3) allow for high-frequency characterization of biological cell cultures using ECIS-8W1E. This will expand the realm of ECIS-8W1E applications from the existing cell adhesion and micromotion applications to high-frequency applications, possibly in the study of biomolecular interactions, where cell-culture-based impedimetric sensing is making inroads [15]–[17]. The model presented in this paper is also applicable to ECIS electrode arrays used with non-ECIS measurement systems such as commercially available two-port impedance analyzers [18], [19], which have been increasingly used in cell-based impedimetric sensing [20]–[22].

II. THEORY

The ECIS-8W1E culture well array consists of eight separate culture wells equipped with individual WEs and a CE, which is common to all eight wells. The eight devices are arranged in two columns with four devices in each column. The measurement points are located at the edge of the substrate, with metal trace lines connecting the WEs and CE to the measurement pads. During impedance monitoring of cell cultures, cell suspension is inoculated into the culture well and impedance is recorded as a function of time and frequency. The electrolyte, which is the cell culture medium, electrically couples the WE and the CE. The polymer-covered region of the WE blocks the ionic pathway and acts as a capacitive barrier; this capacitance is known as the coating capacitance.

The capacitance of a coated metal–electrolyte interface is \( 2 \pi F/cm^2 \) for a 2-µm-thick polymer coating (relative permittivity \( \varepsilon_r = 5 \)), when the coating is dry [23]. This value is a few orders of magnitude less than the double-layer capacitance, which ranges from 10 to 50 µF/cm² [24]. This implies that at low frequencies, current will tend to travel through the exposed regions of the electrode surface. However, due to water \( (\varepsilon_r = 80) \) uptake in polymers as well as the large area ratio between coated and exposed metal, the capacitive reactance of the polymer-coated region becomes comparable to the capacitance of the exposed regions. This is particularly true in the case of microelectrodes, where long lead lines often coated with thin polymer or other insulation layers are used to connect the microelectrode to measurement pads. The lead line area could be several times larger than the area of the microelectrode. In the case of an ECIS-8W1E culture device, a gold pattern of total...
area \( \sim 14.179 \text{ mm}^2 \) \((14.2 \times 10^{-2} \text{ cm}^2)\) is lithographically patterned to achieve a WE area of 0.049 mm\(^2\) \((4.9 \times 10^{-4} \text{ cm}^2)\), leading to an approximately 1:300 of exposed versus coated metal area ratio. The combined effect of: 1) large area ratio of coated and exposed metal and 2) modification of coating capacitance due to water uptake leads to a substantial reduction in coating capacitance from its theoretically calculated value \((\sim 2 \text{nF/cm}^2)\). This leads to impedances of exposed and coated metal regions contributing to the overall impedance at high frequencies.

Fig. 1 illustrates the equivalent circuit model of a partially coated metal electrode. \( R_{\text{exp}} \) and \( \text{CPE}_{\text{coat}} \) are the resistance and capacitance of the exposed and coated areas of the WE, respectively. \( \text{CPE}_{\text{dl}} \) is the double-layer capacitance of exposed portion of the electrode. In most practical electrochemical situations, capacitive effects are represented by CPEs [25]. In fact, it has been stressed that any attempt to characterize the interfacial double layer exhibiting a CPE behavior by electrical capacitance is misleading and that such a simplifying assumption causes a modeling error and influences all parameters of the system [26]. The impedance of a CPE is given by the expression \( 1/(A(j\omega)^n) \), where \( A \) is the magnitude of the element and \( \omega = 2\pi f \) is the angular frequency, where \( f \) is the frequency in hertz. The parameter \( n \) is such that if \( n = 1 \), the impedance of a CPE is that of an ideal capacitor, and when \( n = 0 \), the CPE is a pure resistor. The frequency dependence of interfacial capacitance has been ascribed to a wide variety of effects such as surface planarization, nonuniform current distribution, and distribution of relaxation times [27].

III. MATERIALS AND METHODS

A. Cell Culture Procedures

Ovarian cancer line OVCA429 was provided by Samuel Mok at Harvard Medical School. These cells were grown in M199 and MCD 105 (1:1) (Sigma, St. Louis, MO) supplemented with 10% fetal calf serum (Sigma), 2 mM L-glutamine, 100 units/mL penicillin, and 100 \( \mu \)g/mL streptomycin under 5% \( \text{CO}_2 \) and 37 \( ^\circ \text{C} \) high-humidity atmosphere. Cell culture medium was changed every two to three days, depending on the rate of cell growth, and cells were subcultured at 80% confluence using 5\(< \text{trypsin solution. Forty-eight hours after inoculating cells into electrode wells at } 8 \times 10^{-5} \text{ cm}^2, \text{ confluent cell layers were formed and the normal medium was replaced by Hanks' balanced salt solution (HBSS; Mediatech, Inc., Herndon, VA) without phenol red. Impedance data were obtained at room temperature.} \)

B. Impedance Measurements

An Agilent 4294 A impedance analyzer was used to record impedance of the ECIS array. The ECIS-8W1E was mounted on the Cascade Summit 11000 probe station equipped with micropores with probe tip diameter 5 \( \mu \)m [29]. The measurement setup calibration was carried out by performing open, short, and load compensation according the procedure outlined in the instrument application note [30]. The amplitude of ac voltage signal was 10 mV peak-to-peak, which satisfies the linearity criteria of impedance spectroscopy [31]. Impedance was recorded in the frequency range between 100 Hz and 10 MHz. Device 4 was found to be defective and its parameterization led to poor results; hence, its impedance data have been omitted from the results and analysis. Recorded impedance data were then modeled using the equivalent circuit modeling. The LEVM, complex nonlinear least square (CNLS) simulation and fitting software [32], which uses the Levenberg–Marquardt algorithm [33] for fitting and optimization, was used for impedance data parameterization in this paper. The average normalized parameters are calculated by normalizing the average of the resistances and capacitances determined for all devices to the area of the WE, which is 0.049 mm\(^2\).

IV. RESULTS

A. Measurements With HBSS

The culture wells of ECIS-8W1E were filled with HBSS and allowed to equilibrate overnight before recording impedance between the CE and each of the eight WEs. These measurements serve as control and establish the baseline impedance of the individual WEs in the absence of OVCA429 cells. For a gold–electrolyte system, the expected impedance is that of a combination of an interfacial CPE and solution resistance [9]. The expected Bode diagram of such a system is a slope followed by a plateau, representing a transition from capacitive to resistive behavior from low to high frequency, respectively, which has been widely observed in experiments [9], [13], [34], [35].

Fig. 2 is the overlaid Bode magnitude and phase diagram of impedances of the ECIS-8W1E devices with HBSS only. The Bode diagram of HBSS reveals a dispersion due to the \( R \text{–CPE} \) series combination until approximately 100 kHz, beyond which another dispersion is observed. Dispersion is indicated by a
transition of the phase angle from capacitive to resistive. Two such transitions are observed in the case of HBSS in ECIS-8W1E. The two dispersions are due to the effects of coating (CPE coat) and double layer (CPE dl) in combination with solution resistance ($R_{sol}$) and the spreading resistance of the exposed region of gold WE ($R_{exp}$). This model is illustrated in Fig. 1 and used in parameterizing the HBSS impedance data in the next section. Fig. 3(a) is the complex plane admittance plot of device 3 of the ECIS-8W1E containing HBSS. The complex admittance plot indicates two well-separated time constants contributing to the admittance spectrum. In general, a parallel combination of conductance and capacitance gives rise to a semicircle in the admittance plane [36]. The circuit in Fig. 3(b) (elements connected by solid lines) describes the observed behavior very well, as indicated by the agreement between fit and measured impedance in Fig. 3(a). This agreement is quantified by the fit quality factor, Sigma $\Sigma$, in Table I, which summarizes the estimated parameters of various devices of the ECIS-8W1E. The circuit of Fig. 3(b) (elements connected by solid lines) is used in corrosion measurements for evaluation of coating effectiveness [37]. Pores in the coating allow restricted access to the metal, which is analogous to the present situation with the 250-$\mu$m-diameter exposed metal area representing a macroscopic pore. In addition to the macroscopic pore, microscopic pores are also present throughout the coating, but their effect is masked by the dominant effect of the macroscopic 250-$\mu$m-diameter pore forming the electrode. The polymer-covered model for ECIS is also supported by the data presented by Wegener et al. [9] and Arndt et al. [10]. They used an electrode arrangement similar to ECIS but with the entire WE (TAFE) area in direct contact with the electrolyte. In this case, the second dispersion is not observed.

The expression for the overall impedance of the electrode system with HBSS modeled by the equivalent circuit in Fig. 3(b) (elements connected by solid lines) is given by the following expression:

$$Z_{HBSS} = R_{sol} + R_{lead} + j\omega L + \frac{1}{R_{exp} + \frac{1}{(j\omega)^{n_{coat}} A_{coat}}}$$

where $R_{sol}$ is the solution resistance, $R_{lead}$ is the resistance of the metal trace connecting the WE to the measurement pad, $L$ is the lead inductance, $R_{exp}$ is the spreading resistance of the exposed (250 $\mu$m diameter) WE, $A_{all}$ and $n_{all}$ are the double-layer CPE parameters, and $A_{coat}$ and $n_{coat}$ are coating CPE parameters.

Table I summarizes the model parameters of the equivalent circuit of Fig. 3(b) (elements connected by solid lines). With the exception of some parameters of device 8, all parameters show device-to-device repeatability. This is expected because of batch fabrication of the devices and due to the use of same electrolyte (HBSS) in all wells. The effect of lead resistance has been assimilated into the solution resistance parameter, as evidenced by the increase in the solution resistance with the device number. Assigning two separate resistors, one for solution resistance and another for lead resistance led to poor fitting results, indicates the inability of the solver to resolve these components. The pore resistance is $\sim$1.23 k$\Omega$ for all devices, as expected. The two
CPEs, CPE-dl, and CPE-coat, are also consistent for all devices.

The power factor of coating CPE is close to ideal capacitance (≈−0.95). The double-layer CPE is also close to ideal capacitance (≈−0.975), which is within the range expected for thin-film blocking electrodes such as gold in culture medium [26]. The magnitudes of the two CPEs are separated by roughly one order of magnitude; this is due to water uptake by the polymer as well as a large area of the coated metal, which make the coating capacitance comparable to the double-layer capacitance. Finally, the lead inductance (WE and CE gold track) also displays a significant variation with device number, due to the systematic variation in the length of the WE gold track length. This quantity is well determined (small standard deviation) in all the datasets, which indicates that it is a necessary parameter in the fitting circuit. The parameters of device 8 are inconsistent with the rest of the devices. We believe that this is due to the defect in the device such as polymer delamination or photolithographic defect.

This result reiterates the utility of the modeling presented in our paper as a quality control measure of the 8W1E devices before they could be employed for biophysical investigation.

### B. Measurements With OVCA429 Ovarian Cancer Cells

OVCA429 cells form a confluent layer upon culturing that covers a broad region of the substrate including the WE (coated and exposed) and the CE. The cell layer can be conceptualized as a blanket covering throughout the surface area of the electrodes. Consequently, it is expected that the impedance of the cell layer should be in series with that of the electrode–electrolyte interface. Since the cells form close contact with the electrode, the interfacial impedance parameters will also be altered. The cell layers are usually represented by a parallel resistor–CPE combination [9]. The CPE of the cell layer represents the averaged effect of the membrane capacitance over the area of the electrodes, whereas the resistance is due to cell–cell junction constricting the flow of ions and the intracellular resistance.

Fig. 4 is the Bode diagram of cultured OVCA429 cancer cell layer in ECIS-8W1E devices. Unlike the HBSS–gold electrode, system, there is a substantial low-frequency slope variation in the case of OVCA429. This is due to the formation of intimate contact between the substrate and the cells. From Fig. 4, it is observed that the high-frequency region, i.e., the frequency range where the impedance of polymer coating on the electrode dominates (approximately >100 kHz), is relatively less modified between HBSS data and OVCA429 data. The double-layer slope is substantially shifted upward in relation to the HBSS-only impedance. Further insight can be gained by examining the complex permittivity diagram (Cole–Cole plot) of the OVCA429 dataset.

Fig. 5 is the complex permittivity plot of OVCA429 cell cultures. The complex permittivity is obtained from complex impedance using the relationship: $\varepsilon_r = 1/Z(\omega \text{CC} \varepsilon_0)$, where $Z$ is the complex impedance, CC is the cell constant, $\varepsilon_0$ is the permittivity of free space, and $\omega = 2\pi f$, where $f$ is the frequency in hertz. Data points in the descending order of real axis represent increasing frequency. Three time constants are observed in the complex permittivity plane (“Cole–Cole” plot) of Fig. 5. Fig. 3(b) (all circuit elements) is the fitting circuit used to model the OVCA429-coated electrode data. As indicated earlier, the cell layers contribute an additional $R$–CPE element to the overall impedance of the medium impedance that is in series. This model well represents the data, as indicated by the visual agreement between data and fit as well as the fit quality factor, Sigma $F$, summarized in Table II.

The expression for the overall impedance of the electrode system with OVCA429 cell layers represented by the equivalent circuit with the described CPE parameters is given by:

$$Z_{total} = \frac{1}{\frac{1}{Z_{cell}} + \frac{1}{Z_{environment}}}$$

### Table I

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>DEVICE-1</th>
<th>DEVICE-2</th>
<th>DEVICE-3</th>
<th>DEVICE-5</th>
<th>DEVICE-6</th>
<th>DEVICE-7</th>
<th>DEVICE-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{sol} \times 1E2$</td>
<td>2.07 ± 0.28</td>
<td>2.42 ± 0.27</td>
<td>2.74 ± 0.23</td>
<td>2.23 ± 0.31</td>
<td>2.33 ± 0.29</td>
<td>2.47 ± 0.27</td>
<td>1.84 ± 1.56</td>
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<tr>
<td>$R_{pore} \times 1E3$</td>
<td>1.24 ± 0.21</td>
<td>1.23 ± 0.23</td>
<td>1.23 ± 0.23</td>
<td>1.23 ± 0.25</td>
<td>1.25 ± 0.24</td>
<td>1.24 ± 0.24</td>
<td>0.17 ± 0.19</td>
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<td>CPE(dll) $\times 1E-9$</td>
<td>5.86 ± 0.43</td>
<td>5.66 ± 0.49</td>
<td>5.46 ± 0.48</td>
<td>5.71 ± 0.53</td>
<td>5.63 ± 0.51</td>
<td>5.70 ± 0.51</td>
<td>5.36 ± 1.30</td>
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<td>n(dll) $\times 1E-1$</td>
<td>9.75 ± 0.04</td>
<td>9.77 ± 0.05</td>
<td>9.77 ± 0.05</td>
<td>9.73 ± 0.05</td>
<td>9.75 ± 0.05</td>
<td>9.75 ± 0.05</td>
<td>9.87 ± 0.10</td>
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<td>CPE(coat) $\times 1E-10$</td>
<td>6.10 ± 2.24</td>
<td>5.40 ± 2.56</td>
<td>5.58 ± 2.49</td>
<td>5.32 ± 2.79</td>
<td>5.70 ± 2.66</td>
<td>5.67 ± 2.65</td>
<td>13.04 ± 10.41</td>
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<tr>
<td>n(coat) $\times 1E-1$</td>
<td>9.52 ± 0.16</td>
<td>9.51 ± 0.18</td>
<td>9.50 ± 0.17</td>
<td>9.52 ± 0.19</td>
<td>9.52 ± 0.19</td>
<td>9.47 ± 0.18</td>
<td>9.50 ± 0.18</td>
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<tr>
<td>L$^* 1E-7$</td>
<td>1.87 ± 7.25</td>
<td>2.39 ± 4.65</td>
<td>3.17 ± 3.18</td>
<td>1.73 ± 7.04</td>
<td>2.16 ± 5.39</td>
<td>2.62 ± 4.15</td>
<td>2.84 ± 9.64</td>
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<tr>
<td>Sigma $F$</td>
<td>0.0048</td>
<td>0.0058</td>
<td>0.0056</td>
<td>0.0063</td>
<td>0.0060</td>
<td>0.0061</td>
<td>0.0063</td>
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The notation A/B for each device is the determined parameter value A with a standard deviation (SD) of B×10^C. Resistances are in ohms, the parameter A of CPE is in siemens-second^D, and inductance L is in henry. The parameter n is dimensionless.
TABLE II
SUMMARY OF EQUIVALENT ELECTRICAL MODEL PARAMETERS OF THE CIRCUIT OF FIG. 3(b) (ALL CIRCUIT ELEMENTS), WHICH REPRESENTS THE IMPEDANCE OF ECIS-8W1E CULTURE DEVICE WITH CULTURED OVCA429 CELL LAYER ON THE ELECTRODES

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>DEVICE-1</th>
<th>DEVICE-2</th>
<th>DEVICE-3</th>
<th>DEVICE-5</th>
<th>DEVICE-6</th>
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<td>Rsol*E2</td>
<td>1.93</td>
<td>0.56</td>
<td>2.19</td>
<td>0.90</td>
<td>2.55</td>
<td>1.04</td>
<td>1.88</td>
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<td>3.04</td>
<td>4.18</td>
<td>5.24</td>
<td>4.69</td>
<td>1.63</td>
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<td>1.54</td>
<td>3.78</td>
<td>1.48</td>
<td>7.00</td>
<td>1.59</td>
<td>10.12</td>
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<td>5.11</td>
<td>3.31</td>
<td>3.94</td>
<td>3.46</td>
<td>2.66</td>
<td>3.60</td>
<td>6.51</td>
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<tr>
<td>n(cells)*E-1</td>
<td>8.98</td>
<td>0.75</td>
<td>8.98</td>
<td>0.74</td>
<td>9.43</td>
<td>0.77</td>
<td>8.89</td>
</tr>
<tr>
<td>CPE(dl)*E-1</td>
<td>9.54</td>
<td>8.78</td>
<td>7.08</td>
<td>17.25</td>
<td>12.28</td>
<td>11.99</td>
<td>11.00</td>
</tr>
<tr>
<td>n(dl)*E-1</td>
<td>8.36</td>
<td>1.51</td>
<td>8.42</td>
<td>2.16</td>
<td>7.62</td>
<td>1.28</td>
<td>8.26</td>
</tr>
<tr>
<td>CPE(coat)*E-9</td>
<td>2.43</td>
<td>11.48</td>
<td>4.06</td>
<td>21.42</td>
<td>3.00</td>
<td>29.81</td>
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<tr>
<td>n(coat)*E-1</td>
<td>8.90</td>
<td>0.69</td>
<td>8.56</td>
<td>1.44</td>
<td>8.66</td>
<td>1.98</td>
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<td>1.53</td>
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<tr>
<td>Sigma F</td>
<td>0.0068</td>
<td>0.0085</td>
<td>0.0093</td>
<td>0.0082</td>
<td>0.0112</td>
<td>0.0086</td>
<td>0.0121</td>
</tr>
</tbody>
</table>

The notation A/B is the determined parameter value A with a standard deviation (SD) of B*10^{-1}. Resistances are in ohms, the parameter A of CPE is in siemens-second\(^4\), and inductance L is in henry. The parameter n is dimensionless.

Q4

Fig. 5. Complex plane permittivity (“Cole-Cole”) diagram of cultured OVCA429 cells and equivalent circuit fit from 100 Hz to 10 MHz for a single device. Measured data (dots) and fitted data (line). The three sections of the plot divided by the solid line represent impedance contributions from exposed metal area of the WE and the overall area of gold (TAWE) and the impedance contribution of the cell layer. Fitting was carried out with the equivalent circuit of Fig. 3(b) (all circuit components). The parallel combination of R\(_{cell}\) and CPE cells accounts for the contribution of cells.

Circuit in Fig. 3(b) (all circuit elements) is given by the following expression:

\[
Z_{cell} = R_{sol} + R_{lead} + j\omega L + \frac{1}{R_{pore} + \frac{1}{(j\omega)^{n_{dl}}A_{dl}}} + \frac{1}{(j\omega)^{n_{coat}}A_{coat}}
\]

\[
+ \frac{1}{R_{cells} + (j\omega)^{n_{cells}}A_{cells}}
\]

where R\(_{cells}\) is the resistance due to the cell layers, and A\(_{cells}\) and n\(_{cells}\) are the CPE parameters due to the cell layers.

Table II summarizes the extracted parameters from the circuit model of Fig. 3(b) (all circuit elements). The addition of cell layers has not only contributed two additional impedance elements to the circuit, but also modified the other parameters such as double-layer capacitance and pore resistance. The presence of cells has considerably altered the interfacial space charge distribution of the electrode–HBSS system. This is because the cells form an intimate and adherent layer to the substrate, thereby altering the equilibrium charge density distribution that extends a few micrometers into the bulk of the electrolyte.

C. Comparison Between Control and OVCA429 Impedance

Fig. 6 is the Bode diagram of device 3 (D3) of ECIS-8W1E with OVCA429 cells and with medium only. As indicated earlier, the high-frequency region displays similar characteristics in HBSS and OVCA429 systems, as is evident from the impedance magnitude and phase angle beyond approximately 1 MHz. This implies that this section of the impedance spectra is dominated by the coating impedance. Hence, it is attributed to the polymer-covered portions of the gold WE. The impedance magnitudes and phase angle of cells and medium are considerably different at frequencies below 1 MHz. The cell layer impedance appears as a single slope. This characteristic is due to the overlap of time constants of the RC elements in the equivalent circuit. In such situations, visual analysis of impedance in the Bode plane alone
The generally accepted value of cell membrane capacitance is 1 \( \mu \text{F/cm}^2 \) [28]. Cell membranes are composed of phospholipid bilayers that act as insulators, except for ion channel openings that conduct transcellular currents. Unlike the membrane capacitance, cell layer capacitance indicates the capacitance of a layer of interconnected cells. Cell layers consist of trans-cellular junctions, folded cell walls, and in some cases, multiple cell layers that lead to a capacitance value other than that of a flat sheet cell wall capacitance of 1 \( \mu \text{F/cm}^2 \). Since the cell wall capacitance is in series with the coating and double-layer capacitance in the coated regions, the smallest of the capacitances, namely the coating capacitance, dominates the impedance. Consequently, the effect of the membrane capacitance will be visible in the frequencies higher than those at which the double-layer capacitance appears.

In systems with multiple CPEs, it is important to identify and associate the CPEs with the corresponding physical quantity they represent. Some indicators that help in this differentiation are the CPE parameters, namely, magnitude \( (A_{\text{coat}}) \) and power factor \( (n_{\text{coat}}) \). Due to the thickness of the polymer and its relative permittivity, \( A_{\text{coat}} \) is small compared to the double-layer CPE. The power factor \( (n_{\text{coat}}) \) of the coating CPE is closer to unity in the case of good (void-free) polymer coatings. The power factor \( (n_{\text{dl}}) \) of metal-deposited gold electrodes exposed to simple electrolytes is closer to unity for a smooth deposition of metal. We recall that the power factor of the CPE is an indicator of its fractal nature [38]. For very smooth metal surfaces, the CPE approaches the regime of perfect capacitance with a power factor approaching 1 [39].

VI. CONCLUSION

OVCA429 ovarian cancer epithelial cell layer was characterized in terms of electrical equivalent circuit elements. The trans-layer resistance and capacitance were determined to be 152 \( \pm \) 59 \( \Omega \cdot \text{cm}^2 \) and 8.5 \( \pm \) 2.4 \( \mu \text{F/cm}^2 \), respectively. Equivalent circuit models developed for device containing HBSS and OVCA429 cells indicate that the polymer-coated regions contribute to the impedance spectrum above \( \sim 1 \text{ MHz} \). The model presented in this paper approximates the experimental data very well over a broad frequency range of 100 Hz to 10 MHz. The coated electrode model developed in this paper is comprehensive, taking into account the effects of polymer-coated WE area as well as the electrode-contact pad lead effects. This paper paves the way for future high-frequency studies of cell cultures using the ECIS-8W1E in combination with commercially available impedance analyzers.

REFERENCES

Dielectric Relaxation in Solids


Abdur Rub Abdur Rahman received the B.E. degree in electrical and electronics from the University of Madras, Chennai, India, in 1997, and the M.S. degree in electrical engineering with specialization in microelectromechanical systems (MEMS) from the University of Cincinnati, Cincinnati, OH, in 2000. He is currently working toward the Doctoral degree in MEMS and nanotechnology at the University of South Florida, Tampa.

From 2001 to 2003, he was a MEMS Process Engineer and a Senior MEMS Process Engineer at Form Factor, Inc., and Standard MEMS, Inc., respectively. His current research interests include BioMEMS, fuel cells, and lab-on-a-chip technology.

Chun-Min Lo was born in Chia-Yi, Taiwan, in 1962. He received the B.S. degree in physics from the National Taiwan Normal University, Taipei, Taiwan, in 1985, and the Ph.D. degree in physics from Rensselaer Polytechnic Institute, Troy, NY, in 1994. He was a Postdoctoral Researcher at the University of Toronto and at the University of Massachusetts Medical School. He is currently an Assistant Professor in the Department of Physics, University of South Florida, Tampa. His current research interests include development and application of electric-cell-based biosensor, and the investigation of the effects of substrate rigidity and physical forces on cellular functions.

Shekhar Bhansali (M’98) received the B.E. degree (with honors) in metallurgical engineering from the Malaviya Regional Engineering College (MREC), Jaipur, India, in 1987, and the M.Tech. degree in aircraft production technology from the Indian Institute of Technology (IIT), Chennai, India, in 1991, and the Ph.D. degree in electrical engineering from the Royal Melbourne Institute of Technology (RMIT), Melbourne, Vic., Australia, in 1997.

He is currently an Associate Professor in the Department of Electrical Engineering and the Nanomaterials and Nanomanufacturing Research Center, University of South Florida, Tampa. His current research interests include the areas of bio-microelectromechanical systems (MEMS), sensors, and microsystems.

Dr. Bhansali was the recipient of the National Science Foundation (NSF) CAREER Award.
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