Quantification of the Heterogeneity in Breast Cancer Cell Lines Using Whole-Cell Impedance Spectroscopy

Arum Han,[1] Lily Yang,[2] and A. Bruno Frazier[3]

Abstract

Purpose: Quantification of the heterogeneity of tumor cell populations is of interest for many diagnostic and therapeutic applications, including determining the cancerous stage of tumors. We attempted to differentiate human breast cancer cell lines from different pathologic stages and compare that with a normal human breast tissue cell line by characterizing the impedance properties of each cell line.

Experimental Design: A microelectrical impedance spectroscopy system has been developed that can trap a single cell into an analysis cavity and measure the electrical impedance of the captured cell over a frequency range from 100 Hz to 3.0 MHz. Normal human breast tissue cell line MCF-10A, early-stage breast cancer cell line MCF-7, invasive human breast cancer cell line MDA-MB-231, and metastasized human breast cancer cell line MDA-MB-435 were used.

Results: The whole-cell impedance signatures show a clear difference between each cell line in both magnitude and phase of the electrical impedance. The membrane capacitance calculated from the impedance data was 1.94 ± 0.14, 1.86 ± 0.11, 1.63 ± 0.17, and 1.57 ± 0.12 $\mu F/cm^2$ at 100 kHz for MCF-10A, MCF-7, MDA-MB-231, and MDA-MB-435, respectively. The calculated resistance for each cancer cell line at 100 kHz was 24.8 ± 1.05, 24.8 ± 0.93, 24.9 ± 1.12, and 26.2 ± 1.07 MOhm, respectively. The decrease in capacitances of the cancer cell lines compared with that of the normal cell line MCF-10A was 4.1%, 16.0%, and 19.1%, respectively, at 100 kHz.

Conclusions: These findings suggest that microelectrical impedance spectroscopy might find application as a method for quantifying progression of cancer cells without the need for tagging or modifying the sampled cells.

Currently, breast cancer is diagnosed using a combination of radiological, surgical, and pathologic assessments of tissue samples requiring a microscopic evaluation for diagnosis and prognostic result generation. The methods require significant sample preparation using bench top protocols. The whole process is time consuming, and the risk of a false-positive result is still high (1).

Quantification of the heterogeneity of tumor cell populations is of interest for many diagnostic and drug therapy applications, including determining the cancerous stage of tumors, determining the effects of drug therapies on tumor masses, and customization/monitoring of drug therapies based on tumor heterogeneity. Customization of drug therapies would be possible if an instrument could be developed that quantifies the heterogeneity of tumor cell populations and response of tumor cell populations to therapies.

Recently, studies have been conducted to show that cancer begins shedding neoplastic cells into the circulation at an early stage for some types of cancer (2). It has been also shown that the level of circulating tumor cells can be used as a predictor for patient prognosis (3). PCR and flow cytometry have been the most widely used methods for detecting circulating tumor cells in the bloodstream. These methods typically require the target cells to be modified using methods, such as tagging with fluorescent dyes. Analysis tools that can measure the level of circulating tumor cells (ideally without the need for tagging) would provide a powerful diagnostic tool.

Impedance spectroscopy is a technique mainly used to characterize tissue based on the knowledge of the electrophysiologic properties of tissue in the frequency domain (4). From the various applications of impedance spectroscopy, distinguishing different types of tissues and studying pathologic tissues are of great interest (5). Among the studies relevant to pathologic tissues, one of the more promising areas is the characterization of cancerous tissues. Several studies have been conducted to characterize cancer tissues using impedance spectroscopy (4, 6–11). Although measuring the impedances of tissues can provide valuable information, the measurement is inaccurate and inconsistent because of the complex composition and structure of tissues. It is hypothesized that the

Authors' Affiliations: [1]Department of Electrical and Computer Engineering, Texas A&M University, College Station, Texas; and [2]Department of Surgery and Winship Cancer Institute, Emory University, Atlanta, GA; and [3]School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, GA

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Requests for reprints: A. Bruno Frazier, School of Electrical and Computer Engineering, Georgia Institute of Technology, 777 Atlantic Drive, Atlanta, GA 30332. Phone: 404-894-2030; Fax: 404-894-4700; E-mail: bruno.frazier@ece.tamu.edu.

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measurement and classification of dissociated cell populations instead of intact tissue can provide more accurate and in-depth information on the heterogeneity of the tumor mass.

Impedance measurement on cell suspensions has drawn wide interest, and a variety of single-particle measurement methods that exploit different force effects have been developed over the last 2 decades (12–16). Recent advances in microfabrication and the lab-on-a-chip concept enabled electrical impedance spectroscopy (EIS) devices to detect and analyze a single cell using integrated probes. A single cell is placed inside an electric field that leads to a local distortion of the field, leading to the measurement of the characteristic impedance signature of the cell (17).

A micro-EIS (μEIS) system has the ability to differentiate cells based on cellular properties associated with the plasma membrane and the intracellular contents (18–20). This allows for detection of abnormal cells as well as classification of normal cell types. Due to the sensitivity of the measurement in response to the relative position of a cell between the impedance measurement probes, positioning the target cell precisely at a desired location is important. A microsystem that can manipulate and trap a single cell precisely between the impedance measurement electrodes has been developed by the authors and used for characterizing ion channel activities of single cells (21, 22).

In this article, the development and implementation of a μEIS system for distinguishing cancer cell lines from different cancer stages will be presented. The system was fabricated using various microfabrication technologies.

Materials and Methods

System design. The μEIS system was designed to enable EIS on single cells using integrated, multielectrode configurations within an analysis cavity. The system consisted of an eight-element array of analysis cavities, where each analysis cavity was designed to have a volume slightly larger than the single target cell to be analyzed. The analysis cavity contained a fluid via at the bottom for cell capture, hold, and release steps as well as a backside microchannel and fluid interface to apply negative pressure to the via for cell manipulation. Pairs of opposing electrodes to measure the electrical characteristic of the positioned cell were designed within the analysis cavities in groups of two and four electrodes. For these studies, the electrodes were positioned at one half of the cavity height. A sample delivery channel located above the analysis sites was used to deliver cells to the analysis sites and to control the extracellular fluid environment. Figure 1 shows a schematic illustration of the developed system.

The detailed fabrication steps have been reported previously (21, 22). Briefly, the system was fabricated on a 3-in.-diameter silicon wafer starting with a 1.8-μm-thick thermally grown SiO_2 film. A 2.0-μm-diameter via was etched through the SiO_2 layer followed by a 3.5-μm-thick spin-coated polyimide layer (PI2611, HD Microsystems L.L.C., Parlin, NJ) and served as the bottom half of the cavity. A Ti/Au film was evaporated to a thickness of 250 and 4,000 Å, respectively, and patterned to create pairs of opposing electrodes positioned surrounding the via and inside the analysis cavities. A second layer of 3.5-μm-thick polyimide was spin coated and served as the top half of the cavity and insulating layer for the electrodes. The analysis cavities were formed by dry etching the polyimide. Circles with a diameter of 200 μm were centered on the analysis cavities and etched through the silicon substrate. The backside fluid channels and fluid interfaces were created using stereolithography (23). The fluid interface design incorporated a network of 800-μm-wide fluid channels where each channel covered two analysis cavities. These channels and ports were used to pull a negative pressure through the cavity fluid via to position and capture the cells. The stereolithographically defined parts were aligned to the overlying silicon wafer and bonded. Soft lithography was used to fabricate the frontside fluid channels that are used to guide the cells toward the analysis sites and to control the extracellular fluid environment and bonded on top of the silicon wafer (24). Finally, wires were connected to the bonding pads using conventional soldering techniques.

Sample preparation and experimental setup. Normal human breast tissue cell line MCF-10A, early-stage breast cancer cell line MCF-7, invasive human breast cancer cell line MDA-MB-231, and metastasized human breast cancer cell line MDA-MB-435 were used as the case study representing early to late-stage cancer models. These cell lines were maintained in accordance with the American Type Culture Collection (Manassas, VA) guidelines. Cells were cultured in 37°C, 5% CO_2, and 95% humidity environment in DMEM (DMEM/F12, Invitrogen Corp., Carlsbad, CA). The MCF-7, MDA-MB-231, and MDA-MB-435 cell lines were supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, UT). The MCF-7 line was also supplemented with 10 mg/mL bovine insulin (Invitrogen) and 100 mmol/L sodium pyruvate (Sigma-Aldrich Co., St. Louis, MO). The MDA-MB-231 and MDA-MB-435 cell lines were supplemented with 2 mmol/L L-glutamine (Invitrogen), 100 IU/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). The MCF-10A cell line was supplemented by 5% heat-inactivated fetal bovine serum, 2 mmol/L L-glutamine, 20 ng/mL epidermal growth factor (Invitrogen), 500 ng/mL hydrocortisone (Sigma-Aldrich), 100 ng/mL cholera toxin (Calbiochem EMD Biosciences, Inc., San Diego, CA), and 10 μg/mL bovine insulin.

The fluid tubing from the inlet and outlet of the backside fluid channel was connected to a syringe and a three-way valve, respectively. The syringe was used to pull and capture the cell in position and the three-way valve was used to maintain the negative pressure. The tubing from the outlet of the frontside fluid channel was connected to a 10 μL gas-tight syringe (1701, Hamilton Co., Reno, NV) to manipulate the cells in suspension and guide them toward the analysis cavity. The syringe was either used manually or used with a syringe pump (PHD 2000, Harvard Apparatus, Inc., Holliston, MA) for accurate manipulation of the cell suspension. The electrodes were connected to an impedance analyzer (4294A, Agilent Technologies, Inc., Santa Clara, CA) using soldered wire.

To do impedance measurements, the packaged microsystem was placed under a microscope (Eclipse ME600, Nikon Corp., Melville, NY). A sample drop of D-PBS containing target cells was dispensed at the inlet of the frontside fluid channel and the cells were pulled using the syringe connected to the outlet of the frontside fluid channel. While observing the analysis cavity inside the fluid channel under the

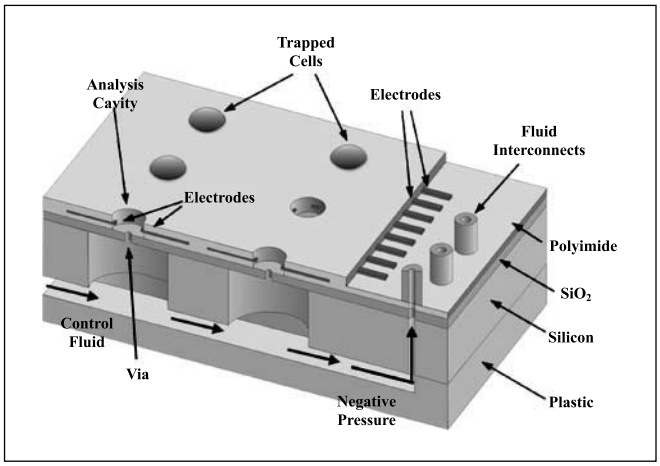

Fig. 1. Illustration of the μEIS system.

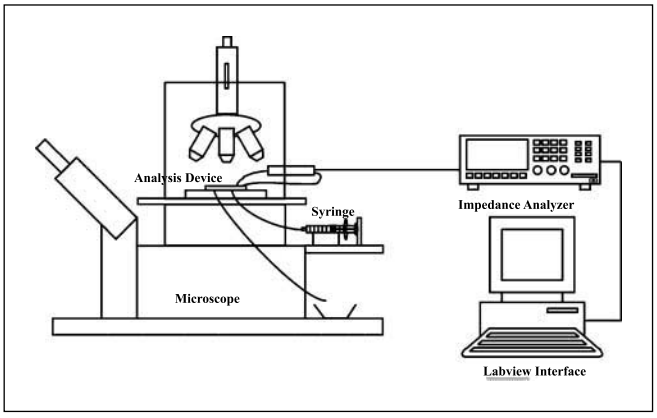

Fig. 2. Experimental setup for whole-cell EIS.

microscope, the flow was stopped when a desired single target cell was in proximity of the analysis cavity. The other syringe connected to the backside fluid channel was used to apply negative pressure through the cavity via to trap the single target cell into the analysis cavity. Once a cell was trapped, the impedance was measured between the two opposing electrodes in the analysis cavity. The impedance spectrums for D-PBS, human breast tissue cell line MCF-10A, and human breast cancer cell lines MCF-7, MDA-MB-231, and MDA-MB-435 were obtained over a frequency range of 100 Hz to 3.0 MHz. Figure 2 shows the experimental setup used.

Results and Discussion

μEIS system. Figure 3A shows a micrograph of the fabricated silicon component. Arrays of analysis sites and electrodes connecting each analysis cavity on a 3-in.-diameter silicon wafer can be seen. Figure 3B shows the analysis region with a 10.0-μm-diameter polyimide cavity, a 2.0-μm via in SiO_2, and two 4.0-μm-wide Cr/Au electrodes. Figure 4 shows the assembled system consisting of the silicon part, backside fluid channel/interface part, and the topside PDMS channel/interface part. In Fig. 4A, the transparent PDMS channel/interface part connected to tubing can be seen. Figure 4B shows the backside fluid interface.

Impedance measurement on cancer cell lines. Figure 5 shows the characteristic impedance signatures for each of the cell lines. The data are given in the form of magnitude and phase to gain insight into the capacitive and resistive nature of the different cell lines. Each measurement shown is an average of 7 to 10 cells tested with the error being approximately the size of each marker. In general, the magnitude decreased as the frequency increased. The phase decreased initially and increased sharply as the frequency increased. In both magnitude and phase, significant differences could be observed between the normal cell line MCF-10A and the cancer cell lines. In addition, when comparing cancer cell lines from the different pathologic states, the impedances in both magnitude and phase could be easily distinguished. The magnitude decreased in the order of MCF-10A, MCF-7, MDA-MB-231, and MDA-MB-435 and the phase increased in the same order.

The membrane capacitance and resistance of each cell line at 100 kHz was calculated by modeling a cell as series of parallel RC circuits. The calculated capacitance of MCF-10A, MCF-7, MDA-MB-231, and MDA-MB-435 was 1.94 ± 0.14, 1.86 ± 0.11, 1.63 ± 0.17, and 1.57 ± 0.12 $\mu F/cm^2$, respectively, at 100 kHz. The calculated resistance of each cancer cell line at 100 kHz was 24.8 ± 1.05 MOhm (1.00 mS/m), 24.8 ± 0.93 MOhm (1.00 mS/m), 24.9 ± 1.12 MOhm (1.00 mS/m), and 26.2 ± 1.07 MOhm (0.95 mS/m), respectively. The decrease in capacitance of the cancer cell lines compared with that of the normal cell line MCF-10A was 4.1%, 16.0%, and 19.1%, respectively, at 100 kHz. It can be seen that that the capacitance decreased for the more pathologically progressed cancer cell lines. As the cancer progresses, the cell membrane becomes more permeable, resulting in decreased capacitance. The change in the resistance was relatively small.

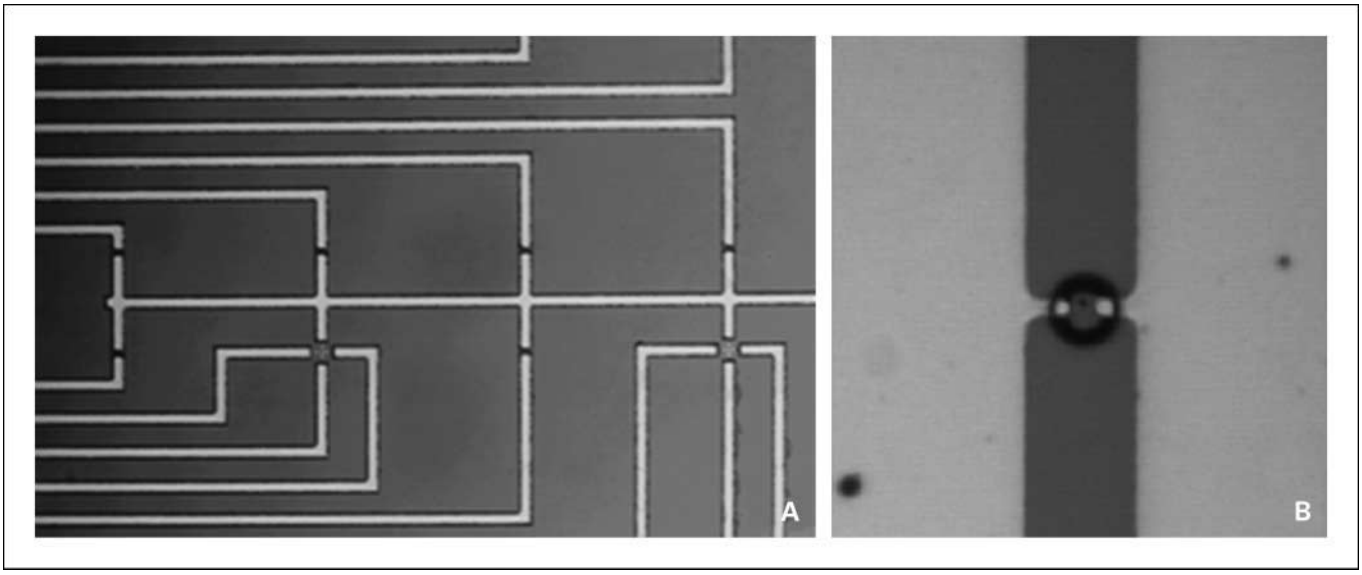

Fig. 3. Micrograph of the enlarged views of the fabricated silicon part. *A,* an array of eight analysis sites with two- and four-electrode configurations. *B,* enlarged view of an analysis site with 10-μm-diameter cavity, 2-μm-diameter via, and two opposing electrodes.

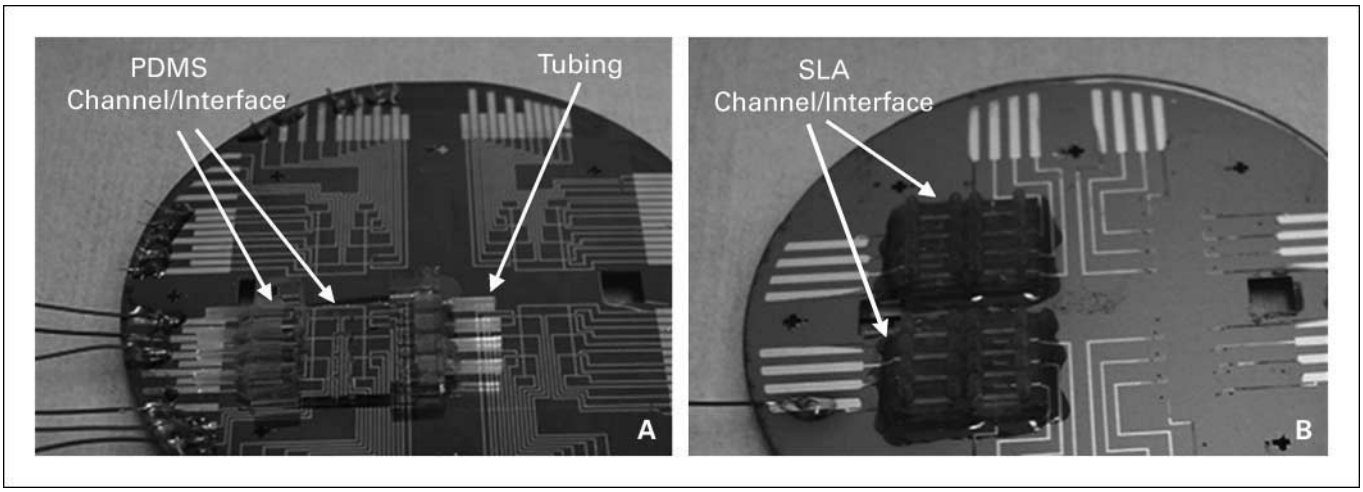

Fig. 4. Micrograph of the assembled device showing the silicon part with PDMS channel/interface part bonded on top of it and connected to tubing and wires (*A*) and backside fluidic channel/interface part bonded on the backside of the silicon part (*B*).

This result shows that the developed μEIS system can be used not only to distinguish cancer cell lines from a normal cell line but also to distinguish cancer cell lines from different pathologic states from early-stage to late-stage cancer models.

Critical analysis and next steps. This novel methodology and miniaturized system with single-cell resolution showed the capability of differentiating cancerous cells from normal cells and it is expected that this technology can possibly enable new classes of diagnosis systems. Although the analysis conducted with our developed system shows promising results as a method for cancer staging, further testing is needed to make sure that the heterogeneity (SD) of the signal within a stage is not greater than the average differences in signal between stages. In addition, with the current data sets collected thus far, it is hard to predict whether we might be able to estimate the time since the tumor initiation event through appropriate calibration. If the change in dielectric properties of cancer cells over time is linear, we expect that the time estimation would be possible. If there is more of an abrupt change in the dielectric properties between different stages, estimating the time would be very challenging. The next phase of study will include redesigning the μEIS system to achieve higher throughput and also the use of multiple cell lines from the same cancer stages as well as normal cell lines to verify the developed capability of the system in accurate cancer staging.

Conclusion

A μEIS system has been developed for whole-cell electrophysiologic analysis. The microfabricated system was used to measure the characteristic impedance signatures for a normal human breast tissue cell line (MCF-10A), early-stage human breast cancer cell line (MCF-7), invasive human breast cancer cell line (MDA-MB-231), and metastasized human breast cancer cell line

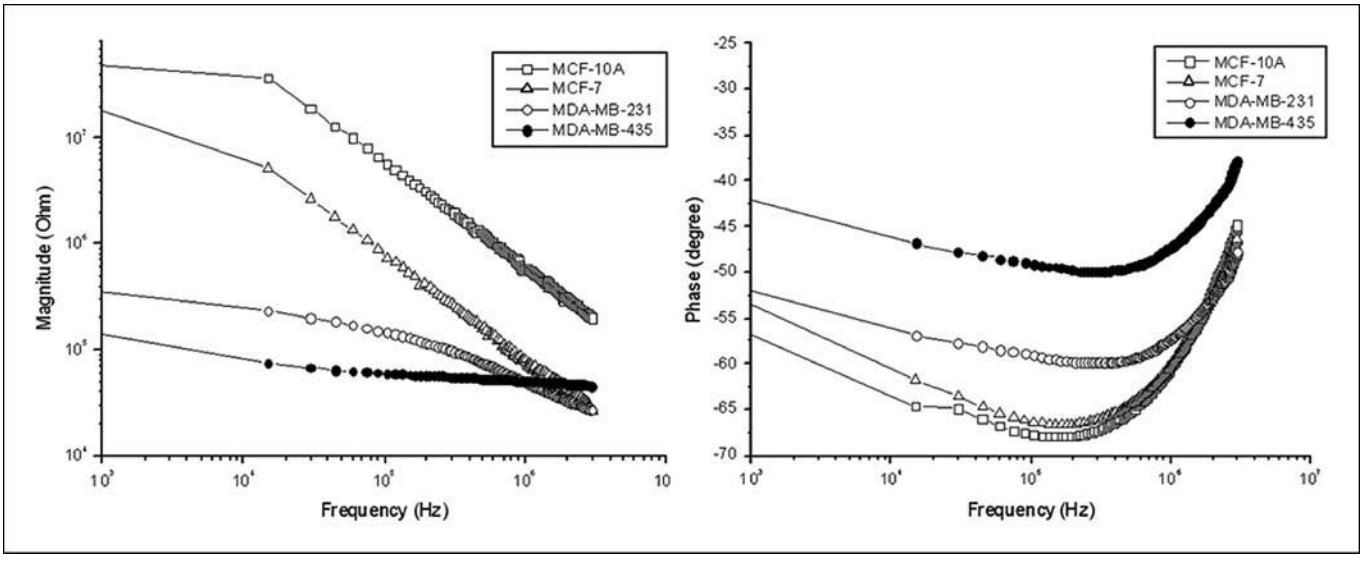

Fig. 5. Impedance measurements of MCF-10A, MCF-7, MDA-MB-231, and MDA-MB-435 over a frequency range of 100 Hz to 3.0 MHz.

(MDA-MB-435). These measurements showed that the impedances of cancer cell lines in both magnitude and phase were significantly different from that of a healthy cell line and one another. The developed system showed robustness to micromachining imperfections and small-medium volume changes. The presented work could lead to development of powerful diagnostic tools for cancer detection and drug therapy based on quantification of tumor heterogeneity at the whole-cell level.

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