A MICRO SYSTEM USING DIELECTROPHORESIS AND ELECTRICAL IMPEDANCE SPECTROSCOPY FOR CELL MANIPULATION AND ANALYSIS

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ABSTRACT

Single cell manipulation and analysis using dielectrophoresis (DEP) and micro electrical impedance spectroscopy (μ-EIS) has been demonstrated on bovine chromaffin cells and red blood cells. Cells were manipulated and analyzed on a micro scale electrophysiological analysis system fabricated using micromachining (e.g. MEMS) technologies. Cells were injected into a microreservoir and collected for μ-EIS analysis using vacuum and DEP. Impedance measurements were taken over a frequency range of 40 Hz to 3.0 MHz. The data obtained for the cells was compared to data obtained for air, and phosphate buffered solution. Data for chromaffin cells was analyzed further, assessing cell size and calcium ion channel activity in relation to impedance spectroscopy.

INTRODUCTION

The study and development of microfluidic systems for biological and chemical analysis has been an area of increasing interest over the past decade. Of particular interest are micro biochemical analysis systems capable of analyzing and processing a variety of biological materials. Microanalysis systems and biosensing systems based on single cells are areas that have benefited greatly by the application of micromachining technologies to biological analysis. The ability to interface cells with microsystems makes it possible to perform analyses which otherwise would not be possible due to system complexity (e.g. multiple precisely defined electrodes, micro scale fluid control). Previous work has shown that interfacing cells with microsystems has made techniques such as electrical impedance spectroscopy (EIS) of single red and white blood cells possible [1,2]. Traditional cellular analysis techniques generally involve fluorescent or radioactive tagging. This is an effective method and yields important information, but requires additional sample preparation and modification of cell biochemistry. The use of micromachining technologies to fabricate micro systems for electrical impedance spectroscopy of single cells enables rapid, parallel analyses and collection of data when compared to conventional techniques for interfacing with cells.

One of the problems with microsystems involved in cellular analysis is precise manipulation of cells. In μ-EIS the requirement is for single cells to be placed in a specific region for analysis. Therefore a precise mechanism for moving cells is desirable. It has been well established that DEP is a viable method for moving cells to a desired location [3], and would fulfill the positional requirements needed in an EIS system. Potential applications for EIS have already been demonstrated in cell sorting and cell-based diagnostic systems using non-contact methods [4].

Thus far, the majority of EIS research has focused on using red or white blood cells. This paper expands the use of EIS to chromaffin cells analyzed using DEP for single cell placement in a μ–EIS system. Chromaffin cells, a hormonal cell found in the adrenal gland, are interesting because they are electrically excitable cells. Using EIS, membrane activity can be studied with regard to ion channel activity and response to chemical stimuli.

In this work a μ-EIS device was fabricated for impedance characterization of bovine chromaffin cells and red blood cells. Dielectrophoresis was used to get precise manipulation of the cells. Cells were separated based on the size and the viability of the cell. In addition chromaffin cells were also studied in relation to calcium ion channel activity. Impedance and phase measurements were compared to air, and phosphate buffer saline (PBS), to investigate the viability of using electrical impedance spectroscopy for chromaffin cell identification, cell sorting and electrophysiological analysis applications.

METHODOLOGY

The micro electrical impedance device was fabricated using standard MEMS fabrication technology. The system was fabricated on a silicon substrate. Initially a dielectric layer of Si_3N_4 was deposited using plasma enhanced chemical vapor deposition, followed by a thin film of Ti (500Å) and Ag (1000Å) for an electroplating seed layer. The film was then patterned to form isolated pairs of opposing electrodes. Next, micro channels were constructed on the surface of the silicon wafer using photo-definable SU-8 material (SU-8-10C, MicroChem Corp.). The microchannels were used as flow channels for routing the individual cells and as micromolds to define the electroplated electrodes. Platinum was electroplated into the micromolds to a thickness of 7.0 μm, with an electrode width of 5 μm. The fluid microchannels were sealed using a glass cover slip with input/output ports.

Figure 1 shows a schematic diagram of two types of μ-EIS systems. Figure 1a shows a system with a single channel and an array of opposing electrodes. Figure 1b

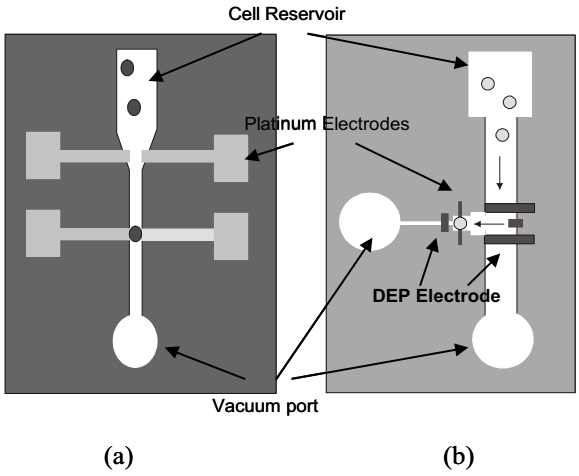

Fig 1. Schematic of a micro electrical impedance device. (a) Cells are pulled into the fluid-filled channel via vacuum and positioned in between platinum electrodes. (b) Cells are pulled into the channel to a T-connection. Using DEP, the cell is positioned into micro chamber for impedance analysis.

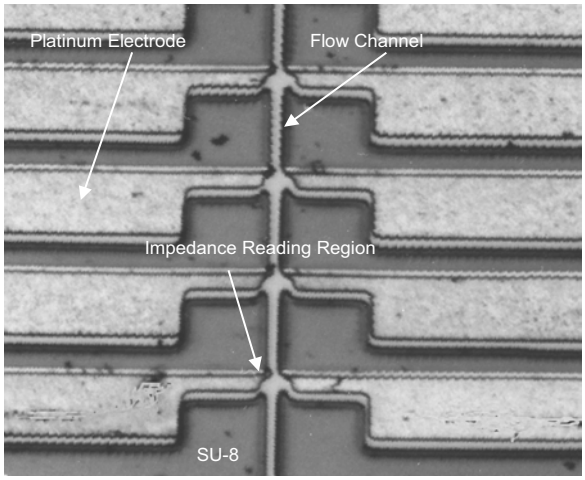

Fig 2. μ–EIS analysis system with 8 μm channels and electroplated platinum electrodes. Cells are brought into the channel via vacuum and place in between opposing electrodes.

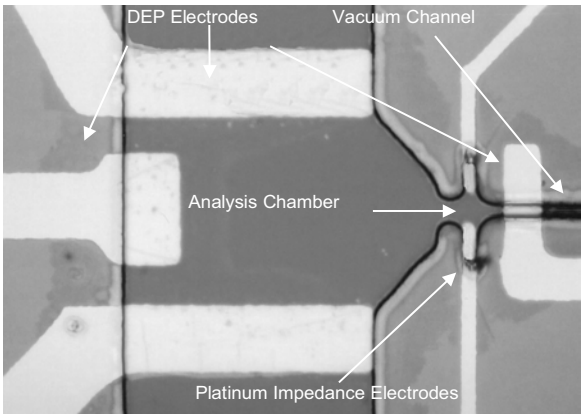

Fig. 3 μ–EIS analysis system with 100 μm wide channel and T-connection leading to 15 μm analysis chamber. A 5 μm vacuum channel is attached to the chamber to ensure proper cell placement after DEP field is turned off. Cell is also removed from the chamber via positive pressure from the vacuum channel.

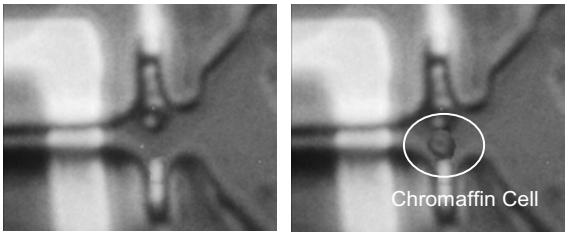

Fig. 4 Images of μ-EIS chamber. (left) chamber with opposing electrodes. (right) Chamber with chromaffin cell being analyzed.

shows a channel with a T-connection and DEP electrodes leading to a chamber where electrical impedance analysis is conducted. Figures 2 and 3 show fabricated devices. Figure 2 depicts a device with 8 μm channel width. Figure 3 shows a 100 μm wide channel that forms a T-connection leading to a 15 μm wide analysis chamber. Analysis chambers of different sizes were fabricated to accommodate the two sizes of chromaffin cells (12 μm and 20 μm). The device in Fig. 2 works via application of a vacuum to move the cells into the flow channel and place them between the opposing electrodes. The device shown in Fig. 3 uses vacuum to bring cells down the flow channel to a T-connection. The T-connection had four electrodes, two perpendicular to the channel and two parallel to the channel. At this intersecion a DEP field was used to manipulate the cell into position. The electrodes perpendicular to the channel serve to trap the cell. Once the cell was trapped on the electrodes, the field was switched off and vacuum was applied via the 5 μm vacuum channel to bring the cell into the analysis chamber. The device was also run in a different mode where a DEP field was applied between the electrodes parallel to the the flow channel. This served to direct the cell into the analysis chamber without the use of vacuum or trapping. The field was then deactivated and the vacuum channel was used to hold the cell against the electrodes for analysis. After the analysis was complete, a positive pressure was applied to the vaccuum channel that pushed the cell out of the chamber.

Red blood cells were taken from a human sample, centrifuged and diluted in a buffered solution for reading in the μ-EIS. Bovine chromaffin cells were separated using a percoll gradient, which separates cells according to size and buoyancy. The adrenal gland was processed and spun at approximately 14,000 RPMs in a test tube with percoll. Layers of cells develop with the smaller diameter cells on the lower layers. Cells were then cultured from the appropriate layer. Dead cells were determined by using Trypan Blue. The cells were suspended in a recording

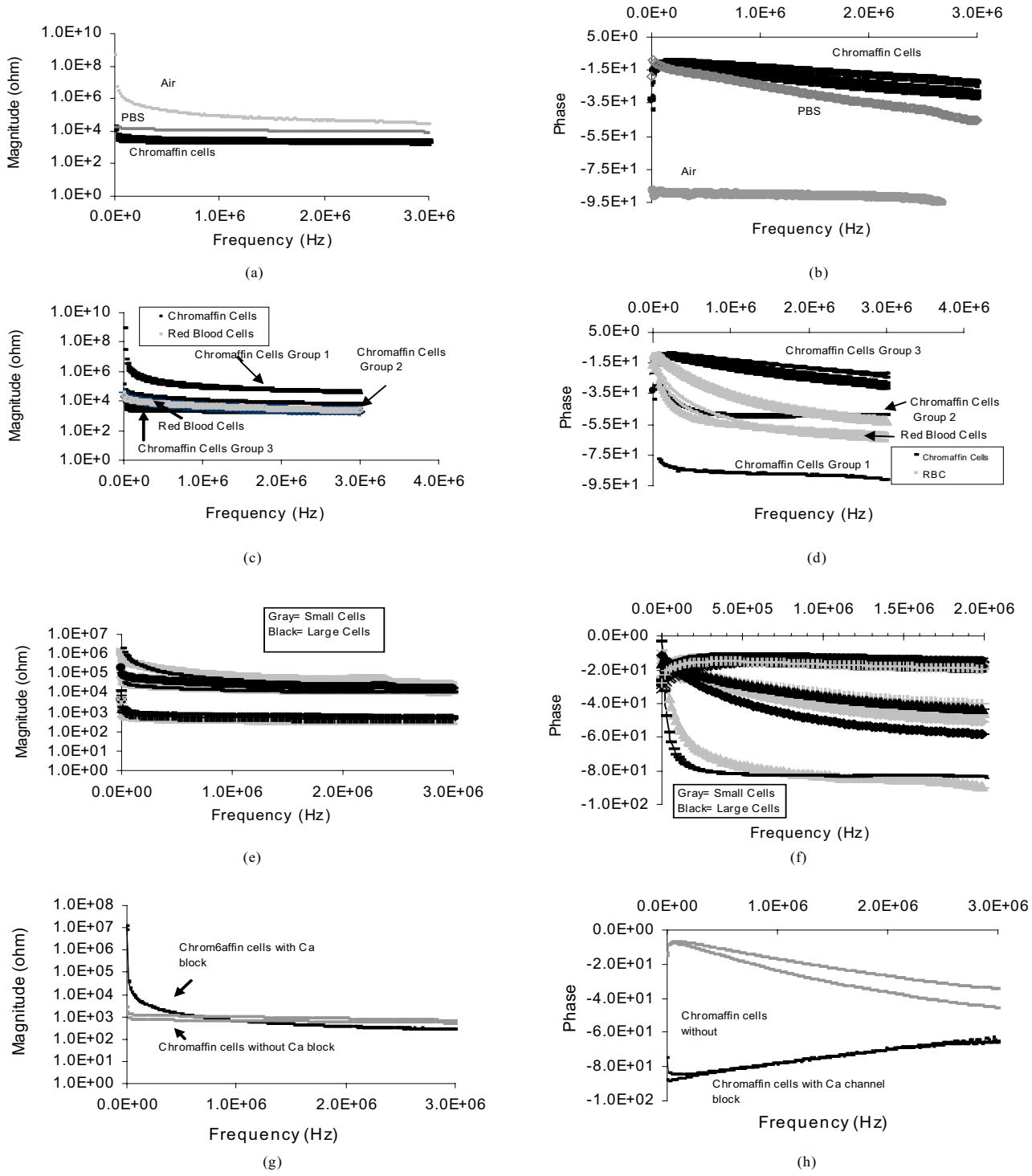

Fig.5 Magnitude and phase measurements of cells. (a) and (b) show magnitude and phase measurements of air, PBS, and most commonly read chromaffin cell. (c) and (d) shows magnitude and phase data for chromaffin and red blood cells. Three different populations of chromaffin cells are shown. (e) and (f) shows magnitude and phase measurements in relation to size of chromaffin cell. (g) and (h) shows preliminary magnitude and phase data for chromaffin cells with Ca ion channel blocked and without block.

medium (Dulbecco's PBS 1x, 1.00 mg/L , D-glucose with 36 mg/L sodium pyruvate, calcium). For calcium ion channel blocking a 1.0 M solution CdCl was added to the solution of chromaffin cells using a ratio of 5 μL to 1 mL.

The μ-EIS system was initially characterized with air, and then PBS to provide a baseline for the impedance data recorded. For devices using DEP, the frequency used was 25 MHz at 8 V_{pp} for trapping and 35 MHz at 8 V_{pp} for

deflection. Figure 4 shows impedance electrodes before and after cell occupation. Impedance readings were taken using an HP4294 impedance analyzer over a frequency range 40 Hz to 3.0 MHz. Magnitude and phase data were taken for each cell. Measurements were done on statistically relevant population of chromaffin cells and red blood cells.

RESULTS AND DISCUSSION

The impedance and phase measurements for air, PBS, red blood cells and chromaffin cells are plotted in Fig 5. Large differences in cell impedance were observed at lower frequencies of the spectrum. At higher frequencies, the curves leveled out to a characteristic impedance value due to the elimination of the membrane capacitive component. Air had the highest magnitude at approximately 5 MΩ over the frequency spectrum. It also had a 90-degree phase shift as expected since the device in air behaves like an ideal capacitor. PBS had a lower magnitude with an average reading just above 10 kΩ across the spectrum. This is expected since PBS is a conductive medium. Figure 5c shows magnitude measurements for red blood cells fell in the range between 10 kΩ to 100 kΩ. Chromaffin cells showed up commonly at three different levels. The first level measured between 1 kΩ and 10 kΩ and accounted for most of the cells observed. Several cells measured between the 10 kΩ to 100kΩ range and other cells fell in between the 100 kΩ to 1000 kΩ range. Distinct phase readings were observed for air, PBS, chromaffin cells, and red blood cells. This is shown in Fig 5d where the chromaffin cells with the lowest magnitude had the lowest phase shift, while red blood cells with a higher magnitude exhibited a higher phase shift. Figure 5e and 5f show the plots of magnitude and phase in relation to chromaffin cell size. Chromaffin cells have average sizes of 12 μm and 20 μm. The magnitude did show some segregation according to size with larger cells having a lower magnitude than the smaller cells. However there was some overlap between the readings making it difficult to see a significant difference. The phase data showed a much more significant different between cell size types with smaller cells generally having a larger phase. The plots shown in figure 5g and 5h show preliminary magnitude and phase data for chromaffin cells with calcium channel blocking compared to normal cells of 20 μm in diameter. At lower frequencies the cells showed a higher magnitude than normal chromaffin cells. The initial readings were in the 10 kΩ range and then dropped to the 1 kΩ range at higher frequencies. The phases for calcium channel blocked cells were significantly different from normal cells. Larger phases were recorded at lower frequencies, and smaller phases were recorded at higher frequencies.

The results have interesting implications in regards to cell characterization and identification. Red blood cells and chromaffin cells clearly have different impedance spectrums. Differences in magnitude between some cell types were not very large but the phase between them was very different (red blood cells and lowest magnitude chromaffin cell population). Therefore the phase data provides added sensitivity for cell identification.

Three different groups of chromaffin cells were observed by the μ-EIS system. Correlating the sizes of the cell to impedance showed that although there was some relation, it did not account for all the differences observed. This may be due to cell viability. The health of chromaffin cells declines soon after sample preparation and can give significantly different readings from healthy cells.

The results of the calcium blocked channel show that the μ-EIS system has some sensitivity to ion channel function, especially when looking at the phase data. This is significant in that the system may be able to detect how ion channel activity of the cell has been affected due to various chemical stimuli or pathogens that alter ion channel functionality.

CONCLUSIONS

Electrical impedance spectroscopy was performed on bovine chromaffin cells and red blood cells with a micro device fabricated using MEMS technology. The system utilized vacuum and DEP for precise manipulation of the cell for analysis. The μ-EIS device is sensitive enough to distinguish between chromaffin cells and red blood cells based on the gain and phase signatures of the impedance spectrum. The system was also sensitive enough to discern changes in calcium channel activity and inactivity in chromaffin cells. Further studies are required to fully understand the role ion channel activity plays in the impedance signature of a cell.

Acknowledgments

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