DETECTION OF BREAST CANCER CELLS IN TRI-CULTURE USING IMPEDANCE SPECTROSCOPY

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ABSTRACT

Impedance spectroscopy is an emerging tool in the analysis of cancer [1-4]. Our goal is to investigate the diagnostic and prognostic capabilities of a MEMS-based micro-bioimpedance sensor. To this end, we report a unique narrow multi-branched electrode system in a micro-cell culture chamber on a silicon chip capable of detecting metastatic human breast cancer cells in a tri-culture comprised of normal epithelial MCF10A, HS68 fibroblast, and cancerous MDA-MB-231 cells to simulate a breast tumor biopsy sample. A bioimpedance signature unique to the metastatic MDA-MB-231 cancer cells is elicited by a chemical stimulus, suberoylanilide hydroxamic acid (SAHA), an experimental anti-cancer agent that exerts selective actions on the cytoskeletal organization of the cancer cells [5]. This is the first time a complex cell culture consisting of three different cell types has been analyzed using MEMS-based impedance spectroscopy.

KEYWORDS: Bioimpedance, Biosensor, Breast cancer, SAHA

INTRODUCTION

Electrode Cell substrate Impedance Sensing (ECIS) was a term coined by Giaever and Keese that refers to the study of cells based on their attachment to the substrate (electrodes) [6]. Because sensing is based on monitoring the interactions between the cells and the substrate, it lends itself to the study of cell properties such as attachment and spreading [6-8], motility [9, 10], growth and proliferation [11]. The method is non-invasive and the ability to conduct real-time analyses to monitor cellular responses to chemical, physical, and biological stimuli offers many opportunities [1, 12].

Recently, there has been considerable interest in the application of ECIS for the study of cancer [1, 5]. Anti-cancer drug actions of cisplatin in human oesophagal cancer cells and drug induced apoptosis of oral squamous cell carcinoma have been monitored using bioimpedance [3, 4]. The prognostic possibilities of bioimpedance sensing are suggested by single-cell analysis of normal, early stage and metastasized breast cells in which differences in the magnitude and phase of the bioimpedance were measured [2].

Our laboratory has demonstrated the use of silicon chip microenvironments for the cultivation and analysis of human breast cell lines [13, 14]. The basis of our biosensor is a MEMS fabrication protocol which produces microelectrodes on silicon chips. In a preliminary report using this sensor we identified SAHA-evoked bioimpedance signals which detected a single cancerous MDA-MB-231 cell in a background of ~ 100 normal MCF-10A cells [15]. We have now introduced HS68 fibroblast cells into the culture mixture to better model a human breast tissue biopsy and to demonstrate the ability of our bioimpedance sensor to handle complexity.

DESIGN AND FABRICATION

The bioimpedance sensor is shown in Figure 1(A). The unique features of this sensor are a large central counter electrode surrounded by four narrow multi-branched sensing electrodes from which electrical connections are made to the measurement system. A cloning cylinder fixed to the sensor creates a 250µl cell culture chamber.

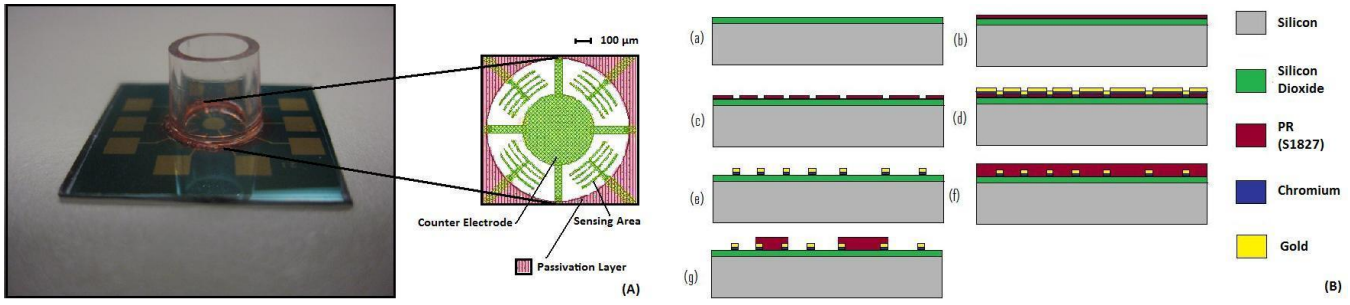

Figure 1: (A) The bioimpedance sensor with an enlarged view of the electrode design. (B) Process flow for the fabrication of the biosensor.

Microfabrication of the sensor follows our previously reported process, in which major steps include Cr/Au electrode patterning using evaporation and lift-off followed by photoresist patterning as passivation layer. The process flow for fabrication of the bioimpedance sensor is shown in Figure 1(B).

EXPERIMENTS

MDA-MB-231 (GFP) and MCF10A (Cherry Red) stably-expressing cells and HS68 cells stained with CellTrace™ calcein red-orange dye were used to obtain fluorescence images in order to correlate bioimpedance responses with the cell type present on each electrode. All cells were counterstained with Hoechst33342 to facilitate cell counting. Cells were seeded and allowed to attach for 20hrs, then SAHA was added; bioimpedance was monitored for another 15hrs, then fluorescence images were taken. Bioimpedance was continuously recorded over 1kHz-12MHz frequency range using an impedance analyzer controlled by a LabVIEW® program [16].

RESULTS AND DISCUSSION

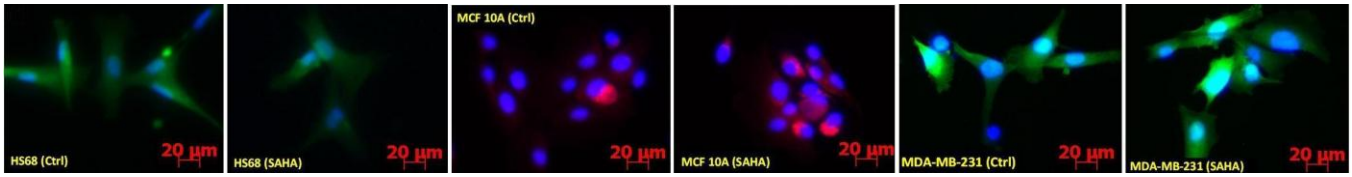

Figure 2: Fluorescence images of the three cell types in monoculture on flat gold without and with 500 nM SAHA

	Before SAHA		**After SAHA**		**% Change**	
	Mean Cell Area (μm^2)	*Peak Bioimpedance. (kΩ)*	*Mean Cell Area (μm^2)*	*Peak Bioimpedance. (kΩ)*	*Mean Cell Area (μm^2)*	*Peak Bioimpedance (kΩ)*
HS68 Cells	1259	454	1260	400	0.09	-11.94
MCF 10A Cells	750	325	751	338	0.08	4.04
MDA-MB-231 Cells	806	374	1425	558	76.72	49.20

Table 1: Comparison of the mean cell area and the peak bioimpedance before and after SAHA action for the three cell types.

Figure 2 shows HS68 cells growing on gold substrate are well spread with or without SAHA. MCF10A cells are cuboidal and maintain cell-cell junctions regardless of SAHA treatment; MDA-MB-231 cells stretch significantly in response to SAHA (Figure 2), and display a significant increase in cell area (Table 1). There was an average 49.2% increase in bioimpedance at the peak frequency after 15 hours in SAHA-treated MDA-MB-231 cells. SAHA did not produce a significant change in bioimpedance in either HS68 (-11.9%) or MCF10A (4%) cells in monocultures (Table 1).

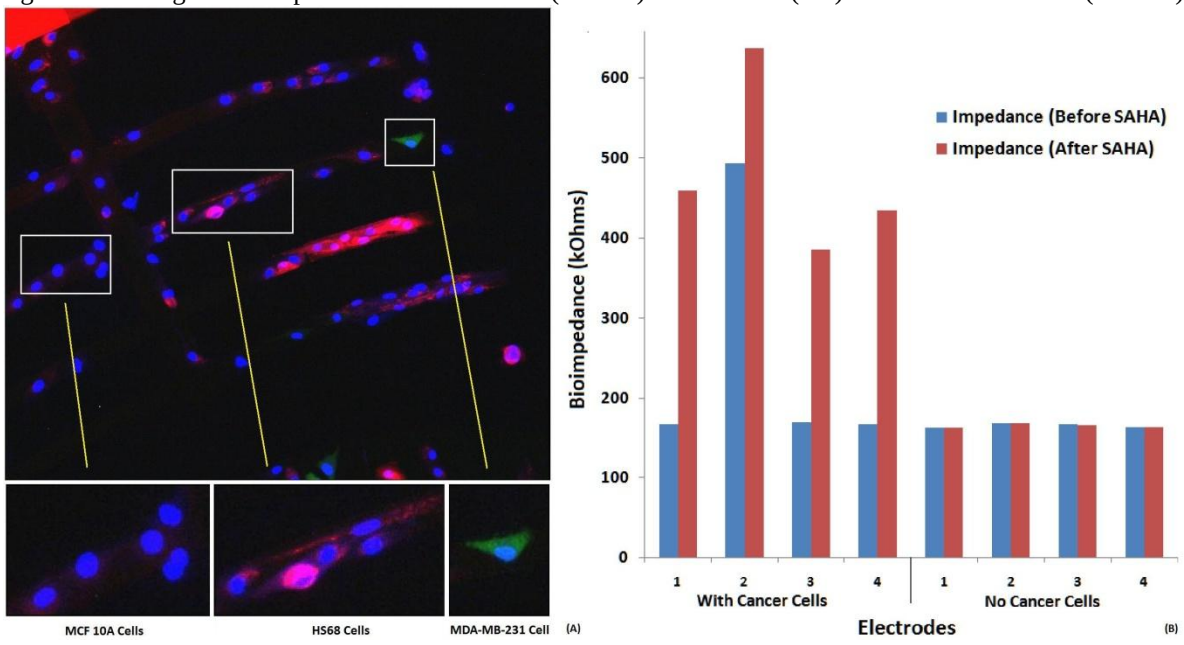

Figure 3: (A) Fluorescence image showing tri-culture of cells adherent to the electrodes during an experiment. (B) Graph showing significant increase in the peak bioimpedance in electrodes on which cancer cells are present in comparison to electrodes on which no cancer cells are present from (n=3) tri-culture experiments.

Because cell contribution to capacitance in the electrical model is directly related to cell membrane area, it is possible that increased MDA-MB-231 cell area after SAHA contributes to the selective impedance response, but other contributions are not ruled out.

Tri-cultures comprised of MDA-MB-231:MCF10A:HS68 in a 2:9:9 ratio were analyzed in the same fashion to evaluate whether the selective bioimpedance response to SAHA by cancer cells was retained in cell mixtures. Analysis of fluorescence images (Figure 3(A)) revealed that ~94% of cells were on the electrodes. Cells preferentially adhered to the gold electrode and spread along the electrode boundary. In this tri-culture, SAHA evoked a 29-174% increase in the peak bioimpedance if at least one cancer cell were present on the electrode (Figure 3(B)). Electrode 2 in Figure 3(B) with cancer cells shows a lower peak impedance rise compared to other electrodes in response to SAHA because it has a larger number of HS68 fibroblasts than cancer or normal cells (see Table 1 for SAHA effect on monocultures). In contrast, the sensor response showed an insignificant decrease in peak bioimpedance if no cancer cells were present on the electrode.

CONCLUSION

A complex tri-culture cell mixture was analyzed using a novel microfabricated bioimpedance sensor for the first time. The ability of the biosensor to detect a small number of cancerous MDA-MB-231 cells among a background of normal MCF10A breast cells and HS68 fibroblast cells was demonstrated.

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