Published in final edited form as:

Sens Actuators B Chem. 2014 August 1; 199: 259–268. doi:10.1016/j.snb.2014.03.065.

Towards an Automated MEMS-based Characterization of Benign and Cancerous Breast Tissue using Bioimpedance Measurements

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Abstract

Micro-Electro-Mechanical-Systems (MEMS) are desirable for use within medical diagnostics because of their capacity to manipulate and analyze biological materials at the microscale. Biosensors can be incorporated into portable lab-on-a-chip devices to quickly and reliably perform diagnostics procedure on laboratory and clinical samples. In this paper, electrical impedance-based measurements were used to distinguish between benign and cancerous breast tissues using microchips in a real-time and label-free manner. Two different microchips having inter-digitated electrodes (10 μm width with 10 μm spacing and 10 μm width with 30 μm spacing) were used for measuring the impedance of breast tissues. The system employs Agilent E4980A precision impedance analyzer. The impedance magnitude and phase were collected over a frequency range of 100 Hz to 2 MHz. The benign group and cancer group showed clearly distinguishable impedance properties. At 200 kHz, the difference in impedance of benign and cancerous breast tissue was significantly higher (3110 Ω) in the case of microchips having 10 μm spacing compared to microchip having 30 μm spacing (568 Ω).

Keywords

MEMS sensor; Inter-digitated Electrodes; Bioimpedance; Breast Cancer; Tissue Micro Array

1. Introduction

In recent years, breast cancer is the most common type of cancer in women and one of the leading causes of female mortality worldwide [1] and the majority of patients having breast cancer diagnosis have the sub-type invasive ductal carcinoma [2]. There is a need to develop newer and better diagnostic tools for the detection of breast cancer and bioimpedance is hypothesized as one such approach in this work. Bioimpedance is defined as the opposition of a biological tissue to current flow. Bioimpedance analysis can be broadly defined as the impedance of biological specimens that includes a wide spectrum of applications ranging from the whole human body measurements to those conducted at the level of DNA. Since the middle of the 20th century, electrical impedance measurement technique has become a standard analytic tool used in the early-recognition of disease onset, 3D monitoring of the cancer progression, detection of pathogenic bacteria in food, and dynamic identification and quantification of cellular changes [3–6]. The utility of this technology originates from the fact that impedance can capture and reflect the rich biological and electrical characteristics exhibited at the whole body, organ, tissue, cell and organelle level [7]. The recent development of micro/nanotechnology has provided additional advantages and introduces new paradigms into the investigation of traditional electrical impedance concepts. The biosensors fabricated using MEMS technology can be used for real-time monitoring of the underlying mechanisms of the onset and progression of cancer [8]. With the advent of micro and nanofabrication technologies, conventional bench-top instruments can be miniaturized [8]. Similarly, microfluidic embedded sensor systems for achieving integrated systems, such as micro total analysis system (μ TAS) is also possible [9]. Miniaturized sensors have significant advantages such as high throughput, small characteristic dimensions, low power consumption, and portability [10]. Drug screening [11], cellular adhesion [12], and cellular kinetics [13] have all been successfully monitored using bioimpedance techniques. Inter-digitated electrodes (IDEs) are implemented in various sensing devices such as surface acoustic wave (SAW) sensors, chemical sensors as well as MEMS based biosensors [14]. MEMS-based bioimpedance measurement are primarily limited to cells [11,12,14]. Different techniques such as microfilters [15–18], microwells [19–21] and dam structure [22] have been implemented for separation of cells. Automated characterization of cancerous cells using micro-cavity array and micromanipulator for exact positioning of cellular spheroids has been studied by other groups [11,23,24]. Ozkan et al. [25] and Umehara et al. [26] showed optical tweezers for manipulation of cells in micro-fluidic devices, including parallel manipulation of cells. Microdevices with modified interior surface by reactive coating and microwells using SU-8 were studied to dock the cells within predefined location [19, 27–29]. Quartz crystal microbalance (QCM) sensor has been used to study the cell-surface interactions and different cell-adhesion behaviors between the healthy and ill-behaved cells [5, 30–31]. Electrode Cell substrate Impedance Sensing (ECIS) is a non-invasive method used to study the cells based on their attachment to the substrate (electrodes) [30]. This method has been recently used to study cell properties such as attachment, spreading, growth and proliferation [30, 32–38]. While IDEs have been optimized for a variety of single cell-based sensing applications, the impedance characterization at the tissue level, in particular, benign and cancerous breast tissue has not been adequately investigated. The advantage of characterizing micro-scale tissues over cells

is that the degree of malignancy as well as the architectural changes that occur during the progression of cancer can be quantified on a larger micro-scale sample to make a deterministic assessment compared to studying a single cell.

An automated measurement system for bio-medical detection has advantages such as higher efficiency, excellent repeatability and statistical reliability [39]. Yamamoto *et al.* developed an automated system for measuring the real part (electrical resistive component) and imaginary part (reactive component) of the impedance [40]. Our goal is to build an automated bioimpedance measurement system using MEMS-based devices capable of measuring bioimpedance of tissue array simultaneously thereby increasing the throughput. To achieve this we have made an array of microdevices (IDE's) [5×6] glass wafer. The semiautomated way of impedance measurement is as shown in Fig. 1. In this work, we have fabricated a microchip having interdigital electrodes inside a SU-8 well to measure the impedance of benign and cancerous breast tissues. To our knowledge, this is the first such study for characterizing the benign and cancerous breast tissues using IDEs and bioimpedance method. It is known that the output signal strength of microchips having IDEs can be controlled through careful design of the active area, width, and spacing of the electrode fingers [41, 42]. In this paper, we investigated breast tissue specimen that originated from a total of ten cases of high-grade invasive ductal carcinoma. The “benign breast tissues” are sampled from normal appearing breast lobules or terminal ducts, and “cancerous breast tissues” are high grade invasive ductal carcinoma. The rest of this paper is organized as follows. In section 2, we provide the experimental details for sensor fabrication, tissue microarray preparation and lumped circuit modeling of the microchip-tissue interface. In section 3, we provide the experimental results and discussion. Finally, in section 4, we make some concluding remarks. The schematics of (a) the MEMS-based bioimpedance sensor for tissue microarray and (b) the semi-automated bioimpedance measurement system is shown in Fig.1.

2. Experimental Work

2.1 Sensor Fabrication

To make the sensor biocompatible, the sensor was fabricated on 1.0 mm-thick glass substrate (Pyrex 7740). The microchip was fabricated using a two-mask process. The fabrication process is shown in Fig. 2. After cleaning glass using a 70:30 solution of H_2SO_4 and H_2O_2 [Fig. 2(a)], Cr/Au ($0.1 \mu\text{m}/0.5 \mu\text{m}$) was deposited using thermal evaporation on glass substrates [Fig. 2(b)]. Using a standard photolithography technique interdigital electrodes ($10 \mu\text{m}$ width with $10 \mu\text{m}$ spacing and $30 \mu\text{m}$ spacing) were patterned [Fig. 2(c)]. SU-8 2007 was used to form reservoir. SU-8 photoresist was spin coated at 2000 rpm for 1 minute on glass substrate to obtain $7 \mu\text{m}$ thickness [Fig. 2(d)] and was patterned using photolithography to form reservoir [Fig. 2(e)]. For using SU-8 on glass substrates, adhesion promoters are not required. The reservoir is made to hold the tissue and medium inside the desired area. The schematic of the microchip is shown in Fig. 3(a) and the reservoir dimensions are shown in Fig. 3(b). Figure 4(a) and 4(b) shows the optical photographs of the fabricated microchips with $10 \mu\text{m}$ and $30 \mu\text{m}$ spacing, respectively. The width in both cases is kept at $10 \mu\text{m}$.

2.2 Tissue Microarray Preparation

Benign and cancerous breast tissue blocks were carefully selected from archival tissue resources at the Histopathology and Imaging Core Facility at Rutgers Cancer Institute of New Jersey. Conventional histology specimen preparation protocol was used in this experiment to fix, slice, and deparaffinize the tissue.

Briefly, fresh tissue is first fixed in formalin – this process preserves tissue from degradation and maintains cellular structure and sub-cellular structure. The fixed specimen is then dehydrated by going through a series of progressively more concentrated ethanol, followed by hydrophobic clearing agent (xylene), infiltration agent (molten paraffin wax), and finally embedded in liquid wax. After cooling to room temperature, the paraffin-embedded specimen can be stored for a very long period of time (a tissue bank in this case), and is hard enough to be sliced at 4 μm for histological purposes. The deparaffinization protocol is also routine histological process, in which the wax is first softened in a baking oven and then gradually replaced with water through the series of ethanol baths. The microchips were deparaffinized as follows: Xylene (5 min, 3 times); 100% Alcohol (5 min, 2 times); 95% Alcohol (5 min, 1 time); 75% Alcohol (5 min, 1 time); rinse in PBS (1–2 times) and immersed in PBS holding solution until impedance analysis. Following the fixation and preparation process described above, the Histology and Imaging Core Facility at Rutgers Cancer Institute of New Jersey archives them and hosts a cancer related tissue bank by storing the formalin-fixed paraffin-embedded (FFPE) specimens at room temperature. Our archive, along with similar tissue banks at other institutes, supported countless experiments in explorative cancer research.

The conventional FFPE method to preserve tissue had been proven effective in reproducing cell morphology and tissue architecture after decades of storage. It is also considered effective in preserving molecular antigenicity for immunohistochemical studies in majority of the cases. Protocols also exist to extract DNA and RNA sample from FFPE specimens for investigative use.

Uniform-sized tissue cores of 0.6 mm in diameter were extracted from each donor tissue block, and inserted into recipient paraffin blocks using Manual Tissue Arrayer (Beecher Instruments) [43]. These single-cored TMAs were rigorously quality-controlled via Hematoxylin & Eosin (H&E) stained sections and the above process was repeated until two characteristic tissue cores were identified from each case. The individual tissue cores were sectioned at 4 μm in thickness and carefully positioned in the center of the interdigitated electrodes. Fig. 5 shows the optical photograph of cancerous tissue placed on the interdigitated electrodes having 30 μm spacing.

As any geometrical variations of tissue samples can affect the impedance measurement, the following quality assurance steps and checks were implemented. The microtome (Reichert-Jung 2030) used in the experiment is regularly maintained by professionals to ensure accuracy. Additional slices of tissue were fixed onto coverglass and examined by SEM imaging. Figure 6 shows the size (diameter) of the single tissue core as being 0.6 mm. A cross-sectional SEM image of the tissue shown in Fig. 7 shows that the thickness of the tissue is $4.0 \pm 0.17 \mu\text{m}$. All specimens in one experimental batch were cut and transferred to

devices in one session and hence the sample preparation condition was constant in terms of microtome thickness setting and water bath temperature. During the course of preparing and conducting the experiment, the tissue cores were quality assured multiple times under the microscope to ensure completeness of the circular shape and flat attachment to the device.

2.3 Circuit Modeling

The microchip interface is modeled using a lumped circuit model as shown in Fig. 8. The equivalent circuit consists of the PBS solution resistance R_{PBS} , the double layer capacitance at the electrode-solution interface C_{dl} and the tissue impedance Z_t in a series connection [44]. The tissue impedance Z_t is modeled as an ohmic resistance R_t and a capacitance C_t element in parallel [45]. The net series impedance is given by:

$$Z_{net} = X_{dt} + R_{PBS} \parallel Z_t + X_{dt} \quad (1)$$

where,

$$Z_t = \frac{R_t}{1 + j2\pi f R_t C_t} \text{ and } X_{dt} = \frac{1}{j2\pi f C_{dt}}$$

Where, f is the frequency, X_{dl} is the capacitive reactance due to the electrical double layer and $j = \sqrt{-1}$

Substituting values of Z_t and X_{dl} in Eq. 1 we get,

$$Z_{net} = \frac{2}{j2\pi f C_{dl}} + \left(R_{PBS} \parallel \frac{R_t}{1 + j2\pi f R_t C_t} \right) \quad (2)$$

Simplifying Eq. 2, we get:

$$Z_{net} = \frac{R_t R_{PBS} (R_t + R_{PBS})}{(R_t + R_{PBS})^2 4\pi^2 f^2 R_t^2 C_t^2 R_{PBS}^2 + 1} - j \left[\frac{1}{\pi f C_{dt}} + \frac{2\pi f C_t R_t^2 R_{PBS}^2}{(R_t + R_{PBS})^2 4\pi^2 f^2 R_t^2 C_t^2 R_{PBS}^2 + 1} \right] \quad (3)$$

The circuit parameters are estimated by fitting Eq. 3 to the experimental data. Optimal parameter estimates should describe both impedance and phase data; hence residuals from both impedance and phase need to be accounted in the fitting approach. We solve the following non-linear regression problem to estimate the circuit parameters:

$$\text{Minimize} = \log \left[\sum_{i=1}^n (Z_t^{exp} - |Z_{net}|)^2 \right] + \log \left[\left(\sum_{i=1}^n (\phi_i^{exp} - \arg |Z_{net}|)^2 \right) \right] \quad (4)$$

Where, Z_t^{exp} and ϕ_t^{exp} are the measured impedance and phase respectively and n is number of data points. The Nelson-Mead simplex algorithm [46] is used to solve the regression problem described by Eq. 4. The logarithm of the sum of squared residuals in Eq. 4 serves the purpose of scaling down the residuals in impedance and phase. This leads to numerical

stability in fitting the experimental data to Eq. 3, and reduces the dependence on accurate choice of the starting point of the simplex fitting algorithm.

3. Results and Discussion

Breast lesions include many different classes of pathologies, among which invasive ductal carcinoma is the most common form of breast cancer. In this work, we investigated differences between high grade invasive ductal carcinoma with tumor adjacent areas and benign morphology. Impedance measurements were performed for the uniform-sized benign and cancerous breast tissue cores placed on IDEs using an Agilent E4980A impedance analyzer. The impedance analyzer was calibrated and operated using the following steps: (a) Connect the probes and turn the power ON, (b) press “Measurement Setup”, (c) press “Correction” and test for open measurement and short measurement, (d) once the open and short measurements are done, press “Measurement Setup” (e) press function key and select “More” option. Select: $z \rightarrow z-\theta d$, frequency $\rightarrow 2$ MHz, Level DC = 10 mV, Trigger $\rightarrow$ manual, measurement time $\rightarrow$ Medium, average = 16, (f) press “Display Format”. Select “List Sweep” and press “Trigger”.

A voltage of 10 mV was applied to the contact pads using gold probe tips. No DC bias was applied. The impedance magnitude and phase were collected over a frequency range of 100 Hz to 2 MHz.

The impedance and the phase shift was measured for benign and cancerous breast tissue on microchip having IDE with 10 μm spacing and 30 μm spacing and are shown in Fig. 9 and Fig. 10 respectively. It was observed that the impedance and phase shift for both benign as well as cancerous breast tissue placed on IDEs with 30 μm spacing were higher than those of tissue placed on IDEs with 10 μm spacing. Furthermore, the differences between benign and cancerous tissues were more prominent when examined with the 10 μm spacing devices than on 30 μm spacing devices (3110 Ω vs 568.0 Ω at 200 KHz respectively).

The slope covering the majority of the frequency spectrum is characteristic of the double layer capacitance at the electrode-solution interface C_{dl} and the tissue impedance Z_t in a series connection [32]. As the impedance is inversely proportional to the capacitance, the impedance decreases when the capacitance increases on increasing the surface area. It is consistent with our observation that the impedance increases when the spacing between the IDE fingers increase.

Impedance measurements were performed on a total of eight specimens, which included 4 benign specimens and 4 cancerous specimens. Two representative cores were extracted from each specimen. Microchip having IDEs with 10 μm spacing was used for a total of 16 measurements. As seen from Fig. 11, benign and cancerous breast tissues have clear differences in their impedance and phase, which can be identified using microchip and impedance analyzer. Note that the benign breast tissues (8023-1, 8023-2) and cancerous breast tissues (3343-7, 3343-8) were acquired from older archived tissues (over 8 years in storage), which may have affected their bioimpedance characteristics.

Prior to our first attempt to study impedance properties of FFPE specimen, we were not sure what affect the preservation method or the length of storage had in change of impedance and we had to hypothesize that it had minimal or limited effect. It can be observed from Fig. 11 that: (i) the duplicated core from each case shows similar characteristics indicating the stability of measurement, (ii) with increase in frequency, the impedance of the tissue decreases and (iii) impedance properties aggregate well within the benign and cancerous group while the distinction are clear between groups.

Our findings are consistent with observations by Halter et al. [47], where they measured the impedance of benign and cancerous prostate tissues. Gabriel et al. reported average increases in conductivity from 0.1 to 0.3 S/m over the frequency range of 1 kHz to 1 MHz for a number of different human tissue types [48].

The Bode plots along with their model fits for all 16 specimens are shown in Fig. 12 and their corresponding circuit parameters are tabulated in Table I. Our initial results indicate that increasing malignancy leads to a corresponding increase in the tissue impedance. The mean tissue resistance R_t in cancerous specimens was 7125.3 Ω compared to 434.19 Ω in benign specimens, while the mean tissue capacitance C_t was observed to be 0.016 nF in cancerous specimens compared to 1.229 nF in the benign specimens. At a frequency of 200 kHz, the mean tissue impedance increased from 236.60 Ω in benign specimens to 7052.0 Ω in cancerous specimens. Since the sample size in our present work is small (8 benign and 8 cancer specimens), we used t-test. We carried out t-test at 200 kHz for benign and cancerous tissue cores and found that the p-value was less than 0.00001667, which indicates that the difference in the impedance value is statistically significant. From Table 1, it is also evident that the fitted circuit parameters C_{dl} and R_{PBS} differ in benign and cancerous breast tissue specimens. This is most likely attributed to alterations in the ionic environment in the vicinity of the breast tissue cores. The results obtained from the present work ($C_{dl} = 0.7$ nF to 10.6 nF, $C_t = 0.027$ nF to 2.935 nF and $R_t = 86$ Ω to 9734 Ω) follows similar trend as reported in literatures focusing on measuring impedance in cells [32].

Many changes occur during the onset and progression of tumor. Morphologically, cancer cells tend to be larger, with increased nuclear-to-cytoplasmic ratio and often basophilic due to heightened protein and nucleic acid synthesis. Biochemists found many disturbed signaling pathways showing either enhanced or diminished activities. Another famous and important phenomenon is that cancer cells loose contact inhibition which implies the usual anchoring and signaling between cell membranes, as well as between cells and extra-cellular matrix, have significantly altered. While it is impossible to prove any direct causation relation at the current stage, we believe that the observed impedance change reflects the overall tissue change in the cancer development.

4. Conclusions

A microchip capable of detecting benign and cancerous breast tissue is successfully fabricated using MEMS technology. The bioimpedance measurement technique was used to analyze benign and cancerous breast tissue cores that are fixed on the microchips. We found that cancerous breast tissue specimens displayed significantly different bioimpedance

characteristics compared to benign breast tissue specimens. It was also observed that by decreasing the electrode spacing, the effective electrode area is increased, thereby increasing the sensitivity of the device. This is our initial attempt to investigate the feasibility of using bioimpedance measurement to assess cancerous tissue based on tissue specimens obtained through tissue microarray technology, which extracts uniform sized tissue cylinders from paraffin-embedded tissue blocks with a hollow needle. Though significant efforts were made to ensure uniform preparation of specimens, we recognize that there may exist minor intraand inter-experimental differences in experimental conditions including tissue heterogeneity, microtome settings, environmental temperature, etc. Further works in this area with more cases, different geometries of the tissues and electrodes, and variety of disease types will allow us to examine these factors and investigate them in more detail to further understand the impedance measurement phenomenon more clearly. We also plan to integrate bioimpedance measurement with mechanical characterization for automated sampling of breast tissue core specimens and apply this technology to investigate a broader set of breast diseases or other organs, especially at different stages of cancer progression.

Acknowledgments

This work was supported by the National Cancer Institute of the National Institutes of Health grant R01CA161375.

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Fig. 1. Schematics of (a) the MEMS-based bio-impedance sensor for tissue microarray and (b) the automated bio-impedance measurement system.

Fig. 2.
Process flow for the microchip.

Fig. 3.
Schematic of the microchip.

Fig. 4. Optical photographs of (a) microchip with IDEs having 10 μm width and 10 μm spacing and (b) 10 μm width and 30 μm spacing.

Fig. 5. Optical photograph of cancerous tissue placed on interdigitated electrodes: (a) 4X and (b) 20X magnification.

Fig. 6.
SEM image of breast tissue cores.

Fig. 7.
Cross-sectional image of the breast tissue core.

Fig. 8. Schematic of (a) bio-impedance measurement of tissues and (b) equivalent electrical circuit.

Fig. 9.

Plots of impedance measurement for (a) benign breast tissue and (b) cancerous breast tissue placed on microchips with IDEs having 10 μm and 30 μm spacing, respectively.

(a)

(b)

Fig. 10.

Plots of phase measurement for (a) benign breast tissue and (b) cancerous breast tissue placed on microchips with IDEs having 10 μm and 30 μm spacing, respectively.

Fig. 11.

Plots of (a) impedance and (b) phase measurement for benign and cancerous breast tissues using microchip having IDEs with 10 μm spacing.

Fig. 12.

Plots of (a) impedance and (b) phase measurement for benign and cancerous breast tissues using microchip having IDEs with 10 μm spacing. Also overlaid are the respective fits in red (for cancerous breast tissue) and blue (for benign breast tissue).

Table 1

Fitted model parameters of the equivalent circuit for benign and cancerous breast tissue specimens shown in Fig. 12

Tissue Label	$R_{pks} (\Omega)$	$C_{dl} (nF)$	$R_t (\Omega)$	$C_t (nF)$	$Z_t (\Omega)$ (at 200 kHz)
Benign 8023-1	222.02	1.777	314.33	0.211	313.24
8023-2	155.81	1.994	393.57	0.177	155.72
26945-3	35.349	8.281	86.564	1.480	35.272
26945-4	31.488	10.654	132.12	2.935	31.278
27122-5	29.871	8.329	284.22	1.114	264.06
27122-6	31.843	6.283	896.81	1.674	419.88
26106-7	47.029	5.288	265.48	0.450	262.54
26106	35.26	6.547	1100.5	1.796	410.86
Mean	73.583	6.144	434.19	1.229	236.60
Cancer 25313-1	851.01	1.617	5821.5	0.023	5735.9
25313-2	971.69	1.781	5710.6	0.013	5682.4
24468-3	1274.4	0.931	5706.9	0.027	5596.9
24468-4	911.39	1.359	8456.4	0.010	8400.4
24531-5	866.06	0.869	9734.5	0.011	9634.5
24531-6	1096.2	0.780	7893.5	0.011	7843.6
3343-7	1166.4	0.705	6968.9	0.018	6882
3343-8	720.7	0.933	6710.7	0.017	6641
Mean	982.231	1.121	7125.3	0.016	7052.0