Dielectrophoretic deformation of breast cancer cells for lab on a chip applications

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1 Introduction

Dielectrophoresis of cancer cells has been widely researched and demonstrated in various applications including control of cell movements [1], electroporation [2] and killing of cells [3]. The advantages of using dielectrophoresis is the label-free targeting of neutrally charged particles that can be sorted towards or away from regions of high electric field gradients in a non-uniform electric field region. Dielectrophoretic (DEP) fields can cause temporary pores to form on a cell membrane in a process known as electroporation and has been demonstrated as a technique for gene delivery, cell lysis and introducing usually impermeable anticancer drugs into targeted tissues in microfluidic devices[4]. Conversely, deformation due to DEP fields refer to the shrinkage and blebbing of target cells subjected to non-uniform electric fields. These cells selectively experience blebbing and

Research Article

Dielectrophoretic deformation of breast cancer cells for lab on a chip applications

This paper presents the development and experimental analysis of a curved microelectrode platform for the dielectrophoretic deformation of breast cancer cells (MDA-MB-231). The platform is composed of arrays of curved dielectrophoretic microelectrodes which are patterned onto a glass slide and samples containing MDA-MB-231 cells are pipetted onto the platform's surface. Finite element method is utilised to characterise the electric field gradient and DEP field. The performance of the system is assessed with MDA-MB-231 cells in a low conductivity 1 % DMEM suspending medium. We applied sinusoidal wave AC potential at peak to peak voltages of 2, 5 and 10 V_{pp} at both 10 kHz and 50 MHz. We observed cell blebbing and cell shrinkage and analyse the percentage of shrinkage of the cells. The experiments demonstrated higher percentage of cell shrinkage when cells are exposed to higher frequency and peak to peak voltage electric field.

Keywords:

Deformation / Dielectrophoresis / Cell shrinkage / Blebbing / Microfluidics / Cancer

shrinkage depending on their electrical properties, where these deformative occurrences may or may not result in the formation of reversible or irreversible micropores in the cell membrane. Irreversible micropores that form can cause apoptosis which is a programmed cell death that is physiological and non-inflammatory[5]. The ability of DEP fields to cause cell death shows that studies of the effect of DEP fields on target cells are crucial in developing viable DEP applications.

Studies of DEP field effects have been demonstrated in terms of estimating the threshold electric field exposure of cells to gauge the viability of cells[6]. Some studies use non-permeable fluorescent dyes such as propidium iodide to indicate apoptosis of cells exposed to electric field [7]. In studies related to morphological changes of the cell, Joshi and Hu [8] demonstrated theoretical calculations of model energy-based analysis of the role of electropores on membrane

blebbing. Meanwhile, membrane blebbing has also been briefly described in a study on the effects of DEP fields on MOSE cells [9]. However, these studies are unable to provide a conclusive link between morphological changes, electric field exposure and the viability of cells exposed to DEP fields in microfluidic platforms.

In this work, we demonstrate the identification of electrical parameters that show cell shrinkage and blebbing on a body of MDA-MB-231 breast cancer cells exposed to DEP non-uniform electric fields. The novelty of this method is the use of sinusoidal AC fields instead of pulsed DC fields that are suitable to induce cell shrinkage and blebbing, which can potentially lead to cell electroporation with cellular transfection applications. The advantage of this approach lies in the ability to selectively shrink the cellular volume and bleb its surface with different amplitude and frequency parameters applied. As healthy cells and cancerous cells have varying dielectric properties (i.e. permittivity and conductivity) in bulk or in surface terms, their response to different amplitudes and frequencies are discernible. This correlates to the study of biophysical, biochemical and cell viability properties towards a standardized template of DEP characterization of cells.

2 Cell Deformation

When an electric field is applied onto a cell, the cell can experience shrinkage or blebbing as a result of the externally applied fields. Shrinkage of cells can be attributed to loss of cell volume from the intracellular media to the surrounding suspension. Conversely, blebbing is attributed to the formation of pores on the cell membrane which may or may not induce a temporary permeation of fluids into and out of the cytoplasm. In the case of temporary permeation, a cell membrane can become permeable in a process known as electroporation [10,11]. The electric fields induces a transmembrane potential (TMP) on the cell membrane and transient pores appear in the membrane when the TMP reaches a critical value [12]. The pores open temporarily and allow diffusion of particles in and out of the cell. Applications of deformation include the release of cytoplasmic proteins, nucleic acids and other metabolites for analysis and the delivery of exogenous reagents such as genes, drugs and nanoparticles with therapeutic purposes or for cellular manipulation [13].

For a cell (of outer radius a and inner radius b), suspended in a physiological medium, the transmembrane potential (TMP) generated by an applied DC field may be calculated according to the Maxwell relationship [14]:

$$\Delta V = 1.5aE_r \cos\theta \quad (1)$$

where E_r is the applied field strength in volts per centimeter and θ is the angle between the field line and a normal from

the center of the sphere to a point of interest on the cell membrane. The conditions for Equation (1) to be valid are the following: a cell of the spherical shape, a much higher resistivity of the membrane than those of the internal and the external media, and a thin membrane compared with the radius of the cell [15]. This relation has been used to describe the TMP for lipid vesicles and cell membrane [16]. A biophysical model of cell membrane deformation is shown in Electronic Supplementary Material (ESI)

3 Materials and methods

3.1 Platform Fabrication

The DEP platform is fabricated using photolithographic techniques [17] on a glass substrate. The DEP microelectrodes are patterned with a gold (Au) and chromium (Cr) film (1500 : 500 Angstroms) deposited using electron beam evaporation. The DEP microelectrodes has a 20 μm spacing between the tips, 150 μm between the widest point at the base of the curved tips, 270 μm between the base and the tips and 300 μm separation between subsequent microelectrodes. The size of the tip of the microelectrode is 0.5 μm . A Clewin drawing of the curved microelectrode is shown in ESI.

Photolithographic patterning is achieved by first spin coating AZ1512 (Microchemicals, UK) with a rotational speed of 3000 r/min followed by soft baking at 90°C for 20 min. The photoresist is then exposed with a chrome mask containing patterns of the curved DEP electrodes. The photoresist is developed in AZ 1:4 developer for 20 s. The metal layers of Au and Cr are etched using wet chemical etching [18] with Aqua Regia ($\text{HNO}_3 + 3\text{HCl}$) and Cr with Cr etchant ($\text{CH}_3\text{COOH} + \text{Ce}(\text{NH}_4)_2(\text{NO}_3)_6 + \text{H}_2\text{O}$) for 15 s and 110 s, respectively. The excess photoresist is then removed using acetone, 1-propanol and deionized (DI) water.

3.2 Cell Culture and Suspension Media

MDA-MB-231 breast cancer cells are cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-deactivated Fetal Bovine Serum (Gibco® Life Technologies, USA) and 1x of Antibiotic/Antimycotic (Gibco® Life Technologies, USA) 37 °C, 5% CO_2 and used for experiments 72 h after passaging. The cells are stripped from the growth medium with a trypsin-based stripping solution, TrypLE dissociation enzyme (Gibco® Thermofisher Scientific, USA) and resuspended in a 1% DMEM media solution which is prepared by diluting 1 ml of DMEM solution with 99 ml DI water. The cell population of 1×10^6 cells/ml is determined and verified by preparing a mixture of 10 μl of Trypan Blue (Invitrogen, USA) with 10 μl of cells suspension and using Countess (Invitrogen, USA). This step also serves to determine the percentage of viable cells. In the absence of electric field exposure, we tested the

viability of freshly prepared cells to be $93.33 \pm 1.76\%$ and $92.92 \pm 1.78\%$ after 5 min. Additionally, viability tests of the cell samples in the absence of electric field exposure after 10 min resulted in reduced cell viability of sub-90% values.

Preliminary experiments with 100% DMEM medium resulted in strong negative DEP forces generated that push the cells away from the microelectrodes tips. Observation of changes on individual cells is difficult as the cells clumped together. It is reported that a low concentration DMEM solution (15% DMEM) has a lower conductivity of 0.03 S/m compared to 1.856 S/m for 100% DMEM solutions [19]. A low conductivity medium was used to reduce the effect of electric fields onto the cells that improves cell survival [20]. More information on MDA-MB-231 cells is provided in Electronic Supplementary Material (ESI)

3.3 Experimental Setup

The platform is cleaned and sterilized with ethanol and placed on an inverted microscope (Olympus CKX 41, Japan) with copper wires taped to the electrode platform and connected to an arbitrary wave form generator (Rigol DG 4102, China). The inverted microscope is mounted with a digital camera (Lumenera Infinity 2-1, USA) and an adapter (Olympus U-TV1X-2, Japan) and connected to a computer where the image from the microscope can be observed and focused. 10 μ l of solution containing MDA-MB-231 cells is pipette onto the platform carefully as a droplet on the focused area using a micropipette (Eppendorf, Germany). No settling time is allocated after the cell sample is pipetted onto the platform as to preserve the cell viability and to minimize clumping of cells at the surface of the platform.

When electric field is applied onto the microelectrode platform, recording of time lapse images of the cell sample at 4 or 6 s is initiated via software (Infinity Analyse, USA). In this work, we generate sine-wave AC fields using peak to peak voltages (V_{pp}) of 2 V_{pp} , 5 V_{pp} and 10 V_{pp} , respectively and two frequencies for each applied V_{pp} at 10 kHz and 50 MHz. The cells are exposed to 5 min of electric fields at each electric parameters to minimize the effects of hyperosmolarity of the suspending medium on the MDA-MB-231 cells. More information on the effects of AC fields and the frequencies of the fields on cell membrane is explained in ESI. With no electric field present, the distribution of cells in the suspension are captured and the cell diameters measured, within and after the 5 min being under observation. It is observed that the average shrinkage reduction of $2.7 \pm 0.2\%$ in cell diameter and an average population of shrunk cells of 11 % (see ESI for details).

3.4 Simulation

A finite element method (FEM) simulation of the electric field distribution of a pair of curved microelectrode tips was performed using the COMSOL Multiphysics software package (ver. 5.3, Los Angeles, CA, USA). The simulations were conducted as two dimensional (2D) approximations as demonstrated in a previous study [21].

A single MDA-MB-231 cancer cell was modelled at varying positions near the curved microelectrode tips for determining the level of electric field exposure experienced by the cell. Some of the simulated positions are presented in Figure 1a-e. Standard electrical and physical properties provided by the software's library were used for the gold electrodes. Assumptions for the values of electrical conductivity and relative permittivity for 1 % DMEM solution is 0.35 S/m [20] and 80 respectively while MDA-MB-231 cancer cells is 9.0×10^{-5} S/m [22] and 49.1 [23] respectively. The tips of the microelectrodes generated the highest electric field with a value of 7.37×10^5 V/m at 10 V_{pp} (without reference particle). From the 2D electric field distribution simulations (Figure 1), we can observe that the electric field density is highest at the tips of each curved microelectrode. On the left side of the pair of curved microelectrode tips, the electric field distribution appears to decrease radially outward that is similar for the outer sides (top and bottom regions in Figure 1) of each curved microelectrode tip. The region in between the tips along the body of microelectrodes is a low electric field density region.

Figure 1. Simulations of the electric field exposure of a single MDA-MB-231 at various locations around the curved microelectrode tips. The electric field values of the cell surface closest to the curved microelectrode tips are labelled.

Figures 1a-c shows a single cell along the middle section in between the pair of curved microelectrode tips at an increasing distance from the tips. The electric field obtained in Figure 1a is 2.63×10^5 V/m compared to 1.31×10^5 V/m (Figure 1b). This shows that the electric field experienced by the cell decreases as it is further away from the microelectrode tips. Figure 1c shows that electric field experienced by a cell on the outer side of the microelectrode

is also high at 2.91×10^5 V/m. Figure 1d shows that if a cell is in between the body of a pair of curved microelectrodes (at a distance of $20 \mu\text{m}$ away from the cell shown in Figure 1a), the electric field exposure is much lesser at 7.33×10^4 V/m.

4 Results and Discussion

This section is divided into few segments. First we describe the physical changes of the MDA-MB-231 cells that have been exposed to electric fields observed in the recorded time lapse images. An example of this time lapse image is shown in Figure 2 where blebbing of the cell membrane is observed and the cells are marked with colored crosses. Blebbing is the formation of bulges on the surface of the cell membrane and is an indication of apoptosis [24].

In Section 4.3, ImageJ (NIH, USA) is used to measure the diameter of breast cancer cells MDA-MB-231 in the recorded time lapse images. The cells that are selected for measurements are of a consistent spherical shape, cells that are not sticking together and cells that are at the bottom of the microelectrode platform and not floating in the droplet. The changes in diameter of the cells at 5 min of electric field exposure are calculated and the data is tabulated as the average shrinkage percentage (ASP) of shrinking MDA-MB-231 cells at the applied electric field voltages of $2 V_{pp}$, $5 V_{pp}$ and $10 V_{pp}$ and frequencies of 10 kHz and 50 MHz. Figure 5 in the ESI shows the population percentage of shrunk MDA-MB-231 cells in the 5 min exposure duration. An additional figure showing the population percentage of shrunk MDA-MB-231 cells in the 5 min exposure duration is added in ESI. In the final section, the findings are presented

by comparing results and simulation and benchmarking against other reported works.

4.1 Blebbing of Cell Membrane

Figure 2a-f shows the images of MDA-MB-231 cells exposed to electric fields of $10 V_{pp}$ 50 MHz on a curved microelectrode platform at 1 min intervals from 0 - 5 min. The cells in the images are marked with colored crosses to show the stages of blebbing of the cells exposed to electric field for 5 min. Cells do not show signs of blebbing in Figure 2a when no electric field is applied. After 1 min of exposure (Figure 2b), we observe minor/early stage blebbing in some cells which are marked with yellow crosses. More cells show blebbing at the 2nd min of exposure to electric fields (Figure 2c) which are mainly around the microelectrode tips. Cells which develop severe blebbing on their cell membrane are labelled with red crosses.

We observed that after the blebbing of some cells gradually diminished after 3 – 5 min of exposure to electric fields (Figure 2d-f). These cells are marked in blue crosses and by visual comparison between the images we observe that the blebs become less obvious as the cell shrink in size. Measurements and analysis of the cell size is conducted in a later section in the manuscript (Section 4.3). The cells with diminished blebs start with cells that are further away from the microelectrode tips (Figure 2d). The cells that still have minor or severe blebbing after 4 – 5 min electric field exposure are mainly concentrated around the microelectrode tips (Figure 2e-f).

Figure 2. Time lapse images at 1 min intervals from 0 – 5 min of MDA-MB-231 cells at $10 V_{pp}$ 50 MHz applied electric field on a curved microelectrode platform. Cells that experience blebbing are marked with a cross (yellow for minor/early stage blebbing, red for severe blebbing and blue for diminished blebbing).

4.2 DEP Induced Cell Movement

Figure 3a-b presents the close up images of Figure 2a-b focusing on the cells around the curved microelectrode tips which are labelled A-E in white. Here we describe the DEP response and movement of the cells observed after the first min of exposure.

By comparing the two images in Figure 3, we observe that they are two cells that move while the other cells in the figure remain in their position. Cell A's movement is shown by red arrow 1 in Figure 3b. At 0 min (Figure 3a), part of Cell A is on top of the curved microelectrode tip. Cell A experiences a slight elliptically offset clockwise rotation and upwards movement to the outer edge of the curved microelectrode tip after 1 min of exposure to electric fields (Figure 3b). Cell B which is located nearer to the microelectrode tip than cell A on the opposite microelectrode does not change its position.

In Figure 3a, cell C and cell D are touching each other at 0 min. Red arrow 2 in Figure 3b shows the elliptically offset counter clockwise rotation of cell C away from the microelectrode tips after 1 min of exposure to electric fields. Cell C continues to remain in contact with cell D during its rotation until it finally stops when it touches cell E.

Figure 3 Close up images of MDA-MB-231 cells exposed to electric field (10V_{pp} 50 MHz) (a) 0 min (b) 1 min (cell movements are shown with red arrows).

4.3 Analysis of Cell Shrinkage

In this section we present the data showing the ASP of shrinking MDA-MB-231 cancer cells within 5 min of exposure to electric fields of varying frequencies and voltages. The diameter of the cells before and during the 5 min of exposure to electric fields are measured and divided by the number of cells shrunk to obtain the ASP. Estimation of the cellular biovolume based on the cell diameter is recognised as an acceptable indication parameter as has been demonstrated previously [25]. A plot of the average shrinkage percentage (ASP) of shrinking MDA-MB-231 cells exposed to 5 min of electric fields at varying frequencies and voltages is presented in Figure 4.

In Figure 4, the MDA-MB-231 cells exposed to higher frequency electric field of 50 MHz experience greater ASP of shrinking cells than the ASP lower frequency electric field of 10 kHz for similar peak to peak voltages of 2 V_{pp}, 5 V_{pp} and 10 V_{pp}. We also observed the trend that the ASP for shrinking MDA-MB-231 cells increases with increasing applied peak to peak voltage in the order of 2 V_{pp}, 5 V_{pp} and 10 V_{pp}. The increase in ASP of shrinking cells is also greater across both frequencies of 10 kHz and 50 MHz when the electric field is increased from 2 V_{pp} to 5 V_{pp} compared to when the electric field is increased from 5 V_{pp} to 10 V_{pp}. The largest diameter reduction observed was at 21 % of the cell's original size from all the experiments. The average size of MDA-MB-231 in our sample is $12.55 \pm 1.67 \mu\text{m}$ in diameter. From the ASP results, the threshold for cell shrinkage depends on the electric field parameters that the cells are exposed to. The threshold is $3.35 \mu\text{m}$, meaning that the cell diameter reduces up to $3.35 \mu\text{m}$ from the average diameter within 5 min of exposure to the DEP fields.

Figure 4. Plot of Average Shrinkage Percentage of Shrinking Cells after 5 min Exposure (%) at peak to peak voltages of 2 V_{pp}, 5 V_{pp} and 10 V_{pp} and frequencies of 10 kHz and 50 MHz.

4.4 Discussion

In this section we compile and discuss the results obtained from experiments in the form of morphological observations of changes of the cells exposed to electric fields over 5 min, electric field distribution simulation of the curved microelectrode configuration and the average shrinkage percentage of shrinking cells. From the observations and discussions, some of the biophysical phenomena as a result of the exposure of cells to dielectrophoretic fields are described and explained.

In Figure 2, we found that cells nearest to the tips of the curved microelectrodes experienced severe or prolonged blebbing while in Figure 3, the movement responses of cells near the tips of the curved microelectrodes are observed in comparison to cells further away from the tips when electric fields are applied. These observations confirmed with the

simulations in Figure 1 where the highest electric field region is located at the tips of the curved microelectrodes. The regions further away from the tips are exposed to lower electric fields hence the slower response due to lower magnitude electric field. MDA-MB-231 cells in 1 % DMEM solution experience positive dielectrophoresis which is confirmed by the responses of Cell A and B in Figure 2 and Figure 3. The movement of cell A as described in Section 4.2 confirms with the electric field simulations in Figure 1 that shows the outer surface of the curved microelectrode tips have higher electric field gradient than the surface in between the microelectrode pair. Positive dielectrophoresis is the phenomena where neutrally charged particles are polarized and attracted to regions of high electric field density [26].

From Figure 3, cell C is already in contact with cell D and rotates towards cell E to form a chain-like formation which is known as pearl chaining [27]. Pearl-chaining is also described as mutual dielectrophoresis [28] and occurs when the electrostatic interactions between polarized cells rebalances their electrical potential energies [29]. Cells naturally have repulsive forces between opposing membrane surfaces which are known as electrostatic repulsion and hydration repulsive forces [30]. Electrostatic repulsion is the net charges between cell membranes while hydration repulsive forces is assumed as the work required to remove water from hydrophilic surfaces as they approach [30]. A cell's membrane surface generates dipoles and becomes polarized when exposed to dielectric fields which forms attractive forces between the cells and is strong enough to overcome the repulsive forces [30]. Unlike cells D and E, there is no cell blocking between the microelectrode tips and cell C which exposes it to higher electric field gradient. This result in cell C experiencing a higher degree of polarization and movement to balance out the electrostatic potential energies described above. We presented in Figure 5 a simulation of the electric field gradient of the approximate positions of cells A- E from Figure 3a to show that the electric field exposure at the cell membrane nearest to the microelectrode tips increases in the order of cells E, D and C.

Figure 5 Electric field simulation of approximate positions of cells C, D and E near the curved microelectrode tips in Figure 3a and labelled with the electric field value of the cell surface nearest to the curved microelectrode tips.

Blebbing of cells observed in Figure 2 and 3 is a result of actomyosin contractions that cause either transient detachment of the cell membrane from the actin cortex or a rupture in the actin cortex resulting in cytosol streaming out of the cell body forming a protrusion in the cell membrane [24]. From Figure 2 and 3, differences in the severity of blebbing are observed between cell A and cell B which are both located at the tips of opposite microelectrodes. In Figure 3b, Cell A is located slightly further away from the microelectrode tip compared to cell B and we try to obtain the approximate electric field values experienced by the cells to determine if there is a difference that causes the blebbing of both cells to be different. For this, we simulated the electric field distribution with the approximate positions of cell A and cell B on the opposing microelectrode tips in Figure 6. We obtained the electric field values at two points on the cell membrane, one at the surface closest to the microelectrode tip (E_1) and one at the surface end across the centre of the cell (E_2). By comparing the intracell electric field gradient difference of electric field exposure within the cell ($|E_2 - E_1|$), we found that cell B has a higher electric field difference of 9.18×10^5 V/m compared with 4.16×10^5 V/m of cell A. Additionally, according to Equation 1, a higher TMP will be experienced by the cell if the electric field experienced is higher. The electric field gradient where it is highest at the nearest point of the cells to the microelectrode tip, E_1 is nearly double in value for cell B (1.16×10^6 V/m) compared to cell A (6.38×10^5 V/m).

Figure 6 Electric field simulations of cell A and cell B from Figure 3 and a table presenting electric field values at the cell surface nearest to the microelectrode tip (E_1) and the opposing surface across the centre cell (E_2) of cell A and B. The difference of electric field exposure ($|E_2 - E_1|$) for both cell A and B is calculated and presented.

From the analysis presented in Section 4.3, we observe that the ASP of shrinking MDA-MB-231 cells is affected by the peak to peak voltage and the frequency of the electric field applied. Figure 4 presents that the ASP for shrinking cells increases with exposure to increasing peak to peak voltage. Cell shrinkage is related to voltage sensitive ion movement regulatory mechanisms of the cell such as ion channels or pumps [31] [32]. A change in the membrane potential much

smaller than the dielectric breakdown potential of a lipid bilayer, known as gating voltage, opens or closes some membrane transport systems giving rise to electroosmotic flow of the ions [33]. The ion movement regulatory mechanism of cell membranes are voltage sensitive and usually have a gating voltage in the range of 10–150 mV that is surpassed by the electric field generated from the microelectrode tips [33]. The cell shrinks and decreases in volume when the ion movement regulatory mechanisms are disrupted by electric fields. From Equation (1), the TMP of a cell is higher when it is exposed to higher electric fields. The electrostatic charges of the cell are temporary and small in magnitude compared to the electric field generated by the microelectrodes [34]. Changes to the microenvironment of the cells are a result of the electric field generated by the microelectrodes and not the movements and shrinkage of the cell.

Figure 4 also shows the trend that the ASP of shrinking MDA-MB-231 cells is higher when exposed to electric fields with higher frequency across different peak to peak voltages applied. A model study the relationship of TMP of sub-cellular bodies within the cell (trans-organelle potential) with electric field frequency demonstrated that electric field frequencies of 50 MHz resulted in higher trans-organelle potential of the cell than at 10 kHz [35]. This shows that electric fields applied at higher frequencies are able to penetrate the cells beyond the cell membrane and reach sub-cellular bodies or organelles of a cell. The volumetric ratio of organelles in a cell, known as the nucleocytoplasmic volume ratio is between 0.179–0.318 depending on the cell type [36]. Higher frequency electric fields could result in the breakdown of organelle membrane and outflow of cellular fluids as that further shrinks the cells.

5 Concluding remarks

In this work, we studied the effect of varying amplitude and frequency parameters of DEP fields to characterize the cell biophysical properties. Interestingly, cell shrinkages are observed as a function of DEP applied electric fields and cell blebbing is the selective rupture of the cell membrane causing swelling known as cytosols. Our study shows that higher frequencies and higher peak to peak voltages results in a higher ASP and higher population of shrinking cells. In addition, high electric field regions close to the microelectrode tips is confirmed via simulation and cells near this region experience prolonged and more severe blebbing. Cell deformation occurs as a possible consequence of these shrinkages and blebbing as the cytoplasmic content of the cells traverse into and out of the cells and the occurrence of electroporation could be confirmed with the use of non-permeable fluorescent dyes such as propidium iodide [37].

The MDA-MB-231 cell line is individually tested because different cells behave differently under the effects of DEP fields. The availability of dielectric properties data for this cell line is simulated and existing experimental studies of permittivity and surface conductance lack in variety of different suspending media. We identified this lack of research and therefore focused solely on one cell line to understand its characteristics. Even so, further work is required on MDA-MB-231 cell line to make it a conclusive DEP characterisation of this cell line. Examples of further studies include the use of different suspending media, cell characterization in flowing liquid on the current platform incorporated with a microchannel and the incorporation of fluorescence dyes into all these studies to understand the biophysical changes. Each of these studies should include detailed biophysical responses of the cell line with respect to the DEP analysis. Investigation of the breast cancer cell line MDA-MB-231 in this study could provide a same DEP microfluidic template can be applied towards characterizing biophysical, biochemical and cell viability properties of other cell lines such as lung, kidney and liver.

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