On Combining the Dielectrophoresis and Microdevices
Investigation of Hippocampal Neuronal Viability after Implementing Dielectrophoretic Positioning on Multi-Electrode Arrays

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Abstract: In this work, we have investigated the viability of embryonic mouse hippocampal neurons after dielectrophoretic positioning on multi-electrode arrays (MEA). We present a systematic evaluation of positive dielectrophoretic conditions, including 1) an investigation of the effect of 10% sucrose (w/v in deionized water), a commonly used, low-conductivity buffer medium, on the viability of mouse hippocampal neurons over different time periods, and 2) a study of the effect of the membrane potential induced by DEP electric field on the integrity of the cell membrane. Post-DEP high neuronal viability was achieved experimentally, and spontaneous neuronal potentials from trapped neurons on the MEA were successfully recorded.

1 INTRODUCTION

Microsystems based on dielectrophoresis (DEP), being flexible and label-free, have been widely used to position, separate, and characterize particles and biological cells (Gagnon, 2011; Li et al., 2014; Pethig, 2010). For instance, various types of cells, such as rat hippocampal neurons (Honegger et al., 2013) and human liver cells and endothelial cells (Ho et al., 2013) have been successfully manipulated and patterned by DEP, while circulating tumor cells (CTC) have been isolated from blood cells using microdevices integrating DEP and microfluidics (Gupta et al., 2012). Likewise, neuronal stem cells, neurons and glial cells have been characterized and separated with DEP microsystems (Prieto et al., 2012).

On the other hand, extracellular multi-electrode arrays (MEAs) have provided reliable platforms for neuronal potential recording because of their non-invasive nature (Berdondini et al., 2009; Hochberg et al., 2006; Spira and Hai, 2013). These MEAs facilitate the study of neuronal physiology and communication through simultaneous *in vitro* recordings and stimulations from multiple neurons. By increasing the number of electrodes, conventional MEAs have been widely used for tissue-level or high-density neural culture and recording (Berdondini et al., 2009; Stevenson and Kording, 2011; Viventi et al., 2011).

Incorporating DEP on MEAs, as demonstrated by the work of Jaber et al., 2009, Rozitsky et al., 2013, and Yoshimura et al., 2014, enables a microsystem to efficiently position individual neurons on single electrodes, as well as to precisely track and investigate electric signals from specific individual neurons. According to Pohl 1978, the polarization of a dielectric particle (e.g. a neuron), when exposed to a non-uniform electric field, drives the cell towards the maximum or minimum of electric field, depending on the relative dielectric and conductive properties of the cell and the suspension medium. Positive dielectrophoresis (pDEP) actively traps cells to the electrodes (maximum of electric field), while negative dielectrophoresis (nDEP) pushes cells away from these areas.

In order to pattern neurons with positive dielectrophoresis (pDEP), neurons have to survive the implementation of pDEP, which attracts them to the electrodes. Two factors influence the viability of

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neurons during the application of pDEP: 1) the DEP cell-trapping solution, because its low conductivity, which is desirable for trapping (pDEP is not possible in high conductivity, standard culture media), is not optimal for cell survival, and 2) electric field. The viability of a few types of neural cells, such as neural cortical cells and neural stem/progenitor cells (NSPCs), in DEP manipulation was investigated previously (Heida et al., 2001; Lu et al., 2012), and high cell viability was achieved for short-term (1 min or less) DEP exposure. In this work, we systematically evaluate the long-term (up to 12 hours) viability of embryonic mouse hippocampal neurons after being actively positioned on the electrodes of a custom-made MEA using dielectrophoresis.

2 MATERIALS AND METHODS

2.1 Experimental Setup for the Study of the Hippocampal Viability in Sucrose

In order for pDEP to take effect, the polarization of neurons should be stronger than that of surrounding media (Jones, 1995), which requires a low-conductivity environment. As a commonly used, low-conductivity buffer medium, sucrose solution is often used as the primary component of a pDEP trapping solution (Huang et al., 2014; Pethig, 2010; Vahey and Voldman, 2009). In our experiments, a 30% cell media DEP suspension medium, which is a mixture of seven parts of 10% sucrose(w/v in deionized water) and three parts of primary neuron culture media, NbActiv1 (BrainBits, LLC.), is used for neuronal pDEP recruiting on MEA. This sucrose/cell media mixture, with a measured conductivity of 0.331 S/m, has low conductivity and appropriate physiological osmolarity for neurons to survive (Jeng et al., 2010) during pDEP trapping.

While cell culture media does not compromise the health of neurons, the viability of embryonic mouse hippocampal neurons, in 10% sucrose, which is the major component of the cell-trapping solution, was investigated. Dissociated hippocampal cells were resuspended in three sterile 15 mL centrifuge tubes, each containing 5 mL 10% sucrose (w/v in deionized water), and in another centrifuge tube of 5 mL cell media NbActiv1 as a control group. The three sucrose samples were placed at room temperature (RT) for 30, 60 and 90 min respectively, and the control tube was placed at RT for 90 min. After each associated time period, 5 mL of phosphate buffered saline (PBS) was added to each of the sucrose tubes, and the samples were centrifuged at 200g for 5 min to harvest the cells. We found the dilution with PBS, above, necessary for pelleting the cells, because otherwise cells remained suspended in the high-viscosity sucrose solution, even after being centrifuged. The viability of harvested cells was assessed through use of Live/Dead™ cell stain, (Invitrogen; Calcein, AM 2□M and Ethdium Homodimer, 1□M in cell media NbActiv1). After 15 min in dark at RT, the cell suspension was transferred to a 35 mm petri dish using a micropipette, and five live/dead fluorescent micrographs were taken at random positions, for each sample, including the control group. Percent cell viability was calculated based on the average of five images. Typically, 30-40 cells were counted on each of the image.

2.2 Simulation and Modeling of the Effect of MEA Electric Field on Hippocampal Viability

Membrane breakdown, or electroporation, is the process where a biological cell membrane is turned into a high-conductivity state because of a membrane potential induced by an external electric field (Heida et al., 2002). This process comes with the creation of pores on the membrane. When induced membrane potential exceeds the threshold level, expansion of membrane pores or creation of more pores leads to membrane breakdown, which can be a fatal effect while trying to attract hippocampal neurons to electrodes using pDEP.

According to Zimmermann and Neil, 1996, in an AC electric field E, the generated membrane potential is given by:

$$V_m = \frac{1.5Ercos\alpha}{\sqrt{1+(2\pi f\tau)^2}} \quad (1)$$

with E the static electric field, r the cell radius, α the angle between the electric field line and a vector from the cell center to an associated point on the membrane, f the electric field frequency, and τ the time constant of cell membrane expressed as (Heida et al., 2002):

$$\tau = rC_m(\frac{1}{\sigma_c}+\frac{1}{2\sigma_m}) \quad (2)$$

where C_m is the effective cell membrane capacitance per unit area, and σ_c and σ_m are the conductivities of cell interior (cytoplasm) and surrounding medium, respectively.

From equation (1), the induced membrane potential is frequency-dependent. Furthermore, the maximum potential is at the membrane point facing an electrode (assuming the cell sitting on top of a planar electrode), where the electric field line is parallel to the vector from cell center to the membrane point, giving $cos\alpha$=1 or $cos\alpha$=-1. The manipulation of cells using DEP requires a non-uniform global electric field, as mentioned above. However, if the local electric field is assumed to be uniform and static (constant E), the induced membrane potential can be calculated based on the protoplast (single-shell) model of a mammalian cell (Jones, 1995; Zhou and Tatic-Lucic, 2012). In this model, the mammalian cell is represented by a homogeneous cell interior (cytoplasm) with ohmic conductivity σ_c, and a thin capacitive cell membrane layer with effective capacitance C_m.

2.3 Post-DEP Hippocampal Viability Verification

The viability of pDEP recruited hippocampal neurons on the MEA was verified, using the same Live/Dead™ cell stain, as described above. Primary neuron culture media NbActiv1, which was the media neurons incubated in after the DEP positioning, was replaced carefully by the live/dead stain with a micropipette. The sample was placed in dark at RT for 15 min, and visualized immediately with a Nikon ECLIPSE E800 upright fluorescent microscope.

3 RESULTS AND DISCUSSION

3.1 Hippocampal Viability in Sucrose

The main objective of our study was to evaluate the effect of the cell-trapping solution on the viability of embryonic mouse hippocampal cells. Cells were exposed to 10% sucrose for various periods of time (30, 60 and 90 min) and their viability analyzed, as previously described in Section 2.1. Since the cell media component of the trapping solution does not compromise the health of cells, their viability was investigated in a more severe (10% sucrose, only) situation.

As shown in Figure 1, all three sucrose treatment samples had acceptable hippocampal viability compared to the control group (cell media); the parameter that should be kept in mind is that the entire pDEP trapping process normally lasts for less than 30 min, thus, that is the duration of exposure to cell trapping solution that has relevance for most of the experimental setups. The viability decreases slightly from 88±2% to 78±3%, as time in sucrose increases (up to 90 min), but the detrimental effect on cell survival is limited, confirming the feasibility of using 10% sucrose as the major component of the cell-trapping solution. In Figure 1, the error bars represent the standard deviations of data from five randomly-taken images.

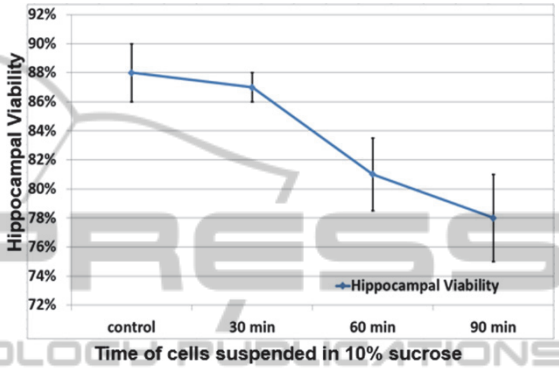


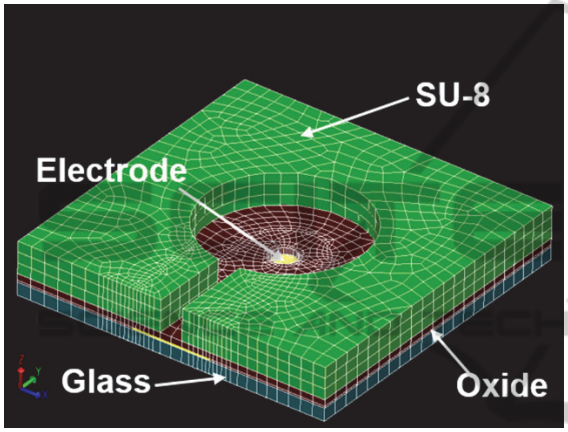
Figure 1: Hippocampal viability assessment after sucrose treatments for various periods of time.

3.2 Neuronal Membrane Potential Simulations on MEA

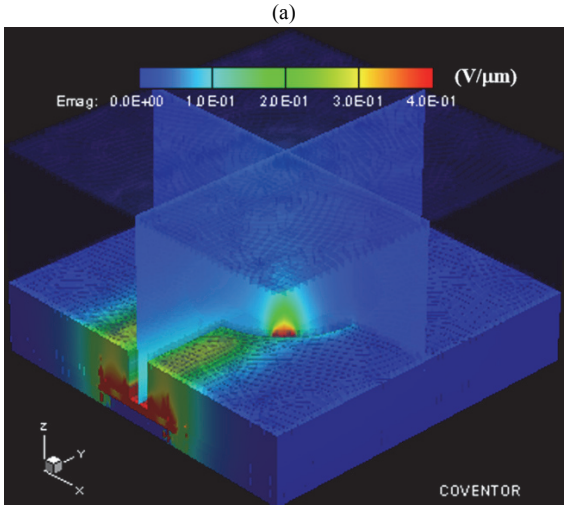
Another facet of this viability study is the investigation of the induced cell membrane potential resulting from non-uniform DEP electric field, which, if it exceeds certain threshold value, could compromise neuronal health; this threshold value is known as the breakdown potential (Heida et al., 2002; Huang et al., 2014). Detailed simulations were performed to ensure cell membrane integrity with the application of a 6 Vpp, 10 MHz AC signal. This signal was selected based on our prior studies on the DEP parameters that secure the attraction of neurons, only, on the electrodes, as opposed to glial cells which are also present in the cell medium (Zhou et al., 2014).

In order to explore the intricacies of pDEP attraction of cells on electrodes, DEP electric field simulation was performed with CoventorWare (Coventor, Inc.) finite element analysis (FEA) software (Zhou and Tatic-Lucic, 2012). A simplified one-electrode model based on targeted device structure was built (see Figure 2(a)). In this model, a single electrode is sandwiched between glass substrate and silicon oxide passivation layer, and a via is etched through the passivation layer to open and define the electrode site. SU-8 epoxy is patterned

above the passivation layer, where different microstructures, including microchambers and microtrenches, are created using photolithography. In the electrostatic simulation, +3V potential was applied to the electrode. The simulated electric field distribution is shown in Figure 2(b). It can be seen that the electric field maximum area is located above the open via, as the central red peak extending above the surface indicates. This means the cell will be attracted to the open via on top of the electrode when pDEP is implemented (Zhou and Tatic-Lucic, 2012).



(a)



(b)

Figure 2: (a) FEA model of DEP MEA electrode structure. (b) Simulated electric field distribution using Coventor.

From this electrode finite element analysis modeling, 2-D electric field data on four different planes of interest was extracted, as shown in Figure 3. These four planes are representative surfaces where hippocampal neurons experience induced membrane potential during pDEP anchoring. The first plane (Z1) is the surface of the silicon oxide passivation layer; the second plane (Z2) is the level of the neuron's center when the neuron lands on the silicon oxide layer; the third plane (Z3) is the surface of the SU-8 layer; and the last plane (Z4) is at the level of the center of the neuron when the neuron is positioned on top of the SU-8 layer. Z1 and Z2 are established when the cell has been trapped on an electrode; Z3 and Z4 represent the cell floating on device surface just before anchored to the electrode by DEP. The position and distance between each plane are based on dimensions obtained from a fabricated DEP MEA and from the measured radii of hippocampal neurons (r= 4 μm).

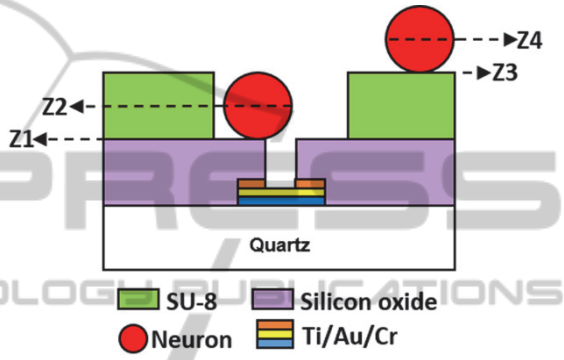


Figure 3: Four planes (Z1-Z4) where electric field data was extracted and induced neuron membrane potential was calculated.

As mentioned earlier, the simulated maximum electric field is above the electrode, which is also the center point of each 2-D plane extracted. Using this electric field maxima and related hippocampal neuronal dielectric properties (Chitwood et al., 1999; Gentet et al., 2000; Heida et al., 2001; Major et al., 1994), the frequency dependence of maximum ($cos\alpha$=1) induced membrane potential V_m on four planes was calculated in Matlab (MathWorks, Inc.). In Figure 4, two graphs indicate the situation where the voltages of 3 V and 4 V were applied to the electrode, respectively.

As can be seen in Figure 4, the induced membrane potential is greater on lower planes (greater electric field strength); nevertheless, the potential on each plane decreases as frequency increases. With different electrode configurations and DEP conditions, Huang et al., 2014 and LaLonde et al., 2014 reported similar results. It was reported that an induced membrane potential below 0.4 V can probably guarantee the survival of cortical neurons, and larger membrane potentials can be tolerated by cortical cells at higher frequencies (Heida et al., 2001; Heida et al., 2002). Assuming 0.4 V is also the membrane breakdown threshold for hippocampal neurons, the membrane potentials are

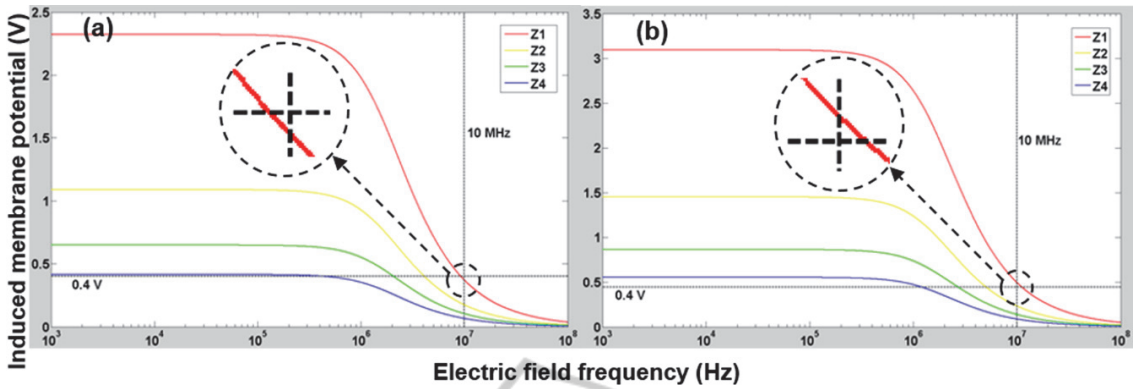


Figure 4: Calculated hippocampal membrane potentials induced by external electric field when (a) 3 V and (b) 4 V is applied on the bottom electrode. Two close-up views for potentials on Z1 at 10 MHz are provided, compared with the 0.4 V threshold.

all below this level at 10 MHz when 3 V is applied, as indicated by the close-up view in Figure 4(a). However, the potential is still above 0.4 V on plane Z1 if 4 V is applied at 10 MHz, which could lead to cell death when the neuron is anchored on top of the electrode. For this reason, 6 Vpp (-3 V to 3 V), 10 MHz AC signal was used for hippocampal neuronal recruiting on the MEA.

It should be mentioned, however, that this membrane potential is not the only factor impacting the viability of hippocampal neurons. For instance, some neurons may have already died during the dissociation process, even before they are exposed to the electric field (Heida et al., 2001). Therefore, approaches to simultaneously track the change of neuronal membrane potential and verify the membrane breakdown-associated cell death are in need, as such necrosis directly relates to external electric field during pDEP implementation.

3.3 Hippocampal Viability Verification and Neuronal Potential Recording

The next step in our work was to experimentally verify the viability of the neurons that were attracted to MEA electrodes and positioned there by pDEP. We used the DEP AC signal of 6 Vpp and 10 MHz, and implemented live/dead staining process to determine whether neurons survived the recruiting procedure. Live/dead staining, which requires media change and manual manipulation, as described in Section 2.3, was not possible immediately following the application of pDEP, because newly placed cells were easily displaced. Therefore, staining was performed 12 hours post-pDEP, for neurons to better attach on the electrode. As can be seen in Figure 5, after 12 hours *in vitro*, pDEP positioned hippocampal neurons on MEA have better than 96% viability (96±2%, n = 7), verifying the integrity of the cell membrane and that neurons stayed alive in the cell-trapping solution during pDEP positioning.

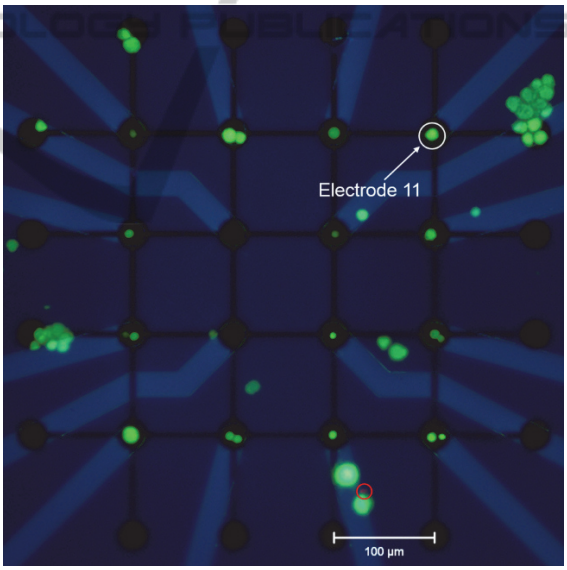


Figure 5: Live (green)/dead (red) stain of hippocampal neurons positioned on MEA at 12 h *in vitro*. Viability better than 96% was achieved.

At the same time (12 hours *in vitro*), spontaneous neuronal potential was successfully detected from neurons anchored on the MEA. In Figure 6, a spontaneous neuronal extracellular potential spike was recorded from electrode 11, as indicated in Figure 5; the spontaneous neuronal spike has an amplitude around 100 µV, which is a reasonable value according to Buzsaki et al. 2012. With the recording of spontaneous neuronal potential, the electrically active properties of pDEP positioned

neurons on the MEA was further verified.

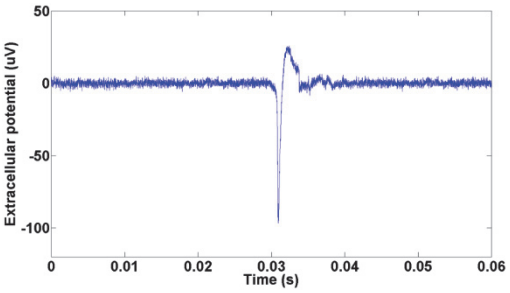


Figure 6: A spontaneous neuronal spike recorded from electrode 11 in Figure 5.

4 CONCLUSIONS

Dielectrophoresis is used, with increasing frequency, in combination with microdevices, to manipulate biological cells. However, it is important to understand the impact the implementation of DEP may have on the viability of cells. In this work we have investigated the viability of mouse hippocampal neurons positioned on the electrodes of microfabricated multi-electrode arrays after the implementation of pDEP. We showed that neurons maintained high viability after short-term exposure to cell-trapping solution, which contained, primarily, 10% sucrose. With electric signal of appropriate frequency and amplitude (such as 6Vpp and 10MHz), neuron membrane breakdown was prevented during the DEP process. Most importantly, we have obtained electrical signals from the neurons positioned on the MEA, 12 hours after using positive dielectrophoresis, further confirming the health and electrically active properties of neurons.

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