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Trapping cortical rat neurons

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Abstract – **Cortical rat neurons were trapped by dielectrophoresis (DEP). Experimental data were compared with theoretically deduced relationships. The neuron was represented by a single-shell model. A planar quadrupole electrode structure was used for the creation of a non-uniform field. The electrode structure was modeled as four point charges. The experimental data did almost completely fit the theoretical yield/time relationship. The theoretical yield/amplitude relationship, however, did only apply for a restricted amount of frequencies. The experimental frequency behaviour (i.e., the DEP-spectrum) did not apply to the theory. A difference in neuronal physiological state can produce different DEP-spectra. For two frequencies (10 kHz and 14 MHz) adhesion to the substrate and outgrowth of the neurons was investigated.**

Key words – **Cortical rat neuron, trapping, Dielectrophoresis, planar micro-electrode plate, neuronal outgrowth, adhesion**

I. Introduction

Dielectrophoretic (DEP) trapping is used to position neurons on particular spots, like an electrode tip in a planar multi electrode array that can be used for stimulation and measurement of neuronal activity. However, a significant requirement for using trapped neurons for stimulation or measurement purposes is that they survive the DEP treatment and stay positioned.

Non-uniform electric fields exert dielectrophoretic forces on polarizable particles [1]. The time-averaged DEP force F_{DEP} in an AC electric field is dependent on the in-phase component of the dipole moment for a particle p, which is suspended in a medium m, and can be written as

$$\vec{F}_{DEP} = 2\pi r^3 \varepsilon_m' \, Re[f_{CM}] \nabla \vec{E}^2_{rms} \qquad (1)$$

where r is the radius of the particle, f_{CM} is the Clausius-Mosotti factor, ε is the permittivity, and E is the electric field with the index rms denoting the root-mean-square value of this field.

A planar quadrupole micro-electrode structure was used for the creation of a non-uniform electric field that could trap neurons in the center of the structure by negative dielectrophoretic forces. Trapping of neurons has been investigated in quantitative and qualitative terms, i.e., the number of neurons trapped in the center, and their physiological state indicated by adherence of the neurons to the substrate and dendritic outgrowth, respectively.

II. Materials & Methods

Photolithographic, plasma deposition and etching techniques were used to create a quadrupole micro-electrode structure on a glass plate of 5 x 5 cm. A titanium-layer was used for the adhesion of the gold-layer on the glass plate. An insulation layer, consisting of a silicon-nitride layer sandwiched between two silicon-oxide layers, was applied to avoid electrochemical processes at the electrode-culture medium interface and to reduce unnecessary heating of the medium. The 'active regions', the regions around and in between the electrode tips were kept free of this insulation layer. The tips were triangularly shaped with angles of 90° and the inter-electrode distance between two diagonally opposing tips was 100 μm. Figure 1 shows the electrode structure.
The micro-electrodes, which lead to the side of the glass plate, were connected via wires to an BNC connector, which was mounted directly on the glass substrate.

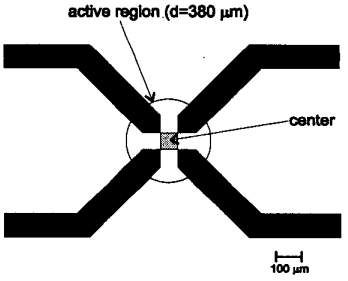

Figure 1. The electrode structure consisting of four electrodes. Indicated are the center (the yield is the number of neurons trapped in this region), and the active region, which is the region kept free of insulation.

For investigation of the viability of the neurons the electrode plate was coated with PEI (Poly-Ethylene-Imine).

Cortical neurons from rat fetuses of embryonic day 18 (E18) and of postnatal day 1 (P1) were used for the experiments in this study. The brains were removed from the decapitated rats, the meninges of the cortexes were taken away and the basal ganglia as well as the hippocampus were removed. The remaining cortexes were collected in a tube with the chemically defined R12 culture medium [2].
Dissociation and centrifugation at a rate of 1200 rpm for 5 minutes loosened the cells. Before using them for the experiments they were counted and medium was added so that

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a suspension of 10^6 neurons/ml was obtained. For each experiment 20 µl was used.

Three different amplitudes (1, 3 V and 5 V) in combination with ten different frequencies (10 and 100 kHz, 1, 4, 8, 12, 14, 18, 30, and 50 MHz) where used as field settings for the determination of the DEP-spectra (the yield as a function of frequency for each amplitude). The experiments were time lapse recorded by taking an image every 10 seconds for the duration of the experiment (30 minutes). For each setting three experiments were performed and the results of these experiments were averaged.
The yield is the number of (E18) neurons that were trapped and stayed trapped in the center while the field was applied for 30 minutes. This parameter was determined for each field setting.

For investigating adhesion and dendritic outgrowth of the (P1) neurons after field application an amplitude of 3 V and two different frequencies, 10 kHz and 14 MHz were used. The field was applied for 1 hour. After this time period the neurons were placed in an incubator (37 °C, 5% CO_2). Three hours after the experiment 2 ml medium was supplied to a ring of 3 cm in diameter that was glued on the glass plate for long term culturing. Adhesion and dendritic outgrowth are two directly observable parameters by which the physiological state of trapped neurons can be determined.

II. THEORY

The so-called *Clausius-Mosotti factor* (f_{CM}) is a mathematical presentation of the interaction between the electric properties (complex permittivities) of the particle as well as that of the medium in which they are suspended. Calculation of this factor gives the frequency-dependency of the dielectrophoretic force as a function of ε_p^* and ε_m^*.

$$f_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \qquad (2)$$

with the complex permittivity/conductivity $\varepsilon^* = \varepsilon' - i\varepsilon'' = \varepsilon' - i\frac{\sigma'}{\omega} = \frac{-i\sigma^*}{\omega}$ $(\omega = 2\pi f)$, where f is the frequency of the electric field.
The real part of this factor ($Re[f_{CM}]$) determines the direction of the dielectrophoretic force (equation (1)). Due to negative dielectrophoresis (*NDEP*) the particles are directed away from the electrodes in contrast to positive dielectrophoresis (*PDEP*).

In order to calculate the Clausius-Mosotti factor for a neuron, the neuron was represented by a *single-shell model*. This model implies that the neuron is a homogeneous sphere (the cytoplasm) enclosed by an insulating shell (the membrane) [3]. With this model the polarization-conduction terms from all possible, and eventually simultaneously active basic molecular mechanisms responding to the external field, are lumped together in one effective complex permittivity [4]. The following parameter values were assumed (or measured) for the neuron: radius r=5 µm, interior conductivity (cytoplasm) σ_{int}=0.75 S/m, interior permittivity $\varepsilon_{int}=80*\varepsilon_0$ (with ε_0 the permittivity of free space), membrane capacitance $c_m=18*10^{-3}$ F/m^2, and the conductivity of the medium in which the neurons were suspended σ_{med}=1.6 S/m.

Figure 2 shows that with media of high conductivity (>0.5 S/m) the real part of the Clausius-Mosotti factor as a function of the frequency is always negative [5].

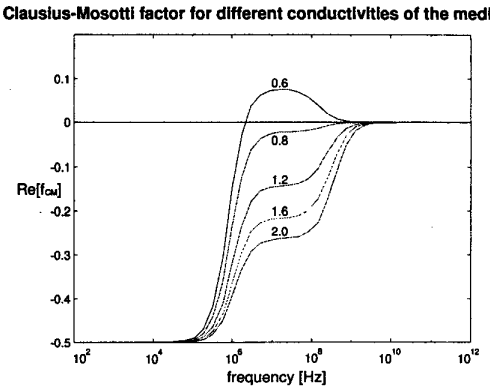

Figure 2. The real part of the Clausius-Mosotti factor for different medium conductivities (σ_{med}=0.6, 0.8, 1.2, 1.6, 2.0 S/m).

Since the dielectrophoretic force is dependent on the electric field and frequency the number of neurons trapped in the center of the electrode structure must have a certain relation with the amplitude and frequency of the field. The frequency dependency is included in the Clausius-Mosotti factor. The other factor determining the dielectrophoretic force is the gradient of the square of the electric field (see equation (1)). By representing the four electrodes by four point charges a simple analytical equation for this factor could be used.
Next to the dielectrophoretic force, a neuron (assuming it to be spherical) also experiences a viscous drag force according to Stokes' law. Once the neurons have precipitated the DEP force and the drag force are the only forces present. Assume that the neurons that precipitated within the center of the electrode structure move with a constant velocity in horizontal direction to be grouped in the middle of this square area. For this situation the following relationship for the yield (the number of neurons trapped in the center) was found.

$$\text{yield} = C_{res}\left(tq^2\,Re[f_{CM}]\right)^{1/3} \qquad (3)$$

Thus, the yield goes with power 1/3 for time, squared voltage, and real part of the Clausius-Mosotti factor (C_{res} is a resultant constant).

IV. Results

Figure 3 shows the yield as a function of frequency after 20 minutes of field application (1 V-experiments were left out of analysis since these were quite comparable to the zero-field situation). For frequencies up to 4 MHz the curves for 3 and 5 V are comparable, but for higher frequencies some peaks occur for the 5 V curve, where only one peak occurs for the 3 V curve.

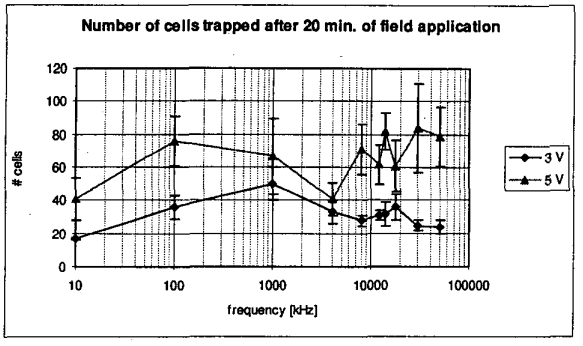

Figure 3. The yield as a function of frequency for 3 V and 5 V after 20 minutes of field application.

After 1 hour of field application (P1) neurons were already adhered to the substrate at 3 V/14 MHz. Figure 4 shows the situation after 4 days in vitro. However, at 5 V/10 kHz a group of neurons was trapped in the center but did not adhere. When medium was applied in the experiment of 10 kHz after 3 hours, most of the trapped neurons were swept away. After 1 day none of the trapped neurons was located at the trapping position anymore and therefore they could not be traced for dendritic outgrowth. Trapping of neurons at 3 V/10 kHz was not so clear (not a distinct group of cells in the center), but again no neurons could be detected in the center anymore after 1 day.

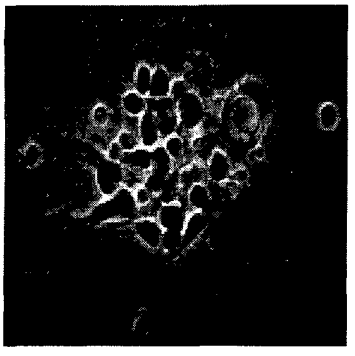

Figure 4. Field application for 1 hour at 3 V/14 MHz did not result in loss of adhesiveness. The image shows trapped neurons after 4 days in vitro (4 DIV), which did show protrusional outgrowth.

IV. Discussion

Comparing the experimental data (the yield as a function of time and frequency) with the theoretically found relationships (equation 3), the yield/time relationship did apply for almost all frequencies, whereas the yield/amplitude relationship did only apply for some frequencies. Figure 5 shows the yield/time relationship for 5 V/4 MHz.

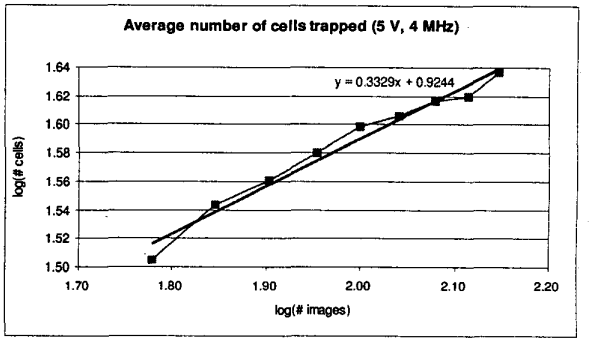

Figure 5. The logarithm of the yield at 5 V/4 MHz as a function of the logarithm of frequency. A linear trend line showed a slope of 0.33.

The theoretical yield/Re[f_{CM}] relationship did not apply to the whole frequency range. The graph of the real part of the Clausius-Mosotti factor (Figure 2) suggests that the number of cells collected should decrease up to about 4 MHz and stay quite stable above 4 MHz.

A decrease was not seen in figure 3 at the lower frequencies. Except for a peak around 14 MHz similarities were seen for the 3 V-curve starting at 1 MHz. This cannot be concluded for the curve showing the yield using an amplitude of 5 V over this frequency range.

The peaks occurring at the higher frequencies can be explained by the electrical behaviour of the electrode plate system. It was found by gain/phase measurement (one electrode was driven with a sinusoidal signal while the other three electrodes were probed one by one with and without medium applied to the culture chamber) that peaks occurred in the gain at frequencies above 1 MHz.

Another aspect playing a role at higher frequencies and amplitudes is the creation of local temperature increments due to the electric field. By measurement of the temperature in the medium just above the electrode structure a maximum temperature rise of 1.6 °C was created at 5 V/50 MHz. With decreasing frequency the temperature rise decreased. The maximum temperature rise for an amplitude of 3 V was 0.6 °C. Due to local heating of the medium a significant fluid flow may arise. In the center of the electrode structure an upward flow occurs and by circulation of the fluid, a flow over the surface of the electrode plate will bring the fluid from the neighbouring regions back to the 'active region'. Therefore, cells that whirled down in the outward regions can be brought from these regions to the center of the electrode structure through the force of this flow. Here, they will also experience a dielectrophoretic force which might keep them trapped there.

This force might contribute to the larger number of cells trapped at 5 V, than theoretically expected.
Adhesion of the neurons to the substrate in the center of the electrode structure is a prerequisite for keeping them localized. It can be concluded that the neurons that were trapped at 10 kHz had different physiological properties than those trapped at 14 MHz. It was already stated that low frequencies (<100 kHz) [6] can cause excessive charging of the cell membrane, which might lead to irreversible changes of the properties of the cell or even to cell death.
Differences in physiological state (like viable versus non-viable [7], or normal and cancerous cells [8]) may result in quite different DEP-spectra. Such physiological differences can explain the difference in expected and experimental DEP-spectrum for low frequencies.

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