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Short communication

Recording electric potentials from single adherent cells with 3D microelectrode arrays after local electroporation

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ABSTRACT

This short communication reports on the innovative method of the local micro-invasive needle electroporation (LOMINE) of single adherent cells. The investigation of cellular reactions in living cell cultures represents a fundamental method, e.g. for drug development and environmental monitoring. Existing classical methods for intracellular measurements using, e.g. patch clamp techniques are time-consuming and complex. Present patch-on-chip systems are limited to the investigation of single cells in suspension. Nevertheless, the most part of the cells of the human body is adherently growing. Therefore, we develop a new chip system for the growth of adherent cells with 64 micro-structured needle electrodes as well as 128 dielectrophoretic electrodes, located within a measuring area of 1 mm^2. With this analytical chip, the intracellular investigation of electro-chemical changes and processes in adherently growing cells will become possible in the near future. Here, we present first intracellular measurements with this chip system.

1. Introduction

The cell represents the smallest living unit. The basic structure of the cell membrane consists of a phospholipid double layer, a variety of functional proteins and other bound molecules (Singer et al., 1972). Active and passive ion transports as well as free diffusion through the membrane result in a potential difference between extra- and intracellular space. External electrical fields may induce pores with a size depending on the intensity of the applied field strength (Olofsson et al., 2003). Extracellular compounds may cross the membrane via these pores affecting cell metabolism. The physiological transmembrane potential V_m is described by the Goldman equation (Goldman, 1943).

In living cells, absolute values of V_m vary around −65 mV, depending on organism and cell type. Accurate measurements of absolute values of V_m are complex even with penetrating electrodes. However, it is feasible to measure voltage alterations. Up to now, transmembrane potential changes in adherently growing single cells can only be examined by the classical patch clamp approach (Hamil et al., 1981), invented by Erwin Neher and Bert Sakmann (Nobel prize for physiology or medicine, 1991). By their work, the measurement of membrane potentials and ion channel currents of cells became possible. Later developments permitted the automated analysis of single cells in suspension. Regarding adherent cells, the work of Neher and Sakmann in principle are still state-of-the-art. In the network state, these adherent cells cannot be analyzed with present commercial patch-on-chip systems, i.e. today's patch clamp setups for adherent cells (95% of the body cells) in principle function like 34 years ago (Neher and Sakmann, 1976) and are still the gold standard for ion channel analysis with adherent cells (Milligan et al., 2009).

At the same time, commercially available patch-on-chip systems are limited to cell suspensions (Stett et al., 2003; van Stiphout et al., 2005); nevertheless, most of all cell types of the human body are growing adherently on biological (e.g. nerve or liver cells) or artificial biocompatible matrices (e.g. polystyrene). However, all these methods, except for whole-cell patch clamp, are mainly used to investigate ion channel currents. In the following, we present a new method for the measurement of whole-cell V_m alterations after local electroporation of single cells growing on silicon chips. Since a measurement of absolute V_m values is not feasible, V_m alterations induced by pharmacological test substances or drugs shall be detected by our new Local Micro-Invasive Needle Electroporation (LOMINE). The additional use of dielectrophoresis (Gimsa, 2001) can help positioning single cells at specific sites of our biochip.

The patented LOMINE method (Baumann et al., 1998a, 1998b) hybridizes local electroporation with a patch-on-chip system. The LOMINE method uses voltage pulses applied to micro-structured needle electrodes (MNEs). In contrast to the classical electroporation-setup, where all cells experience an increased

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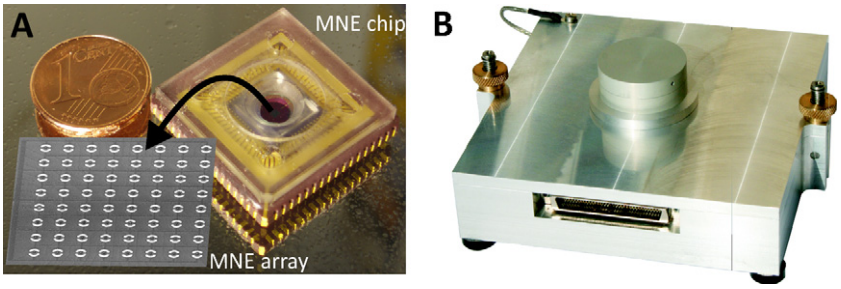

Fig. 1. The MNE chip and the chip system. (A) The MNE array with circular dielectrophoretic electrodes and (B) hardware setup.

transmembrane voltage leading to the formation of many membrane pores, LOMINE allows for a local electroporation of the membrane of a single attached cell that overgrows a single MNE. This overgrowing should not be left to chance.

In order to increase the number of successful LOMINE measurements, different techniques could be applied. Defined networks with small numbers of cells are a prerequisite for reproducible signal acquisitions and the observation of specific information exchange during LOMINE. This is difficult and complex in non-defined cultures. Additional methods have to be applied for culturing single cells, cell aggregates or networks in a predetermined way. Photolithographical techniques (Shu et al., 2004) and dielectrophoresis (DEP) can be applied to allocate cells to a specific adhesive site or to electrically conducting chip structures (Heida et al., 2001; Prasad et al., 2003). For cells, DEP was first described in the 1950s (Pohl, 1951) and was chosen to be the best suitable method for LOMINE because it is easily applicable. Uncharged particles or cells can be moved by DEP induced in a non-uniform AC field of a given frequency when their effective polarizability exceeds that of the suspension medium (Gimsa, 1991; Gimsa, 2001), i.e. the polarizability of the particle or cell is predominantly responsible for the dielectrophoretic effect. This polarizability is the frequency dependence of the dielectric constant of the particles or cells. This dependence is already known under different conditions and for different cell types for a long time (Schwan, 1957). The field-induced dipole moment leads to a net movement of the whole cell into (positive DEP; pDEP) or away from (negative DEP, nDEP) regions of higher field strength (Gimsa et al., 1996). Next, the choice of buffer is important for successful pDEP at frequencies in the MHz-range. The external buffer conductivity must be lower than that of the cytoplasm for pDEP, and higher for nDEP, respectively. Accordingly, the cytoplasm permittivity must be higher than that of the external buffer for pDEP and lower for nDEP. Choosing the right buffer conditions, the scientist must balance between high-nutrition buffer with high conductivity and good pDEP performance in low conductive buffer. High-nutritive medium will result in nDEP (cell repulsion). Low conductive buffer will lead to cell damage, weak cell adhesion, poor neuronal sprouting, cell starvation and finally cell death during pDEP. Higher voltages must be applied for larger electrodes and/or electrode distances. However, higher voltages and/or lower frequencies (nDEP) may cause cell damages. The movement depends on factors like cell shape, cell parameters, and medium conductivity (Gimsa, 1991; Jones, 1995). The dielectrophoretic force (F_{DEP}) (Gimsa, 1991) of a spherical cell is given by:

$$F_{DEP} = \frac{2}{3}\pi \cdot \varepsilon_0 \cdot \varepsilon_e \cdot r^3 \cdot \mathrm{Re}(K) \cdot \nabla E^2$$

here ε_0, ε_e, and r stand for the dielectric constant of the vacuum, the relative dielectric constant of the surrounding medium and the radius of the cell, respectively. Re(K) and E stand for the real part of the Clausius-Mossotti-Factor, i.e. the frequency-dependent part of the induced dipole moment and the electric field (Gimsa, 2001).

Practically, DEP is easily applicable because the same electrodes can be simultaneously used for cell allocation and electric detection. In this short communication, pDEP was applied to special DEP electrodes to allocate L929 cells at the MNEs of the PoreGenic® chips.

2. Materials and methods

2.1. PoreGenic® – a bioanalytical micro needle chip

The PoreGenic® chip comprises different relevant compounds. In short, a 68-Pin-CLCC (Ceramic Leadless Chip Carrier) carries a silicon chip comprising 64 MNEs arranged in an 8 × 8-microelectrode array (MEA; Fig. 1A). Chip layout and production were adapted to the requirements imposed by the LOMINE (Trautmann et al., 2004; Held et al., 2008b). The chip is completed by a fluidic chamber that is glued on the silicon chip. The chip allows for long-term culturing of adherently growing cells, simultaneous local electroporation of independently selectable single MNEs and measurement of intracellular transmembrane potentials of cells. The fabrication process of the MNEs is given elsewhere (Held et al., 2008a).

2.2. System setup

The starting point of our technical development was a suitable equivalent circuit reflecting the substantial relations between cell, bioanalytical chip and electronic setup (Tautorat et al., 2007). The cell overgrows the MNE in the chip and this MNE is controlled and analyzed by the electronic circuitry. Our preliminary test version allows for the parallel individual control of 16 MNEs (Fig. 1B). The electronic periphery of each electrode contains circuits for cell manipulation and the measurement of the needle potential. Progressive rotation of the chip in the socket by 90° allows us to contact 16 electrodes in each of the four positions and to use all 64 MNEs. Programming of the process control under LabVIEW® and the use of a multifunction data acquisition card reduced developmental time and effort. Two different circuits were used for intracellular potential measurement. 12 voltage-followers and 4 current-to-voltage converters were implemented for the needle potential measurements. While voltage-followers are limited to voltage measurements, the advantage of current-to-voltage converters is the possibility to apply the voltage clamp mode. A defined test pulse can be applied if necessary. These properties allow for an examination of the electrical characteristics of the electroporated cell, e.g. testing of the Gigaseal quality (R_{seal}). The measuring signals of all 16 electrode channels are sampled at 15 kHz with a resolution of 0.1 mV and accordingly 0.6 nA. The present test application permits the creation of rectangular single pulses (bi- or unipolar) with a minimum pulse interval time of 5 μs. Amplitude, duration, and polarity of the pulses are user-defined. We use special reed-relays and tri-state circuitry to minimize stress effects for the cell caused by leak currents, etc. Our convenient soft-