INTRACELLULAR POTENTIAL MEASUREMENTS OF ADHERENTLY GROWING CELLS USING MICRO-NEEDLE ARRAYS

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ABSTRACT

We present a new sensor chip system for intracellular potential measurements of adherently growing cells using micro-structured needle electrode (MNE) arrays. Existing methods for intracellular investigations are time-consuming, tedious or limited to the analysis of suspended cells. However, most biological cells grow adherently. To overcome these methodological limitations a novel technique, local micro-invasive needle electroporation (LOMINE) in MNE arrays, has been developed. LOMINE opens the cell membrane for introducing a MNE into the cytoplasm. This paper describes the fabrication process of the MNE-array chips and first cell electroporation experiments.

KEYWORDS: electroporation, patch-clamp, membrane potential, lab-on-a-chip

INTRODUCTION

The patch-clamp technique developed by Neher and Sakmann [1] is the classical method for studying electrophysiological properties of cells. In the *whole-cell* configuration, patch-clamp allows for intracellular recordings [2]. The minimal patch-clamp setup comprises a microscope with a micromanipulator placed on a vibration isolated table within a Faraday cage, a highly sensitive amplifier, a pulse generator, and data-recording devices. Due to this technical complexity, an experimenter needs a considerable experience to perform these experiments. On the other hand, *patch-on-chip* systems simplify, miniaturize and parallelize patch-clamping [3]. However, these systems are limited to the analysis of ion channel currents of suspended cells. Motivated by these facts, a small-sized measuring system has been developed containing a silicon chip with 64 *micro-structured needle electrodes* (MNEs) for parallel intracellular recordings from adherently growing cells. The MNEs are arranged in arrays of 8×8 electrodes with a pitch of 100 µm. For the proof of principle, we developed an electronic setup with 16 electrode channels (Fig. 1c). It allows for the signal acquisition from all 64 MNEs applying a sequential chip rotation by 90°. A commercial data acquisition card (NI PCI-6221) is used for signal digitization and system control. The graphical user interface and the fully automated process control are based on a LabVIEW® program.

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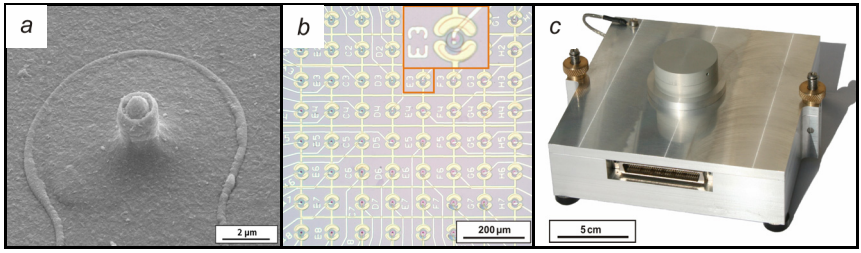

Figure 1. Sensor chip system: (a) Scanning electron micrograph (SEM) of a MNE; (b) micrograph of MNE-array with kidney-shaped DEP electrodes and on-chip electrode labeling; (c) 16 channel head-stage.

MATERIAL AND METHODS

The biological cell represents the smallest living unit. Cells are confined by a thin (approx. 6 nm) membrane consisting of a phospholipid double layer, a variety of functional proteins and other molecules [4]. Active and passive ion transports as well as ionic leak fluxes through the membrane generate a potential difference between the extra- and intracellular bulk media. The resting transmembrane potential is approx. -40 mV, depending on organism and cell type. As a first system application, we aim at the detection of transmembrane potential alterations, e.g. induced by pharmacological test substances or drugs.

The novel technique of *local micro-invasive needle electroporation* (LOMINE) is designed to establish an electrical connection between an individual MNE and the cytoplasm of a single cell. An electrical pulse applied to the MNE, induces high local field strength at the MNE-tip which modifies the lipid structure of the membrane, i.e. the binding forces are overcome allowing for a reassembling of the phospholipids. As a result, the electrode may penetrate the cell due to *local electroporation* (LEP), as illustrated in Fig. 2. Appropriate LOMINE parameters for field strength and pulse duration are required to obtain membrane resealing around the MNE after LEP and gigaseal formation [1]. A stable gigaseal between the cell membrane and the MNE is absolutely essential for defined intracellular measurements.

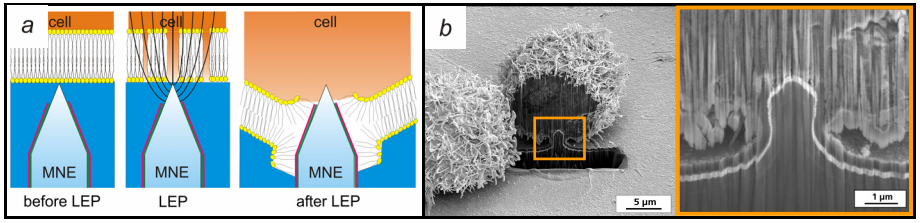

Figure 2. LOMINE: (a) Schematic cross-sectional view; (b) FIB preparation and SEM cross-section of an electroporated L929 cell with penetrated MNE.

The MNE fabrication technology is based on a three-step silicon dry etching process combined with the insulation, metallization and passivation of the silicon

micro-needles using standard micro electromechanical systems (MEMS) processes. The passivation layer at the tip of the MNE is opened by means of reactive ion etching in order to expose the metallization [5-7]. Depending on the three-steps etch sequence, MNEs with diameters as small as 1 µm, heights below 5 µm and different geometries can be realized [8]. The chip design, as reported in [6], was extended by integrating 128 planar kidney-shaped dielectrophoresis (DEP) electrodes [7] as shown in Figs. 1b and 3a. In this context, dielectrophoretic cell positioning is a useful tool to increase the probability of cell location at the needle electrode and to lower the required cell count used in the experiments.

RESULTS AND DISCUSSION

Preliminary experiments have shown that human skin fibroblasts, L929 cells and primary cells of mice (glia, neurons) are successfully positioned using DEP and grow on MNE-arrays (Fig. 3a). First LEP experiments were accomplished and results were investigated by means of fluorescence microscopy as well as by scanning electron microscopy (SEM) together with focused ion beam (FIB) preparation [10]. For the FIB technique, the cells on the MNE chip were prepared by conventional critical point drying. Cross sections of the cell-chip interface were prepared with FIB to observe the penetration of the MNEs through the cell membrane. Fig. 2b shows a respective FIB-SEM cross-section of an electroporated L929 cell in two magnifications, whereas Fig. 3b illustrates LEP results in a fluorescence microscopy image.

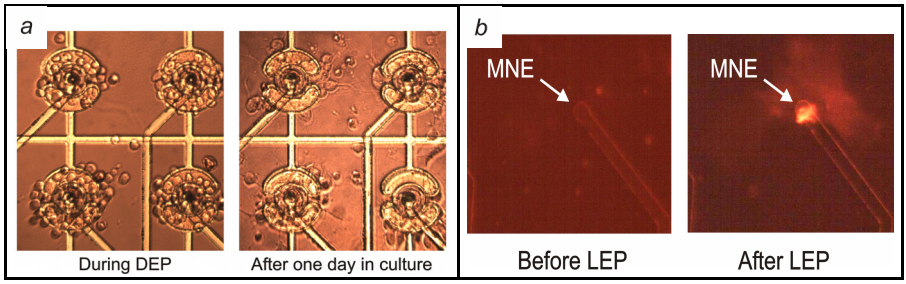

Figure 3. DEP and LEP experiments: (a) Positioning of prenatal murine neurons using DEP (MNE pitch 100 µm) and (b) fluorescence microscopic view of an electroporated human skin fibroblast (fluorescent stain: rhodamine phalloidine).

In addition, LEP-pulse experiments in cell-free chips were performed to investigate superimposed effects from the electronic and electrochemical properties of the system (electrode polarization, local-element effects, time constants of the circuitry, etc.). It will be essential to eliminate these effects for defined intracellular potential measurements.

CONCLUSIONS

Our sensor chip system is a new approach for parallel intracellular measurements in adherently growing cells. DEP and LEP were successfully demonstrated by fluorescence microscopy and FIB-SEM images. Our next goals are to increase the

electrochemical stability of the MNEs and to improve the electronic circuitry and system control of the LEP-sequence.

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