Nichrome Electrode Technology for Cell Culture Monitoring

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***Abstract*—The aim of this work was to present a method of tissue culture research by measuring the impedance of cells cultured in the presence of nichrome. For this purpose, the Electric Cell-substrate Impedance Sensing (ECIS) system was used with the substrate consisting of nichrome electrode arrays. The electrodes were made using a thin film magnetron sputtering. In the experimental part, the culture of cells of mouse fibroblasts on the prepared substrate was performed.**

Keywords-BioMEMS; ECIS; nichrome (NiCr); thin film.

I. INTRODUCTION

A local heating of biological substances in Biomedical MicroElectroMechanical Systems (BioMEMS) devices can be performed using a contact or noncontact method. For the contact method, the heating element is mainly fabricated using thin film deposition techniques (e.g., thermal evaporation or magnetron sputtering). The selected material should be biocompatible and should not react with the active substance. Due to the ability to withstand high temperatures, good chemical stability and biocompatibility, platinum is the most commonly used material for contact heating [1]. However, alternative metals are used for many biomedical applications, in particular for disposable structures, or for short-term applications. Some other metals offer different chemical, physical and electrical properties than platinum, which is also an expensive material. In biomedical microdevices, the heaters are also made of nickel, aluminum, tungsten, silver alloys, aluminum alloys and Indium-Tin Oxide (ITO) [2]. However, one of the most interesting materials is nichrome (Ni-Cr 80/20 wt. %), due to its high stability of electrical properties, high resistivity, low Temperature Coefficient of Resistance (TCR), adequate price and technological simplicity [3][4].

The main problem of the choice of material for the heater in biomedical applications is the assessment of the influence of its presence on the cells or substances tested [5]. The aim of this work is the presentation of the extension of the Electric Cell-substrate Impedance Sensing (ECIS) method [6] to study the activities of cells grown in tissue culture in the presence of nichrome.

The paper consists of 4 sections. Section II describes the fabrication approach of the nichrome electrode array. The results of monitoring the cells behavior in tissue culture are presented in Section III. We conclude the work in Section IV.

II. TECHNOLOGY OF NICHROME ELECTRODE ARRAY

The original ECIS method was used for the first time in 1984 by competing with microscopic methods. This impedance-based cell monitoring technology uses sterile, disposable arrays of gold electrodes placed on a biocompatible substrate [7][8]. Based on standard 8 well arrays, a mask was designed with eight electrodes located on a single substrate to work with ECIS instruments. Single electrodes were designed as comb capacitors in which the width of a single finger was 200µm.

A 2mm thick polycarbonate was used as the substrate. The key step in the sequence of technological processes was the fabrication of the metallization layer by magnetron sputtering using the Kurt J. Lesker NANO 36™ deposition tool. The next step was to obtain shapes in the lithography process and to etch the nichrome layer. Special polystyrene wells were placed on the electrodes and fixed using biocompatible silicone (Figure 1).

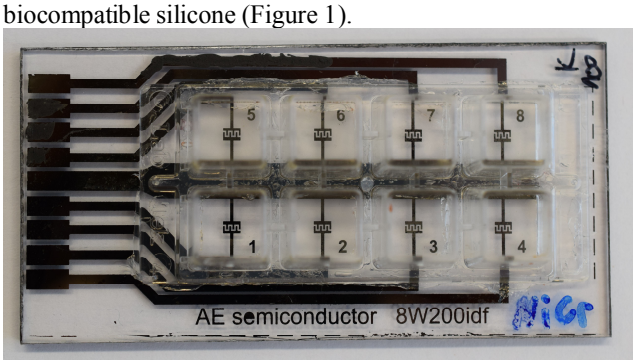

Figure 1. Polycarbonate substrate with 8 wells and electrodes made of nichrome

Each of the 8 wells with a volume of 600µL and a substrate area of 0.8cm^2 contains a single comb active electrode. For the whole setup to be sterile, ready-made

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substrates with attached wells are subjected to bactericidal ultraviolet radiation.

In the experiment, cells of mouse fibroblast cell line, - NCTC clone 929 [L cell, L-929, derivative of Strain L] (ATCC® CCL-1™) derived from ATCC organization were cultured according to the instruction manual in complete Eagle MEM medium (Sigma Aldrich) supplemented with 10% Fetal Bovine Serum (FBS) Good HI, in an Galaxy 170R incubator, under controlled growth conditions, constant humidity and air saturation of 5% CO_2. After (approx. 7–14 days) the culture reached at least 75% confluence, the next stage was culturing the cells on the tested nichrome electrode array. Inoculation of arrays was carried out by 300 microliters per well of cell (L929) suspension at ~1.2×10^5cell/ml. Every cell type has its characteristic adhesion and growth curve that can be manipulated by, e.g., varying seeding density or other stimuli like concentration of substances in the medium [9].

III. RESULTS OF EXPERIMENT

During the experiment, it was found that the resistance increased, reaching 4750 ohms, during the initial 10 hours of cells culturing (Figure 2). This indicates good cell viability and proliferative potential. The following drop in resistance indicates that the nichrome electrode used in the system makes it difficult to achieve stabilization in culture. After the time of 20 hours, resistance begins to fall, which should be interpreted as a progressive cell death. However, it should be noted that despite the difficulties, the fibroblast cells used in the study, as a result, maintain the growth and proliferation process (as evidenced by a stable resistance value of over 2500 ohms) in the environment of the nichrome electrode.

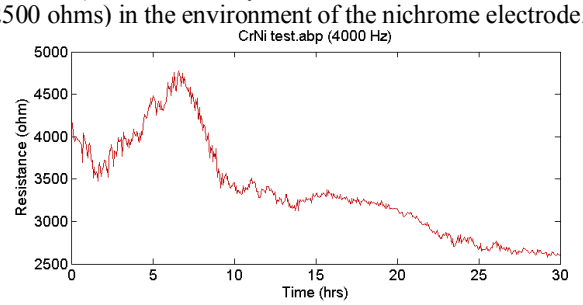

Figure 2. Resistance response measured by an ECIS sensor array at 4kHz.

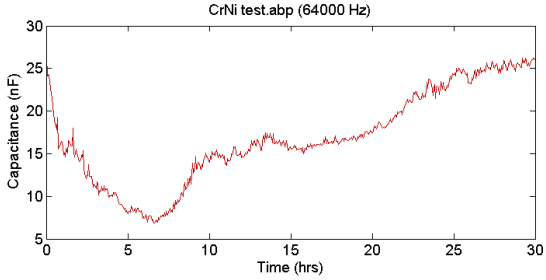

Figure 3. Capacitance response measured by an ECIS sensor array at 64kHz.

Similar fluctuation was observed for the high-frequency capacitance. The resistance represents the quality and function of the cell barrier and therefore takes into consideration the resistance towards para- and trans-cellular current flow. Capacitance provides an overall measure of electrode coverage [10]. The decrease in capacitance (Figure 3) reached during the initial 10 hours indicates fibroblast cell proliferation while the increase in the resistance should be interpreted as cell proliferation and for that matter both values complement each other and should be analyzed in parallel as a standard.

IV. CONCLUSION AND FUTURE WORK

In this paper, we demonstrated that the nichrome electrode had a significant effect on the resistance and capacitance of L929 cell line but did not kill them, that indicates the possibility to use the examined medium. However, as the studies have been carried out on cells characterized by stable growth, it is necessary to test the nichrome electrode for other types of cells, including cancerous ones.

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