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NICHROME CAPACITORS ON POLYCARBONATE SUBSTRATE FOR MONITORING CELL CULTURE USING IMPEDANCE SENSING TECHNIQUE

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 system was used with a prototype substrate containing comb capacitors made of nichrome. Magnetron sputtering, photolithography and etching processes were used to produce the thin-film electrodes. In the experimental part, cells of mouse fibroblast cell line L929 were cultured according to the instruction manual in complete medium, under controlled growth conditions. Inoculation of arrays was carried out by 300 microliters per well of cell suspension at ~1.2×10^5 cells/ml. The results of the monitoring cells behavior in tissue culture indicate good cell viability and proliferative potential.

Keywords: bioimpedance, ecis, sputtering, nichrome, fibroblast

1. Introduction

Lab-on-a-Chip (LOC) devices used in cellular engineering allow for cell culture under conditions similar to those *in vivo*. Their basic advantages include low consumption of substrates, minimal risk of sample contamination, precise dosing of reagents and controlled reaction conditions. The small sample volume determines a small amount of waste after analysis and guarantees quick temperature changes with low energy consumption. As a result, the cost of testing using analytical microsystems can be reduced compared to traditional systems. Miniaturization enables mass production, which positively influences the price, the availability of specific tests and the use of disposable devices.

The microdevices can include a resistor for local heating of biological samples. One of the problems of the choice of material for the heater is the need to assess the influence of its presence on the substances tested [1]. The grouping of materials according to the histopathological studies of implants, including their impact on the living organism, it is possible to identify three main collections: non-reactive, reactive and toxic materials. In the case of the contact method, the heating element is usually made of platinum (non-reactive) using thin film deposition techniques [2]. An alternative material for heating applications is nichrome (Ni-Cr 80/20 wt. %), which is considered to be reactive materials [3]. However, this material has very good electrical properties, is technologically simple and is much cheaper than platinum, which significantly affects the price of the final device. Analysis of the advantages of the nichrome allows qualifying it to materials that do not affect living organisms or whose influence is negligible. The issue is the effect of the use of nichrome on the biological sample, because the corrosive properties of metals and their biotolerance are not yet fully understood [4-6], especially for devices that are introduced into the body. An allergic response reflects the state of hypersensitivity after exposure to a specific substance. An allergic reaction is an exaggerated behavior of the body's defenses [7]. Therefore, it is important in biomedical devices to understand the effect of NiCr on a biological substance.

2. Methods and materials

2.1. Electric cell-substrate impedance sensing (ECIS)

Measurement of the impedance of live biological cells is increasingly used because it is a non-invasive and quantitative analytical method for assessing cell status. Typical methods are electric cell-substrate impedance sensing (ECIS), impedance flow cytometry and electric impedance spectroscopy [8]. The

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Electric Cell-substrate Impedance Sensing method is based on the non-invasively monitoring of the frequency-dependent electrical impedance of the culture cells covered the gold-film electrodes along the time course of the experiment. This unique, monitoring technique was created by Dr. Ivar Giaever and Dr. Charles R. Keese, the founders of Applied BioPhysics Inc. It is an *in vitro* method that allows the analysis of cell activity based on morphology, ability to reproduce, division or movement. It is an excellent alternative to studies using optical microscopes, where the results are obtained on the basis of observations [9].

The main element of the sensing device is a small gold electrode deposited on the bottom of tissue culture vessels. When cells attach and spread on this electrode, it affects the current flow, which changes the measured impedance. The impedance changes mean an important dynamic information about the shape and movement of the cells. The standard test substrate is made of PET or polycarbonate.

The measurement is performed by switching the alternating current <1 mA, which flows through the cells. At frequencies below 2 kHz, a significant amount of current flows intercellular spaces, providing information on the adherence of cells. The use of a relatively high frequency, approx. 40 kHz, causes the current to flow directly through the cell membrane. As a result, information about the amount of electrode coverage by cells is obtained. For simplicity, the cells can be presented as a parallel connection of a resistor with a capacitor. The duration of the experiment depends on the user and can take a few seconds or several days. The software supplied with the ECIS device allows measurements to be carried out at well-defined values of the signal frequency in the range of 62.5 Hz to 64 kHz. Data obtained from a typical fibroblast L929 culture are shown in Fig. 1.

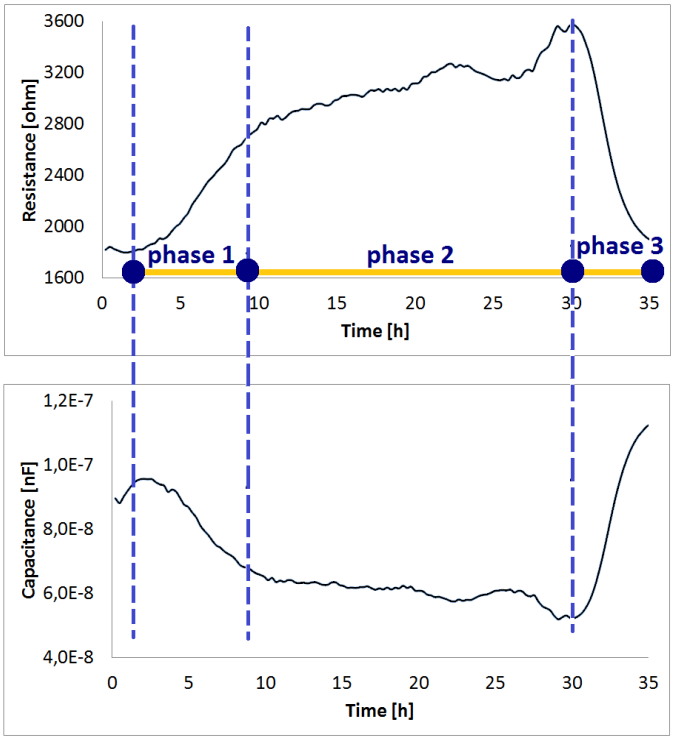

Fig. 1. Resistance (top) and capacitance (bottom) response of mouse fibroblast cells on measured by an ECIS sensor array with golden electrode at 4 kHz and 64 kHz, respectively

These are the characteristics obtained during the culture of mouse fibroblast cells on a standard substrate. Each parameter reading is plotted as a point, in ohms for resistance or nanofarads for capacitance, verses time. As cells grow and cover the electrodes, the current is impeded in a manner related to the number of cells covering the electrode, the morphology of the cells and the nature of the cell attachment (and resistance grows – it is a phase 1). Phase 2 shows the stabilization of the cell culture. In phase 3 the cells die, their adhesion decreases, therefore the resistance decreases and the capacity is growing.

2.2. Technology of nichrome electrode array

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 200 µm.

A 2 mm 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 (Fig. 2).

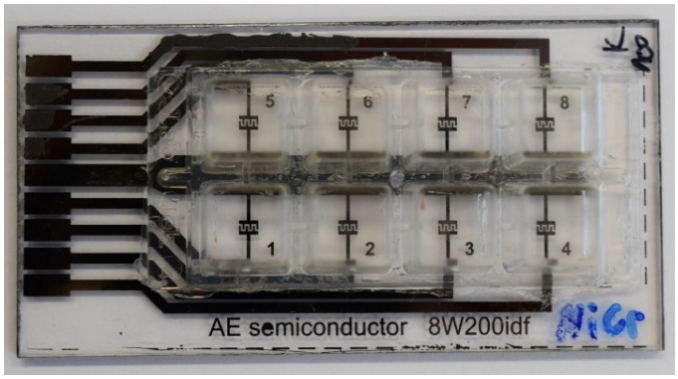

Fig. 2. Polycarbonate substrate with 8 wells and electrodes made of nichrome

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

2.3. Cells used for the experiment

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 ~1.2×10^5 cell/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 [10].

3. Results of experiment and discussion

During the forty-hour experiment, it was found that the resistance began to grow after 5 hours of cell inoculation (Fig. 3). This indicates good cell viability and proliferative potential. The low dynamics of resistance increase indicates that the nichrome electrode used in the system makes it difficult to achieve stabilization in culture. After 30 hours, in the well W3 the resistance begins to fall, which should be interpreted as progressive cell death. In the well W4 after 40 hours there is still a stable state. However, it should be noted that despite the difficulties, the fibroblast cells, as a result, maintain the growth and proliferation process (as evidenced by a stable resistance value) in the environment of the nichrome electrode.

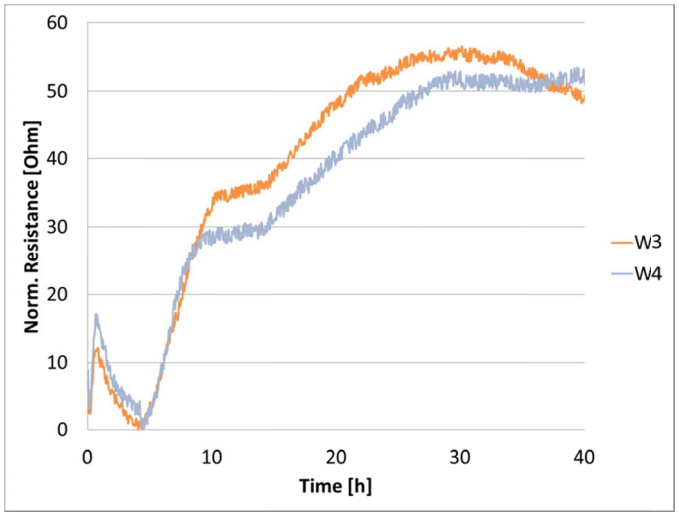

Fig. 3. Resistance response measured by an ECIS sensor array with NiCr electrode at 62.5 Hz

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 [11]. The decrease in capacitance (Fig. 4) reached during the 30 hours in well W3 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.

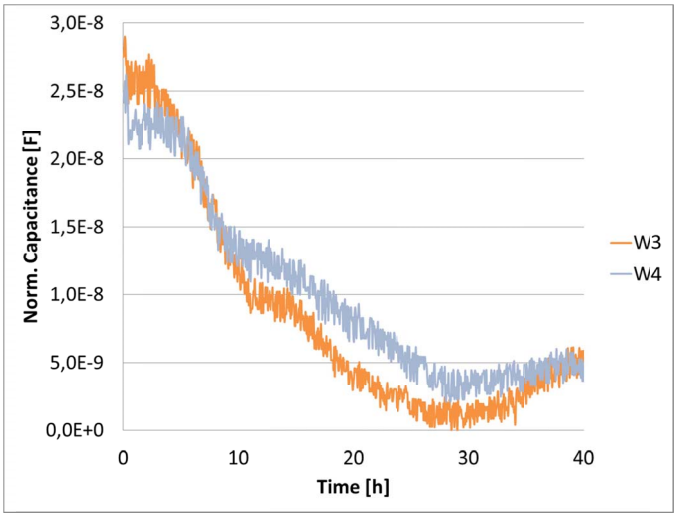

Fig. 4. Capacitance response measured by an ECIS sensor array with NiCr electrode at 64 kHz

Fig. 5. The dead cells on one of the electrodes after the experiment

The study indicates that it is possible to use deposition of thin layers of metallization for the analysis of the influence of the presence of metal on the cell culture. This allows extending the measurement capabilities of the ECIS system with the function of assessing the development of the cells cultured on electrodes made of various materials. The cell culture carried out for 40 hours in the presence of nichrome allowed to observe the growth phase, stabilization and the beginning of the cell death phase. After the experiment, a photo was taken with an optical microscope on which fibroblasts are clearly visible (Fig. 5).

4. Conclusion

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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