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Nickel comb capacitors for real-time monitoring of cancer cell cultures

Abstract. *The work is devoted to the technology of biocompatible substrates with nickel electrodes for in vitro impedance cell culture studies. The legitimacy of this subject was tested by conducting measurements using a system based on the Electric Cell-Substrate Impedance Sensor method. A device for cell bioimpedance testing, made in thin-film technology, has been described. Parameters and applications of the material used for construction, which is commonly used nickel, are discussed. The results of preliminary studies on melanoma cancer cells from the A375 cell line were presented, during which the already used measurement matrices were used. An analysis of the observed changes and obtained results was carried out.*

Streszczenie. *Praca poświęcona jest technologii biokompatybilnych podłoży z niklowymi elektrodami do badań impedancji hodowli komórek in vitro. Zasadność podjęcia tej tematyki przetestowano przeprowadzając pomiary przy użyciu systemu opartego na metodzie ECIS (ang. Electric Cell-Substrate Impedance Sensor). Opisano przyrząd służący do badań bioimpedancji komórek, wykonany w technologii cienkowarstwowej. Omówiono parametry i zastosowania wykorzystanego do budowy urządzania materiału, którym jest powszechnie stosowany nikiel. Przedstawiono wyniki wstępnych badań nad komórkami nowotworowymi czerniaka z linii komórkowej A 375, do których użyto wykonane matryce pomiarowe. Dokonano analizy zaobserwowanych zmian i otrzymanych rezultatów. (**Kondensatory grzebieniowe z niklu do monitorowania hodowli komórek nowotworowych w czasie rzeczywistym**).*

Słowa kluczowe: Bioimpedancja, Urządzenie biomedyczne, Nikiel, Cienkie warstwy.
Keywords: *Bioimpedance, Biomedical devices, Nickel, Thin film*.

Introduction

The development of microelectronic devices contributes to increased comfort in many areas of life. It is also an opportunity to learn new possibilities for protecting human life and health. Analytical biomedical devices of new technologies allow to diagnose various types of diseases at the point of care.

This work presents measurement matrices made in thin-film technology, enabling measurements of electrical parameters of culture cells. Nickel was used to make capacitor electrodes on test polycarbonate substrates. It is a material with interesting parameters, but with a much lower biocompatibility than gold, platinum or titanium. However, the possibility of using nickel in many applications means that there is a need for research into the effects of nickel on cells. The device described in this work is compatible with the ECIS (Electric Cell-Substrate Impedance Sensor) measuring system for monitoring the vital functions of cells cultured in vitro. The use of this technique allows measurements to be made in real time that reflect the life cycle of cells (multiplication, development and death).

In many biomedical applications, the replacement of platinum or gold with a material with lower biocompatibility and a lower price can significantly reduce production costs. Especially if they are disposable devices or for short-term applications and the impact of the presence of a given metal on chemical and biological substances will be negligible. However, it should be investigated whether less favorable culture conditions will not significantly affect cell adhesion to the medium, their proliferation, and even death in early life.

The device will allow to monitor the vital parameters of cells cultured in the presence of nickel. The use of nickel electrodes can be an alternative to testing the level of biocompatibility and the impact of the presence of this material on living organisms.

Nickel

Nickel (Ni) plays a very important role in nature. It is a trace but necessary nutrient in plants and some animal species [1]. It has not been shown that its presence is necessary for the proper functioning of the body. Thanks to its unique physical and chemical properties and above average corrosion resistance, nickel it is a frequently used material [2]. It is used in many industry sectors, i.e. fuel and energy, chemical, metallurgical, electro-machine, printing and food. It is one of the main raw materials for the production of batteries, catalysts and also nano-materials [3]. However, the popularity of nickel also raises concerns about its impact on the human body. This issue is not well known by scientists [2]. Unfortunately, while short-term contact with trace amounts of nickel does not cause negative effects, long-term exposure or contact with excessive amounts of this element can lead to serious consequences like dermatitis, damage to the nervous system, and various types of cancer, including lung, liver and kidneys [2]–[5]. According to many dermatologists, skin changes are most often caused by long-term effects of products containing components made of nickel. These include surgical instruments, implantable medical devices, piercing accessories and jewelry. However, their nickel concentration is relatively low and can only cause non-cancerous health effects. If an increased dose of nickel is applied directly to the body, dangerous neurological disorders can occur. The first signs of severe poisoning include nausea, vomiting, blurred vision, pain dizziness, and coughing. Then, when inhaled or swallowed, nickel enters the bloodstream. In this way, the susceptible to toxins nerve center is damaged by inducing nickel-induced cytotoxicity in various types of nerve cells [6], [7]. This is an extremely dangerous situation. However, among the mentioned above, the biggest problem is carcinogenicity, which is diagnosed in both humans and animals [8]. Statistically, cancer cases were more often observed among employees who were exposed to nickel in the workplace [9]. Nickel levels in their blood, urine and tissues were significantly increased. This is extremely interesting, especially since generally oversensitivity affects only 2% of men and about 10% of women. Based on this, it can be argued that nickel may have carcinogenic properties. As a result, nickel is classified as toxic material to organisms [10]–[12]. These observations contributed to the increased interest in the impact of nickel on human health, which explains the fact that in 2008 nickel won the infamous title of allergen of the year [13]. Despite many research data, not all sources of

nickel have been known. Its impact on the human body brings many questions that remain unanswered till now. Therefore, work in this field should be continued in order to determine in detail the effect of nickel on living organisms.

Bioimpedance

Currently, methods based on microscopic observation and spectroscopy are most commonly used to assess the effect of elements on living organisms. After adding the mixture containing the element to the culture, the behavior of small organisms (e.g. Daphnia magna, Dugesia tigrina) or microorganisms (e.g. L929 murine fibroblast cell line, human lung adenocarcinoma A549 cells, human malignant melanoma cell line A375, luminescent bacteria e.g. vibrio fischeri) is monitored using a microscope [14]–[19]. The main criteria for assessing the effect of a given material on organisms are: LD 50 (Median Lethal Dose, defined as the amount of the substance required to kill 50% of the test population) and LC 50 (Lethal Concentration, that refers to the concentration in air or water that will kill half of the sample organisms that are exposed to it). At the same time, the following methods can be used: IC 50 (half maximal Inhibitory Concentration, which is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function) and EC 50 (Median Effective Concentration, being the concentration of a substance in an environmental medium expected to produce a certain effect in 50% of test organisms in a given population under a defined set of conditions). [20]. Using spectroscopy, spectra are interpreted. They are obtained as a result of the interaction of all types of radiation on the biological sample, marked with a given contrasting element [21]. Depending on the level of absorption, information is obtained on the amount, morphology, proliferation and apoptosis of the test sample. These types of methods include atomic absorption spectrometry, atomic fluorescence spectrometry and atomic emission spectrometry [22]–[25]. The alternative for these tests is bioimpedance measurement. This method is free from such disadvantages as the need to use microscopes to observe changes and the need for additional chemical compounds. It is used to assess the condition of tested cells, without chemical labeling that can affect the measurement obtained results, making it a non-invasive method. It allows to quantify cell populations, observe morphological changes and monitor biological processes. The most popular methods based on impedance measurement include impedance measurement of cells located on the surface of measuring electrodes (ECIS), impedance flow cytometry and impedance spectroscopy (EIS). In the work the method based on ECIS real-time cell impedance measurement was described in detail [26],[27].

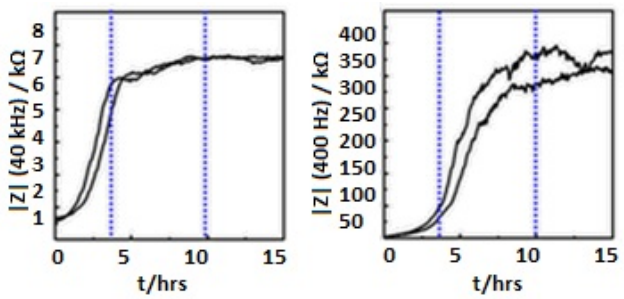

Fig. 1. Expected characteristics on the example of impedance measurement of Canine Kidney epithelial cells [37].

ECIS (Electric Cell-substrate Impedance) is a method of measuring cell impedance in real time. It enables the study of the activity of various cell cultures, referring to changes in their morphology, ability to reproduce and spread. This method has been used in toxicological tests, epithelial tissue function tests and diagnosing the invasive nature of cancer cells. Using the ECIS-based system, it is possible to observe the cell cycle taking place, ranging from proliferation to the dying of cell culture [26]–[28]. The ECIS measurement technique uses a single-shell model, which is based on Maxwell's theory of mixtures. He describes that the cell consists of a conductive sphere and an insulating coating. When measuring at frequencies below 2kHz, the current flows under and between adjacent cells. The use of frequencies above 40kHz causes direct flow through cell membranes. The experiment begins with the preparation of a plate with detection electrodes which are made of a thin layer of metallization. Special wells located above them are used to place inside them a medium containing the medium necessary for cell culture. Cell growth and migration causes a change in the measured impedance value. During the measurement, an excitation signal is supplied to the system in the form of alternating voltage. Then the system response is measured, which is the electric current. The ECIS system software, using special mathematical transformations, generates results and plots the characteristics of impedance change. Each value read is plotted as a point in Ohms (Ω) or nanofarades (nF), per unit of time. The duration of the experiment is set by the user and can last from a few seconds to several days. The differences between the measurement values are transferred for analysis, determining the effect of individual factors and other external stimuli on the properties of cells [29]–[34].

Experiment and results

As a part of the work, measuring matrices were made with nickel electrodes on a biocompatible polycarbonate substrate. Nickel was chosen because of its wide application range in various fields. It is worth to mention, that further research in order to determine its influence on living organisms is still being conducted. The possibility of performing experiments with its use would open new directions for research.

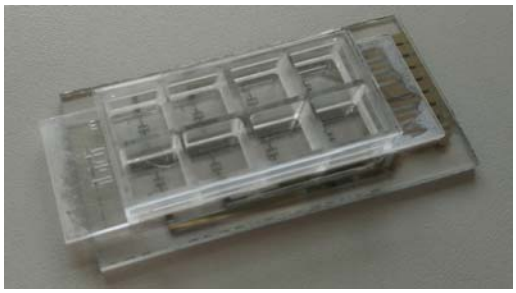

Fig. 2. Test matrix for measuring bioimpedance with eight-chamber ibidi GmbH plate with polymer bottom, compatible with ECIS measuring system.

Based on commercial 8W10E plates, a measuring matrix with a set of eight electrodes was designed. Each pair of electrodes is in the form of comb capacitors with dimensions of 200μm × 200μm. A 100nm thick nickel layer was deposited in the magnetron sputtering process, using a NANO 36™ sputtering system from Kurt J. Lesker. The obtained geometry was the result of the use of positive photolithography [35-36]. A chambered coverslip (ibidi GmbH) with 8 wells for cell culture were placed on the electrodes and fixed using biocompatible silicone (Fig. 3). Additionally, in order to ensure sterility of the designed matrices, all of the developed the structures were disinfected using UV light before use.

The aim of the work was to culture cancer cells and measure electrical parameters that change over time. The expected nature of the changes should have a course comparable to that obtained after conducting an experiment using commercial measuring matrices with gold electrodes. Measurements were carried out for different signal frequency values. The changes of chosen electrical parameters in the culture of human melanoma cells A375 line were measured. The presented graphs show the course of the measured value of resistance and capacity (Fig. 3) and impedance (Fig. 4) in the time domain.

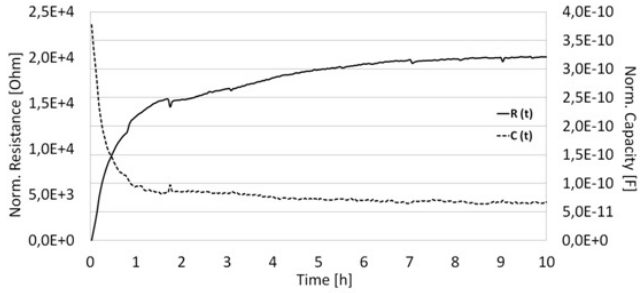

Fig. 3. Resistance response measured at 62.5Hz and capacitance response measured at 16kHz by a nickel sensor array for 10 hours.

The nature of changes was close to expected. The results indicate that cell proliferation has occurred. This is demonstrated by an increase in the impedance value to a level of about 20kΩ, at a frequency of 2kHz. At the same time, a decrease in the capacity value is visible, which confirms the correctness of conducting the test. Initially, the cells are in suspension. The impedance value is the lowest. Then the process of cell adhesion to the substrate begins. When cells are at the bottom of the well, they increase in size and multiply. This occurs when the environment does not adversely affect the cell population, or when the effect is not significant for the reproduction process. The impedance value increases until the stabilization visible around 10 hours, which means that the cells have reached maximum confluence.

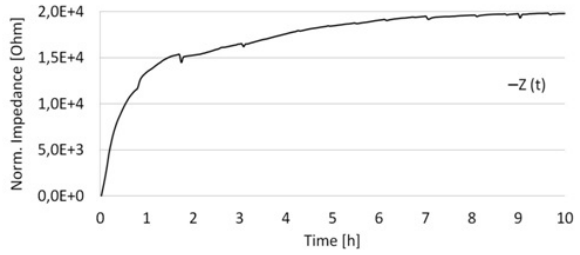

Fig. 4. Impedance response measured by a nickel sensor array at 2kHz for 18 hours.

The posted photos show the electrode on the measurement matrix used. Based on the obtained data, the discussed characteristics have been plotted. Pictures were taken after the experiment was carried out using a Keyence VHX 5000 digital microscope. As can be seen, the examined cells are visible on its surface. This confirms that cell proliferation has occurred. Also, it can be noticed that nickel did not negatively affect the cells that occupied the entire bottom surface of the well.

Conclusions

The article lists the methods currently used to study the effects of various factors on cell culture. The measurement method used during the experiment based on the ECIS system is described in detail. The electrical properties of A375 cell line were measured. The resistance, capacitance and impedance values are shown in the charts. Analysis of the obtained data was carried out. Photographs of measuring electrodes containing examined cells after the experiment were shown. The presence of nickel electrodes did not interrupt cells in a typical life cycle. Nickel, although toxic, did not cause cell death. It was possible to carry out a test culture. Promising results were obtained for the electrical parameters of the cells. Both electrical measurements and culture photos indicate that cell proliferation and multiplication occurred. This means that the substrate made with nickel electrodes can be used for further tests. It is possible that in the future the examined test matrix will be used to expand the research capabilities of the currently used ECIS measuring system by analyzing the impact of the presence of the material on living organisms.

Fig. 5. Nickel capacitor electrode with cells after made experiment (magnification ×100)

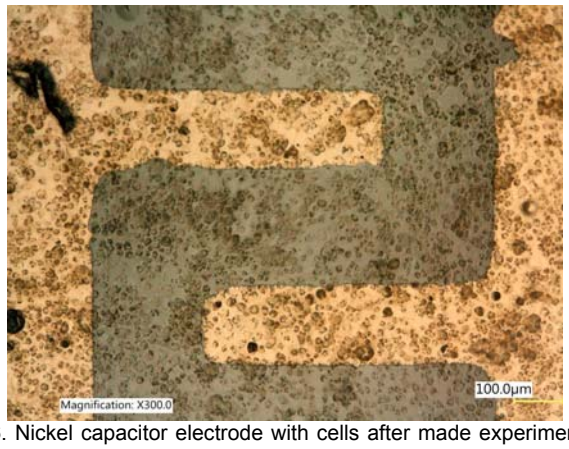

Fig. 6. Nickel capacitor electrode with cells after made experiment (magnification ×300)

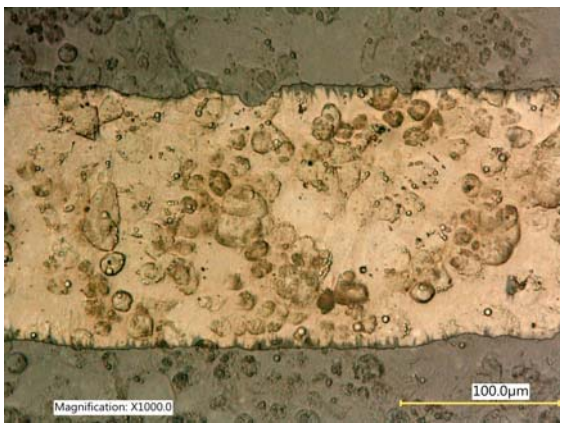

Fig. 7. Nickel capacitor electrode with cells after made experiment (magnification ×1000)

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