MONITORING VITAL FUNCTIONS OF A-375 MELANOMA CELL CULTURES VIA THIN-FILM NICKEL CAPACITORS

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Abstract

This article deals in the constantly developing branch of microelectronic devices used in various fields of medicine, i.e. diagnostics and treatment of previously incurable human diseases. A method for assessing and monitoring the vital functions of living cells by measuring cellular impedance in real-time using the ECIS® system and a commercial culture substrate is presented. The goal was to develop a substrate significantly less expensive than a commercial substrate that would be suitable for multiple uses and compatible with the ECIS® measurement station. Moreover, thanks to the use of a material with electrochemical properties other than the biocompatible material (gold or platinum) it is possible to observe the cells behavior with regard to the toxic agent. For this purpose, a culture substrate with nickel comb capacitors was used. To make the electrodes, a thin metal layer was sputtered on polycarbonate plates in the magnetron sputtering process. Prior to the next stages, technological masks were designed so as to fit in the ECIS® measuring station. Subsequently, the microelectronic processes of photolithography and etching the metal layer were performed. Finally, the wells were glued onto the culture medium with a biocompatible adhesive. The completed substrates were transferred to the Department of Human Physiology, Medical University of Lublin, for the culture test on A-375 human melanoma cells. The results of the experiment determined the usefulness of the device for monitoring cell culture vital functions by means of impedance measurement.

Keywords: *ECIS, nickel, thin layers, melanoma, magnetron sputtering*

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Introduction

Over the last few years, there has been much progress in the development of medical measuring devices due to the increasing number of incurable diseases in the modern world. The novel microelectronic technologies and strategies are to limit animal testing and yet allow monitoring cellular changes at the molecular level resulting from external stimuli or administered pharmacological agents [1-3].

One of the methods to assess the condition of cell culture is to measure its electrical parameters. This is a completely non-invasive technique, performed *in vitro*, which enables the relative determination of the number of cells in all the tested samples. Therefore, the obtained test results are accurate and quantitative [4].

The ECIS® impedance detection between the cell and the electrical substrate is a popular technique based on measuring the ratio of voltage and current in AC circuits using a sinusoidal signal [5-6].

This paper presents the results of a study conducted on a nickel electrical circuit with a suspension of A-375 melanoma cells deposited on its surface. The ECIS® technique consists in measuring the amount of the current flowing and the amount blocked by the culture cell membranes adjacent to the electrode surface. Due to the life cycles of cells and their ability to migrate, the experiments results changed proportionally to the impedance changes. Due to the possible long-term monitoring of the vital cells functions, the ECIS® method is gaining popularity among biological and chemical laboratory tests [7].

Materials and Methods

ECIS® apparatus in the study of cells vital functions

The ECIS® cell impedance measurement consists in using a biocompatible substrate on the surface of which there are 8 wells with thin-film comb capacitors. This technique has been widely used in *in vitro* studies to perform the cells behavior qualitative assessment. Microorganisms are tested in terms of multiplication, migration, barrier functions, transduction effects, cell invasiveness, cell response to toxins, electroporation effect, and many others [1]. The capacitors and cells can be converted to an equivalent electrical circuit containing a resistor and a capacitor. The capacitance of the capacitor surface can be treated as the capacitance of the well containing the cell culture medium. The electrode resistance prevails over the well resistance, whose value is oppositely proportional to the capacitor surface area. A simplified mathematical model of the ECIS® system is further discussed in the publication [8].

Tests performed on the ECIS® system begin with applying a culture medium onto the electrode surface. Then the cell culture is introduced and it enters phase 1 of its life cycle. In this phase, cells stick to the bottom of the well and begin to grow and multiply, thanks to a properly selected medium. The impedance increase depends on the decreasing free space on the capacitors surface occupied by non-conductive cell membranes [6]. In phase 2, the cells stabilize, which results in a virtually unchanging impedance value. The minimal increase or decrease in the value comes from the culture movement and the cells finding free spaces on the electrode surface. In phase 3, there is a decrease in resistance and, similarly, an increase in capacity due to cell death and the loss of their adhesive properties with the substrate [7]. The three phases of the cell life cycle are shown in FIG. 1 by the measured values of resistance and capacity during 35 h of the culture.

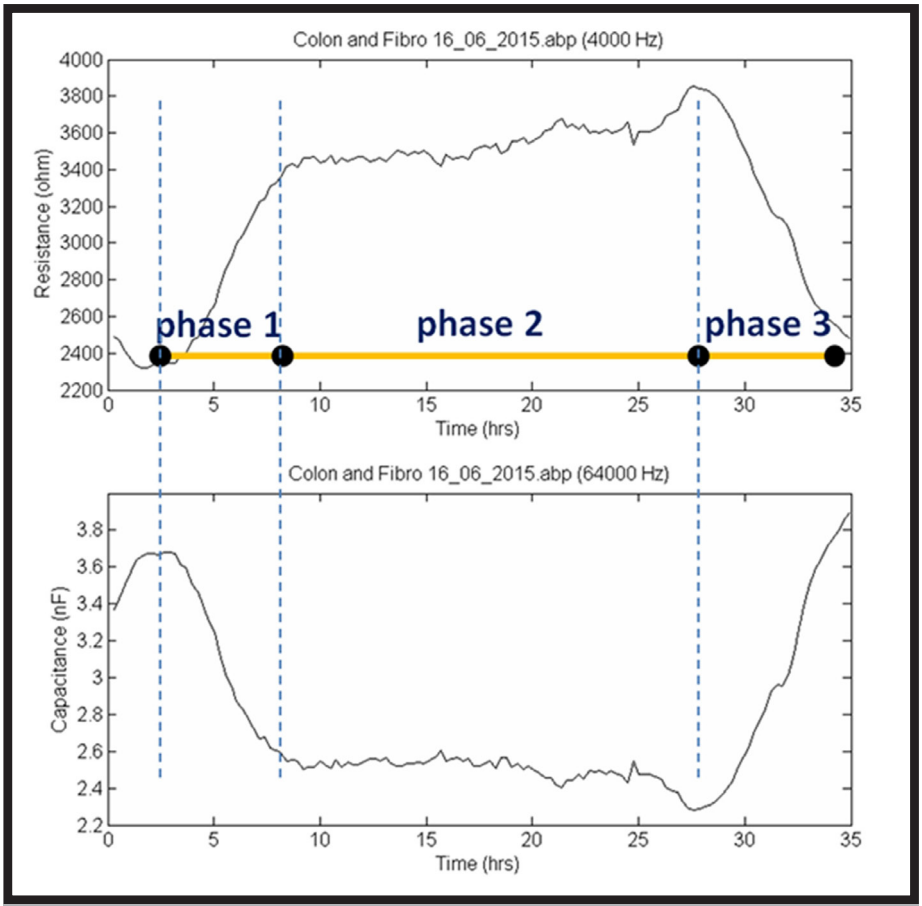

FIG. 1. Results of measurements of resistance (f = 4 kHz) and capacitance (f = 64 kHz) measured using a commercial ECIS® substrate [7].

Nickel

In order to obtain the test substrate, it was necessary to match the electrode size of the comb capacitor to the cells size. Commercial ECIS® plaques only use polyethylene terephthalate (PET) or polycarbonate (PC) substrates with gold or platinum measuring electrodes. The ECIS® apparatus also makes it possible to study the effects of heavy metals, as their properties are different from those of biocompatible metals measured on living cells. However, in order to carry out such tests, it was necessary to create dedicated measurement structures from other metals [9-11].

Biocompatibility is crucial in the case of prolonged contact with metal, e.g. implants. However, in many applications (e.g. qualification tests) the material has a short-term direct contact with the patient or organic material. In this paper, the aim is to create test substrates with nickel electrodes used to monitor the vital cells functions using the ECIS® measuring station and thus replace much more expensive commercial substrates.

Nickel and its alloys, due to their electrical properties, have been widely used in electronic microcircuits. Due to its high resistance to elevated temperatures, nickel is readily used in electroplating processes [12]. Advances in technology have led to the development of metal neural probes. One of such probes consists of a nickel shaft made via the electroplating process [11]. Additionally, diagnostic purposes often require elevated temperatures. In order to meet these requirements, a nickel micro-heater has been developed, which can maintain the operating temperature of 250°C throughout the duration of the experiment [13]. Nickel is also used in the CellTracks cell analysis system [14].

Metallization

In order to deposit a thin layer of metal, the magnetron sputter deposition of nickel was carried out on a 2 mm thick rectangular polycarbonate plate. This technique is based on the evaporation of material particles coming from the source (target) under the influence of ionized electromagnetic field energy of inert gases (in this case argon). The process temperature did not exceed 70°C so as not to damage the substrate material. Before initiating the atomization process, it was necessary to obtain a sufficiently high vacuum of 10^{-7} Torr. As a result, a nickel layer with a thickness of ~100 nm was obtained.

Design of technological masks and the etching process of photosensitive emulsions and metal

As part of the experiment, the test substrates with nickel electrodes were made. The first step was to design technological masks that were modeled on a commercial electrode system with eight wells. Each pair of electrodes was made as a 6-finger comb capacitor, the width of a single finger and the distance between them measured 200 µm. The second step was to apply a photosensitive emulsion to the sputtered nickel layer. In the photolithography process, the photosensitive emulsion was exposed to UV radiation through the mask. Then, the etching of the emulsion and metal layers was performed with chemical reagents. The final step was to clean the substrate in an ultrasonic washer, fix the wells to the culture media with biocompatible silicone, and sterilize them with ultraviolet radiation. FIG. 2 shows the electrode array with 8 wells.

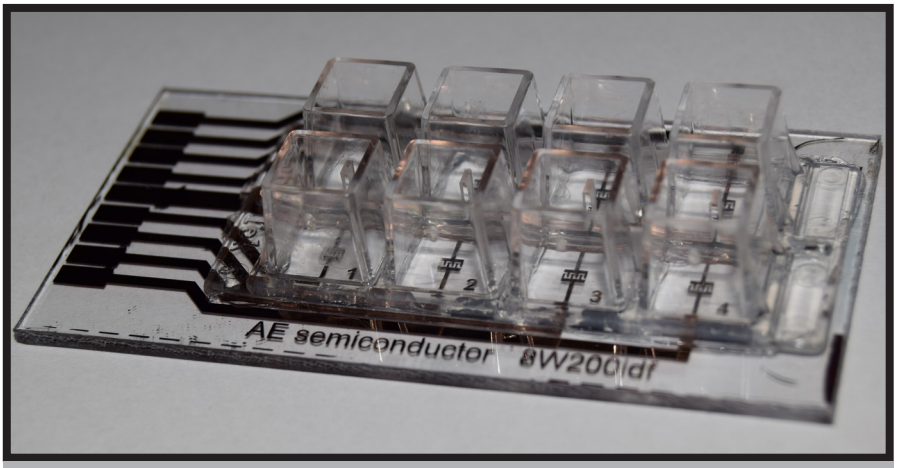

FIG. 2. Ready-made nickel cell culture medium.

The course of the experiment on cell culture of A-375 melanoma

The substrates were used for the experimental cell culture at the Department of Human Physiology of the Medical University of Lublin, using melanoma tumor cells derived from human skin tissue. The culture came from the American Type Culture Collection (ATCC) organization and the medium was Dulbecco's Modification of Eagle's Medium (DMEM) [15]. The cells were grown under controlled growth conditions with constant humidity and 5% CO_2 air saturation.

600 µl of the DMEM culture medium was applied to every well to check their good leakage. Then the matrices were fixed in the measuring station and placed in an incubator under appropriate conditions. The electrical parameters calibration was performed at 4 kHz. After 24 h, the culture medium was removed from the wells and replaced with 540 µl of the fresh medium along with bovine serum and 60 µl of the cell suspension in six wells. The pure reference medium was introduced in the remaining two wells. The cell culture matrices were placed in the ECIS® measuring station for over 160 h (FIG. 3).

FIG. 3. Nickel matrices with A-375 melanoma cell culture (left) in the ECIS® measuring station.

Results and Discussions

In our work, we determined the possibility of using innovative nickel electrodes to measure changes in electrical parameters in the culture of A-375 human melanoma cells. Using the ECIS® system we recorded important changes in resistance, impedance, and capacitance in the A-375 cell culture.

Monitoring the impedance changes in cell cultures is of great importance in biological sciences, medicine but also in technical fields, regarding the biomaterials development and modification for implants [16] or cell–semiconductor interfaces [17] where the cell attachment is an important parameter. Accordingly, the experimental means to monitor the attachment and spreading of animal cells to artificial surfaces are most valuable [18].

There are two electrodes in the ECIS® device: a large common reference electrode and a small working electrode. In our experiment, the A-375 line cells were cultured in a holder and measured using nickel electrodes. During the culture, the cells attachment and spread on the electrode surface change the impedance value in such a way that morphological information of the attached cells can be inferred. That is why the cells behavior is an important factor for this type of biosensor [18]. Adherent cells, e.g. melanoma, attach to a surface before they grow and proliferate. Having attached, the cells are no longer spherical, they become flat [19], which affects the impedance.

The obtained results prove that melanoma cells effectively grow on the electrodes used in the study (FIGs. 4-7). Depending on the cells type (a cancer cell or a normal cell), their growth, proliferation and then death are reflected in the changes in impedance and capacitance over time. One of the major difficulties to study those processes is to differentiate between adhesion, spreading, and proliferation. Wegener et al. [18] described in detail the use of a combination of resistance and capacitance to distinguish between those parameters. Our results coincide with Wegener's observations and complement each other. The obtained impedance values (FIG. 5) show an upward trend throughout the almost entire experiment duration (nearly 160 hours). The hours 10-90 reflect the proliferation and migration of cells on the electrode surfaces. The resulting impedance values increase from about 7010 Ω to 7090 Ω during this period. As it can be noted in FIG. 4 there are clear impedance fluctuations expressed in small peaks, which reflects the cells movement. This strongly corresponds to the previous observations presented in FIG. 1 representing fibroblasts movement and growth on gold electrodes in the ECIS® device. Alternations in the cell behavior after attachment (spreading, proliferation, micromotions) result in impedance changes [20]. Electrical impedance is defined as the opposition to an electrical current within a circuit. In direct current systems, the impedance is simply the resistance. In alternating current systems, the changing electric and magnetic fields create additional and varying opposition to the applied current [21]. This is consistent with the resistance measurement results (FIG. 5) obtained during our experiment.

The decrease in capacitance (FIG. 6) and the resistance increase correspond to the cell proliferation, the recorded values complement each other. The measured capacitance at 64 kHz decreases with the increasing surface coverage, which is useful for assessing the cells spread on the electrode surface. These results are consistent with the results of Wegener's [18] studies on MDCK cells (epithelial cell line derived from the canine kidney) on gold electrodes.

The obtained results of electrical parameters are reflected in the microscopic image presented in FIG. 7 with an almost confluent layer of cells on the electrodes surface. However, it should be noted that the experiment was conducted on melanoma cells that have high metastatic potential. The reason for using this type of cells was their high viability *in vitro*. Having improved the technology of non-gold electrodes production, the more demanding cells characterized by the less stable growth may be used for future tests.

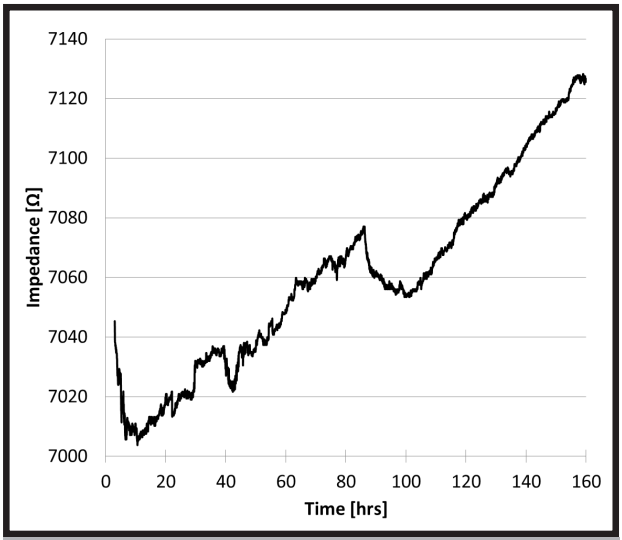

FIG. 4. Impedance measured with a nickel matrix at 16 kHz for over 160 h.

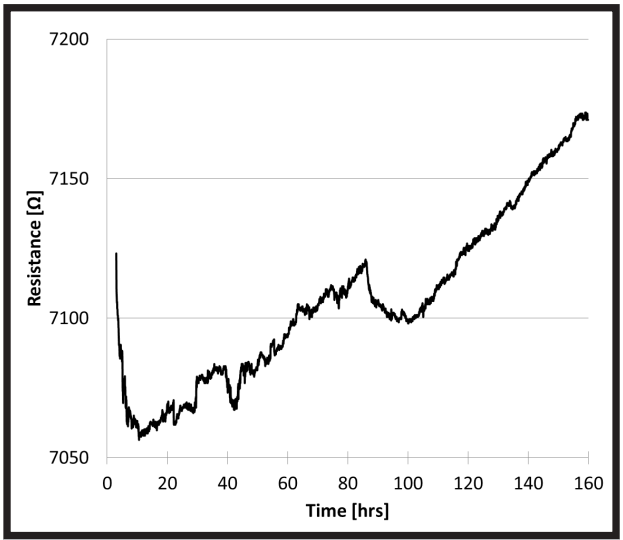

FIG. 5. Resistance measured with a nickel matrix at 4 kHz for over 160 h.

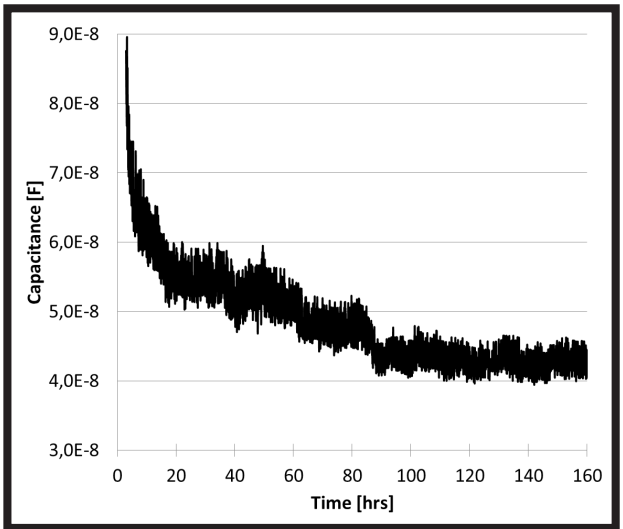

FIG. 6. Capacitance measured with a nickel matrix at 64 kHz for over 160 h.

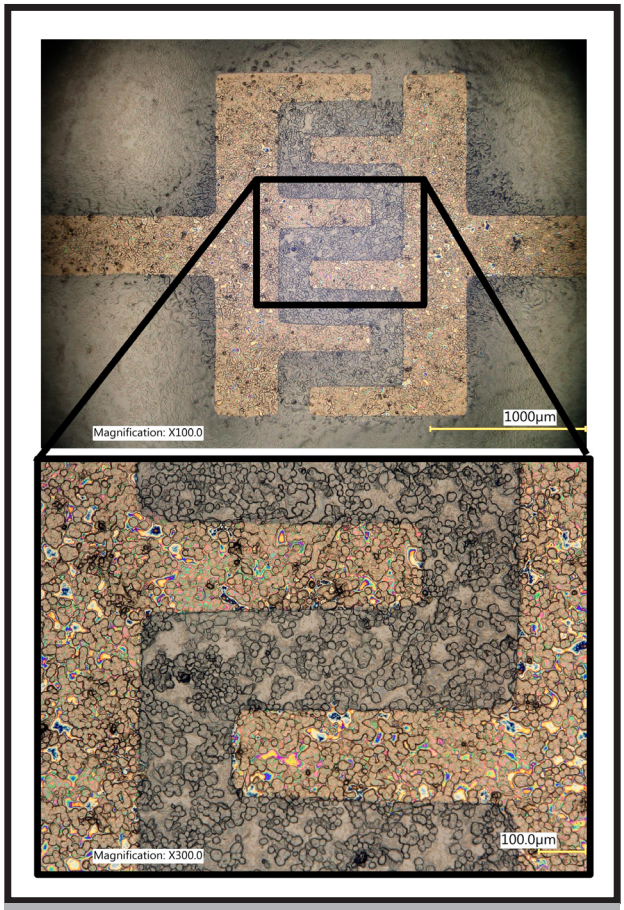

FIG. 7. Nickel capacitor electrode with cell culture after more than 160 h of the experiment.

Conclusions

The above article presents a non-standard technology to obtain the measuring medium with thin-film nickel comb capacitors applicable in the ECIS® apparatus. Prior to performing the test cultures, the substrates were checked for compatibility with the measurement station. Due to its high *in vitro* viability, the A-375 melanoma cell culture was used for the experiment. Thanks to this, it was possible to check whether the toxic nature of nickel would allow monitoring the vital functions of cells. In this experiment, we found that nickel electrodes can be used to study cells. Nickel electrodes in the ECIS® system do not inhibit proliferation and are not cytotoxic to A-375 cells. The correct course of the cell cycle indicates that nickel electrodes may be used to study the material's influence on living cells. In addition, our results prove that nickel can be used as a cheaper material for many applications that have direct contact with the living organism.

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References

[1] https://biophysic.com [access: 12.12.2020]
[2] Rack H.J., Qazi J.I.: Titanium alloys for biomedical applications. Materials Science and Engineering C 26 (2006) 1269–1277.
[3] Chalklen T., Wuinhshen J., et al.: Biosensors Based on Mechanical and Electrical Detection Techniques. Sensors 20 (2020) 5606.
[4] Xu Y., Xie X., Duan Y., Wang L., Cheng Z., Cheng J.: A Review of Impedance Measurements of Whole Cells. Biosensors and Bioelectronics 77 (2016) 824–836.
[5] Giaever I., Keese C.R.: A Morphological Biosensor for Mammalian Cells. Nature 366/6455 (1993) 591–592.
[6] Voiculescu I., Toda M., Inomata N., Ono T. Li F., Nano and Microsensors for Mammalian Cell Studies. Micromachines 9 (2018) 439.
[7] Kociubiński A. et al.: Real-time Monitoring of Cell Cultures with Nickel Comb Capacitors. Informatics, Control, Measurement in Economy and Environmental Protection 2 (2020) 32–35.
[8] Caide Xiao, John H.T. Luong: A simple mathematical model for electric cell-substrate impedance sensing with extended applications. Biosensors and Bioelectronics 25 (2010) 1774-1780.
[9] Crowell L., Yakisich J., et al.: Electrical Impedance Spectroscopy for Monitoring Chemoresistance of Cancer Cells. Micromachines 11 (2020) 832.
[10] Arias L.R., Carla A.P., Yang L.: Real-Time Electrical Impedance Detection of Cellular Activities of Oral Cancer Cells. Biosensors and Bioelectronics 25(10) (2010) 2225–2231.
[11] Scholten K., Meng E.: Materials for Microfabricated Implantable Devices: A Review. Lab on a Chip 15(22) (2015) 4256–4272.
[12] Serek A., Budniok A.: Otrzymywanie i własności elektrolitycznych warstw kompozytowych na osnowie niklu zawierających tytan. Wyd. Uniwersytetu Śląskiego, Instytut Fizyki i Chemii Metali 3 (2002) 63–67.
[13] Bhattacharyya P., Basu P.K., Mondal B., Saha H.: A low power MEMS gas sensor based on nanocrystalline ZnO thin films for sensing methane. Microelectronics Reliability 48 (2008) 1772–1779.
[14] Kagan M. et al.: CellTracks: Cell Analysis System for Rare Cell Detection. Proceedings SPIE, Clinical Diagnostic Systems: Technologies and Instrumentation 4625 (2002) 20–28.
[15] https://www.lgcstandards-atcc.org/products/all/CCL1.aspx#culturemethod [access: 13.12.2020]
[16] Vogler E.A.: Thermodynamics of short-term cell adhesion in vitro. Biophysical Journal 53 (1988) 759–769.
[17] Parak W.J. et al.: Electrically excitable normal rat kidney fibroblasts: A new model for cell–semiconductor hybrids. Biophysical Journal 76 (1999) 1659–1667.
[18] Wegener J., Keese C.R., Giaever I.: Electric cell–substrate impedance sensing (ECIS) as a noninvasive means to monitor the kinetics of cell spreading to artificial surfaces. Experimental Cell Research 259 (2000) 158–166.
[19] Mohanty S.P.: Biosensors: A Survey Report. Citeseer (2001) 1–15.
[20] Xiaoqiu H., Nguyen D., Greve D.W., Domach M.M.: Simulation of microelectrode impedance changes due to cell growth. IEEE Sensors Journal 4/5 (2004) 576–583.
[21] Coffman F.D., Cohen S.: Impedance measurements in the biomedical Sciences. Analytical Cellular Pathology 35 (2012) 363–374.