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Author of the doctoral dissertation: Elżbieta Jarosińska MSc. Eng.

Supervisor: prof. Anders Martin Jönsson-Niedziółka Ph.D., D. Sc.

Auxiliary supervisor: Emilia Witkowska Nery Ph.D.
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Title of the doctoral dissertation: Development of electrochemical sensor arrays for monitoring cell cultures

Abstract

The development of *in vitro* cell culture methods is one of the most important achievements of experimental biology in the last century. It allows cells to be isolated from tissues and cultured under physiological conditions outside the parent organism. Research conducted to date using cell cultures has led to the discovery of many cellular mechanisms and has contributed to the testing of new drugs and the development of many vaccines. Commonly used cell culture methods in biomedical research do not reflect *in vivo* conditions, but they do allow for reproducible results at a relatively low cost.

Currently, one of the biggest challenges is to develop a three-dimensional (3D) cell culture model that will allow for a more accurate representation of physiological conditions *in vivo*. The analysis of cell cultures is primarily based on optical methods, microscopic analysis of cell morphology, staining, and endpoint tests. An important aspect is the possibility of using sensors that allow, among other things, the measurement of extracellular signalling molecules throughout the entire culture. In recent years, the use of electrochemical methods in cell culture analysis has been increasing due to the possibility of miniaturization and integration of electrochemical sensors in microfluidic systems.

This dissertation concerns the use of electrochemical oxygen sensors in the analysis of metabolic parameters in static cell cultures. An additional topic was anti-fouling coatings, which enable long-term research in cell cultures while maintaining signal stability.

In the first stage of the work, an oxygen reduction reaction catalyst was identified that enabled measurement at low overpotential on various types of electrodes, including sputtered gold electrodes and 3D printing electrodes. The gold electrodes in the developed 8-electrode sensor array were characterized by a sensitivity of 76.8 ± 6.5 nA/% in the low concentration range (2-18% O_2).

At the same time, an anti-fouling layer was identified that enabled long-term measurements in cell cultures using carbon electrodes. Measurements were carried out in culture medium using more than 11 different compounds with anti-fouling properties. Syringladazine was used as a model catalyst layer, exhibiting a characteristic linear dependence of the reduction/oxidation peak potential on the pH of the solution and stability in buffer solutions. Measurements using cyclic voltammetry and differential pulse voltammetry showed that silicate-based modification is the most effective anti-fouling material for carbon electrodes in

cell culture. The signal of the tested indicator layer was visible throughout the experiment, and AlamarBlue tests and microscopic analysis showed no significant toxic effects on the HeLa cell line.

In the second stage of the work, experiments were conducted using a developed sensor array of 8 sputtered gold electrodes. In HepG2 cell culture, changes in oxygen and glucose concentrations and impedance were analyzed during incubation with DMSO and acetaminophenone. The results of the AlamarBlue test after incubation with acetaminophen showed significantly reduced viability of HepG2 cells compared to the test using a 96-well plate.

In the third stage of the work, the developed sensor arrays were used in measurements in a three-dimensional culture of huCho and HepG2 cells on hydrogel scaffolds under static conditions. A half-synthetic GelMa hydrogel was used for cell encapsulation. An analysis of oxygen change in 3D culture was performed under reduced oxygen conditions (5% O_2) and during incubation with acetaminophenone. Due to the large diameter of the measurement system used and the diameter of the hydrogel matrix, dissolution of the hydrogel matrix was observed during 72 hours of culture. The study indicated necessary changes in the architecture of the measurement system used, which will allow for more accurate analysis of metabolic parameters using electrochemical methods in the future.

Keywords: electrochemical oxygen sensors, sensor arrays, antifouling layers, HepG2, 3D cell cultures

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