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Title of the doctoral dissertation:

ITO microelectrodes and microelectrode arrays for the analysis of cell cultures and biomedical applications

Abstract

This thesis explores the potential of electrochemistry as a non-intrusive, highly sensitive, and cost-effective analytical tool for investigating cellular processes. Emphasizing its broad applicability, the study underscores the ethical advantages of electrochemical biosensing based on cell cultures, presenting a viable alternative to animal testing in medicine, biotechnology, and environmental science.

The long-term objective of this research is to develop a multielectrode design that enables cell growth and electrochemical analysis at various points of 3D cell cultures. Such a setup will be used for drug screening through viability studies by analysing glucose and oxygen consumption in cell cultures of immortalised hepatocytes. In this thesis, I successfully developed low-cost indium tin oxide (ITO) microelectrodes and MEAs, demonstrating their versatility, availability and stable performance. The work also explores the synthesis of osmium polypyridyl complexes and Prussian blue analogues as mediators for glucose sensing.

The first chapter highlights the significance of cell culture-based biosensing, overviews the important factors in electrode fabrication, basic electroanalytical methods and glucose sensing. The second chapter explores different electrode fabrication techniques. The main focus of this chapter features the comprehensive procedures and characterisation of ITO electrodes prepared using a CO_2 laser plotter. In the third chapter, electrochemical impedance spectroscopy (EIS) is used as a tool to illustrate an application of these cost-effective, simple, easy-to-use electrodes. The ITO microelectrode arrays are employed to characterise HeLa, HepG2 and mouse hepatocyte cell cultures through the EIS technique. The measurements showed

conductivity variations and surface modifications of the electrodes in response to the attachment and detachment of the cell cultures.

Chapter four discusses biosensing and my current interest in developing a glucose biosensor that can be adaptable for lactate and glutamate measurements. This chapter also describes the importance of mediators in glucose biosensors. Mediators are employed in glucose sensors to enhance the effective flow of electrons between the electrodes and the glucose oxidase enzyme. Therefore, they enhance the sensor's sensitivity and enable precise monitoring of glucose levels. In this work, simultaneously, two strategies are used for the synthesis of mediators. One of the approaches is using a mediating redox hydrogel that contains an osmium polymer complex with the general formula cis-$[(Os(N\text{-}N)_2(PVI)_{n+1}Cl]^+$(n = 9). The synthesis of Prussian Blue analogues of the general formula $A_x[R(CN)_6]_{1-y}\cdot wH_2O$ using flash light sintering is the other approach.

In conclusion, the research underlines the potential of electrochemistry in advancing biosensing for cellular studies and biomedical applications. The successful development of low-cost ITO microelectrodes and MEAs marks an important milestone in this study. They can be employed as any normal working electrodes in the market. As an example of their application, electrochemical impedance spectroscopy was used to study cell culture dynamics. The promising results obtained from the ongoing efforts in synthesizing mediators also open exciting possibilities for glucose biosensing.