Nafion and Polylysine treated PEDOT mammalian cell biosensor.

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Abstract

The present study describes a cell-based biosensor utilizing PEDOT electrodes coated with Nafion and Polylysine for combined conductivity, cellular adhesion and proliferation. Neuroblastoma N2a cells were seeded on top of PEDOT electrodes treated with Nafion and Polylysine.

Cellular attachment and viability were assayed and chronoamperometric measurements were taken to evaluate H_20_2 toxicity. Cells exhibited relatively hight viability compared to those seeded in tissue culture plates.

Chronoamperometric responses also provided preliminary evidence of the possible use of this assembly as a toxicity biosensor.

Introduction

Cell-based sensors utilize the ability of cells to selectively respond to complex mixtures of signals in a way that makes them highly attractive for detection of chemical and biological analytes, for detection of environmental toxins and for drug screening screening.

Electrochemical biosensors using monolayer mammalian cell cultures on electrode surfaces require controlled environments for survival, functionality and reproducibility.

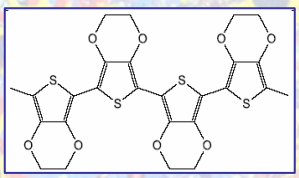

Fig. 1. The chemical structure of PEDOT

PEDOT is one o f the most important and successful conducting polymers synthesized in the field of organic electronics. Biocompatibility testings have shown that cells proliferate and differentiate successfully on top of PEDOT films. The present study describes a cell-based biosensor utilizing PEDOT electrodes coated with Nafion and Polylysine.

The combination of conducting polymers with perfluorosulfonic acid membranes like Nafion has attracted intense interest.

Experimental Setup

PEDOT electrodes (Dropsens, Ref. P10), consist of a 4mm PEDOT working electrode, a carbon counter electrode and a silver reference electrode. Nafion and Polylysine were deposited on top of PEDOT electrodes throught evaporation. Cells were seeded on top of the electrodes with the use of a cut-off pippete tip which formed a 4mm culture well (Fig. 2) at a density of $40x10^4$ cells/mm^2.

A Uniscan PG 580 potentiostat was used for assessing the chronoamperometric responses of the N2a cells seeded on the PEDOT/ Nafion/Polylysine electrode.

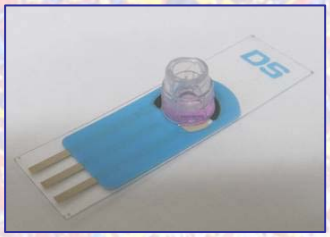

Fig. 2. Cell culture well on top of PEDOT electrode consisting of a cut-off pipette tip (polystyrene, diameter 4mm)

The disposable electrode was connected to the potentiostat throught a Dropsens DSC interface (Fig.3). The working solution was 60μl and the applied potential was +100mV.

Fig. 3. Chronoamperometric measurement of N2a cells seeded on PEDOT/Nafion/Polylysine working electrode with a Carbon Counter Electrode and a Silver reference electrode. Measurments were taken with a Uniscan PG580 potentiostat.

Results

The suitability of the electrode substrate for cell culture was investigated through the adhesion of cells on the coatings. Tissue culture plates commercially available for cell culture were used as control. Attachment rates in different time intervals are presented in Table 1. Four hours after cell seeding 93.82 (±2.91)% of cells have attached. This rapid attachment shows that the surface is probably suitable for the growth of cells.

Table 1. Attachment of N2a cells on the modified electrodes 30min, 1 hour, and 4 hours after seeding.

Time after seeding (hours)	0.5	1	4
Attachment rate %	61.23	84.66	93.82
Standard deviation	4,25	3.94	2.91

The results of the chronoamperometric response of the modified electrodes seeded with N2a cells are presented in Figure 4. At +100mV applied potential bare electrodes showed no difference in current response, while electrodes with cells produced decreases from initial currents. These results suggest that the electroactivity of the PEDOT/Nafion/Polylysine electrodes covered with the cellular monolayer is higher than the uncovered ones.

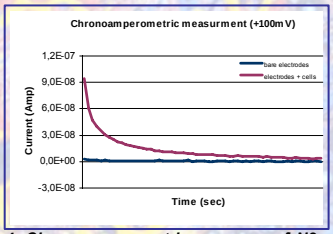

Fig. 4. Chronoamperometric response of N2a cells at +100mV in PBS (60μl). Bare PEDOT/Nafion/Polylysine electrodes showed no difference in current response, while cells produced sharp decreases from their initial currents.

In order to assess the chronoamperometric response of the cells in relation to hydrogen peroxide toxicity cells were treated with two H202 concentrations and the average current decreases are presented in Figure 5. The current decreases exhibited a linear response against the three concentrations of the toxicity factor suggesting its potential as a mammalian cell toxicity biosensor.

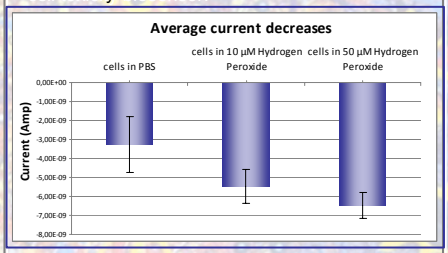

***Fig. 5.** The chemical structure of average current decreases in the chronoamperometric responses of N2a cells treated with and without hydrogen peroxide (10μM, 50μM), data are presented as the mean±standard deviation(n=6).*

Conclusion-Future Work

In summary the PEDOT/Nafion/Polylysine electrode surface showed interesting affinity with cellular monolayer allowing cell adhesion and relatively high viability. These results suggest that these modified electrodes could be a promising electroactive material for application in the field of biosensors, especially in view of the physiological (bioactivity) information provided by the cellular responsive elements.

Future experiments could focus on the investigation of the possible analyte-specific pattern of biosensor response, as well as the limit of detection of a given analyte. In addition, various operational parameters should be elaborated, including, for example, the density of attached cells, temperature, etc

Acknowledgements

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