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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.

Keywords: PEDOT ; Nafion ; cell-based biosensor

1. 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 [1]. Electrochemical biosensors using monolayer mammalian cell cultures on electrode surfaces require controlled environments for survival, functionality and reproducibility [2]. In the last few years, there has been an increasing interest in conducting polymer surfaces that have been shown to support cell adhesion and growth. Anchorage-dependent cells growing directly on electrode surfaces result in compact cell-sensor interfaces that offer the advantages of non-destructive and potentially long-term coupling between sensor and cells [2]. PEDOT is one of the most

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important and successful conducting polymers synthesized in the field of organic electronics. PEDOT's relatively high conductivity and optical transparency enables fluorescence/spectroscopic applications simultaneously performed with electrochemical measurements. Biocompatibility testings have shown that cells proliferate and differentiate successfully on top of PEDOT films. [3] The combination of conducting polymers with perfluorosulfonic acid membranes like Nafion has attracted intense interest. Nafion is a cation-exchange polymer which can be used as a protective layer formed on the surface of electrodes improving their stability and ionic conductivity [4]. Nafion coatings reject negatively charged components, improve the biocompatibility of biosensors and provide protection against biofouling [5].

2. Experimental Setup

2.1. Electrode preparation

PEDOT electrodes were purchased from Dropsens (Ref. P10). Each disposable electrode consists of a 4mm PEDOT working electrode, a carbon counter electrode and a silver reference electrode. The overall dimensions of the electrode are L33 x W10 x H0.5 mm and the electric contacts are made of silver. Nafion was deposited on top of PEDOT electrodes thought evaporation, one drop of Nafion 0,25% in ethanol was placed on top of the electrode surface and allowed to air dry for two hours. Polylysine coatings were then prepared by placing 10 μl of Polylysine solution (50μg/ml) on the electrode and letting it dry at room temperature. Excess polylysine was removed by rinsing the electrode with PBS.

2.2. Cell culture

N2a cells were routinely cultured in 25 cm^2 cell culture flasks at 37 °C in a humidified atmosphere of 5% CO_2:95% air in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. When cells reached confluence they were harvested by trypsinization with 0.25% trypsin and 0.02% EDTA. The density of cells was determined using a hematocytometer. PEDOT/Nafion/polylysine electrodes were placed in petri dishes and sterilized using ultraviolet radiation under a laminar flow cabinet. Cells were seeded on top of the electrodes with the use of a cut-off pippete tip which formed a 4mm culture well (Fig. 1) at a density of $40x10^4$ cells/mm^2. After seeding the Petri dishes were placed in a humidified incubator (37 °C, 5% CO_2) and the cells were allowed to settle, attach and grow on the substrates. The viability of cells cultured on the electrode was determined by the Trypan Blue exclusion assay. Cell adhesion was assayed after 1h, 4h and 24h after seeding. Electrodes were gently rinsed with fresh medium and both nonadherent and adherent cells were counted. Attachment was calculated as the percentage of the number of adherent to the total number of cells.

2.3. Electrochemical experiments with cells

A Uniscan PG 580 potentiostat was used for assessing the chronoamperometric responses of the N2a cells seeded on the PEDOT/Nafion/Polylysine electrode. The disposable electrode was connected to the potentiostat throught a Dropsens DSC interface (Fig.2). The working solution was 60μl and the applied potential was +100mV.

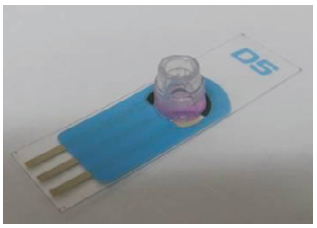

Fig. 1. Cell culture well on top of PEDOT electrode consisting of a cut-off pipette tip (polystyrene, diameter 4mm)

Fig. 2. 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.

3. 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 cell seeding	30 min	1 hour	4hours
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 3. At +100mV applied potential bare electrodes showed no difference in current response, while electrodes with cells produced decreases from the initial current. These results suggest that the electroactivity of the PEDOT/Nafion/Polylysine electrodes covered with the cellular monolayer is higher than the uncovered ones.

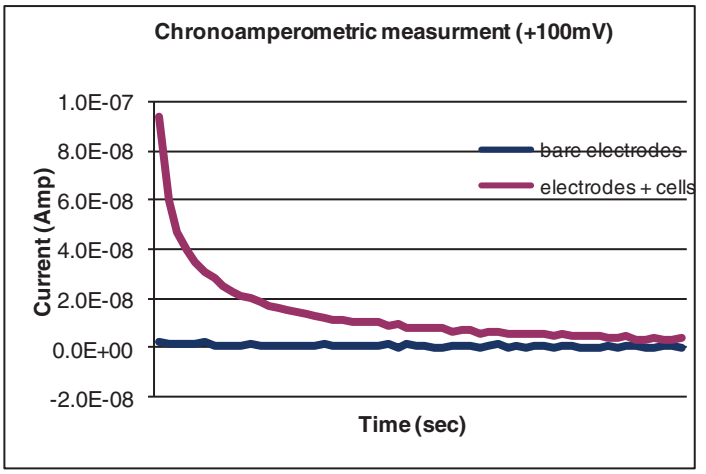

Fig. 3. Chronoamperometric response of N2a cells at +100mV in PBS (60µl). Bare PEDOT/Nafion/Polylysine electrodes showed no difference in current response, while cells produced sharp decreases from their initial currents.

In order to assess the chronoamperometric response of the cells in relation to hydrogen peroxide toxicity cells were treated with two H_20_2 concentrations and the average current decreases are presented in Figure 4. The current decreases exhibited a linear response against the three concentrations of the toxicity factor suggesting its potential as a mammalian cell toxicity biosensor.

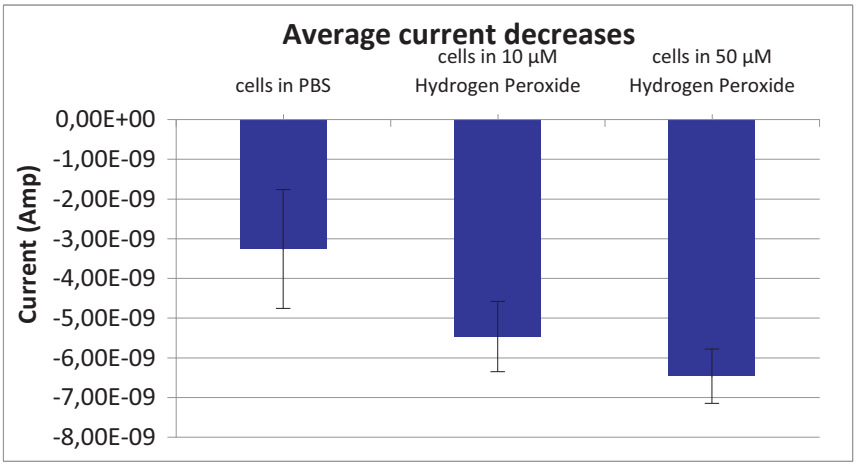

Fig. 4. Average current decreases in the chronoamperometric responses of N2a cells treated with and without hydrogen peroxide (10µM, 50µM), data are presented as the mean±standard deviation(n=6).

4. Conclusion

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.

4. Future work

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.

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