Microfluidic devices for cell culturing and electrochemical sensing of hydrogen peroxide and nitrite

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Abstract

Integration of electrochemical detection (ED) in cell culture is an advantageous strategy to detect the secretions from cultured cells. This is especially interesting for unstable species since they are not diluted prior to detection, so the minute quantities of species secreted be detected and quantified. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are a group of compounds of special interest because of their critical role in physiological processes like cellular signalling and immunological activity. However, an overproduction may cause the so-called oxidative stress (OS) which is able to cause damage to lipids, proteins or DNA. These alterations promote pathophysiological conditions such as diabetes, cancer, Alzheimer's and Parkinson's disease. To study this phenomenon, Hydrogen peroxide (H_2O_2) and nitrite (NO_2^-) were selected as indicators of ROS and RNS respectively for their stability and for being the stable end-products for each group. In this work, the ED of this compounds has been studied employing different electrode materials like Pt, carbon black (CB) and Prussian Blue. The microfluidic system coupled with ED was further employed to culture different cell lines and to follow their response in terms of H_2O_2 and NO_2^- concentrations released after treatment with calcimicyn.

Introduction

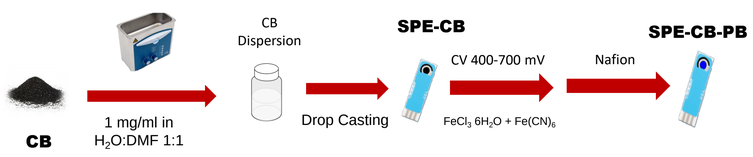
Oxidative Stress is defined as an imbalance between oxidant stressors and antioxidant defences, this physiological status leads to several diseases such cancer, ischemia, atherosclerosis, Alzheimer's and Parkinson's disease (PD). Therefore, there is a growing interest in the quantification reactive oxygen species (ROS) and reactive nitrogen species (RNS). Hydrogen peroxide and nitrite are commonly used as indicators of oxidative stress due to its relative stability in contrast to superoxide, nitric oxide or peroxynitrite. Different analytical strategies have been proposed for their detection such as chemiluminescence, fluorescence, and electrochemical techniques. Among these, electrochemical sensors are very appealing for their simplicity, speed, sensitivity, miniaturization and cost-effectiveness. Microfluidic systems allow the real-time measurement of this response on modified PB electrodes. Therefore, the combination of electrochemical detection with microfluidic devices are very appealing for fast and decentralized analysis in cell cultures. As cellular model, macrophages are commonly chosen as a cell line for oxidative stress studies because they are a natural barrier against infection and inflammation. In order to neutralize their targets they usually produce and secrete powerful radicals to the extracellular medium which facilitate the study of their response towards oxidative stressors.

Objectives

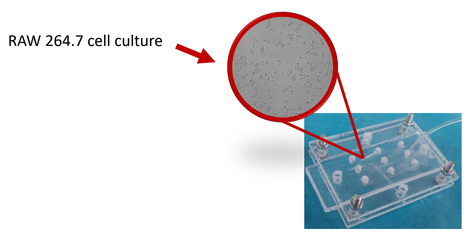
- To develop a new microfluidic platform able to perform electrochemical on-line measurements in cell cultures.
- To test the developed platform towards the detection of H_2O_2 and NO_2 released by living cell populations in a integrated platform.

Experimental

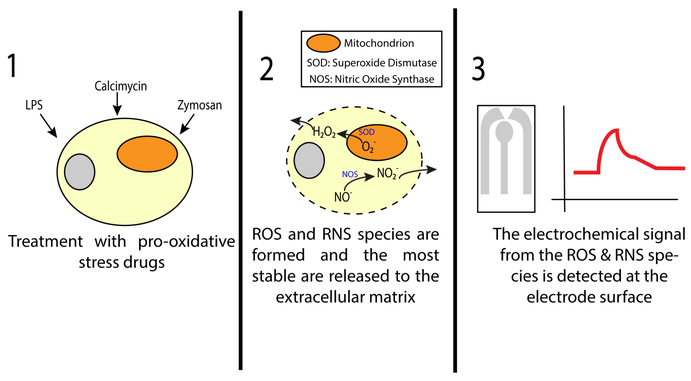
CB-PB electrodes preparation



Microfludic device

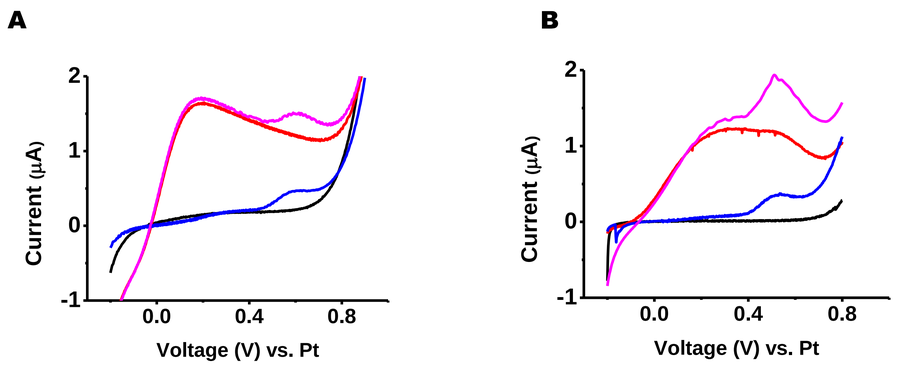


Assay workflow



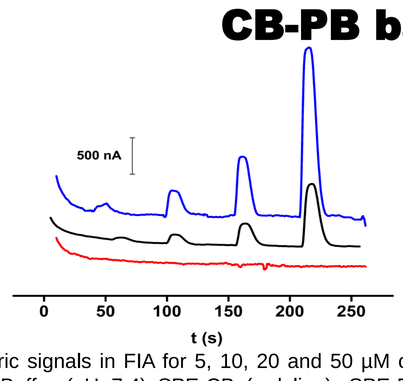
Results

Pt based electrodes

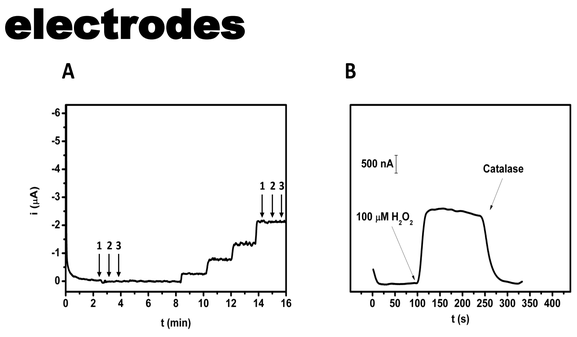


A) LSV of PBS (black), H_2O_2 (red), NO_2 (blue) and an equimolar mixture (magenta) at 1 mM concentration B) HDV electrode PBS (black), H_2O_2 (red), NO_2 (blue) and an equimolar mixture (magenta) at 1 mM concentration. Flow rate 50 µL min^{-1}

CB-PB based electrodes

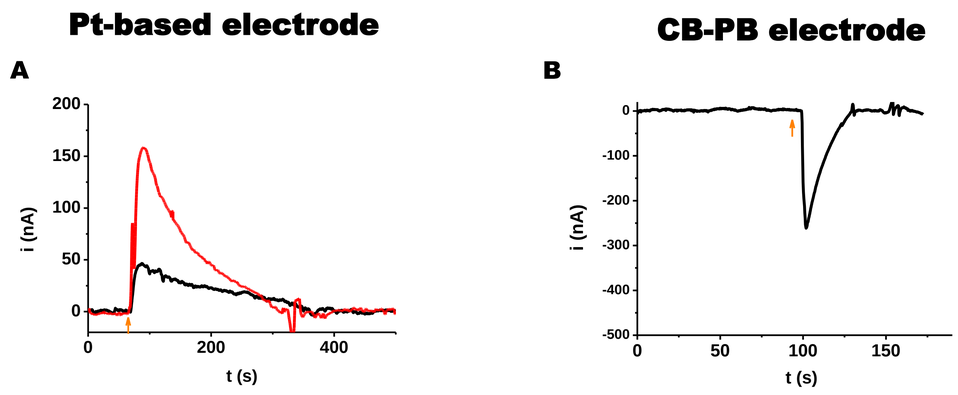


Amperometric signals in FIA for 5, 10, 20 and 50 µM of H_2O_2 in Phosphate Buffer (pH=7.4) SPE-CB (red line), SPE-PB (black line) and SPE-CB/PB (blue line).



A) Amperometry signals due to the addition of FBS (1), L-Glu (2) and P/S (3) in DMEM medium B) Selectivity of the electrode towards 100 µM of H_2O_2 spiked in the cell culture without cells. E=-50 mV vs Ag

RAW 264.7 cells responses to calcyomicin



Responses of RAW 264.7 cell population towards 5 µM of calcimycin recorded with A) Pt electrodes at 0.3 (black) and 0.85 (red) V vs Ag B) CB-PB electrodes at -0.05 V vs Ag

Conclusions

- Secretions of Raw 264.7 cells produced by calcimicyn were successfully detected using two different electrodes.
- Pt-based electrode is able to measure the end products of ROS (H_2O_2) and RNS (NO_2^-)
- PB-based electrode allowed the interference free detection of hydrogen peroxide.
- These results pave the way for an optimization of the microfluidic device to perform (bio)chemical assays in different cellular conditions.

References

[1] Rojas, D.; Della Pelle, F.; Del Carlo, M.; D'Angelo, M.; Dominguez-Benot, R.; Cimini, A.; Escarpa, A.; Compagnone, D. Electrodeposited Prussian Blue on Carbon Black Modified Disposable Electrodes for Direct Enzyme-Free H2O2 Sensing in a Parkinson's Disease in Vitro Model. *Sensors Actuators, B Chem.* **2018**, *275*, 402–408.

[2] Liu, H.; Weng, L.; Yang, C. A Review on Nanomaterial-Based Electrochemical Sensors for H2O2, H2S and NO inside Cells or Released by Cells. *Microchim. Acta* **2017**, *184* (5), 1267–1283.

Further information about Rep-Eat Project and this work can be foud at: repeat.unite.it

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