**Electrochemical sensor for H2O2 released from human THP-1 macrophages**

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**1.Introduction**

Reactive oxygen species (ROS) are by-products of aerobic metabolism. The superoxide anion (O2- ), hydrogen peroxide (H2O2) and hydroxyl radicals (OH-) are part of the ROS. ROS are usually indicative of oxidative stress, and high ROS concentration induces disease by damaging lipids, proteins and DNA[1][2][3][4].

Among ROS species, hydrogen peroxide is of interest due to its unique physiological property. H2O2 can cross biological membranes and is therefore more damaging to DNA than hydroxyl radicals[5]. For this reason, it is very important to measure and estimate oxidative stress, especially H2O2 levels. Current methods to measure ROS in cell cultures use fluorescent probes, colorimetric/fluorometric biochemical assays, or liquid chromatography coupled with mass spectrometry-based approaches[6][7]. These methods have limitations, such as the need to stain cells. In addition, fluorescence methods are not specific for a particular ROS, so they do not allow absolute quantification[8]. Recently, the electrochemical detection of H2O2 has been of great interest due to its many advantages, such as ease of use, low-cost instrumentation, suitability, high sensitivity and selectivity, rapid response and low cost chemical instruments[9][10][11][12][13]. Several electrode materials have been tested [14][8][15][16][17] and in particular, graphene oxide (GO) modified with metal nanoparticles (NPs) has been widely studied as active material for H2O2 detection[18][19]. In the present study, we show the fabrication and calibration of a nanostructured electrochemical sensor, based on rGO and Au-NPs, to measure H2O2 released from human THP-1 macrophages exposed to different treatments.

**2. Methods**

The fabrication of the rGO/Au-NPs-based electrode is realized by the method detailed in a previous study[20]. In particular, Indium tin oxide coated PET (ITO-PET) sheet was used as a substrate for sensor fabrication. ITO-PET was ultrasonically washed in pure isopropanol and deionized water for 10 min each. ITO-PET was used as working in an electrochemical cell manufactured by a stereolithography 3D printer (3D Form 3+ Low Force Stereolithography (LFS)™). The 3D printed cell is equipped with two channels with a diameter of 1 mm that allow the connection with a peristaltic pump which recirculates the electrolyte with a speed of 50 ml/min. All electrochemical measurements were carried out using a potentiostat/galvanostat (Solartron CellTest® System). The electrochemical co-deposition of rGO and Au-NPs was carried out in acetate buffer solution (ABS), containing 0.5 mg/ml GOx and 0.5 mM KAuCl4. A constant cathodic potential of 0.8 V vs. SCE was applied for 200 s. The electrodes were characterized using scanning electron microscopy (SEM, FEG-ESEM, FEI QUANTA 200), energy dispersive spectroscopy (EDS), X-ray diffraction and Raman spectroscopy. For the electrochemical detection a three-electrode cell was used where the working is the ITO-PET/rGO-Au-NPs electrode, the counter is a platinum wire and the reference is an SCE. The sensor was tested and calibrated using cell growth medium mixed with PBS as a blank in a 1:1 ratio.

**3. Results and discussion**

The co-electrodeposition of rGO and Au-NPs from an aqueous solution containing GO and HClAu4 as precursors, occurs following the reactions proposed in previous studies[21][22].

Figure 1 shows the SEM image of the rGO/Au-NPs-based electrode. The co-electrodeposition led to the formation of Au-NPs of about 33 nm that uniformly covered the electrode surface. The presence of rGO and Au NPs was confirmed by Raman spectroscopy and X-ray diffraction.

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**Figure 1. A)** SEM images of the rGO/Au-NPs-based electrode; **B)** calibration curve of sensor for H2O2 detection.

Calibration was performed in blank solution where different concentration of H2O2 were inserted. The detection was carried out by chronoamperometric test at -0.8 V vs SCE. For each investigated H2O2 concentration three different tests were performed. Figure 1B shows the calibration curve of the electrode. A linear range from 10 to 1000 μM was obtained with a LOD and a sensitivity of 3.3S/b and 0.0392 μA μM− 1 cm-2, respectively. Sensor was also validated to measure H2O2 released from human THP-1 macrophages exposed to different treatments.

**4. Conclusions**

In this work an electrochemical sensor based on rGO and Au-NPs were used for the to detect H2O2 released from human THP-1 macrophages. The results are significant because they support the possibility of direct quantification of extracellular H2O2 release in order to monitor intracellular ROS levels with a fast, easy, reproducible and low-cost electrochemical method. This electrochemical sensor may have an easy use to implement in research laboratories where direct measurement of H2O2 in cell supernatants can replace current expensive and time-consuming biochemical and flow cytometry-based approaches. In addition, the future development of a sensor based on gold nanowires or nanotubes is envisaged in order to develop a sensor with a larger surface area and hypothetical higher sensitivity.

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