Electrochemical quantification of oxidative stress in airway epithelial cells

B. Patella[1], S. Di Vincenzo[2], M. Ferraro[2], M. Buscetta[3], E. Pace[2], C. Cipollina[2,3], G. Aiello [1], L. Bollaci[1], R. Inguanta[1]

Affiliations
[1] Department of Engineering, University of Palermo - Palermo (PA) (Italy); [2] Institute for Biomedical Research and Innovation (IRIB), National Research Council of Italy (CNR) - Palermo (PA) (Italy) ;[3] Ri.MED Foundation - Palermo (PA) (Italy)
bernardo.patella@unipa.it

Oxidative stress occurs due to an imbalance between reactive oxygen species (ROS) generation and the antioxidant capacity of our body. This imbalance leads to an increase of ROS that contributes to aging, inflammation and to the pathogenesis of most chronic lung disorders [1]. ROS include superoxide anion, hydroxyl radical and hydrogen peroxide. Among them, hydrogen peroxide has the ability to cross biological membranes and damage the DNA as well as to diffuse in the extracellular space [2]. Thus, a common and useful way to quantify oxidative stress is the quantification of hydrogen peroxide in cellular supernatants. Nowadays hydrogen peroxide quantification is carried out by different lab-based techniques such as fluorimetric and colorimetric assays and liquid chromatography. However, different drawbacks are associated with these techniques: they are expensive, time consuming, require highly skilled personnel and, even more important, cannot be carried out in situ and in real time. Electrochemical sensors are perfect candidates to overcome all these drawbacks keeping good features in terms of sensitivity, selectivity and limit of detection. In our previous work, we have developed a nanostructured electrode made of reduced graphene oxide and gold nanoparticles to detect hydrogen peroxide (by chronoamperometry) released by human macrophages (THP1 cells) grown in RPMI medium [3]. Here, using the same type of sensor, linear scan voltammetry was employed as electrochemical detection technique obtained a much higher sensitivity (of about 3 times higher) compared to chronoamperometry. In particular the sensor was used to quantify the hydrogen peroxide released by human primary bronchial epithelial cells (PBEC), a human bronchial epithelial cell line (16HBE) and an adenocarcinoma alveolar basal epithelial cell line (A549). The effect of different stimuli, both pro-oxidant such as cigarette smoke extract and anti-oxidant such as resveratrol, on H_2O_2 release was studied. The results were compared with those obtained by flow-cytometry using the same cells stained with Carboxy-H_2DCFDA and MitoSOX Red, which detect intracellular ROS and mitochondrial superoxide, respectively. Results obtained with the electrochemical sensorwere consistent with those obtained by flow-cytometry. In particular, with both techniques a significant increase of the quantified hydrogen peroxide was found in the case of cells exposed to CSE while resveratrol reverted this effect. Thus, the proposed sensor guarantees high sensitivity and selectivity, short response time (<60 sec) and can be applied for real time, in situ and easy monitoring of hydrogen peroxide release.

Acknowledgment:

This work was supported by Fondazione Ri.Med, University of Palermo and Italian National Research Council, and partially financed by the Project “SeNSO” (n. 082651290364, 1.1.5 P.O. FESR Sicilia 2014/2020).

References:

[1] G. Ganguli, U. Mukherjee, e A. Sonawane, «Peroxisomes and Oxidative Stress: Their Implications in the Modulation of Cellular Immunity During Mycobacterial Infection», *Front. Microbiol.*, vol. 10, pag. 1121, giu. 2019
[2] M. Waghray *et al.*, «Hydrogen peroxide is a diffusible paracrine signal for the induction of epithelial cell death by activated myofibroblasts», *FASEB j.*, vol. 19, n. 7, pagg. 1–16, mag. 2005
[3] B. Patella *et al.*, «Electrochemical sensor based on rGO/Au nanoparticles for monitoring H2O2 released by human macrophages», *Sensors and Actuators B: Chemical*, vol. 327, pag. 128901, gen. 2021