Monitoring Oxidative Stress in Osteoblast-Like Cells Using Quartz Cristal Microbalance with Dissipation

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Introduction:

Reactive Oxygen Species (ROS) are very unstable entities that can be produced in organisms by endogenous and exogenous sources such as hydrogen peroxide (H_2O_2). At a normal physiological state, an organism possesses biological antioxidants that can prevent the uncontrolled formation of these ROS. However, when the generation of ROS overwhelms the capacity of antioxidant removal from the organism, an oxidative stress (OS) can occur[1]. It is well known that OS can promote diverse biological responses associated with several disorders including aging and cancer. Hence, OS is a great source of interest in medical community. Quartz Crystal Microbalance with Dissipation (QCM-D) is an acoustic surface sensitive technique, which provides simultaneous, real-time information on mass and structure of thin films. In the past decade QCM-D has been successfully established as a dynamic and sensitive tool to monitor living cell incubation and cell attachment to the electrode surface[2], but so far, no complex biological phenomenon has been studied by this technique. This study sought to demonstrate that QCM-D can be used as a novel and efficient tool to monitor complex biological process such as oxidative stress in human osteoblasts-like cells.

Methods:

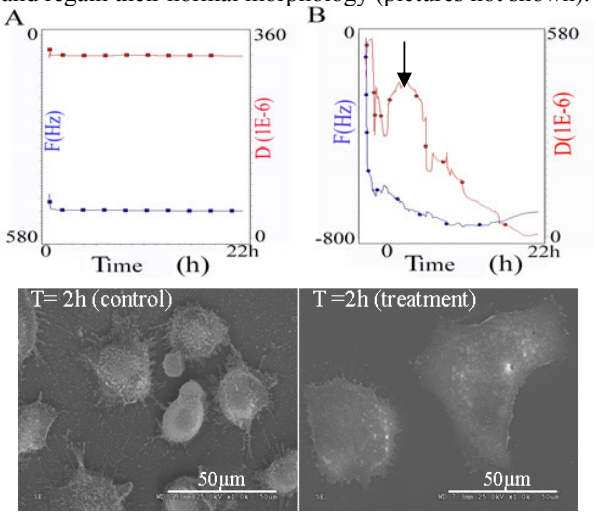
Quartz Crystal Microbalance with Dissipation Studies

QCM-D measurements were performed with a Q-Sense E4 unit (Q-Sense AB, Goteborg, Sweden) by simultaneously monitoring the changes in frequency (Δf) and energy dissipation (ΔD) of a 5 MHz polystyrene-coated QCM-D crystal (QSX-301). The QCM-D crystal was excited to oscillate at its fundamental resonance frequency (f_0 = 5 MHz) or at overtones (n = 3, 5, or 7) by applying a radio-frequency voltage across the electrodes.

Cell culture. MG-63 (ATCC, Manassas, VA) osteoblasts-like cells were grown in supplemented DMEM medium (10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin) at 37 °C in a humidified 5% CO_2 atmosphere. After trypsinization (0.25% trypsin/0.03% EDTA, Sigma-Aldrich, Oakville, ON, Canada), cells were resuspended in fresh medium, and cell concentration was determined using a hemocytometer. A population of 1×10^5 cells was used for QCM-D analyses. Within 10 min after the final resuspension, cells were injected into the flow chamber, and left to adhere for 40 min. To investigate OS, a solution of 10 µM H_2O_2 was injected in the flow chamber and left to incubate with cells for 30 min. After this, cells were washed for 12 min with fresh medium. For the control, fresh medium was used instead of H_2O_2. Changes in Δf and ΔD were monitored for 22 h. For scanning electron microscopy **(SEM)** analysis,MG-63 cells were seeded on a polystyrene cover slip in 24 well plates at 1×10^5 cells/ml for 0, 2 and 4 h at 21°C without (control) or with 10 µM of H_2O_2. Samples were fixed in 2.5% gluteraldehyde, dehydrated in graded alcohol and amyl acetate solutions, and critical-point dried with CO_2. Samples were sputter-coated with a gold–palladium layer and examined using a Hitachi S4700 SEM at an accelerating voltage of 2.0 kV.

Oxidative stress was measured fluorometrically using 2,7 Dichlorofluorescein diacetate with a Cytofluor 2300 plate reader, after the same treatment (with or without H_2O_2) previously described.

Results: In QCM-D, adsorption of mass on a sensor induces a decrease in the resonant frequency (f) which for a rigid substance translates into the change of mass according to the Sauerbrey relation: $\Delta m = -C\Delta f/n$ (n= 3, 5, 7 is the overtone number)[3]. For cells, a change in the dissipation (D) of the crystal provides the evolution of their viscoelastic properties. Results showed that production of ROS is 3 folds higher at 2 h post-treatment than for the control group (results not shown). Besides, the D pattern is radically different and varied with time for treated cells (Fig 1-A versus 1-B), suggesting changes of viscoelastic properties of these cells, especially between 1 h and 4 h post-treatment. SEM pictures at 2 h post-treatment, show morphological changes in osteoblasts, which corresponds to the peak indicated by an arrow on QCM-D measurement (Fig 1, B). These cells are much larger and showed flatten morphology. After 4 h, treated cells seem to recover from the oxidative stress and regain their normal morphology (pictures not shown).



Conclusion: This study is the very first to demonstrate that QCM-D can be used as an efficient tool to monitor a complex biological phenomenon like oxidative stress. Ongoing work aim at investigating the role of ROS on the proteins of MG63 cells cytoskeleton to explain these morphological changes observed by QCM-D.

References: 1:Tkaczyk C. J Biomed Mater Research A. 2010;94:419-425. 2: Modin c. Biomaterials. 2006;8:1346-1354. 3 Steinbach D. Blood. 2003;1:1493-1498.