SIMULTANEOUS QCM-D AND MICROSCOPY MONITORING OF CELL ADHESION WITH THE Q-SENSE WINDOW MODULE

INTRODUCTION

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. The mass of an adsorbed adlayer is sensed as resonance frequency of the sensor movement (Δf) and the viscoelastic properties are deduced from the damping of the sensor movement (ΔD). QCM-D can be combined with optical microscopy by using the QCM-D window module (Figure 1). A system of interest can thus be simultaneously sensed from the surface beneath and observed from above.

This application note presents how such a combined set-up enables unique analysis of cell behavior at a solid support.

BACKGROUND

Controlling interactions between living and non-living matter is essential in designing medical implants, cell-based biosensors and in tissue engineering. It is therefore crucial to identify and understand events taking place at the living/non-living interface. Consequently, tools are needed for evaluation of cell-substrate interactions, especially where microscopy alone is insufficient. In this context, the QCM-D technique provides powerful means for real-time in-situ analysis of cell attachment and cell membrane rearrangement. In particular, with the window QCM-D module enabling visual access to the sensor, QCM-D can be directly combined with microscopy (Figure 1). By doing so, changes in mass and viscoelastic properties sensed with QCM-D can be directly correlated to real-time observation of cell number and morphology.

APPROACH

In this particular example, initial cell adhesion and spreading were studied on protein pre-coated surfaces. More specifically, tantalum and oxidized polystyrene coated QCM-D sensors were covered with proteins either promoting (fibronectin or serum) or prohibiting (albumin) receptor mediated cell adhesion. The adsorption of proteins onto the two substrate types was evaluated with QCM-D. Subsequently, fibroblasts were allowed to interact with the pre-coated substrates. Simultaneous QCM-D monitoring and fluorescence microscopy observation of the cells was employed with the window module.

RESULTS AND DISCUSSION

QCM-D analysis showed that both tantalum and oxidized polystyrene substrates attracted comparable amounts of fibronectin, serum proteins and albumin, respectively.

Upon initial attachment of cells an increase in mass and viscoelasticity of the adlayer was sensed by QCM-D. This could be correlated to increased number of cells at the sensor surface, which was verified with fluorescence microscopy. With time, the QCM-D responses developed differently depending on the type of protein coating and the underlying substrate, indicating different cell attachment and spreading processes on the different substrates. The general QCM-D trend on the cell

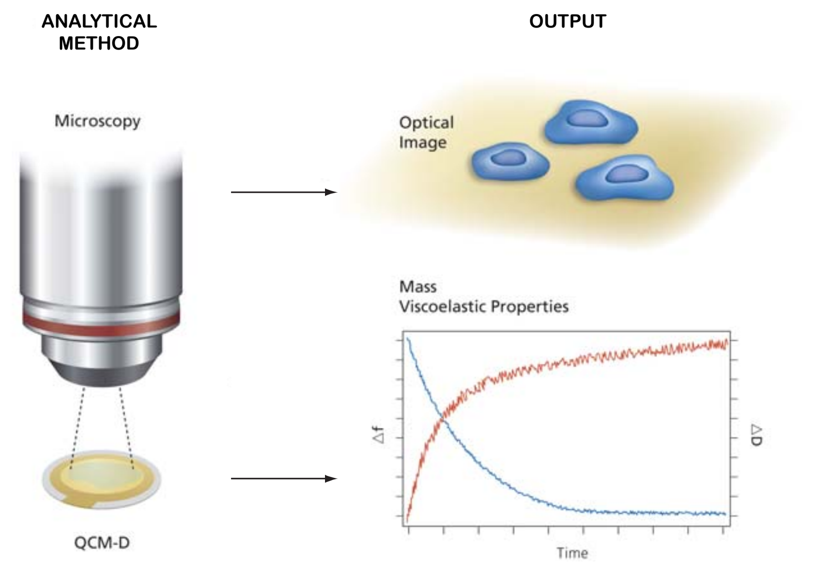

Figure 1 *- QCM-D in combination with microscopy provides information on mass and viscoelastic properties as well as an optical image.*

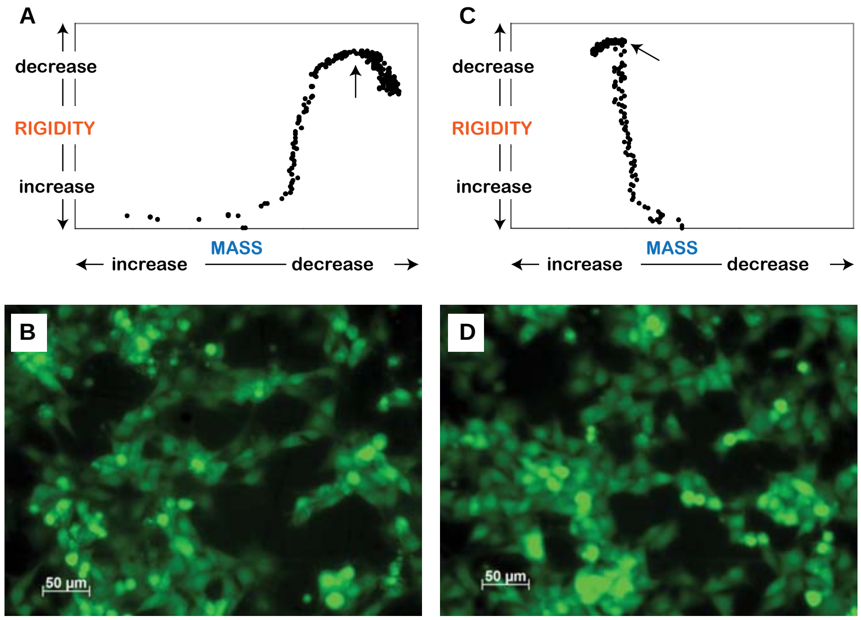

Figure 2 *- QCM-D data (A, C) and fluorescence microscopy images (B, D) of 2 hour cell attachment and spreading on oxidized polystyrene (A, B) and tantalum (C, D) precoated with serum. Arrow indicates 1 hour post cell seeding.*

adhesive fibronectin and serum proteins indicated stiffening of the adlayer with time, which corresponded well with cell spreading observed with fluorescence microscopy (Figure 2). In the specific case of serum proteins on oxidized polystyrene cell spreading was accompanied by decreased mass response (Figure 2A), which did not reflect detachment of cells as confirmed by micrographs (Figure 2B). Instead, the loss of mass during spreading was attributed to changes in cell-surface contacts, such as extra cellular matrix remodeling or rearrangement of focal adhesions/cytoskeleton, resulting in decreased cell-sensor contact area. Interestingly, such a trend was not observed on serum proteins deposited on tantalum substrates (Figure 2C), even though the microscopy images of cells on serum protein coatings were comparable independent of the underlying substrate (Figure 2B and 2D). Similarly, cell attachment was comparable on fibronectin coated substrates judging from the micrographs, however the QCM-D responses were distinctly different. On the non-adhesive albumin coated substrates only small changes in mass and viscoelastic properties were observed during interaction with cells, which correlated well with poor adhesion and rounded cell morphology observed with microscopy.

CONCLUSIONS

QCM-D was able to sense events associated with cell adhesion and spreading, which were not detectable with microscopy. More generally, the combined analytical technique approach enabled real-time correlation of QCM-D analysis and microscopy offering unique information.

REFERENCE

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