QCM-D IN COMBINATION WITH LIGHT MICROSCOPY TO FOLLOW CHANGES IN LIVE CELL MORPHOLOGY

The morphology and mechanical properties of living cells are connected to central cell fate processes such as cell mobility, mitosis, adhesion and apoptosis. By combining QCM-D and light microscopy, complementary data can be collected to relate cell morphology to changes in mechanical properties at the cell-surface interface.

INTRODUCTION

Changes in cell morphology are regulated by cell signaling pathways that can be triggered by both external and internal stimuli. The dynamic nature of these morphology changes can be readily analyzed by real-time, label-free techniques to reveal new time-resolved information in addition to microscopy methods that usually provide end-point measurements. Specifically the Quartz Crystal Microbalance with Dissipation monitoring technology (QCM-D) enables analysis of the mechanical properties at the cell-surface interface. Here, QCM-D is combined with light microscopy to track morphological changes in NIH3T3 fibroblasts and human derived fibroblasts.

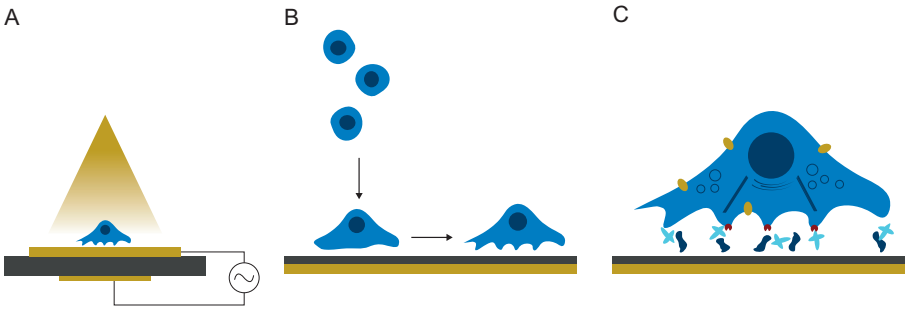

Figure 1. Schematic illustration of the experimental design, using (A) a combined setup with a Q-Sense QCM-D window module and a microscope. (B) The cells were seeded on the QCM-D sensor and were allowed to attach and spread. Their reversible responses due to rearrangements in the cytoskeleton at the cell-surface interface (detailed in C) were followed in real-time.

APPROACH AND EXPERIMENTAL SETUP

All measurements were carried out using a Q-Sense E1 chamber equipped with a Q-Sense Window Module mounted on a Leica DM4000M microscope. Images were taken using a 10X objective. QCM-D sensors coated with silicon dioxide were functionalized with collagen I to act as a cell adhesive layer through binding to integrin receptors on the surface of the cells. Two different types of fibroblasts were used; i) a standard NIH3T3 fibroblast cell line and ii) human derived fibroblasts (HS483.T). Both fibroblast types showed similar QCM-D results in changes in resonance frequency (Δf, mass changes) and energy dissipation (ΔD, viscoelasticity) while the HS483.T cells were larger in size which was beneficial for the microscopy imaging. Cells (2-5 x 10^5 cells/mL) in CO_2 -independent media containing 10% fetal calf serum were injected under flow until a stable baseline was obtained. All measurements were carried out at 37 °C and a water bath was used to preheat all solutions to 37 °C.

After adhesion the cells were subjected to cytochalasin D (cyt-D) that hinders the polymerization of actin in the cytoskeleton which induces dramatic changes in cell morphology. By using the combination of QCM-D and light microscopy the changes in cell morphology could be linked directly to changes in the viscoelastic properties at the cell-surface interface.

RESULTS AND DISCUSSION

By coating the sensor with collagen I and subsequent serum proteins, a cell adhesive layer was formed before cell addition (Fig. 2, 0-30 min). The results indicate this as a soft and hydrated layer with corresponding $\Delta f \approx$ -80 Hz and $\Delta D \approx$ 10*10-6. Cell seeding and attachment (Fig. 2 A-B) resulted in a large positive ΔD and also a small positive Δf. The observed f and D changes are caused by a collective response of the cells interacting with the substrate including intracellular changes close to the cell-surface interface.

Cell mobility was followed by microscopy to confirm that the cells were alive and active. After adhesion the cells were allowed to spread for 1 hour (Fig. 2 C) which resulted in a small negative shift in Δ*D* but no shift in Δ*f*. This negative shift in Δ*D* can indicate the formation of adhesion points between the cells and the surface resulting in a more rigid cell-surface interface.

Addition of cyt-D resulted in a negative shift in Δ*D* that was reversible upon rinsing (Fig. 2, D and D'). Light microscopy images were taken at the same time points and show a significant change in cell morphology upon cyt-D exposure (Fig. 2, II). Since very small frequency shifts are connected to the cyt-D treatment there are no significant mass changes at the cell-surface interface connected to these morphological changes but rather dramatic changes in the viscoelastic properties of the cells. Since QCM-D is sensitive to changes close to the sensor surface it is most likely that the detected changes in viscoelasticity are largely connected to changes in the properties of the cell-surface contacts during cyt-D treatment since these are also rich in actin. These fine details in the properties of the cell-surface interactions were not possible to reveal by microscopy alone and this emphasizes QCM-D as a powerful tool to use as a complement to traditional cell assyas.

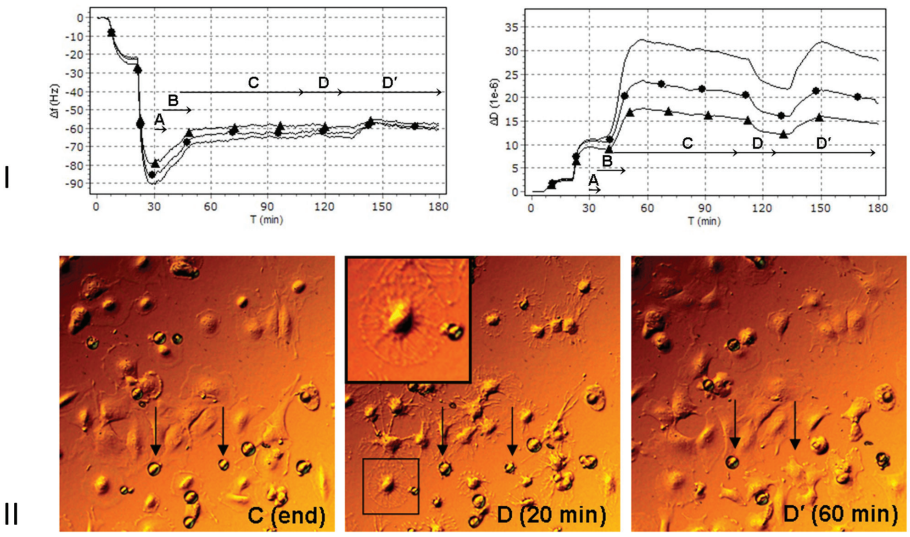

Figure 2. QCM-D frequency and dissipation versus time curves for 3T3 fibroblasts (I), the 3rd (line), 5th (circle), and 7th (triangle) overtones are shown. Live cell images of human derived fibroblasts (II) on collagen coated sensors (500 x 500 µm crop). (A) cell seeding, (B) cell attachment, and (C) cell spreading, after which the effect of (D) the addition of cytochalasin D (2 µg/ml) followed by (D´) rinsing with medium and cell recovery was monitored. Arrows indicate the position of two cells throughout C-D' to guide the eye and the square indicate which part of D that is magnified.

CONCLUSIONS

Morphological changes in human derived and NIH3T3 fibroblasts upon cytochalasin D exposure were detected by QCM-D as negative dissipation shifts that were reversible upon rinsing. By combining QCM-D and light microscopy the obtained QCM-D responses could directly be validated to be caused by changes in cell morphology. The unique information gained from using QCM-D revealed fine details in the cell-surface interactions that were not possible to resolve from only the microscopy images.

ACKNOWLEDGEMENTS

The presented work was originally performed by assistant professor Sofia Svedhem and co-workers [1] at Chalmers University of Technology, Göteborg.

REFERENCES

[1] Tymchenko, N., Nilebäck, E., et al., *Reversible Changes in Cell Morphology due to Cytoskeletal Rearrangements Measured in Real-Time by QCM-D.* Biointerphases, 2012. 7(1): p. 1-9.

INSTRUMENTATION

Q-Sense E1 chamber with Window Module, QWM 401 and Silicon dioxide coated QCM-D sensors, QSX 303.

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