Application Note

Monitoring Cellular Response to Bioactive Agents

Applying bioactive agents to living cells causes the cells to divide, migrate or die. The resulting cells' mass redistribution and alteration of viscoelastic properties can be detected in real time by QCM device.

Summary

Monitoring cellular response to an agent in real time using a mass measuring device reveals unique information relevant to the biomechanicals of living cells. Using a quartz crystal microbalance (QCM) device incorporating living cells, the real time response of adherent cells to a bioactive agent can be measured [1]. The QCM device (qCell T) is housed in a temperature controlled flow cell and responds with a frequency shift to changes in the cellular mass during treatments. Living adherent cells when added in growth media to the chamber above a gold-coated sensor will stably attach, couple to and equilibrate on the sensor surface. Subsequently, the response of the cell measured as changes in viscoelasticity or cellular mass rearrangement can be measured in response to bioactive molecules by monitoring changes in crystal oscillation frequency.

Background

In conventional QCM biosensor research, antigen–antibody recognition reactions were studied. In these applications an antibody is coupled to the QCM surface and candidate antigens bind the specific antibody to that antigens epitopes, producing an increase of coupled mass and oscillation frequency decrease, indicative of an antibody–antigen recognition event [2]. Cells as a mass coupled to the crystal surface are capable of responding and reacting in a wide variety of ways to its environment, and by coupling the cell to QCM the cell is effectively replacing the antibody as the sensing agent of a biosensor. Now instead of having specificity to just one agent, the cell provides a specific response unit to any agent that causes changes in the cell biomechanics (Figure 1) [3].

The cells' mass and viscoelastic elements link and communicate the cells' internal cytoskeleton to the cells' external environment via integrin–extracellular matrix interactions. Once cells reach equilibrium of attachment, if agents are applied that causes the cells to divide, migrate, or die, the resulting cells' mass redistribution and alteration of viscoelastic properties can be detected in real time by the QCM as changes in frequency. Previously, we showed that cell-based QCMs could be used to detect whole cell mass alterations as suggested above [1].

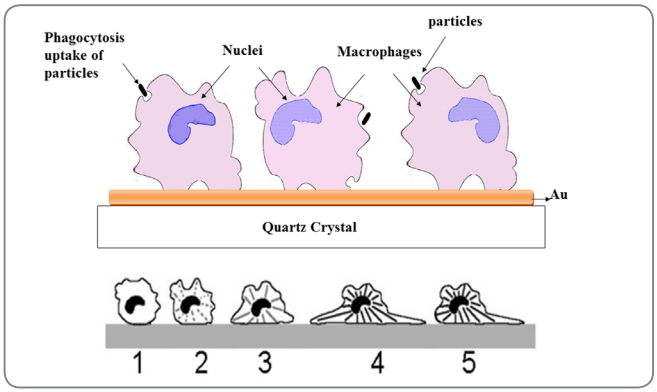

Figure 1. Schematic description of real time response of a QCM sensor by example of different stages of stably attached macrophages on QCM sensor when polystyrene beads or Zymosan A are added and the cells phagocytose this material [2]. In the process of attachment (1) and spreading (2-4) the synthesizing and polymerization of elements of the cell´s cytoskeleton result in changes in resonant frequency of the QCM sensor. While round cells will cause a maximal decrease in frequency, increased spreading and distribution of cellular mass cause an increase in frequency from step 1 thru 4 and reach a cell number dependent level of homeostasis. This stable frequency status could be perturbed by stimulating the macrophages to phagocytose. They will exhibit increased migration, involving re-rounding (5).

Strategy

The cells are attached to the gold surface of the quartz chip using the flow setup. Once the cells have stably attached and adjusted to flow, the system is ready to test the cellular response to an agent added to the culture media. Once testing is complete the cells can be removed from the crystal using trypsin washes, demonstrating a return to the original frequency and proving all cells have been removed.

Method

The experiment is divided into two parts, the cell deposition procedure and subsequently the treatment procedure. The qCell T is set at constant temperature of 37°C, and phosphate buffered saline (PBS) and the cell culture media ideally should be at least at room temperature before use. The experiment is carried out using the flow temperature unit which allows the agent to be thermostated in the flow. The quartz chip must be primed with PBS per the manufacturers

specification. The peristaltic pump is halted and the tubing is switched over to a vessel with the cell culture media and the peristaltic pump is started again. Once the quartz chip within the flow cell is covered with cell culture media, the peristaltic pump is halted again and the data acquisition is started. We used a waiting period of at least 2 hours, which allows for stable serum protein deposition on the quartz chip. A stable trace must be acquired before the cells can be added as seen in Figure 2.

During this waiting period, cell culture media is passively perfused with CO_2 in the cell culture incubator. The cells for use in the experiment are subcultured from stock cultures and suspended in the CO_2 perfused media at densities which will result volumetrically in a 70% coverage of the quartz chip. The peristaltic pump tubing is submerged into the microfuge tube containing the cells and then cells are introduced to the system at 80µl/min to ensure coverage of the quartz chip without harming the cells. The peristaltic pump is then deactivated for at least 18 hours to allow for stable cell attachment and equilibration onto the quartz chip.

Once the cells have stably attached they can be perturbed using an agent of interest. This allows for the real time observation of the cellular response. Before the agent is added, two containers of fresh media are again passively perfused with CO_2 in the cell culture incubator. The peristaltic pump tubing is submerged into the cell culture media vessel and the pump is activated at a flow rate that provides shear forces that mimic physiological flow rates for vascular cells or minimizes shear forces for non-vascular cells, as shown with the pump activation arrow in Figure 3. Once the cells have reached equilibrium, the agent is then added into the second cell culture media at desired concentrations, and with

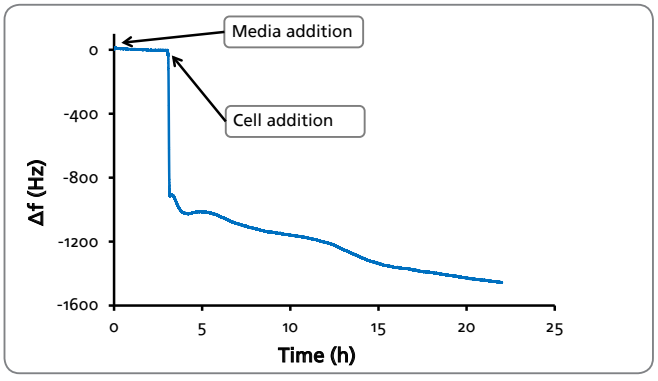

Figure 2. I. Cell deposition trace. The cells are added once a stable media trace is established. The cells take approximately 18 hours to bind the gold and reach equilibrium before experimentation can commence.

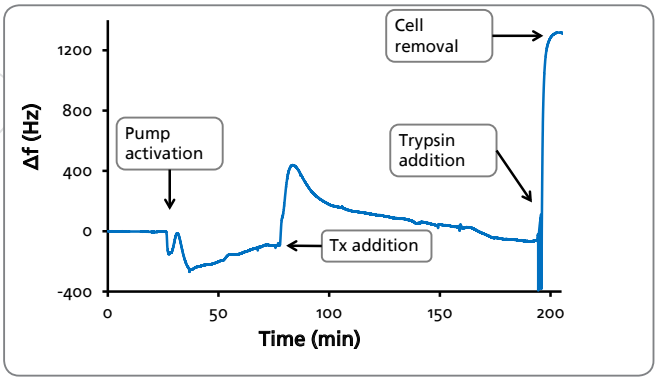

Figure 3. Treatment trace. Once the cells have reached equilibrium, the frequency is set to 0 again and the experiment can commence. First the cells need to adjust to the flow and once they have stabilized treatment (50mM NaN_3) can be added. With the addition of trypsin the cells lift and the frequency returns to the original baseline.

a brief halting of the peristaltic pump the vessel is attached to the flow temperature unit to start the treatment (TX) portion of the experiment as demonstrated in Figure 3.

Once the treatment time course is complete, the peristaltic pump is briefly halted and the vessels switch to PBS, to rinse the cells. The peristaltic pump is halted again and a vessel with trypsin is switched in and then at a flow rate of 5000 µl/min trypsin cell removal is achieved using the peristaltic pump. The pump is then halted for 15 min, and then two additional trypsin washes are performed to completely remove cells. The effluent can be collected to perform cell counts.

Conclusion

The qCell T using adherent cells attached to the quartz chip provided unique continuous monitoring of cellular responses of bioactive agents on living cells. The detection of peak effects can guide investigators to time points of interest to determine the mechanism of action agents have on living cells.

References

[1] Dewilde AH, et al. Quartz crystal microbalance measurements of mitochondrial depolarization predicting chemically induced toxicity of vascular cells and macrophages. Analytical Biochemistry 2013;439(1):50-61.
[2] Wang G, et al. A living cell quartz crystal microbalance biosensor for continuous monitoring of cytotoxic responses of macrophages to single-walled carbon nanotubes. Part Fibre Toxicol 2011;8:4.
[3] Park I-S, Kim W-Y, Kim N. Operational characteristics of an antibody-immobilized QCM system detecting Salmonella spp. Biosensors and Bioelectronics 2000;15(3–4):167-172.

Acknowledgement

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3T analytik GmbH & Co.KG
take-off GewerbePark 4
D-78579 Neuhausen ob Eck

Tel.: +49 (0)7467-947-66-0
Fax: +49 (0)7467-947-66-29
E-Mail: info@3t-analytik.de
www.3t-analytik.com