Application Note

Monitoring Ligand-induced Cellular Response

Cellular response induced by epidermal growth factor (EGF) is mediated through the epidermal growth factor receptor (EGFR), which regulates cell growth, proliferation, motility, and differentiation. Real time Monitoring of EGF-induced cellular response can be provided by a flow cell set-up of qCell T.

Summary

Cellular response induced by epidermal growth factor (EGF) is associated with embryonic development, wound repair and regeneration, inflammatory response and tumor cell metastasis [1, 2].
We have successfully monitored changes in the EGF-induced cellular response of a monolayer of A431 cells using a flow cell set-up of the qCell T. The results obtained with the qCell T are comparable to those obtained with an established QCM technique, the quartz crystal microbalance with dissipation monitoring (QCM-D).

Background

The QCM is a label-free, noninvasive, and highly sensitive acoustic sensing device. In the past decade, the QCM has emerged as a powerful bioanalytical tool that is capable of detecting the response of live cells that adhere to the crystal surface [3]. Such detection is by large based on the altered cell attachment to the crystal surface in respond to various environmental stimuli. The resulting measurement can provide information on both extracellular and intracellular interactions. Cellular response induced by EGF is mediated through the epidermal growth factor receptor (EGFR), which regulates cell growth, proliferation, motility and differentiation [1]. Abnormal expression of EGFR and deregulation of EGFR signalling are known to be associated with the development of epithelial malignancies in humans [4]. A example of the three main downstream pathways of EGFR signalling is given in Figure 1 [5]. Real-time monitoring of EGF-induced cellular response can provide the physiological status of cells, which may have a significant medical implication.

Strategy

The cells are allowed to adhere onto the surface of the sensor crystal under favorable growth condition. The cell-attached sensor is mounted onto the QCM instrument and the entire measurement is then conducted at 37°C with a constant liquid flow. Once the baseline is stabilized, the stimulus (EGF) is introduced and signals in both frequency and damping are recorded to reveal the changes in cellular response.

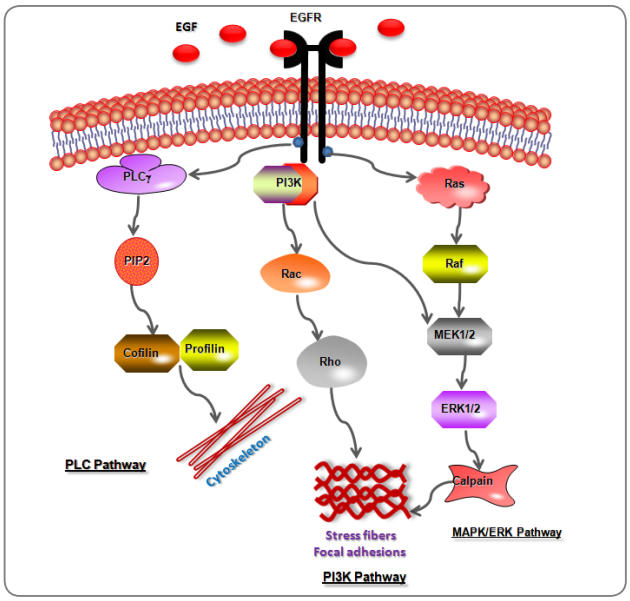

Figure 1. The three main downstream pathways of EGFR signaling that are potentially involved in regulation of cell de-adhesion of MCF10A cells [5].

Method

I. Cell Culture. A431 cells are cultured in T175 Corning culture flasks and maintained under a humidified atmosphere at 37 °C and 5% CO_2 in DMEM containing 10% FBS, 100 IU/mL penicillin, and 100 µg/ml streptomycin. The cells are harvested at 90% confluency.
II. qCell T Sensor Preparation. First a gold-surfaced sensor is rinsed with water and ethanol and then exposed to UV-ozone for 20 min. The sensor is then stored in the tissue culture hood under UV light. A431 cells are harvested from the T175 culture flask and then seeded onto the top side of the sensor in a sterile petri dish. The cells are allowed to adhere and grow under a humidified atmosphere at 37 °C and 5% CO_2 overnight. The following day, cells on the sensor are washed with serum free medium and starved in serum free medium for additional 18 h before the QCM measurement.
III. QCM Measurement. A QCM (qCell T, qCell) is used to record resonant frequency (f) and damping (D) as a function of time at the fundamental frequency. On the day of the QCM measurement, the sensor with the adhered cells

is carefully rinsed with the assay buffer (20 mM HEPES in HBSS buffer, pH 7.2) and the back side and edge around the quartz crystal is dabbed with Kimwipe to remove residual buffer. The sensor is then mounted onto the qCell T. The assay buffer is allowed to flow over the quartz crystal at 410 µl/min at 37 °C until the flow cell is completely filled. The flow rate is then changed to 10 µl/min. The recording of baselines is initiated with the reset of the data acquisition program. Once a stable baseline is achieved, a solution that contains a specific concentration of EGF in assay buffer is introduced. The signals in both frequency (f) and damping (D) are recorded simultaneously and continuously for 3 h.

Results

The traces measured with the presence of 0 nM and 75 nM of EGF are shown in Figure 2. The difference between the two traces lies in an initial sharp decrease between 10 and 20 min, which represents the cellular response induced by 75 nM of EGF.

Comparison to the QCM-D Measurement. The QCM-D (BiolinScientific, Inc.) has previously been used to detect cellular response mediated by EGFR [5]. The traces of the QCM-D detection, which is based on the change in energy dissipation factor of EGF-treated cells, are shown in Figure 3. The QCM-D traces in Figure 3 are very similar to the QCM traces in Figure 2 in both shape and time-dependence, suggesting that the qCell T technique is capable of performing cell studies at the level of sensitivity and time resolution that is comparable to the established technique such as the QCM-D.

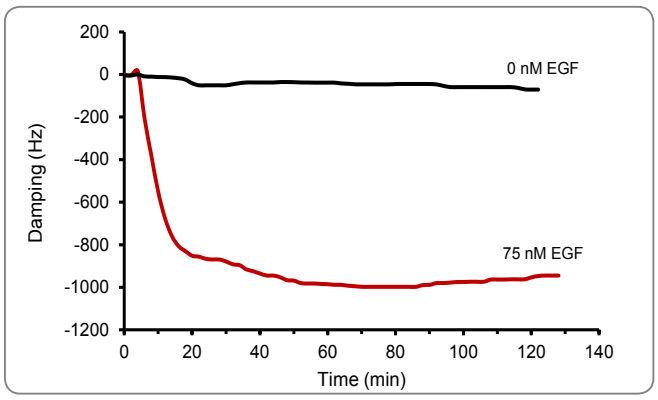

Figure 2. Damping traces measured with the qCell T. Recording conditions: 0 nM (black trace) and 75 nM (red trace) EGF in A431 cells for 3 h at a flow rate of 10 µL/min and temperature of 37 °C.

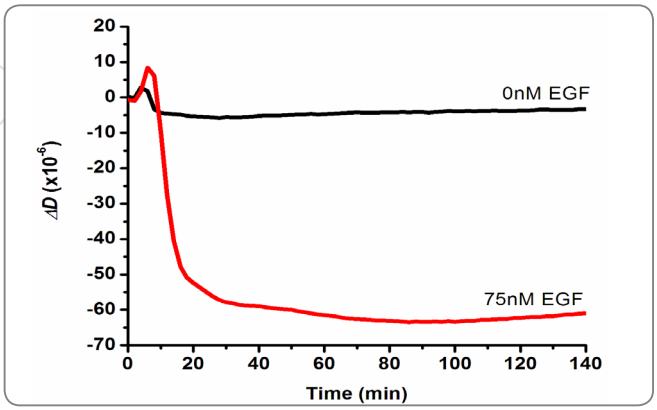

Figure 3. Change in dissipation factor (ΔD) traces measured with the QCM-D. Recording conditions: 0 nM (black trace) and 75 nM (red trace) EGF in A431 cells for 3 h at 37 °C.

Conclusion

The qCell T provides real-time monitoring of the responses of EGF treated live A431 cells. The results of the QCM measurement are comparable to those obtained with the QCM-D technique. The qCell T has the capability of examining ligand-induced cellular response.

References

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Acknowledgements

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