Make the invisible visible

Multi-day real-time cell culture health monitoring in physiological conditions using the Discovery-Q

Abstract

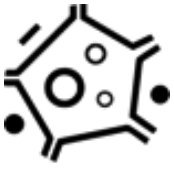
The Discovery-Q is a 4-well miniplate designed for cell-based research, that provides a real-time method for quantitative phenotypic investigation. The device is designed to function in a cell culture incubator to ensure physiological conditions for cell research over multiple days.

The Discovery-Q is a living whole cell biosensor that detects measurable changes in cellular biomechanics: attachment, mass redistribution, viscoelasticity, attachment, proliferation, death. The Discovery-Q sensor is created using a 10Mhz quartz crystal microbalance with a biocompatible gold coating that the cells grow on. This precision measurement system has been used in chemistry and physics applications. However the innovation of the Discovery-Q is the creation of a standard multi-well system for use with cell cultures. The Discovery-Q uses a sterile consumable well plate with an embedded sensor, and the well size matches a standard 24 well culture plates.

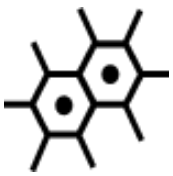
The Discovery-Q is a non destructive assay that also does not require specialized media or reagents. Once cells have been added to the media, the Discovery-Q in real-time, creates reproducible and robust experiment data and can be used with any adherent and semi-adherent cell type. The technique is label free, rapid, and sensitive, and it gives unique kinetic information when an agent interacts with the bound cells. The data generated is numerical and requires significantly less storage than microscopy systems. This allows for cell health monitoring once every second, with readings regarding mass/attachment mechanics and viscoelasticity of the cells at each time point over multiple days.

The device enables identifies time points for research ranging from cell death mechanism, mitochondrial perturbations, chemotherapeutic agent mechanism of action. Additional research areas are co-culture interactions, disease and regeneration modeling with diverse extracellular matrix, liver toxicity, liver fibrosis, and immune cell behavior and response. With the specific research area, cell type and agents, InVitroMetrix can create biokinetic signature patterns to assist in recognition of cell events at any time point during the experimental course.

Increase repeatability and accuracy of research



Organelle & Cell changes due to agent



Cell to cell interactions

Adherent & Semi-Adherent cells

Monitor Real–time Cell Health

Introduction

Quartz Crystal Microbalance (QCM)

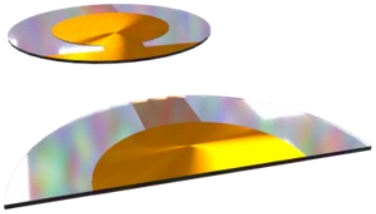


Figure 1. Discovery-Q quartz electrode with a biocompatible gold electrode.

A quartz crystal microbalance (QCM) is a precision measurement device developed for chemistry and physics research[1]. Following the development of the Sauerbrey equation in 1959, oscillation changes in frequency could be correlated to change in mass on the QCM's surface and was subsequently used to measure gas or elements in a vacum[1; 2]. A QCM is made up of some basic components: an AT cut quartz crystal and metal electrodes (Fig. 1)[1-3] On the quartz crystal a thin layer of gold is attached to act as the sensing electrode, as well as the surface for cell adherence.[1; 3; 4]

QCM in Biochemistry

Subsequent modifications of the QCM device occurred to enabled it to function in liquid (Fig. 2)[1; 3]. These modifications enabled the QCM's use in chemistry and biochemistry, where liquid is needed above the electrode. This enabled the measurement of enzymatic reactions, antibody binding. More recently the QCM has been used to measure changes in anchorage dependent whole cells[5-13]. Previous research has shown that a QCM device is able to measure anti-body based immunoassay by direct or sandwich methods, detection of nucleic acids, glucose detection via hexokinase binding, real time measurement of cell metabolism and division rates, and immunological detection of microbes[5-13]

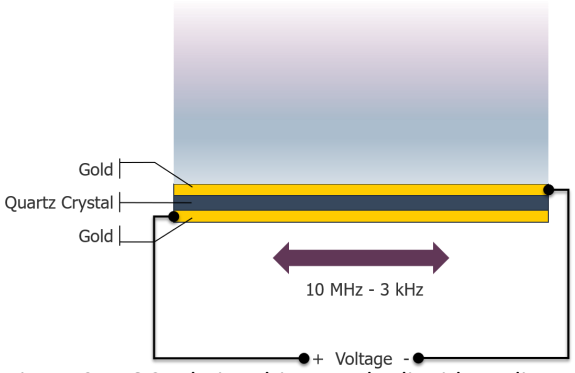


Figure 2. A QCM being driven under liquid conditions. Some energy of the motional resistance is lost into the liquid requiring an increase in voltage. Changes in the frequency relate to lass and changes in resistance equates to loss of energy.

Whole Cell Biosensor

The Discovery- Q, a modified QCM multi-well device that can monitor real time changes occurring within living cells while maintaining physiologically appropriate temperature, humidity, and CO_2 by working in a cell culture incubator. The Discovery-Q can detect when there is an attachment of mass to the sensor, which decreases the frequency at which the crystal oscillates, such as in the case

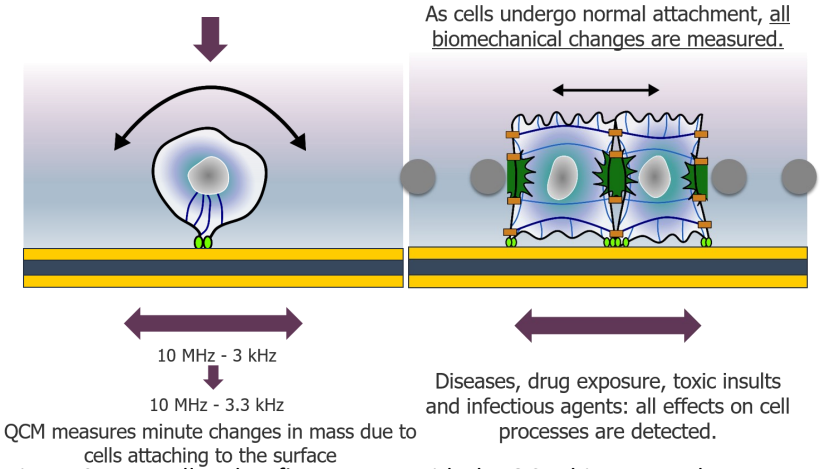


Figure 3. As a cell makes first contact with the QCM biosensor, the sensor detects the addition of mass and the sway of the cell in real time as binding progresses.

of antibody binding. However with cells the sensor detects not just the mass of the cell that is forming an attachment, but also the sway of the cells as they attach to the sensor (Fig. 3).

Discovery-Q

The Discovery-Q, while functioning in a cell culture incubator has the ability to measure all changes with the added cells from the moment they are added until the experiment is stopped, and has been used up to 8 days continuously. Using the Discovery-Q during research ensures repeatable experiments and accurate reproducible data generation during research. It also enable trouble shooting when unexpected results are obtained with cultures.

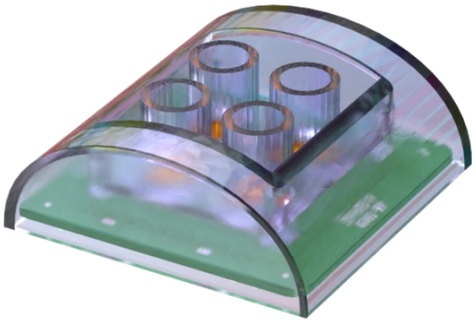


Figure 4. The Discovery-Q consumable well plate that is attached to the base in the incubator to acquire real time cellular data.

While the Discovery-Q is in the incubator cells can be added to the mini plate, in an identical fashion as a cell culture plate (Fig. 4). There are no special culture media's or reagents needed to run the experiment. If a biofilm is needed, the sensor can be coated as per the manufacture's instructions before cell addition.

Calibration of the Discovery-Q

All the devices we provide have been pre-calibrated using a sucrose calibration curve. Following system assembly, QC procedures are run using a sucrose mass-calibration solution to determine sensor-to-sensor variability. Correction algorithms are then applied to remove variability, though such variability is negligible. This ensures that any variability that is measured by the researcher is due to cell plating variability during the experiment.

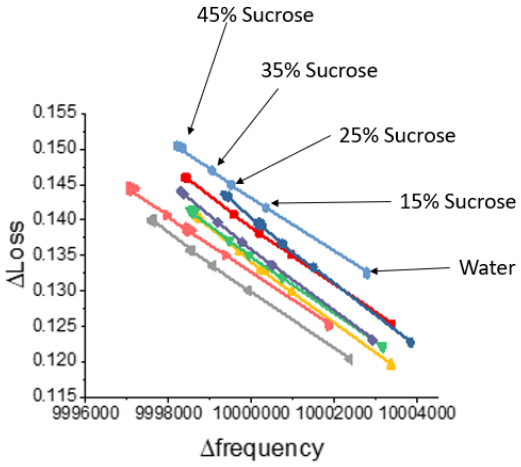


Figure 5. An example of a sucrose calibration curve of a Discovery-Q. Each sensor has a slight variability post manufacturing. Each sensor is tested for tuning frequency. A series of sensors with the closest frequencies are selected for a mini plate and variability is eliminated with the software and the data sored in the plate.

Historically the largest source of measurement error when using QCM's is the differences in tuning, contaminants on sensor and/or scratching on the sensor. To eliminate this issue with self made systems, each Discovery-Q consumable disposable well plate is calibrated, sterilized for cell culture and ready for use without having to worry about these issues.

Every mini plate is assembled with sensors that are matched and subsequently calibrated for use. The calibration data is stored on the well plate and it read by the system and incorporated used by the software while acquiring data during an experiment. This method removes well to well and plate to plate variation.

Cell addition to the QCM- ensuring experimental repeatability and accuracy

The Discovery-Q can start recording cell behavior as soon as cells are added into the well plate and the device is recording. The Discovery-Q can measure the biochemical and biophysical changes in cells while adhering to the sensors. Prior to cell addition the media is added and allowed to heat up and stabilize for 20-30 minutes. Subsequently the change of frequency is zeroed and the time is set to equal zero. This enables measurement of all changes from the time of cell addition. Each cell type has a mass per cell dependent frequency decrease[14].

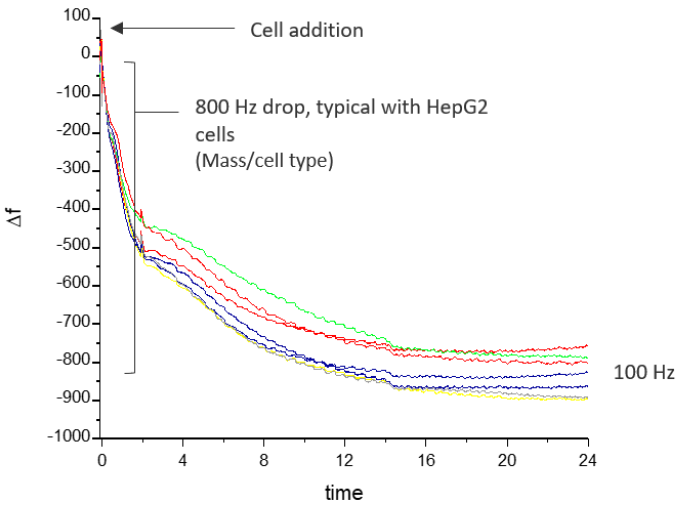


Figure 6. At time 0, 125,000 HegG2 cells were plated into each culture well at the same time. The real time data was collected regarding the cell attachment curve over 24 hours.

As seen in Figure 6, once cells are added the plating success can be monitored in real time and the variability between well plating can be accessed. Since a cell number dependent mass change is expected, in this experiment the 100Hz variation is equivalent to 15,000 cells from the highest to the lowest reading[14]. Well to well and experiment to experiment variability can be minimized.

When working in situations that use expensive cells such as in primary cells, or expensive experimental drugs in the drug discovery phase, the health of the cells can be assessed before the experiment is continued or the agent is added. This ensures that the quality of the data that is obtained is of the highest quality increasing reliability of the experiment.

The Discovery-Q can ONLY use cell lines that are adherent or semi-adherent (such as macrophages). Suspension cells can only be used if a biofilm to ensure adhesion is previously applied. Any biofilm or culture coating can be applied directly on the gold coating of the sensor as per the manufacturer's instructions.

The Discovery-Q can use primary cell lines that are vendor purchased, or primary cell lines that are created from fresh isolates. With a mixed population the mass/cell calculation changes due to the cell to cell influence of the different cell types and the 3D architecture that the cells assume. However in this circumstance, measurements of the tension, viscoelasticity and polarity of the cultures can be interrogated with the Discovery-Q. For more information regarding this application, please refer to the primary liver culture application note.

Culture maintenance and monitoring of health

Since the Discovery-Q can provide real time readings over multiple days, the wells are treated like a conventional culture plate. Every 24 hours if needed, the media can be changed, allowing for the collection of media samples for additional biomarker assay.

The changing of media can be seen by the lines in Figure 7, indicating where the cells were momentarily dry before fresh media was added. In proliferating cell lines, every division cycle results in the increase of mass which corresponds to a decrease in frequency. The Discovery-Q can monitor the rate of proliferation when agents such as growth factors are added into different wells, or agents to inhibit proliferation. When culturing non dividing cells, such a primary hepatocytes, once the cells have settled after 24 hours, the cells can be monitored to see that a healthy stable monolayer has been acquired. For more information regarding this application, please refer to the primary liver culture application note.

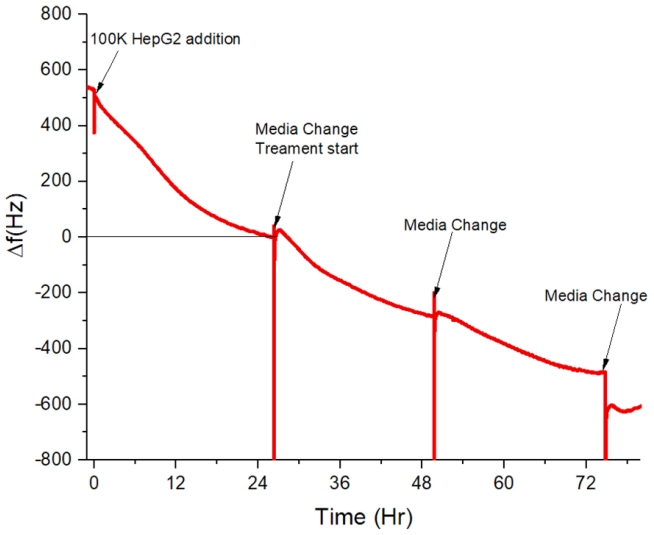


Figure 7. After 14 hours of cell deposition the health of the culture has been established with the appropriate decrease in frequency in relation to the number of cells added. The frequency is then zeroed out to measure pro-

Monitoring of time and mechanism of action of agents

With the Discovery-Q, real time data regarding drug response mechanism and time can be obtained. In addition multi-day repeat dose experiments can be conducted as seen in the experiment in Figure 8. In the experiment HepG2 liver epithelial cell line was cultured over 3 days. After 24 hours of allowing the cells to settle, the media was changed in all wells and treatment was added in a series of wells. This experiment tested a repeat treatment scenario with two doses and a vehicle control of phosphate buffered saline. The agent is Bromfenac, a nonsteroidal anti inflammatory drug designed as an ophthalmic solution to be used post eye surgery[15]. The drug was removed from the market following numerous reports of idiosyncratic hepatotoxicity[15]. The experiment was able to show that even at a low doses, repeat treatments of Bromfenac on the liver cells were inducing cell death[15].

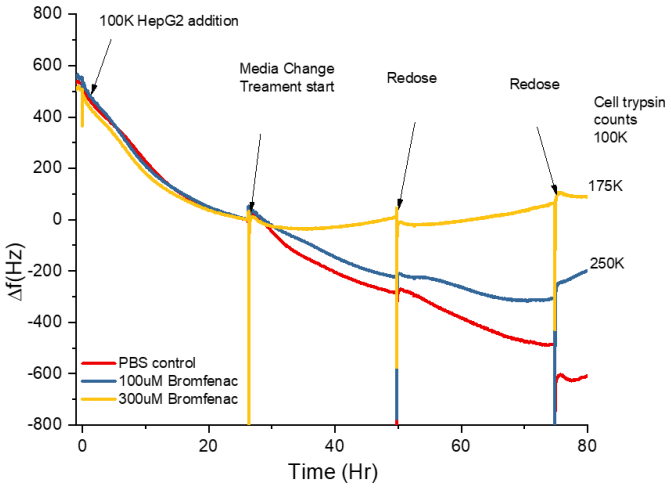


Figure 8. The Discovery-Q was plated with 125,000 HepG2 liver epithelial cells, and 24 hours post plating Bromfenac was added to test toxicity. Every 24 hours a repeat treatment was conducted to measure cumulative redose toxicity.

Biokinetic signatures and time point identification

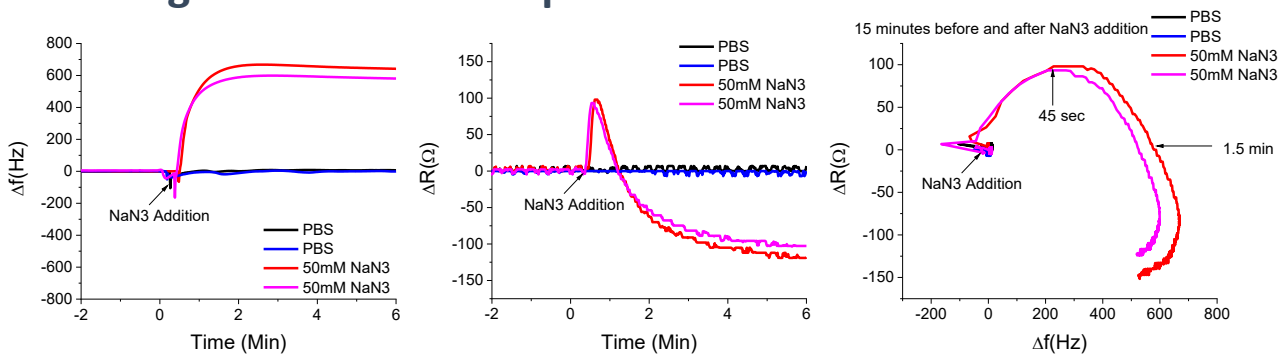


Figure 9. Macrophages were exposed to sodium azide to induce cell death. The frequency shows a cellular change in mass right after addition. The resistance shows a transient change in viscoelasticity or swelling that then falls below the untreated baseline. The two traces are combined to create unique biokinetic signature for the mechanism of death.

The Discovery-Q has the ability to monitor for real time changes in cells in response to the addition of agents. This unique ability allows for the identification of time points where the mechanism of action relating to a change is occurring[16]. In the example in figure 9, the addition of sodium azide to macrophages caused apoptosis after 24 hours. However with the Discovery-Q changes in the biokinetic signatures can be investigated to determine the cause[16]. The frequency detected a change of mass of the cells when the agent is added, but no subsequent mass change[16]. However, the resistance measured a change in viscoelasticity or swelling.[16] The resistance trace detected a transient swelling, followed by a decrease below baseline all within 15 minutes of adding the agent[16]. Once the mechanism time point was identified, an investigation into the cause determined that the sodium azide resulted in a mitochondrial membrane permeability change, first allowing for the hyperpolarization and subsequently the depolarization of the mitochondrial which initiates apoptosis[16]. The combination of the signatures creates a biokinetic signature identifies this specific mechanism of cell death for future comparison against. The mechanism of action was confirmed using mitochondrial membrane permeability dyes. This proves how the biokinetic information provided by the Discovery-Q can advance research into the mechanism of action of agents being researched[16].

Discovery-Q capabilities

The Discovery-Q can detect biomechanical changes in the cells as they respond to agents. Previously detected mechanisms are:

- cell death (necrosis and apoptosis)
- changes in cytoskeletal elements (actin and microtubules)
- mitochondrial membrane hyper-polarization and depolarization
- changes in cell shape and rearrangement of mass
- cell-to-cell interaction
- motility
- immune cell behaviors (phagocytosis and macrophage activation)

The Discovery-Q was created for live cell research and is designed based on cell culture wells instead of fluidics systems. The novel multi-well live-cell sensor monitors the cells in real time over multiple days if needed. It is the only method that can provide direct real-time second-to-second information of cells' mass distribution and viscoelastic properties resulting in unique discoveries regarding cells behavior and health.

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Product information

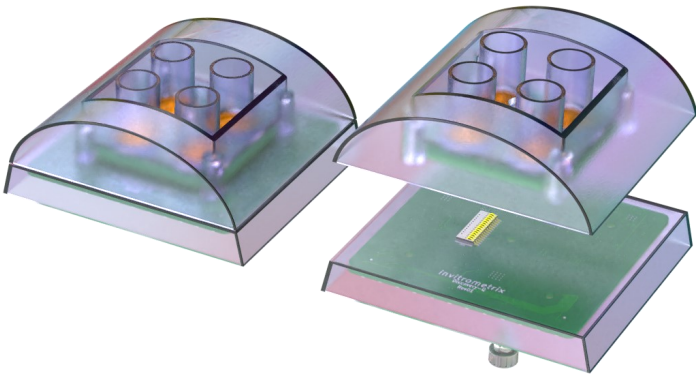
Platform: Discovery-Q

- Compatible with adherent and semi-adherent cell lines
- Simultaneous real time readings of frequency and resistance (dissipation)
- Minimum cell number per well: 1,000
- Label free detection, **specialized media or serum not required**
- Network based system
- Remote operations on web browser
- Data is date and time stamped and downloadable as CSV files

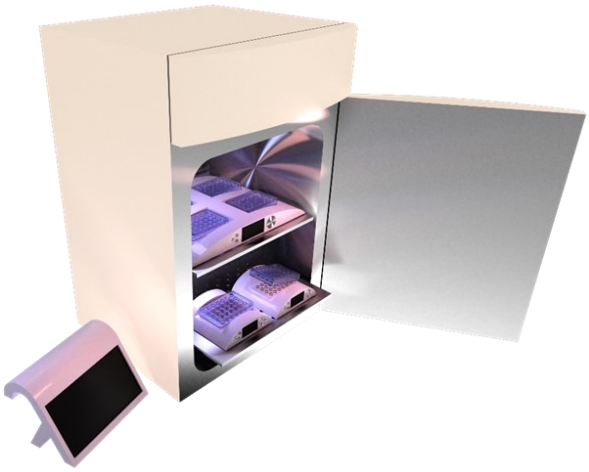
Cell culture types tested: Primary cell lines (cryopreserved plateable hepatocytes, stellates), liver, breast, lung, umbilical, macrophage, primary tumors

Cell culture lines tested (not a comprehensive list): HepG2, Sk-Hep-1, DH82, SK-Br-3, MDA-MB-231, HUVEC, BAE, BAEC, HL-60, HT-29, HMEC, NHBE, HMVEC-L, HMVEC-BL, HepRG, Hs578t, FaDu, MCF-7, MCF-12A,

For information regarding pricing of units or any other matters please contact: info@invitrometrix.com



The Discovery-Q and consumable well plate



Invitro-Q units working in a cell culture incubator.

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