Modified citrus pectin slows migration of triple negative breast cancer cells in an impedance-based scratch assay

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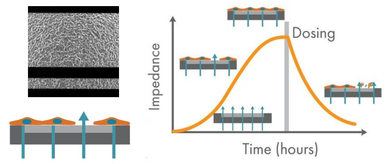
Maestro Z: Dynamic Cell Tracking

Impedance Technology

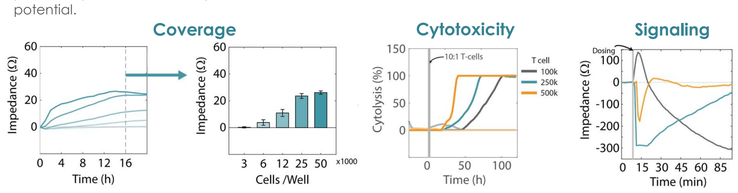
The flexibility and accessibility of cell-based models has allowed complex human biology to be reproduced *in vitro* for applications in toxicology, disease-in-a-dish modeling, and drug/therapy discovery.

Triple-negative breast cancer (TNBC) is an aggressive type of cancer that has a poorer prognosis than other types of breast cancer due to limited therapeutic options and a high risk of metastatic reoccurrence. A cell migration/wound healing assay can be used to assess metastatic potential of cancer cells and evaluate potential antimetastatic therapies.

The Maestro Z impedance assay is a sensitive and nondestructive method to continuously monitor cancer cells, allowing assessment of both the kinetics and degree of migration after scratch, providing a powerful quantification of key factors in metastatic potential.

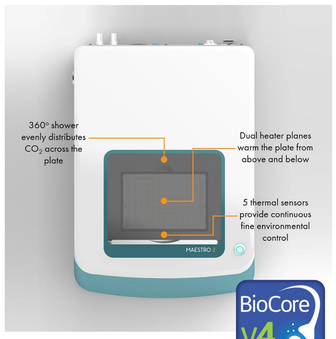


Impedance is measured by passing small electrical signals through the electrodes at the bottom of the well. When the electrode is uncovered, electrical signal easily passes (arrows) and the impedance is low. When cells cover the electrode, less electrical signal passes and impedance is high. If a perturbation causes cells to die or detach, the impedance will decrease.



Impedance-based cell analysis provides measurements related to the attachment, proliferation, and coverage of attached cells, as well as the dynamics of cytotoxicity or changes in cell conformation and morphology as a result of cell signaling.

The Maestro Z Platform



- **Label-free, non-invasive tracking** of cultured cells
- **Integrated environmental control** provides a stable environment for short- and long-term studies with a small benchtop footprint
- **Automatic and continuous cell monitoring** from 96 wells simultaneously
- **"One button setup"** automatically docks the plate and adjusts temperature and CO_2 levels
- **Powerful data analysis** to focus on the science, while AxIS Z handles the details with simple setup and automatic experiment tracking
- **See your cells** with the viewing window included in each well of the CytoView Z 96-well plate
- **State-of-the-art electrode processing chip (BioCore v4)** enables advanced endpoints, such as barrier index, to further characterize cell-based models

Impedance assays measures diverse cell properties

Assessing the metastatic potential of cancer cells *in vitro* is often done via imaging assays, which are subjective and often limited to snapshots in time. Axion BioSystems' Maestro Z platform offers impedance-based cell analysis for real-time, label-free monitoring of cells in an easy-to-use benchtop system. The Maestro Z uses impedance technology to characterize cells cultured directly onto electrodes embedded at the bottom each well, with sensitivity to each of the following:

- **Coverage** – the change in impedance is directly related to the number of cells covering the electrode affording opportunity for migration/wound healing assays.
- **Cytotoxicity** – dynamic monitoring of cell viability compared to untreated controls facilitates measurements of the degree and speed of cytotoxic activity.
- **Morphology** – the size, shape, and intercellular tight junctions of adherent cells significantly impact the measured impedance signal.
- **Signaling** – small changes in cell shape or cytoskeleton organization are detected in response to intracellular signaling events

Maestro Z Scratch Assay

Experimental Methods

(A) MCF-7 (Cat. HTB-22) and HCC1806 (Cat. CRL-2335) cells were obtained from ATCC. A CytoView-Z 96-well plate was coated with 50 µL PDL and incubated for 30 minutes at room temperature. 100uL of DMEM complete medium was added to each well for a baseline measurement. MCF-7 cells (27,000 cells/well) and HCC1806 cells (30,000 cells/well) were added to 28 wells each. **(B)** Impedance measurements were acquired continuously with the Maestro Z platform. After 72 hours, cells were switched to low-serum media to transition them into a reduced proliferative state. 24 hours later, the plate was removed from the Maestro Z and a pipette tip was used to gently scratch away cells in the center of each well (n=20 wells per cell type), creating a gap in the monolayer of cells. Immediately after the scratch, PectaSol-C and in-house MCP were each added to 5 wells per cell type to examine effects on cell migration. **(C)** Cell migration into the gap was assessed in real-time on the Maestro Z for 80 hours. All analyses were performed with AxIS Z software. Migration extent was corroborated with microscopy.

Breast cancer cell lines exhibit different cell profiles

Different forms of breast cancer exhibit differences in growth and metastatic potential. Here, MCF-7, a hormone-receptor positive line, and HCC1806, a triple-negative breast cancer (TNBC) line were seeded on a CytoView-Z 96 well plate. Both MCF-7 and HCC1806 attached and spread, resulting in a steady increase in impedance. HCC1806 reached confluency by 60 hours, while MCF-7 continued to proliferate for a higher maximum impedance. At 72 hours, cells were switched into low-serum media and scratch and treatment applied 24 hours later.

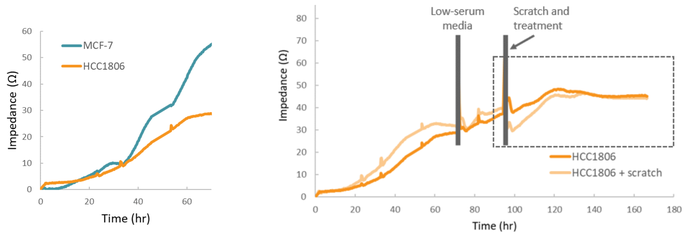


Figure 1: (Left) Growth curves for MCF-7 (teal) and HCC1806 (orange) cells illustrate distinct rates of proliferation. (Right) After reaching confluence, cells were switched to low-serum media. A scratch was then applied to some wells. Scratched wells showed a clear reduction in impedance. Subsequent migration was then monitored for 72 hours, with and without treatment.

Scratch assay confirmed visually

After 24 hours in low serum media, a scratch was applied to a subset of MCF-7 and HCC1806 wells. Scratches were confirmed visually through the viewing window on the CytoView-Z 96 well plate. Both cell types showed a large, consistent gap through the cell monolater. MCF-7 showed little migration into the gap at 24 hours post-scratch. In contrast, HCC1806 showed migration into the scratch, significantly filling in the scratch gap by 24 hours. These results are consistent with the greater migration and metastatic potential of TNBC observed clinically.

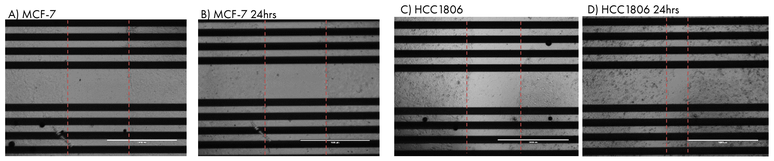


Figure 2: Image of MCF-7 cells taken just after the scratch (A) and 24 hrs later (B). Image of HCC1806 cells taken just after the scratch (C) and 24 hrs later (D). Orange vertical dashed lines approximate the cells' position at the edges of the scratch at each timepoint. Scale bar is 1000 µm.

Migration and Metastatic Potential

TNBC migration post-scratch reflects greater metastatic potential

Both cell types exhibited a large, consistent decrease in impedance post-scratch. After scratch, Maestro Z impedance readily detected differences in cell migration and rate of migration post-scratch between the two breast cancer cell lines. The normalized impedance of MCF-7 remained consistently decreased over 72 hours post-scratch (Figure 3), suggesting little to no cell migration into the scratch gap, which was consistent with visual inspection (Figure 2).

In contrast, HCC1806, TNBC with high metastatic potential, showed a rapid increase in impedance post-scratch. Over time, normalized impedance returned to near unscratched levels, suggesting significant migration into the scratch gap. Again, this was consistent with visual inspection.

The differences in migration detected by impedance reflect the differences in metastatic potential of the two lines, with TNBC exhibiting much greater metastatic potential compared to hormone positive breast cancer.

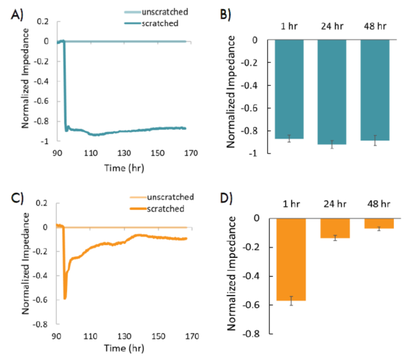


Figure 3: Normalized impedance after scratch for MCF-7 cells (A,B) and HCC1806 cells (C,D).

Modified citrus pectin slows TNBC migration

Modified citrus pectin (MCP) is a polysaccharide that shows potential for reducing metastasis. Treatment with MCP reduced migration of both breast cancer cell lines, resulting in a consistently lower impedance reflecting less migration into the scratch gap. For TNBC, HCC1806 cells, in-house MCP was slightly more effective compared to PectaSol-C MCP, as evidenced by a lower impedance by and after 35 hours post-scratch.

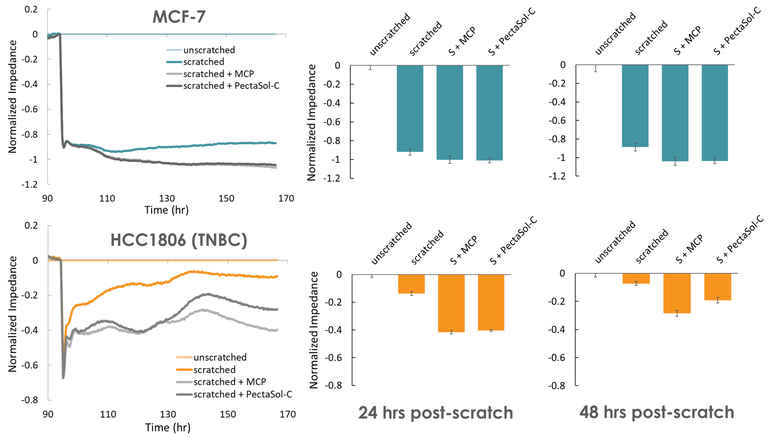


Figure 4: Normalized impedance after scratch for MCF-7 (teal) and HCC1806 (orange). Wells treated with MCP and PectaSol-C are shown in gray and dark gray respectively. Bar plots show the normalized impedance values for each treatment at 24 and 48 hours post-scratch.

Conclusions

- The Maestro Z platform enables dynamic, label-free, impedance-based cell tracking to quantify attachment, proliferation, and migration.
- The Maestro Z readily distinguished differences in migration that accurately reflected clinical metastatic potential of breast cancer cell lines.
- Modified Citrus Pectin (MCP) reduced the migration of HCC1806 cells, offering a promising therapeutic potential for TNBC with limited options. The Maestro Z impedance-based scratch assay was sensitive to small differences in antimetastatic MCP potencies and dynamics, confirming its value for assessing potential antimetastatic therapies.

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