Monitoring Cell Motility Using CytoTronics' Microplate

ABSTRACT

Cell motility is required for many biological processes such as embryonic development, axon guidance, wound healing, and tissue regeneration. In tumors, increased motility and migration can be predictive of more aggressive subtypes and increased metastasis. While optical imaging for measuring cell motility and migration at high throughputs presents challenges – primarily due to the time needed for a microscope to scan each well in a microplate – CytoTronics' technology offers a streamlined solution. Its built-in measurement electronics and electrodes in each well ensure consistent readings over time. Furthermore, the ability to simultaneously capture multiple functional and morphological measurements allows for a holistic understanding, linking motility to other cell functions.

In this note, we establish that CytoTronics' technology can, in real time, monitor cell motility and provide examples of its unique insights. We show that different cell types (A549, HepG2, and U-2 OS) have different motility profiles and demonstrate pharmacological modulation of motility using Cytochalasin D, a cytoskeletal inhibitor, and Bosutinib, a multikinase inhibitor.

TECHNOLOGY

Using the vertical field (VF) 4 kHz measurement, the CytoTronics platform can accurately map cell positions on the electrode array. To quantify motility, we then compare changes in cell positions from consecutive measurement frames and aggregate the signal across all electrodes using a root mean square (RMS) formula (Figure 1). Our technology's high spatial resolution electrode arrays combined with ability to do fast measurements, enables motility monitoring with single-cell resolution[1].

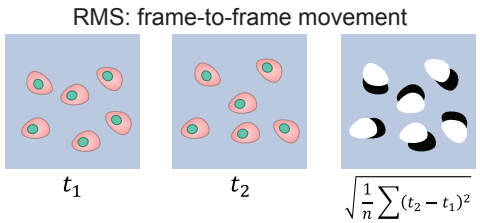


Figure 1. Schematic describing the RMS calculation derived from the VF 4 kHz measurement. The difference between two consecutive VF 4 kHz images (t1 and t2) is aggregated across all electrodes using a root mean square (RMS) formula.

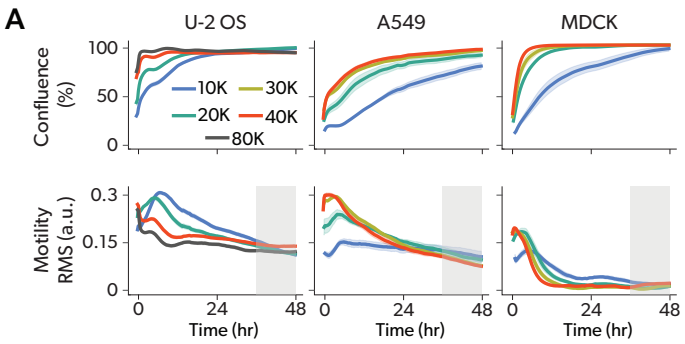
RESULTS

Inherent motility properties of cells

We characterized three cell lines with distinct motility characteristics using the CytoTronics' platform: A549 (a lung adenocarcinoma cell line) U-2 OS (an osteosarcoma cell line), and MDCK (a primary kidney epithelial cell line). Cells were plated at various starting densities and both confluence and motility were monitored over time (Figure 2A). After initial attachment, MDCK cells showed virtually no motility, measuring just above the electrode background signal (Figure 2B). Their epithelial nature to grow in packed sheets with a characteristically-strong attachment to their substrate supports this low measured movement. In contrast, U-2 OS and A549 cells, both derived from tumors, demonstrated high motility. However, their motility decreased as confluence increased, suggesting that as cell density rises, available space for movement diminishes.

Pharmacological inhibition of motility

To demonstrate a pharmacological modulation of motility, we used Cytochalasin D, a known inhibitor of cell motility due to its inhibition of actin polymerization[2,3]. Upon treating U-2 OS and A549 cells with 0.1 µM Cytochalasin D, we observed a marked decrease in motility within 6 hours compared to DMSO control (Figure 3, bottom). We additionally explored disease relevant



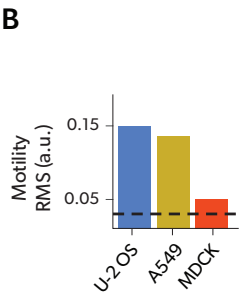


Figure 2. (A) Confluence (top) and motility (bottom) of three cell lines at various densities measured from seeding to 48 hours. Steady state motility (max of shaded region) was used for (B). Shaded areas around the curves represent the standard error of well replicates. (B) Motility at steady state. The dotted line represents the no-cell electrode signal, illustrating the minimum detectable motility.

pharmacological changes to motility. We found that cells treated with 1 µM Bosutinib, a Src inhibitor shown to reduce cell motility and invasion in breast cancer cell lines4, also had a rapid decreased motility in both A549 and U-2 OS cells (Figure 3. bottom).

We then extended our analysis beyond motility to include cell attachment (Figure 3, top). Interestingly, we observed a decrease in attachment in A549 cells treated with Bosutinib, while Cytochalasin D caused an increase in attachment. U-2 OS cells also showed distinct changes in attachment in response to both compounds. Such outcomes underscore the benefit of measuring multiple parameters simultaneously: both Cytochalasin D and Bosutinub similarly decrease motility but affect other cell parameters like attachment quite differently, arising from their different mechanisms of action.

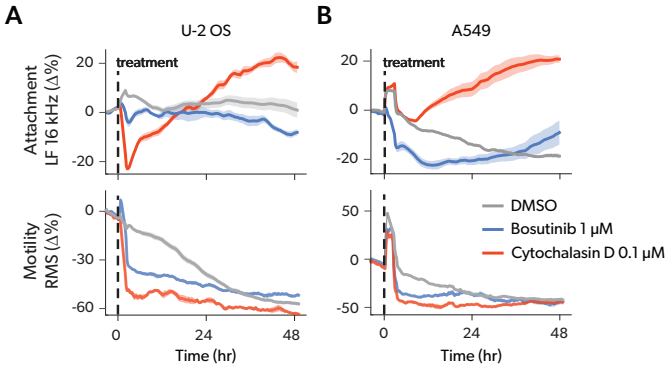


Figure 3. Changes in attachment and motility for U-2 OS (A) and A549 cells (B) treated with DMSO (grey), 0.1 µM Cytochalasin D (orange), or 1 µM Bosutinib (blue). Measurements were time-normalized to 2 hours before the treatment.

CONCLUSION

CytoTronics' impedance technique provides a precise real-time assessment of cell motility dynamics. It offers accurate measurements of motility across a variety of cell types and facilitates the real-time monitoring of motility changes, whether induced by compound treatments or innate cellular processes such as cell growth and increase in cell density.

METHODS

Cell lines

All cell lines were obtained from ATCC and maintained in a humidified incubator at 37°C and 5% CO_2. A549 (CCL-185), MDCK (CCL-34), and U-2 OS (HTB-96) cells were cultured in DMEM supplemented with 10% FBS.

Treatment and measurement

Impedance measurements were taken at 0.25, 1, 4, and 16 kHz inside a humidified incubator at 37°C and 5% CO_2. For cell growth experiments, cell lines were seeded between 10,000 and 80,000 cells per well with impedance measurements taken every 15 minutes over 48 hours. For the Anisomycin experiment, MDCK cells were seeded at 30,000 cells per well and grown for 24 hours. Anisomycin (Selleck Chem S7409) was added at 0.1 µM with a 0.5% (v/v) DMSO control. Compounds in media were temperature and CO_2 equilibrated prior to addition. Impedance measurements were taken every 15 minutes.

Data analysis

The well median of each measurement (motility and confluence) was plotted over time, with the standard error calculated across three technical replicates. Confluence was calculated as a percentage of electrodes occupied by cells. To determine occupancy, impedance response of electrodes without cells was measured. When impedance response increases above the bare electrode due to attachment of cells, the electrode is considered occupied. Relative motility was calculated by normalizing motility to its value one hour before compound addition with the relative change plotted over time. To compare motility across cell lines, the average steady-state movement between 36- and 48-hours post cell seeding was calculated.

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38 Wareham St, Boston, MA 02118

contact@cytotronics.com

cytotronics.com