APPLICATION NOTE

Monitoring of Cell Viability in Adherent and Suspension Cells Using Electrical Imaging

INTRODUCTION

Cell viability is a key readout for many biological assays. In oncological applications, cytotoxicity is the primary measure of drug efficacy, while for other drugs, it is crucial to assess unintended cytotoxic effects for safety purposes. Though a variety of assays are used to evaluate cell proliferation and cell death, including imaging and luminescence assays, most rely on destructive end-point readouts, limiting their ability to study response kinetics.

In this note, we establish that CytoTronics' technology[1] can non-destructively monitor cell proliferation, viability, and cell death in both adherent (e.g. A549) and suspension (e.g. K562) cells in real time throughout an experiment. This approach delivers in-depth kinetics of both growth and death phases in a single high-throughput assay. We further demonstrate the ability to monitor dose-dependent changes in cell viability for chemotherapy compounds across both adherent and suspension cell lines.

RESULTS

Assessment of Cell Death in Adherent Cell Lines

Optical imaging methods use confluence as a measure of cell number. Similarly, we define confluence as the percentage of electrodes that are occupied by cells. To demonstrate accuracy of the CytoTronics' Pixel in measuring kinetics of cell death, we used A549 cells, a lung adenocarcinoma cell line, that is often used in high-throughput cell death assays. A549 cells were treated with Paclitaxel, a common chemotherapeutic drug for lung cancer that inhibits microtubule depolymerization. As seen in Figure 1A and 1B, a dose-dependent decrease in cell confluence was observed upon Paclitaxel treatment.

To confirm the impedance-based results from the CytoTronics' platform, we then performed a traditional end-point cell viability assay using CellTiter-Glo (CTG). Dose responses from the CTG and impedance experiments were used to calculate

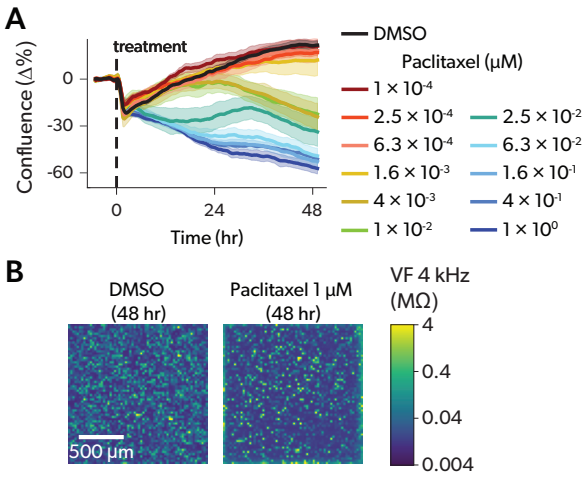

Figure 1. (A) Time-normalized confluence of A549 cells treated with DMSO or Paclitaxel. (B) Impedance images showing confluence for A549 cells treated with DMSO or 1 μM Paclitaxel after 48 hours. (C) Confluence dose-response at 48 hours (3 technical replicates per concentration). Measured IC50 is 8 nM (dashed line). (D) CellTiter-Glo (CTG) assay dose-response of Paclitaxel at 48 hours (3 technical replicates per concentration). Measured IC50 is 3 nM (dashed line).

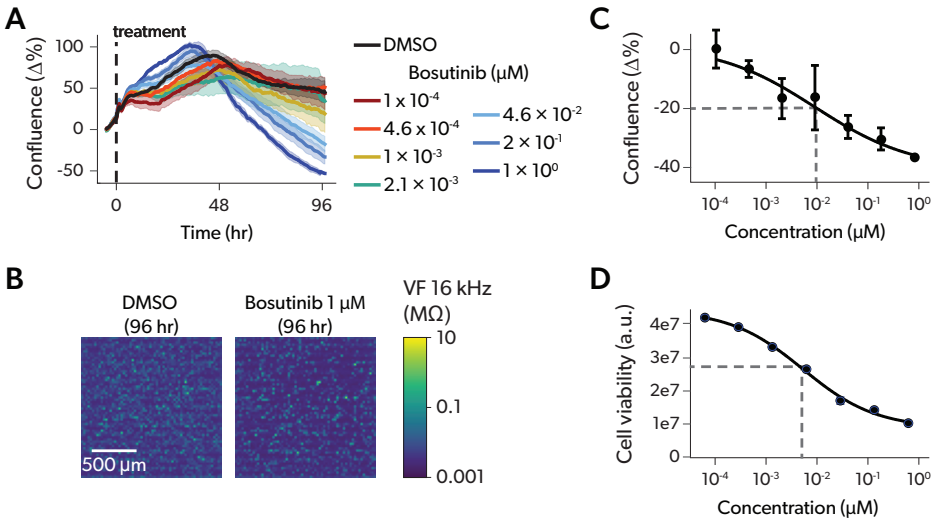

Figure 2. (A) Time-normalized confluence of K562 cells treated with DMSO or Bosutinib. (B) Impedance images showing confluence for K562 cells treated with DMSO or 1 μM Bosutinib after 96 hours. (C) Confluence dose-response at 96 hours (3 technical replicates per concentration). Measured IC50 is 10 nM (dashed line). (D) CellTiter-Glo assay dose-response at 48 hours (3 technical replicates per concentration). Measured IC50 is 5 nM (dashed line).

IC_{50} values for Paclitaxel (Figures 1C,D). The results showed a strong correlation both between the assays, and with previously published data.[2]

Assessment of Cell Death in Suspension Cell Lines

Suspension cells are challenging to study by optical imaging, as confluence cannot directly be used as a measure of cell number. In the CytoTronics' microplate, cells are immobilized close to the electrode surface, enabling a calculation of confluence, which is defined as number of electrodes that are occupied with cells. We have previously demonstrated that this method has a wide range of quantifiable and accurate confluence measurements which increase linearly with an increase in cell number.[1] In this study, K562 cells, isolated from the bone marrow of a patient with a chronic myelogenous leukemia (CML), were treated with Bosutinib, a multi-kinase inhibitor approved for treatment of CML. As seen in Figure 2A,B, a dose-dependent decrease in cell confluence was observed upon Bosutinib treatment.

To confirm the impedance-based results from the CytoTronics' platform, we then performed a traditional end-point cell viability assay using CellTiter-Glo (CTG). Dose responses from the CTG and impedance experiments were used to calculate IC50 values for Bosutinib (Figure 2C,D), The values showed a strong correlation both between the assays, and with previously published data.[3]

CONCLUSION

CytoTronics' high-resolution impedance assay provides an exceptional real-time evaluation of cell proliferation and viability kinetics for both adherent and suspension cells. It offers accurate measurements of confluency across a variety of cell types and facilitates the real-time monitoring of cell number and viability changes, whether induced by compound treatments or innate cellular processes such as growth.

The combination of response kinetics as highlighted here and measurement of other morphological parameters, such as attachment, flatness, barrier, and motility, can further provide information not only about cell viability, but also into the mechanisms of cell death.

MATERIAL AND METHODS

Cell Lines

Cell lines were obtained from ATCC and maintained in a humidified incubator at 37°C and 5% CO_2. A549 (CCL-185) cells were maintained in DMEM supplemented with 10% FBS and 100 U/mL Penicillin-Streptomycin. K562 (CCL-243) cells were maintained in IMDM supplemented with 10% FBS and 100 U/mL Penicillin-Streptomycin.

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 every 15 minutes throughout the experiment.

A549 cells were seeded at a density of 20,000 cells per well and cultured for 24 hours prior to compound treatment. Paclitaxel (Selleck Chem S1150) was dissolved in DMSO and added at concentrations from 0.1 nM to 1 μM with a 0.5% (v/v) DMSO control.

K562 cells were seeded at a density of 30,000 cells per well on PDL-coated plates and cultured for 24 hours prior to compound treatment. Bosutinib (Selleck Chem S1014) was dissolved in DMSO and added at concentrations from 0.1 nM to 1 μM with a 0.5% (v/v) DMSO control.

Compounds in media were temperature and CO_2 equilibrated prior to addition.

Cell Titer- Glo

CellTiter-Glo (Promega) assay was performed as per the manufacturer's instructions. Cells were plated on 96-well white-walled plates at equivalent densities to the impedance experiments. Cells were grown for 24 hours and then treated with either Paclitaxel or Bosutinib as described above. The CellTiter-Glo kit was applied and incubated either 48 hours (A549) or 72 hours (K562) after drug addition. Luminescence was measured using a Glo-Max Plate Reader.

Data Analysis

The well median of the confluence measurement 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 confluence was calculated by normalizing confluence to its value one hour before compound addition with the relative change plotted over time. Curve fitting for IC50 curves and calculation of IC50 values was performed using GraphPad Prism using a dose-response model.

REFERENCES

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3. Sang, F. et al. Structure–Activity Relationship Study of Rakicidins: Overcoming Chronic Myeloid Leukemia Resistance to Imatinib with 4-Methylester-Rakicidin A. *J Med Chem* **59**, 1184–1196 (2016).

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