Quantifying Proliferation and Cytotoxicity of Suspension Human Cancer Cell Lines Using the Maestro Z

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Cytotoxicity

Abstract

The ability to quantitatively correlate drug potency with target cell killing is critical for immuno-oncology studies. Endpoint assays only provide a snapshot in time, and are limited in their ability to assess dynamic target cell response. Here, we used the Maestro Z to record real-time, label-free impedance measurements to generate cell proliferation curves and examine the cellular response to cytotoxic chemotherapeutic compounds. Several target cancers for immunotherapy are liquid (suspension) and require antibody-mediated immobilization on plates. Thus, we assessed the tethering of suspension cancer cell lines, Daudi and K562, via cell-type specific antibodies on CytoView-Z impedance plates. The Maestro Z was able to detect antibody tethered cells versus untethered cells, resolve impedance changes across a range of cell densities, and quantify cell death after addition of cytotoxic compounds, doxorubicin and mitoxantrone.

Introduction

Most cancer immunotherapies rely on the use of monoclonal antibodies or cancer vaccines, but a new approach using the patient's own immune cells to kill target cells is progressing rapidly. This therapeutic approach requires the ability to investigate quantitative changes in cellular physiology and health *in vitro*. The Maestro Z allows for the continuous monitoring of cellular impedance, enabling the study of immune cell-mediated cytotoxicity, and providing a powerful means of assessing immune cell potency (Figure 1).

Approximately 10% of all new cancer diagnoses are blood cancers, which do not form solid tumors, and thus require tethering to the surface of the plate with antibodies for impedance measurements.[1] Here, we investigated the tethering and proliferation of two different suspension cancer lines (Daudi and K562). Daudi is a human B-cell lymphoma cell line commonly used as a model to study the mechanisms of leukemogenesis.[2] –K562 cells are a human chronic myeloid leukemia cell lines often used for *in vitro* cytotoxicity studies.[3]

In this study, Daudi and K562 cells were continuously monitored using the Maestro Z to quantify the dynamics of suspension cell tethering, proliferation, and cytolysis.

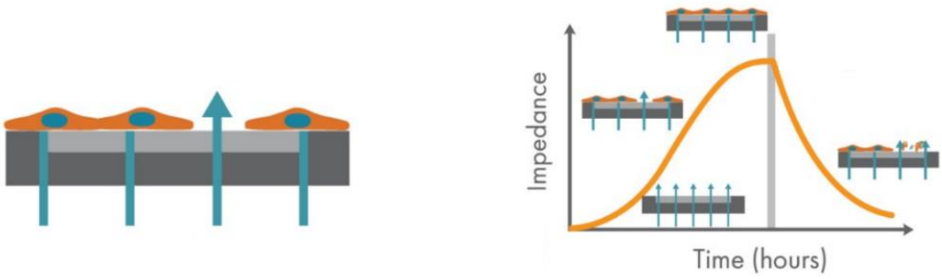

Figure 1. Impedance measures how much electrical signal (teal arrows) is blocked by the electrode-cell interface (right). When the electrode is uncovered, electrical signal easily passes and the impedance is low. When cells cover the electrode, less electrical signal passes and the impedance is high. When cells die or detach, the impedance decreases.

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Materials and Methods

Materials

Cells and reagents

Daudi (cat. CCL-213) and K562 (cat. CCL-243) cells were obtained from ATCC (Manassas, VA). Human anti-CD40 antibody (cat. AF632) was obtained from R&D Systems (Minneapolis, MN). Human anti-CD71 (cat. MAB24741-SP) antibody was obtained from R&D Systems. Daudi culture media was composed of RPMI-1640 medium (Gibco, cat. 11879020), 10% fetal bovine serum (FBS, Gibco, cat. 16000044), and 1% penicillin/streptomycin (Gibco, cat. 15140122). K562 culture media was composed of either RPMI medium (ATCC, cat. 30-2001), 10% FBS (GE Healthcare, cat. SH30071.03) or TexMACS™ phenol-red free medium (Miltenyi, cat. 170-076-307). Doxorubicin (cat. 15007) and mitoxantrone (cat. 14842) were purchased from Cayman Chemical.

CytoView-Z 96 plate

CytoView-Z 96-well plates (Axion BioSystems) were used for the impedance experiments. The plate is composed of a polyethylene terephthalate (PET) surface with a gold recording electrode embedded in the culture surface of each well. Humidity reservoirs on the plate were filled with deionized water to maintain humidity.

Maestro Z Impedance-based assay

The Maestro Z (Axion Biosystems) uses impedance measurements (ohms, Ω) to quantify the presence and behavior/morphology of cells on the electrode. To measure impedance, small electrical signals are delivered to the electrode. Cell attachment, spreading, and cell-cell connections block these electrical signals and are detected as an increase in impedance. Impedance is also sensitive to subtle changes in cell conformation, such as those caused by receptor-mediated signaling or cell morphology. Since impedance is non-invasive and label-free, impedance assays can be used to quantify dynamic cellular responses over minutes, hours, and days. In addition, the Maestro Z has built-in environmental control, finely regulating temperature and CO_2 throughout the experiment.

Methods

Plate preparation

The surface of the plate was coated with 50 µL of either anti-CD40 antibody (4 µg/mL) for Daudi cells or anti-CD71(1x) for K562 cells, diluted in PBS. Some wells were left uncoated to compare cell attachment between wells with and without the antibody coating. The plate was then incubated at 4°C overnight. After incubation, the antibody solution was aspirated from the plate, washed twice with PBS, and 100 µL of complete RPMI medium was added to each well.

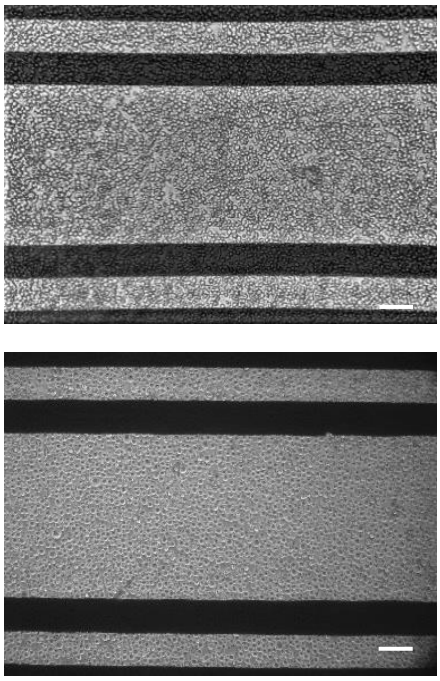

Figure 2: (Top) Images of Daudi cells with anti-CD40 antibody immobilization 48 hours post seeding at 150k cells/well on a CytoView-Z plate. (Bottom) Images of K562 cells with anti-CD71 antibody immobilization 48 hours post seeding at 50k cells/well on a CytoView-Z plate. The viewing window in each plate permits visualization of cells. Black horizontal bars are the recording and ground electrodes. Scale bar is 400 µm.

It is critical that antibodies targeted for specific antigens on suspension cells are optimized prior to performing an impedance assay. Antibodies produced by separate vendors and for different assays may differ in their effectiveness to tether suspension cell lines to the surface of the plate.

Media-only recording

The plate was docked in the Maestro Z and either a media-only or media plus antibody coating baseline was recorded to provide a reference for the data collected during the rest of the experiment.

Cell culture

Daudi and K562 cells were thawed and cultured in accordance with supplier recommendations until sufficient numbers were present for the assay. Flasks of cultured suspension cells were removed from a 37°C incubator, and the suspension was mixed with a serological pipettor. A sample of the cell suspension was used for counting using a hemocytometer and trypan blue solution. The cell suspension was transferred to a 15 ml conical tube for centrifugation. The supernatant was aspirated and diluted in complete RPMI medium to a working concentration of cells per 100 µl. The working

concentrations ranged from 50,000 to 300,000 cells per well for Daudi cells and 12,500 to 50,000 cells per well for K562 cells. Cells were seeded onto the CytoView-Z plate at 100 µl per well (see Figure 2). Sterile DI water (8 mL) was added to the humidity reservoirs on the edges of the plate. After cell addition, the plate was left to rest in the biosafety cabinet for one hour at room temperature.

Impedance recordings

The plate was docked in the Maestro Z and the automatic environment controls were set to 37°C and 5% CO_2. Impedance measurements were recorded continuously. All impedance graphs are displayed as the change in impedance from baseline and media-corrected (i.e. average impedance of media-only control wells were subtracted).

Results

Antigen-specific antibody tethering of suspension cancer cells enables optimal impedance detection

For a new cell line or plating procedure, comparing the impedance profiles across several densities is recommended to identify the optimal cell density to reach full confluence within 24-48 hours. Multiple densities of Daudi cells were plated on a CytoView Z-plate and their impedance was monitored by the Maestro Z for at least 48 hours. In this experiment, Daudi cells exhibited the most favorable tethering and proliferation results using an anti-CD40 antibody purchased from R&D Systems. An alternative anti-CD40 antibody purchased from a different vendor showed no difference in impedance values as compared to untethered Daudi cells (data not shown).

As shown in Figure 3, Daudi cells tethered to the anti-CD40 antibody (orange) exhibited a striking difference in overall impedance values as compared to untethered cells (teal), which exhibited little to no changes in their impedance values (≤1 Ω) over the entire time course. Tethered Daudi cells showed significant increases in impedance (1-5 Ω) within 2 to 10 hours after cell addition and continued to show higher impedance values as the cells proliferated over time (Figure 3, top). These results indicate that suspension cells, such as Daudi, require tethering to the surface of the plate via antibodies to produce robust changes in impedance signals that continue to increase over time as cells proliferate.

Impedance correlates with the tethered cell density

Changes in impedance were sensitive to differences in cell density, with higher cell densities showing a faster rise in impedance and higher final impedance values. The highest density of Daudi cells (300,000 cells per well) reached full confluence by 40 hours, as

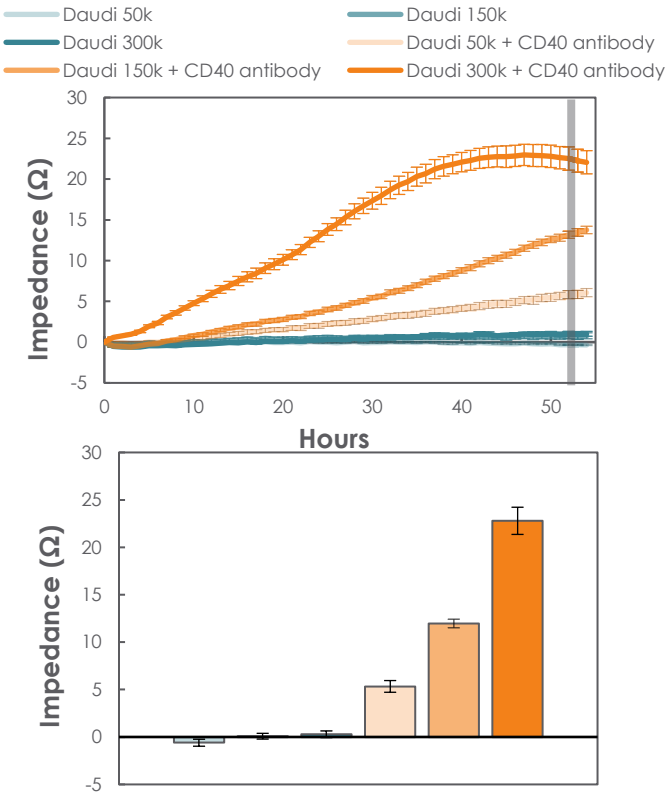

Figure 3: (Top) Growth curves for Daudi cells seeded at 50k, 150k, and 300k cells/well, with or without CD40 antibody, and monitored on the Maestro Z during the attachment, spreading, and proliferation phases. (Bottom) At 48 hours, the highest cell density (300k) had the largest impedance, whereas lower densities had lower impedance as they continued to proliferate. When no antibody was used, no impedance change was observed.

indicated by the plateau in impedance (Figure 3, top). The lower cell densities (50,000 and 100,000 cells per well) did not reach confluence by 40 hours, as shown by a continued increase in impedance. The differences between cell densities were only observed in tethered wells (Figure 3, bottom).

K562 cells were tethered with anti-CD71, and changes in impedance were measured at different densities for 120 hours. Similar to the Daudi cells, K562 cells displayed differences in impedance values between different cell densities, with higher cell densities showing a faster rise in impedance and higher overall final impedance (Figure 4) but with a more rapid increase during the initial tethering phase (0-2 hours). K562 cells continued to show a slow increase in impedance values, increasing from approximately 7 to 18 Ω. Overall, impedance is sensitive to, and correlates with, the density of tethered suspension cells.

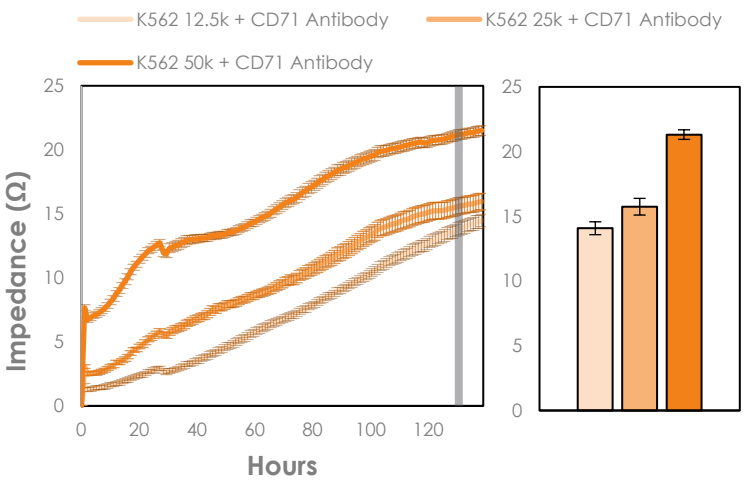

Figure 4: (Left) Growth curves for K562 cells seeded at 12.5k, 25k, and 50k with anti-CD71 antibody, and monitored on the Maestro Z during the attachment, spreading, and proliferation phases. (Right) At 130 hours, the higher cell density (500k) showed a larger impedance as compared to the lower cell density (12.5k).

Real-time tracking of K562 cytolysis

Approximately 30 hours post plating, K562 cells were treated with 0.01 or 1 uM doxorubicin, or 0.1 or 10 uM mitoxantrone. Some wells were left untreated, while others were treated with 1% Tergazyme, to serve as no treatment and full lysis controls, respectively. Tergazyme immediately lysed the cells, reflected in an impedance decrease to near zero. Cells responded to doxorubicin and mitoxantrone in a dose-dependent manner with cell death measured by decreasing impedance values (Figure 5).

Conclusion

The Maestro Z enables simple, label-free, non-invasive monitoring of cellular profiles. With the appropriate cell-type specific tethering antibodies, the Maestro Z impedance assay is compatible with multiple suspension cell lines. The Maestro Z impedance assay is sensitive to cell number, proliferation, and captures the real-time killing kinetics of chemotherapeutic treatments as demonstrated in K562 cells. By applying the same techniques demonstrated in this assay may be expanded to test other cancer therapies as well, such as killer T-cells or oncolytic viruses.

References

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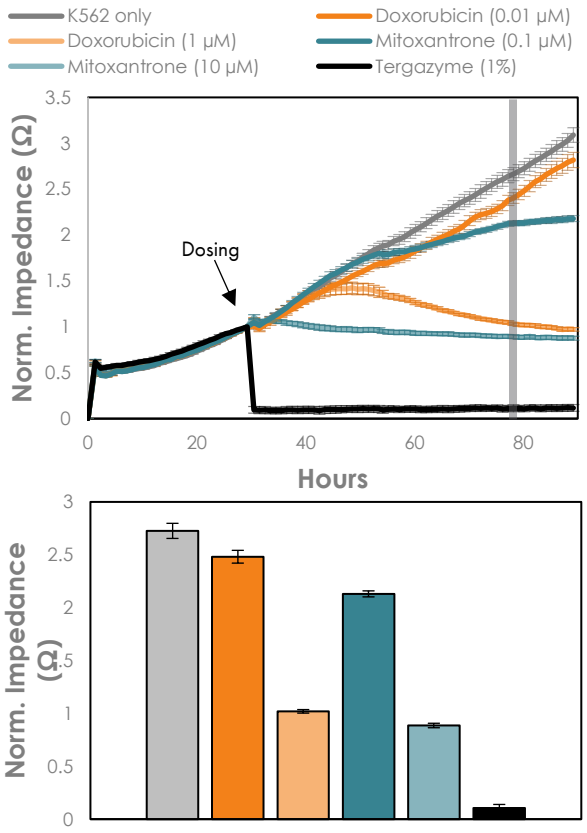

Figure 5: (Top) K562 cells proliferated on the CytoView-Z 96 well plate and were subsequently dosed with doxorubicin, mitoxantrone, or Tergazyme (black, positive control) at 30 hours post plating. Wells dosed with Tergazyme showed an immediate decrease in impedance, reflecting complete cell death. (Bottom) At 48 hours post drug dosing, wells (n = 6) treated with doxorubicin and mitoxantrone exhibited a dose-dependent decrease in impedance, reflecting more cell death with increasing doses of the cytotoxic compounds.