Quantifying Cellular Profiles of Non-Adherent Human Cancer Cell lines using the Maestro Z

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Abstract

Immune cell-mediated killing of target cells is a promising approach for cancer treatment. Several target cancers for immune-therapy are liquid (non-adherent), posing a challenge for traditional assays that require cells to be adhered to the surface of the plate. We assessed the tethering of multiple non-adherent cell lines (Daudi, K562, and Nalm6) via cell-type specific antibodies using the Maestro Z. Non-adherent cell proliferation curves were generated using the Maestro Z to record real-time, label-free impedance measurements. The Maestro Z was able to detect antibody tethered cells versus untethered cells, as well as quantify impedance changes between different cell densities.

Introduction

Presently, most cancer immunotherapies rely on the use of monoclonal antibodies or cancer vaccines, but a new type of approach that involves using the patient's own immune cells to kill target cells is progressing rapidly. This type of therapeutic approach requires the ability to investigate quantitative changes in cellular physiology and health *in vitro* using label-free and nondestructive methods. 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 cancer cells are non-adherent and thus require tethering to the surface of the plate using antibodies for impedance measurements.[1] Here, we investigated the tethering and proliferation of three different non-adherent cancer lines (Daudi, K562, and Nalm6). Daudi is a human B-cell lymphoma cell line commonly used as a model to study the mechanisms of leukemogenesis.[2] K562 is a human leukemia cell line widely known as a sensitive *in vitro* target to examine natural killer cell activity.[3] Nalm6 is a B-cell precursor leukemia cell line and is often studied to understand the mechanisms governing tumor growth metastasis, as well as evaluating the effect of various drugs or therapies.[4]

In this study, Daudi, K562, and Nalm6 and pelleted by cells were continuously monitored using the Maestro Z to quantify the dynamics of non-adherent cell tethering via cell-type specific antibodies and proliferation.

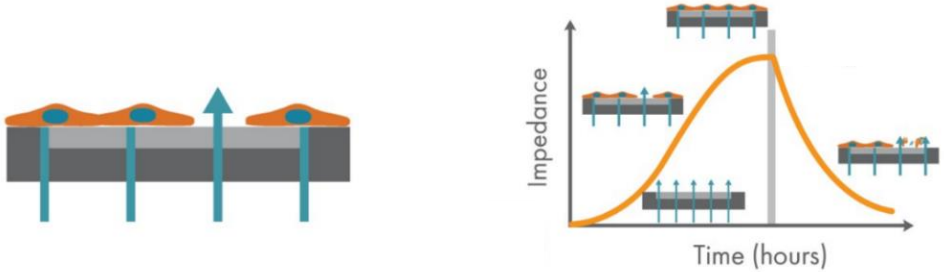

Figure 1. Impedance measures how much electrical signal (teal arrows) is blocked by the electrode-cell interface (right). When the electrode is uncovered, electrical signal easily passes and the impedance is low. When cells cover the electrode, less electrical signal passes and the impedance is high.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

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Materials and Methods

Materials

Cells and reagents

Daudi (cat. CCL-213), K562 (cat. CCL-243), and Nalm6 (cat. CRL-1567) cells were obtained from ATCC (Manassas, VA). Human anti-CD40 antibody (cat. AF632) was obtained from R&D Systems (Minneapolis, MN). Human anti-CD9 and anti-CD71 antibody were obtained from a collaborating lab. 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 and Nalm6 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).

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 controlling 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, anti-CD71(1x) for K562 cells, or anti-CD9 antibody (1x) for Nalm6 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. It is critical that antibodies targeted for specific antigens on non-adherent 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 non-adherent 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, K562, and Nalm6 cells were thawed and cultured in accordance with supplier recommendations until sufficient numbers were present for the assay. Flasks of cultured non-adherent 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. Cells were seeded onto the CytoView-Z plate at 100 µl per well (see Figures 1 and 2). Sterile DI water (8 mL) was added

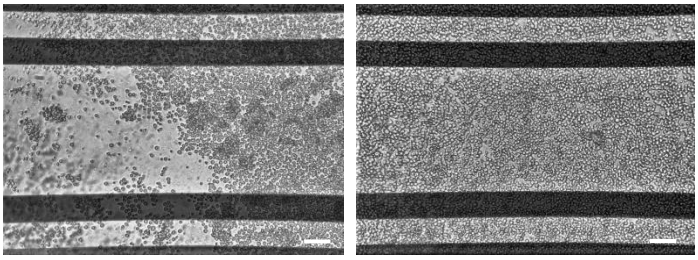

Figure 1: Images of Daudi cells without (left image) or with (right image) anti-CD40 antibody immobilization 48 hours post seeding at 150k cells/well on a CytoView-Z plate. The viewing window in each plate permits visualization of cells. Black horizontal bars are the recording and ground electrodes. Scale bar is 400 µm.

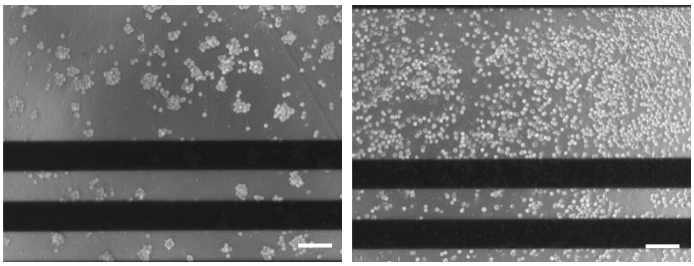

Figure 2: Images of K562 with anti-CD71 antibody (left image) and Nalm6 cells (right image) with anti-CD9 antibody, respectively, on a CytoView-Z plate at 48 hours post seeding. Scale bar is 400 µm.

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 set the chamber 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 removed).

Results

Antigen-specific antibody tethering of non-adherent 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. 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 by 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 the entire time course as compared to untethered cells (Figure 3, top). These results indicate that non-adherent 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) showed the fastest increase in impedance during the tethering and proliferation phases, reaching full confluence by 40 hours, as indicated by the plateau in impedance (Figure 3, top). The two lower Daudi cell densities (50,000 and

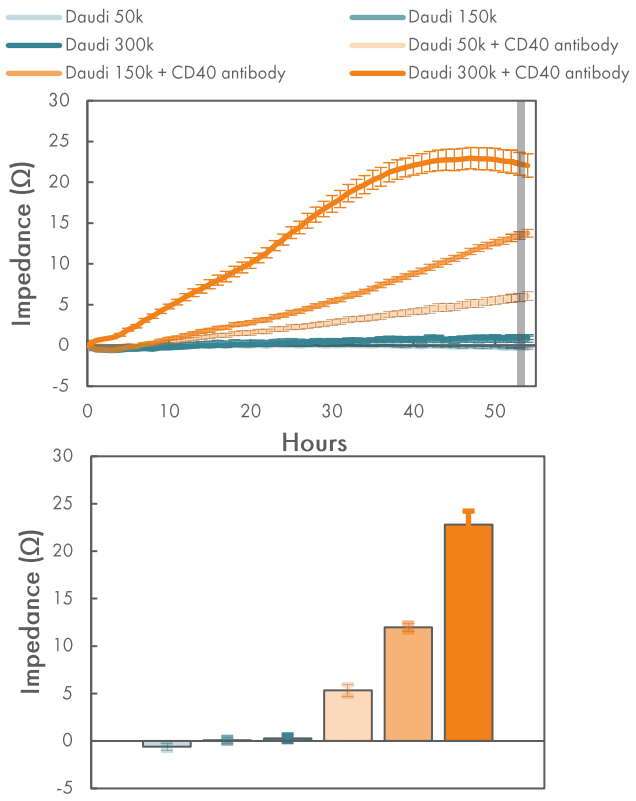

Figure 3: (Top) Growth curves for Daudi cells seeded at 50k, 150k, and 300k cells/well, with or without CD40 antibody, and monitored on the Maestro Z during the attachment, spreading, and proliferation phases. (Bottom) After 48 hours, the highest cell density (300k) had the largest impedance, whereas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

100,000 cells per well) continued to display an increase in impedance over time, but at lower impedance. The differences between cell densities after 48 hours are further illustrated in the bottom graph of Figure 3, as higher cell densities resulted in larger changes in impedance.

In addition, non-adherent cancer cell lines (K562 and Nalm6) were also tethered by their respective antibodies, anti-CD71 and anti-CD9, and changes in impedance were measured at different densities for at least 48 hours. Similar to the Daudi cells, K562 and Nalm6 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 (Figures 4 and 5, top). In contrast to the Daudi cells, both K562 and Nalm6 cells showed a greater initial increase in impedance values during the tethering phase (0-2 hours), representative of cells binding to the antibody. After the tethering phase, K562 cells continued to show a slow increase in impedance values, increasing from approximately

7 to 18 Ω until plateauing around 30 hours. In contrast, Nalm6 cells displayed minimal changes in impedance after the initial tethering phase, indicating minimal cell proliferation occurred over the time course. Overall, results demonstrate that impedance values correlate to increasing densities of tethered non-adherent cells.

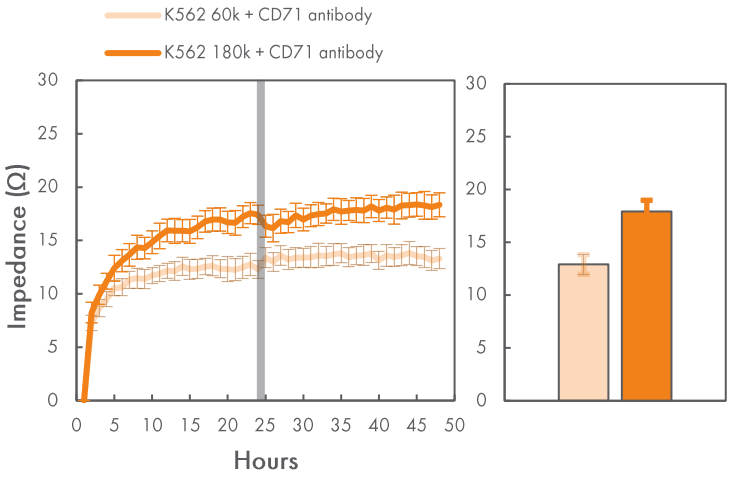

Figure 4: (Left) Growth curves for K562 cells seeded at 60k and 180k with anti-CD71 antibody, and monitored on the Maestro Z during the attachment, spreading, and proliferation phases. (Right) After 24 hours, the higher cell density (180k) showed a larger impedance as compared to the lower cell density (60k).

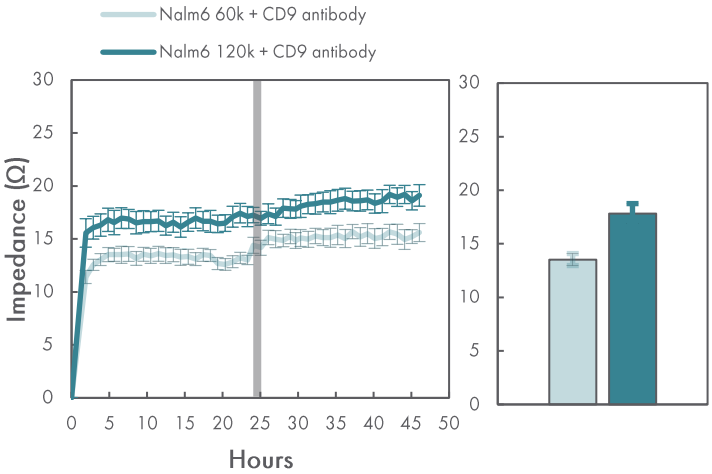

Figure 5: (Left) Growth curves for Nalm6 cells seeded at 60k and 120k with anti-CD9 antibody, and monitored on the Maestro Z during the attachment, spreading, and proliferation phases. (Right) After 24 hours, the higher cell density (120k) showed a larger impedance as compared to the lower cell density (60k).

Conclusion

The Maestro Z enables simple, label-free, non-invasive monitoring of cellular profiles. By using cell-type specific antibodies, non-adherent cells can be tethered to the surface of the plate to track cell proliferation. Here, we showed that the Maestro Z impedance assay readily distinguished the profiles of three different non-adherent cell lines (Daudi, K562, and Nalm6) and was able to differentiate between various cell seeding densities. In addition, the Maestro Z was able to detect changes in impedance between tethered and untethered cells, allowing for future studies that are focused on immune cell-mediated killing of non-adherent cancer cells.

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