>> Development of an in vitro potency assay of immune effector cell-mediated cytotoxicity and kinetics

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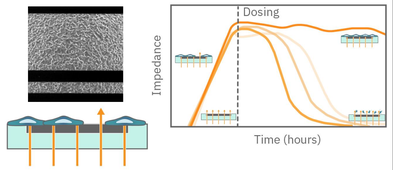
Maestro Z: Dynamic Cell Tracking

Impedance Technology

Immune effector T cells are a promising cancer therapy due to their innate cytotoxicity. In addition, engineering chimeric antigen receptors (CAR) to target tumor-associated or neo-antigens can lend high specificity. Assessing the efficacy and potency of such T cell therapies label-free, *in vitro,* and at high throughputs is vital for the preclinical development of these promising therapies.

Axion **BioSystems'** Maestro Z platform offers impedance-based cell analysis for real-time, label-free monitoring of cell viability, morphology, cytolysis, and signaling. Here, we measured cytotoxicity data from several different potency assays using a variety of target cells and immune-effector cells.



The impedance is measured from electrodes embedded in the bottom of each well. As cells cover more of the electrode, impedance increases in proportion to the number of attached cells. If a perturbation kills the attached cells, impedance decreases as the cells lyse.

The Maestro Z Product Family

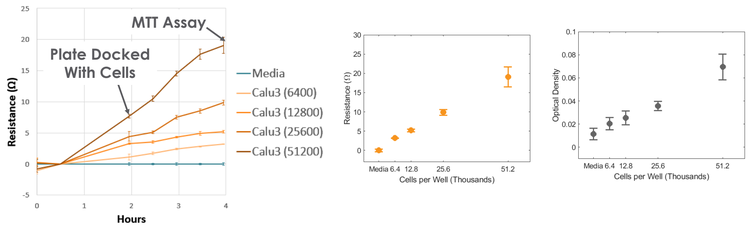
Features	*Maestro Z*	*Maestro TrayZ*	*Maestro ZHT*
Throughput:	96-well	Up to 8 x 96-well	384- and 96-well
Environmental Controls:	Built-in	External	Built-in
GxP Compatible:	✓	Coming soon	✓
Barcode Plate Tracking:	✓	✓	✓
Automation API:	✓	No	✓
Dimensions *(WxDxH)*:	280 x 413 x 225 mm	440 x 450 x 60 mm	280 x 452 x 225 mm

- ***Label-free, non-invasive tracking*** *of cultured cells or spheroids/organoids*
- ***Integrated environmental control*** *provides a stable benchtop environment for short- and long-term toxicity studies*
- ***Automatic and continuous cell monitoring*** *from 96 or 384 wells simultaneously*
- ***"One button setup"*** *automatically docks the plate and adjusts temperature and CO2 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)*** *offers stronger signals, ultra-low frequency content, and enhanced flexibility*

BioCore v4

Direct Correlation of Impedance Assay with Cell Number

To validate impedance-based monitoring of cell viability, Calu-3 cells were added to a CytoView-Z plate with varying number of cells per well and monitored for four hours on the Maestro Z platform. The change in resistance was correlated with the number of cells initially seeded, and the resistance continued to increase as the cells adhered and flattened on the surface. At four hours post-seeding, the plate was removed and an MTT assay was performed in the CytoView-Z plate. The resistance measured with the Maestro Z platform was linear with respect to cell number and directly correlated to the MTT assay readings from the same wells.

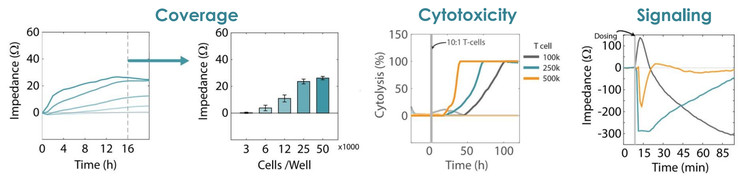


Real-time, Label-free Cytotoxicity Assay

Impedance Assay Measures Diverse Cell Properties

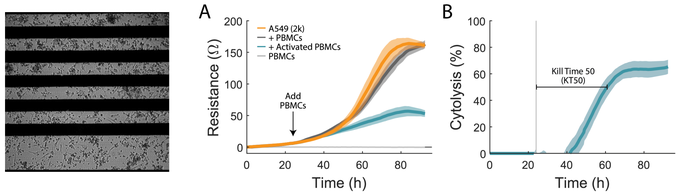
The Maestro Z records impedance at multiple frequencies simultaneously, enabling a thorough characterization of cell behavior, including:

- Coverage/Density – the change in impedance is directly related to the quantity of cells in a 2D and 3D culture covering the electrodes.
- Cytotoxicity – dynamic monitoring of cell viability provides measures of the degree and speed of cell death.
- Morphology – cell size, shape, and intercellular tight junctions significantly impact the measured impedance.
- Signaling – small changes in cell shape or cytoskeleton organization are detected in response to intracellular signaling events



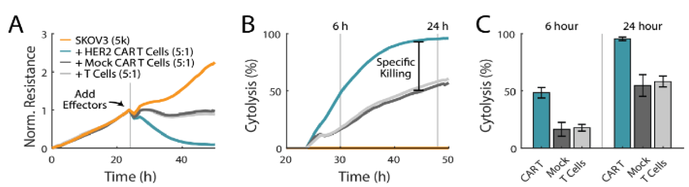
Impedance-Based Assay for Cell-Mediated Cytotoxicity

The impedance measurement is sensitive to the attachment of adherent or tethered target cells, but not the presence of non-adherent immune effector cells. In this way, the assay is naturally sensitive and specific to target cell attachment and cytotoxicity. The attachment and proliferation of the A549 target cells (orange) is measured via the resistance over time. At 24 hours, the PBMCs were added across various conditions. First, the PBMCs were added to some wells alone (light gray), and did not affect the resistance measurement, confirming that the measurement in this assay is specific to the target cells. PBMCs were also added to wells containing the target cells, with (blue) and without (dark gray) anti-CD3 and IL-2 to activate the immune effector cells. The resistance measure was significantly lower when activated PBMCs were added to the target cells, indicating immune cell-mediated cytotoxicity. The dynamics of the cytotoxicity were quantified as the kill time 50 (KT50), defined as the time duration required for 50% cytolysis of the target cells. In this example, the KT50 was 39 ± 3 hours for activated PBMCs added at a 10:1 effector to target ratio.



CAR T-cells Demonstrate Antigen-Specific Killing

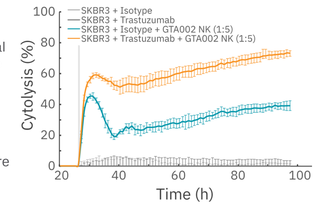
CAR T-cell therapy uses genetically engineered T cells that express a chimeric antigen receptor that binds to a specific antigen on tumor cells. In HER2-overexpressing SKOV3 cell line, donor-matched mock CAR T cells, which lack the tumor antigen-recognizing domain, and non-transduced T cells were used to separate non-specific killing from specific CAR T-cell killing. HER2-targeted CAR T groups demonstrated approximately twice as much target cell killing as mock CAR T cells and non-transduced T-cells as shown by the (A) resistance and (B) cytolysis time courses for SKOV3 killing by CAR T cells and the (C) comparison of %cytolysis at 6 and 24 hours following effector cell addition at E:T = 5:1.



Potency with Stem Cell-Derived Models

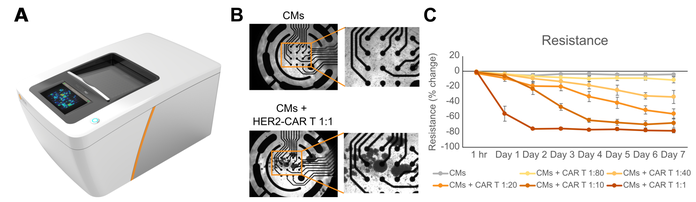
Trastuzumab enhances cord blood-derived NK cell-mediated cytotoxicity

The functional cytotoxic capacity of GTA002 NK cells against SK-BR-3, a breast adenocarcinoma cell line expressing the tumor-associated antigen HER-2, was determined using an impedance-based cytotoxicity assay. SK-BR-3 cells were seeded at the optimal seeding density of 50,000 cells/well in a CytoView-Z 96-well plate and incubated for 20 hours. After initial SK-BR-3 proliferation, either human IgG1_k isotype (control Ab) or Trastuzumab (anti-HER-2 Ab; Bioconnect) was added to the target cells at a concentration of 1 µg/ml and incubated for 30 minutes at room temperature. To assess the antibody-dependent cellular cytotoxicity (ADCC) capacity of GTA002 NK cells, effector cells were then added (gray line) to the target cells and the co-culture was incubated for an additional 48-96 hours. Higher cytolysis of the target cell was observed when trastuzumab was added in addition to the GTA002 NK cells.

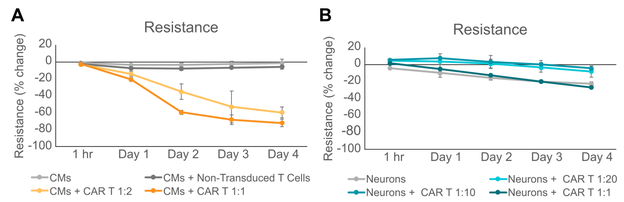


Evaluation of On-Target, Off-Tumor Effects with iPSC-derived CMs

The receptor tyrosine kinase HER2 is expressed in several tissues throughout the body including the breast, skin, respiratory tract, and heart. Therefore, therapies targeting HER2-expressing cancers can also damage these normal, healthy HER2-expressing tissues. We confirmed the expression of HER2 in iPSC-derived cardiomyocytes (iPSC-CMs) using flow cytometry (data not shown) and then monitored killing of iPSC-CMs by HER2-CAR T cells at effector-to-target (E:T) ratios ranging from 1:80 to 1:1 on the Maestro Pro (A). Imaging showed that at 7 days post-dose, CAR T cells formed clusters, indicating significant cell activation after culture with the iPSC-CMs (B). MEA Viability (resistance) measurements from the iPSC-CMs decreased in a CAR T cell dose-dependent manner from baseline over 7 days (C). In the highest dose (1:1), significant decreases in resistance were seen as early as one day post-dose. At 7-days post dose, all treatment groups had lower resistance than the untreated control, including the lowest dose (1:80).



After showing that HER2-CAR T cells killed iPSC-CMs, we wanted to confirm that this cytotoxicity was due to the CAR T cells targeting the HER2 antigen on iPSC-CMs rather than general, non-specific T cell killing. Therefore, we treated iPSC-CMs with either HER2-CAR T cells or non-transduced control T cells. As expected, CMs dosed with HER2-CAR T cells at 1:2 and 1:1 ratios led to significant decreases in iPSC-CM resistance, while dosing with non-transduced T cells led to a much smaller decrease in resistance (A). We further tested for antigen-specific killing of HER2-CAR T cells by dosing iPSC-derived neurons which do not express HER2. There was no significant difference between the changes in resistance of untreated neurons and those treated with the 1:1 CAR T cell dose (B).



Conclusions

- The Maestro Z allows for simple, non-invasive, real-time monitoring of immune-cell mediated killing of target cells, providing a sensitive, quantitative assay for evaluating potency *in vitro.*
- Continuous monitoring of cord blood-derived NK cell-mediated killing provides a detailed look at the influence of anti-tumor antibodies like trastuzumab.
- The Maestro Pro can effectively measure the on-target, off-tumor effects of CAR T cells in an *in vitro* model with iPSC-derived target cells.