Application Note

Investigating cell lines derived from hematological malignancies via impedance

Topic:
liquid tumor cells
Cells:
K562 (lymphoblast), Jurkat (T lymphocyte)
Tools:
AtlaZ

Summary

Cancer remains one of the most prevalent and lethal diseases globally. Liquid tumor (hematological cancer) cell lines are vital in studying blood cancers and developing therapies. Immunotherapy, particularly chimeric antigen receptor (CAR) T-cell therapy, has shown great promise, improving outcomes in hematological malignancies [1]. Since 2017, the FDA has approved six CAR T-cell therapies for blood cancers, including lymphomas, leukemia, and multiple myeloma [2].

Advancing CAR T-cell therapy requires basic mechanistic research, bioengineering, and clinical trials to enhance T-cell biology and improve CAR T-cell proliferation and durability. CRISPR-Cas9 technology offers new opportunities for genome-wide screening to identify genes that can boost CAR T-cell resilience [2].

This application note describes how liquid tumor cells can be monitored and how effects of cytolysing reagents or effector cells such as CAR T-cells can be quantified using an impedance-based assay employing the AtlaZ platform. We used K562 and Jurkat cells which are widely used cell lines in research to model specific types of hematological malignancies due to their distinct characteristics. K562 cells are derived from a patient with chronic myeloid leukemia (CML) in the blast crisis phase. They are classified as erythroleukemia cells and exhibit characteristics of erythroblasts [3]. Jurkat cells are an immortalized human T lymphocyte line used to study acute T-cell leukemia, T-cell signaling, and drug/radiation susceptibility [4].

The strength of the AtlaZ assay approach with regards to liquid tumor cells is its (i) continuity of monitoring, (ii) the fact that it is label-free, (iii) throughput and (iv) it enables quantitative analysis allowing for easy comparison between different treatments or conditions. A crucial advantage over standard assays that rely on labels for optical or radiometric detection is the independency of potentially disruptive dyes or labels.

Results

We used a tethering approach to adhere liquid tumor cell lines on the surface of gold biosensors embedded in the bottom of

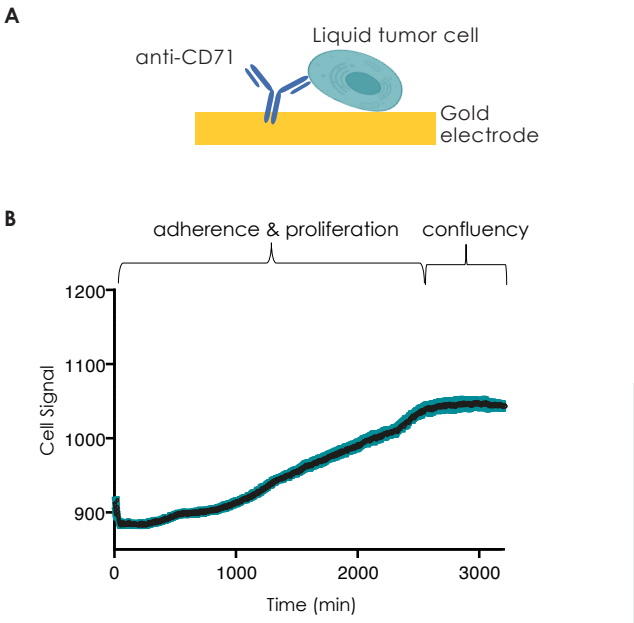


Figure 1: ***Monitoring liquid tumor cells. A*** *Precoating the wells of the NSP-Z Plates with anti-CD71 enables the liquid tumor cells K562 to be detected by the impedance sensors.* **B** *Adherence and proliferation of K562 cells, until reaching confluency.*

the recording plate (AtlaZ NSP-Z plate). Using this approach, the lymphoblast cell line K562 was successfully tethered to the surface, resulting in a robust impedance signal as recorded with the AtlaZ system. After the tethered cells attained a certain level of growth and confluence, the prototypic cytotoxic immune cells CD8 T and NK were added, resulting in a decrease in the impedance signal (Cell Signal), representing cytolysis. For the assay to be accurate, it is important that the tethering reagent is selective for the target cells, precluding any impedance signal derived from the effector cells. Next to the K562 cells, T cell acute lymphoblastic leukemia (Jurkat) cells were investigated. We show that anti-CD3 and anti-CD28 antibodies play important roles in the activation and proliferation of Jurkat cells, furthermore fibronectin enables cell adhesion to the gold electrodes. Here, adherence and proliferation was monitored.

To facilitate K562 cells to adhere, plates were coated with human anti-CD71 antibodies. After an initial adherence phase (Figure 1), the Cell Signal was reaching a plateau phase indicating that the surface of the measurement plate reached full confluency. Next, effector CD8 T cells and NK cells were added and the measurement was continued for another 24 hours (Figure 2). Both types of cells are prototypic cytotoxic immune cells and we observed a killing of K562 cells in both cases (Figure 2). A 100% cytolysis was reached at t = 7.8 h and t = 4.6 h in case of CD8 T cells and NK cell, respectively (Figure 2B).

In the context of Electric Cell-substrate Impedance Sensing (ECIS), investigating capacitance can offer more informative insights into cell adherence and spreading compared to impedance alone. This is because capacitance specifically relates to the properties of the cell membrane, which plays a crucial role in cell attachment and spreading processes. Impedance is a composite measure that includes contributions from resistance and capacitance. Capacitance directly quantifies the insulating effect of cells on the electrode, simplifying its interpretation as a measure of coverage. As cells attach and spread on the electrode, the capacitance decreases in a way that is directly proportional to the electrode coverage, thus a quantification of the confluency is possible (Figure 2C). When cells are damaged or the cell monolayer is disrupted, the capacitance is increasing, indicating a reduction in cell coverage, as seen here with K562 cells treated with effector cells (Figure 2C).

Next, we investigated Jurkat cell adherence. After pre-coating the surfaces with fibronectin, Jurkat cells were seeded on the recording plate. In the absence of a coating substrate, after 2 hours, the Cell Signal was 50% in amplitude compared to the fibronecting coated wells (Figure 3A). Fibronectin allowed a significant and more rapid increase of the Cell Signal (cell adherence), suggesting a more efficient adhesion induced by the presence of this coating substrate (Figure 3A, B). As seen in Figure 3C, the spatial distribution correlated with the capability of fibronectin to facilitate efficient adhesion of the leukemia cells. Thus, cell distribution emulates the typical monolayer growth, resembling the close configuration found in the bone marrow or in the lymph nodes. In wells without fibronectin coating, a typical clumping of cells was observed.

To investigate antibody activation of Jurkat cells, the cells were starved in RPMI 1640 supplemented with 2% FBS for 2 hours. Next, the cells were then seeded onto NSP-Z plates which were coated with goat-anti-mouse IgG antibodies. Cell seeding was immediately followed by the addition of either the negative control antibody IgG 2_{AK} or a mixture composed of functional anti-CD3 and anti-CD28 antibodies with the concentrations as indicated (Figure 3D, E). The Cell Signal increased after Jurkat cells were stimulated with a mixture of free anti-CD28 and anti-CD3 functional antibodies, peaking within 10 minutes. A concentration of 1 µg/mL for the anti-CD28 and anti-CD3

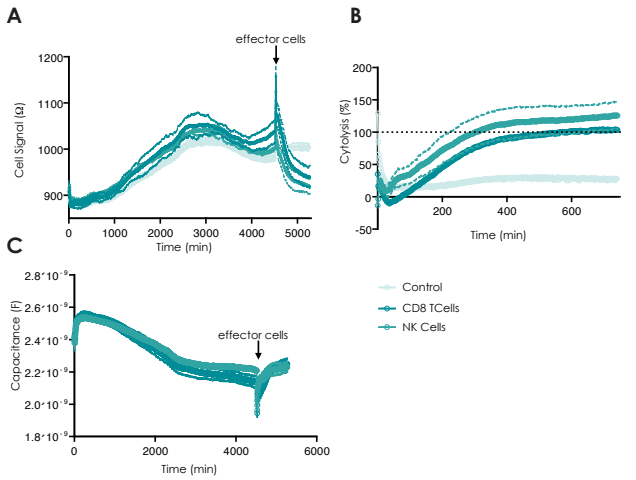
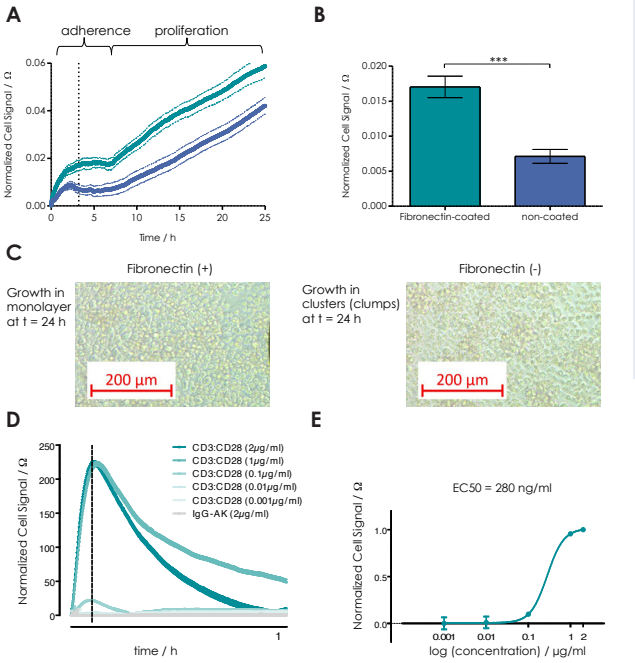


Figure 2: A *K562 cell adhesion and growth, (seeding at t = 0). Prototypic cytotoxic immune cells (CD8 T and NK) induce cytolysis (**A** and **B**), **C** Quantification of capacitance, where adhesion is represented by initial plateau phase. Proliferation, in linear relation to the electrode coverage, is represented by the slope of the curve and is completed after aprox. t = 3000 min. Then a confluency phase is reached and effector cells were added.*

Figure 3: ***A,*** *Fibronectin coating of plates facilitates adhesion of Jurkat cells. In the first 2 h the cells attached, then a stabilization phase (2 - 7 h) was observed.* ***B*** *After t = 3 h (dashed line in A) significantly more cells are adhered in coated wells.* ***C*** *Fibronectin is able to efficiently attach Jurkat cells.* ***D*** *After coating plates with goat-anti-mouse IgG antibodies, Jurkat cells were seeded in the presence of indicated concentrations of anti-CD3 and anti-CD28 functional antibodies. Dashed line indicates the time point used to generate the concentration response curve (**E**).*



mixture was the lowest concentration that elicited the most robust increase in cell signal using the AtlaZ system. Hence, in the AtlaZ label-free assay and as described earlier [5], stimulation with agonistic antibodies promotes increased adhesion and cytoskeletal rearrangement, leading to an increased surface area of contact with the gold electrode on the well surface, which subsequently results in a rise in measured impedance.

In conclusion, the AtlaZ system offers a highly automated approach capable of studying liquid tumor cells and the killing effect of e.g. effector cells. In addition, the system does not require labeled reagents and provides data in real-time. Straightforward assay design, minimal artifacts, and short assay time, contribute to higher and more efficient throughput.

Methods

AtlaZ platform

The AtlaZ platform (Nanion) provides quantitative live-cell analysis by measuring the impedance (Ohm, Ω, recorded at frequencies ranging from 0.1 kHz - 100 kHz) displayed as Cell Signal, of adherent cells grown on 96-well plates with embedded planar gold-film electrodes (Nanion). The methodology, Electrical Impedance Spectroscopy, provides a unique richness of information from cells. Depending on the data acquisition frequency, the system detects predominantly the resistive part of cell-cell and cell-matrix contacts at lower frequencies or predominantly capacitive currents across the cell membranes at higher frequencies as an indicator for electrode coverage. The latter is the basis for cell adhesion or migration assays. Thus, real-time impedance data provide insights into various cell phenotypes, such as cell morphology changes as a result of signaling, proliferation, lateral migration or cytotoxicity even over prolonged periods of time.

The measured impedance is made up of components including capacitance and resistance. Measurements of capacitance at a higher frequency such as 80 kHz (Figure 2C) provide insights on adhesion and spreading.

Calculation of cytolysis and Kill Time 50

Percent (%) cytolysis was used to quantify cell death and is calculated by AtlaZ Control software as follows:

$$Cytolysis\ (t, \%) = \left[\left(\frac{\Delta_{kt}}{\Delta_{G_{\text{no effector cells}}}} \right) - \left(\frac{\Delta_{kt}}{\Delta_{G_{\text{target + effector cells}}}} \right) \right] \cdot 100$$

where $\Delta_{k,t}=Z_t-Z_0$ and $\Delta_G = Z_0-Z_{min}$. Z_t is the impedance value after the addition of the treatment and Z_0 is the last impedance value before treatment addition. Z_{min} is the smallest impedance value in the region before treatment addition. This means that Cytolysis (t, %) is the effect of the effector cells on the target cells at a certain time t, displayed in percent. $\Delta_{k,t}$ (k=killing; t= time) contains information on the time-dependent cell death based on the impedance change before and after treatment and Δ_G considers the cell proliferation and growth (g = growth) post-seeding. Next to Cytolysis (%) AtlaZ Control software also displays Kill Time 50 values which are as well determined through the impedance measurements.

K562 assay

Firstly, NSP-Z plates were coated with 5 µg/ml human anti-CD71 antibodies over night in an incubator at 37°C. After removal of coating solution and 1x washing with PBS, 1.5 x10^4 K562 cells/ well were seeded and monitored for three days until a plateau phase of the Cell Signal was reached, representing confluency. As a next step 2x10^5 effector CD8 T cells or 1x10^5 NK cells were

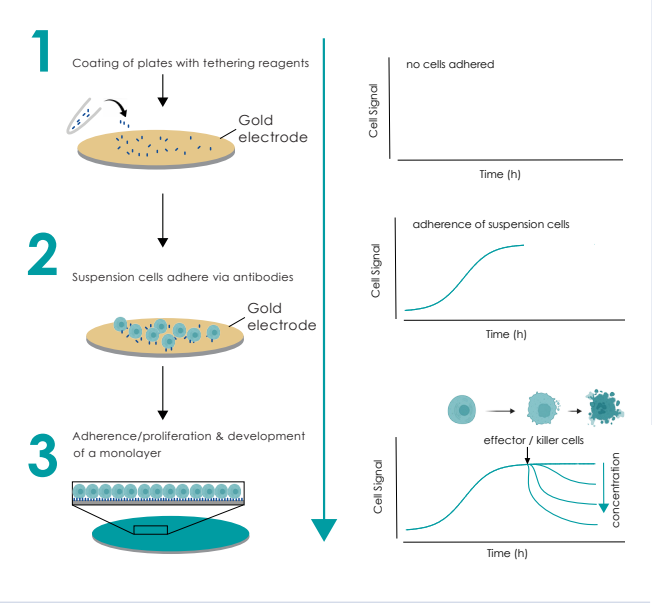


Figure 4: *Workflow for the use of liquid tumor cells (suspension cells).* *Precoating the wells of an NSP-Z AtlaZ plate with a tethering reagent enables liquid tumor cells to adhere and proliferate. (1) Coating of plates with antibodies or fibronectin. Once target suspension cells adhere via antibodies and spread out (2), pharmacological/ killing experiment can be conducted. Cytolysis of target cells upon treatment with killer cells can be monitored (3). The Cell Signal is monitored continuously over time, revealing the kinetics of stimulus-induced effects.*

applied to K562 cells, and measurements were continued for 24 hours.

Jurkat assay

After pre-coating the surfaces (1 hour) with 50 µl per well of 1:100 diluted fibronectin with PBS (fibronectin stock 1 mg/ml), Jurkat cells were seeded at 120 k cells/well on the recording plate. To investigate adherence via Antibodies, plates were coated with 50 µl per well of 5 µg/ml goat-anti-mouse IgG (Cat. No. ab6708 from Abcam) in PBS for 3 h in an incubator at 37°C. After removal of coating solution and 1x washing with PBS, $2.5x10^5$ Jurkat cells/well were seeded, followed immediately by adding either the negative control antibody IgG 2_{AK} or a mixture composed of functional anti-CD3 and anti-CD28 antibodies (Cat. No. 16-0037 and16-0289, respectively, from from eBiosciences (USA)) with the mass ratio as indicated. The readout, displayed as the Cell Signal, represents raw impedance values, and was recorded at 10 kHz. For capacitance recordings we have chosen a 80 kHz recording frequency, which is best suited for cell adherence investigations. In general, the optimal recording frequency is cell-type specific and the value is displayed in the AtlaZ software.

Key findings

- Our results demonstrate the capability of AtlaZ to detect adherence and proliferation of suspension cells and to monitor killing via effector cells.
- AtlaZ allows for cellular research on cell adhesion, proliferation and cytotoxicity, label-free and in real-time.
- Recordings can be performed for up to 6 x 96 = 576 samples either simultaneously or independently.

Acknowledgements

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