Application Note

Monitoring cell – attachment and tight junctions in the same assay

Topic:
TEER (Transepithelial Electrical Resistance)
Cells:
MDCK-II epithelial cells
Tools:
AtlaZ

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Summary

The numerous different cell types in the human body are greatly specialized and often require a conjunctive action of a population of cells, for example in tissues. Cells are interconnected via cell junctions, multiprotein complexes found in the cell membrane of animal cells, and such cell junctions allow for a mechanical, chemical or electrical transmission of signals. These junctions can be subdivided into (I) tight junctions, (II) anchoring junctions or (III) gap junctions. Defects in cell–cell junctions give rise to a wide range of tissue abnormalities that disrupt homeostasis and are common in genetic abnormalities and cancers (1).
The so-called tight junctions form the barrier in endothelial and epithelial cells. Classical transepithelial electrical resistance measurements are performed using microelectrodes, where trans- and para-cellular conductivities can be calculated (2). Here the cells are grown on a porous filter membrane which is placed between two fluid compartments. Flux of solutes from one compartment to the other must pass the interfacial cell layer then, and this is determined by the functional porperties of the tight junctions.

Electrical impedance spectroscopy (EIS) (3, 4) as the methodology behind the AtlaZ system, is based on the opposition of current flow, on measuring the impedance of a system while it is excited with a low ampltidue alternating current or voltage. One important property of EIS is its noninvasiveness, as the technique relies entirely on low-amplitude currents and voltages that sensure damage-free examination with a minimum disturbance of the cells or tissues being investigated (5).

Here, we used MDCK-II cells, which are a well established epithelial cell line used to study tight junctions (6). After completion of cell adhesion and formation of a confluent monolayer, the barrier forming tight junctions between adjacent cells are developed (Figure 1). The formation of tight junctions requires fully established cell-surface contacts.

The platform used here, AtlaZ, is a quantitative live-cell analysis system and allows for cellular research on cell adhesion and proliferation, cytotoxicity, GPCR, morphology and barrier function, label-free and in real-time. Recordings can be performed in up to six 96-well plates simultaneously or independently.

The impedance of planar gold-film electrodes that are used as growth substrate for adherent cells reveals changes in electrode coverage (Figure 1) or cell behavior. Real-time impedance data provide insights in cell morphology,

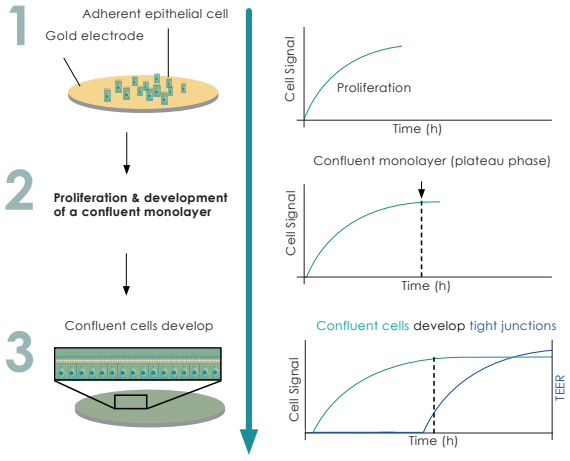


Figure 1: *Workflow for TEER assay. Tight junction forming cells proliferate after seeding. Once a confluency of cells is reached, cells start forming tight junctions which are sealing the paracellular gap. The TEER signal then is recorded in parallel to cell adherence.*

proliferation, lateral migration or cytotoxicity even over prolonged periods of time.

A crucial advantage over standard assays, e.g. filter based methods using labels and either an optical or radiometric detection technique, is the continuity of cell monitoring.

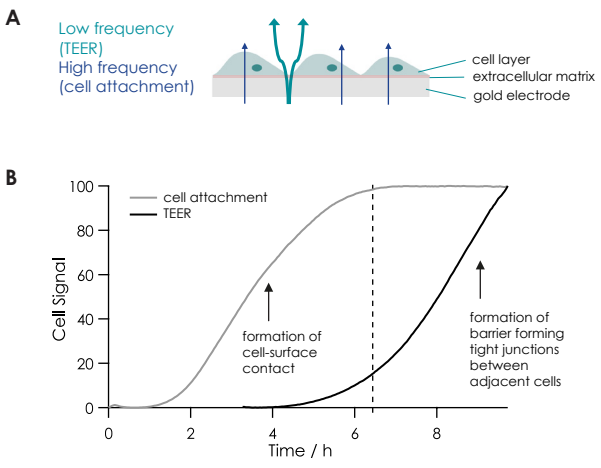
Unique culture plates with integrated electrodes as used in the AtlaZ system enable long-term measurements over several weeks. Thus real-time data on cell adhesion, proliferation and barrier integrity can be aquired.

Results

Here MDCK-II cells were used which are a well established epithelial cell line developing tight junctions. Cells were seeded on non-coated AtlaZ sensor plates at time zero (Figure 2B). The initial cell density was sufficiently high so that a confluent monolayer could form within 6 - 7 hours. A plateau phase of the Cell Signal represents full coverage of the surface with adherent cells. From the timepoint at which the cell adhesion was complete (dashed line in Figure 2B), the transepithelial electrical resistance (TEER) began to increase considerably for the next hours, indicating the formation of barrier forming tight junctions between adjacent cells.

In general, the Cell Signal recorded with AtlaZ is dominated by the paracellular cell layer resistance or capacitive currents across the cell membranes – depending on the readout frequency (Figure 4). Figure 2A shows a schematic depicting low and high frequency impedance recordings for para- and trans-cellular resistance, representing cell coverage and strength of barrier formation, respectively.

The data in Figure 2B show that the formation of tight junctions requires fully established cell-surface contacts.



It is well known that the formation of cell-matrix (surface) junctions is dependent on the presence of either Ca^{2+} or Mg^{2+} (or Mn^{2+}), whereas tight junction formation is known to depend on the presence of Ca^{2+}, which cannot be substituted by a different cation. To prove this, and, furthermore to show that the increase in TEER in Figure 2B is due to the development of tight junctions, we next cultured cell populations in either Ca^{2+} or Mg^{2+} containing buffer (Figure 3). In the presence of only Mg^{2+}, the tight junctions could not develop, whereas in the presence of Ca^{2+} the TEER due to tight junctions increased until a plateau phase was reached and then the junctions remained stable (Figure 3).

Taken together, the results show that it is possible to monitor the formation of cell – surface and cell-cell contacts in one and the same cell population (well) by utilizing non-invasive electrical impedance spectroscopy measurements in real time.

In summary, AtlaZ allows for label-free and real-time cellular research on cell adhesion, proliferation and TEER measurements. The AtlaZ system provides a versatile tool for in vitro cell monitoring addressing the demands for versatility, physiological relevance and throughput.

Methods

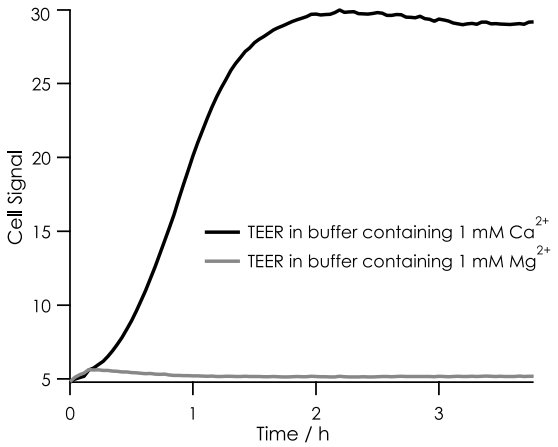
AtlaZ platform

The AtlaZ platform (Nanion Technologies) provides quantitative live-cell analytics by measuring the impedance (Ohm, Ω) of adherent cells as grown on 96-well plates with embedded planar gold-film electrodes (Nanion Technologies).

A crucial advantage over standard assays is the continuity of cell monitoring. Continuous measurements reveal the kinetics of cell behavior and allow an in-depth mechanistic understanding without the need for time- and labor-intensive endpoint assays. For example, one of the crucial advantages of data derived from the AtlaZ platform is the possibility to analyse dose-responses at any time during the experiment. Advanced information content is obtained by using multi-

Figure 2: A *Low and high frequency impedance recordings for cell-cell contact or cell attachment measurements.* **B** *Cell coverage, measured at 50 kHz increases over time (grey) and TEER, measured at 1 kHz (black) reveals barrier formation.*

Figure 3: *In the presence of only Mg^{2+}, the tight junctions cannot develop, whereas in the presence of Ca^{2+} the cell-cell contacts via tight junctions build an increasing TEER. The resistance increases and then reaches a plateau phase and remains stable after approx. 2 h after cell seeding.*



frequency impedance readouts, which is possible with the AtlaZ Control software. With impedance readouts at 1000 different frequencies (here a spectrum of 0.1 kHz – 100 kHz, see Figure 4) it is possible to further dissect physiological response into deeper levels, and thus zooming in on changes in membrane topography, cell-cell or cell-matrix junctions is achievable. G-protein-coupled receptor (GPCRs) activation is one example here.

Workflow of AtlaZ TEER assay

Target cells are seeded in AtlaZ sensor plates and they adhere on the surface with embedded gold electrodes (Figure 1). Each well of the 96-well plate contains 1 center gold electrode with 0.6 mm in diameter and one reference electrode. After the target cells being adhered, the population starts to proliferate. When the cells reach confluency, cells start forming tight junctions which are sealing the paracellular gap. The TEER signal then is recorded in parallel (Figure 4) to the cell attachment, proliferation, cell death or morphology changes in the presence of drugs or stimuli of interest.

Acknowledgement

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References

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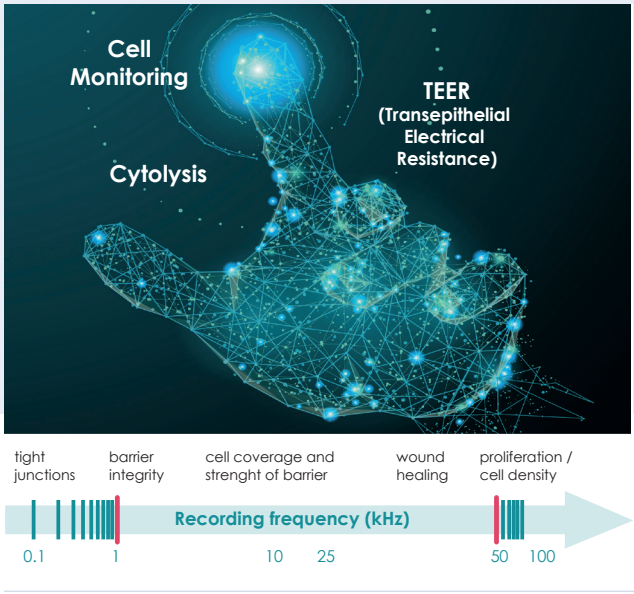

Figure 4: *Electrical impedance spectroscopy. AtlaZ allows to detect impedance signals at 1000 different frequencies, ranging from 0.1 kHz – 100 kHz. TEER signals representing barrier integrity can be recorded in parallel to cell adherance when analyzing the data output at 1 kHz or 50 kHz, respectively. Predefined recording modes can be chosen for ease-of-use.*

Key findings

1. Our results demonstrate that cell-cell and cell-surface contacts can be recorded in parallel.
2. AtlaZ allows for cellular research on cell adhesion, proliferation and cytotoxicity, label-free and in real-time.
3. Recordings can be performed in up to six 96-well plates simultaneously or independently.

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