Application Note

Barrier integrity changes in retinal endothelial cells in the presence of VEGF-A_{165} and test compounds

Topic:
VEGF-A_{165}
Cells:
iBREC
Tools:
AtlaZ

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Summary

Retinal endothelial cells form the inner blood–retinal barrier (BRB), which is essential for maintaining retinal homeostasis and visual function. Barrier integrity depends on specialized intercellular junctions, including adherens and tight junctions, with proteins such as claudin-5 playing a key role in restricting paracellular permeability[1].

Elevated levels of vascular endothelial growth factor-A (VEGF-A_{165}) are known to increase membrane permeability of retinal endothelial cell layers and thereby contribute to the development of vision-threatening macular edema[2].

Clinically, these effects can be counteracted by intravitreal administration of VEGF-binding antibody derivatives[3]. *In vitro* studies using immortalized bovine retinal endothelial cells (iBREC) have demonstrated that VEGF-A_{165} alters the expression and/or subcellular localization of proteins involved in regulating para- and transcellular transport as well as proteins mediating adhesion to the extracellular matrix[4,5,6]. Previous investigations further revealed that VEGF-A_{165} induces a multiphasic cellular response that persists for at least six days[5,6] and is modulated by additional growth factors such as angiopoietin-2 (ANG2)[7].

Although VEGF-binding proteins rapidly reverse cell morphology changes caused by VEGF-A_{165} stimulation, this recovery is only transient, with barrier function subsequently declining again and not returning to control levels despite continued presence of the antagonist[6].

In the present study, we aimed to monitor the effects of VEGF-A_{165} on iBREC in the presence of different compounds and to identify associated changes in barrier integrity and overall cell morphology. Measurements performed using the AtlaZ platform enable quantitative assessment of barrier integrity, cell-cell junction stability, and cell-substrate adhesion.

Methods

iBREC cells

iBREC were used as an *in vitro* model of the inner blood-retinal barrier. Cells were cultured under standard endothelial growth conditions and exposed to VEGF-A_{165} either alone or in

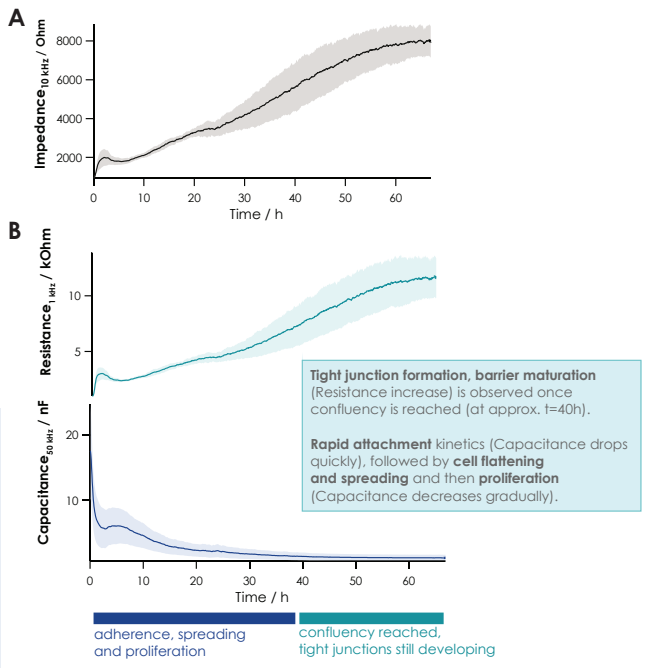


Figure 1: *iBREC cells develop tight cell-cell contacts within approx. 30 h after reaching confluency.* ***A,*** *Proliferation curve as recorded via impedance.* ***B,*** *Cell adhesion and proliferation are more clearly reflected by capacitance (a component of impedance), whereas the component resistance primarily reports tight-junction formation. n = 8 +/- SD.*

combination with ANG2 or additional test compounds. Barrier integrity and cellular responses were monitored over time to investigate both acute and long-term effects of growth factor exposure.

Changes in endothelial barrier function were assessed by continuous monitoring of impedance values using the AtlaZ measurement platform. Particular attention was given to alterations associated with para- and transcellular permeability, cell-cell junction stability, and adhesion of endothelial cells to the plate surface. Temporal changes induced by VEGF-A_{165} and combinatorial treatments were analyzed to characterize the multiphasic response of retinal endothelial cells.

Monitoring barrier integrity

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

The methodology, Electrical Impedance Spectroscopy (EIS), 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.

Thus, real-time impedance data provide insights into various phenotypic changes in cells, such as cell morphology changes, or alterations in barrier integrity as a result of signaling, growth, or upon compound application.

Technically, resistance and capacitance are the model components that generate impedance, and biologically they often change in opposite directions as cells attach, spread, and form barriers (see Figures 1 and 4).

Results

The effects of VEGF-A_{165}, ANG2, and an inhibitor of proteasomal proteases (IPP) on the cellular barrier of iBREC cells were asessed by impedance, resistance, and capacitance measurements, demonstrating overall morphology, barrier integrity and cell adhesion, respectively.

1. Adherence and proliferation of iBREC cells were monitored over a 72-hour period. The measurements revealed that confluency was reached after approx. 40 hours, followed by tight barrier formation over the subsequent 30 hours. Adherence and proliferation were effectively tracked by capacitance readouts, whereas tight junction formation was reflected by resistance values (Figure 1).

2. VEGF-A_{165} (1.3 nM) caused a decrease in resistance, suggesting increased membrane permeability and disruption of tight junctions (Figure 2A). Cell viability remained stable since adherence (attachment strength) was largely unaffected (Figure 2B). A drop in resistance was observed in PBSd control cells from approximately 125 hours onward. VEGF-A_{165} and VEGF-A_{165}+ANG2 disrupt normal barrier formation by delaying or reversing the increase in resistance typically seen before this time point.

3. Upon application of IPP, a concentration dependent effect could be seen (Figure 3A,B). At 2.3 nM, IPP induced a persistent barrier dysfunction, reflected by reduced resistance values. However, cells recovered after 10 days, showing an increasing resistance going back to control values (Figure 3A). The highest concentration of IPP, 4. 5 nM induced an instantaneous and non-reversible drop of the Impedance signal back to baseline (value like before cell seeding),

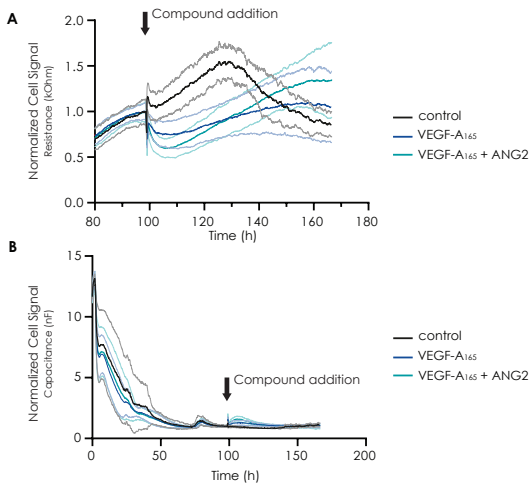
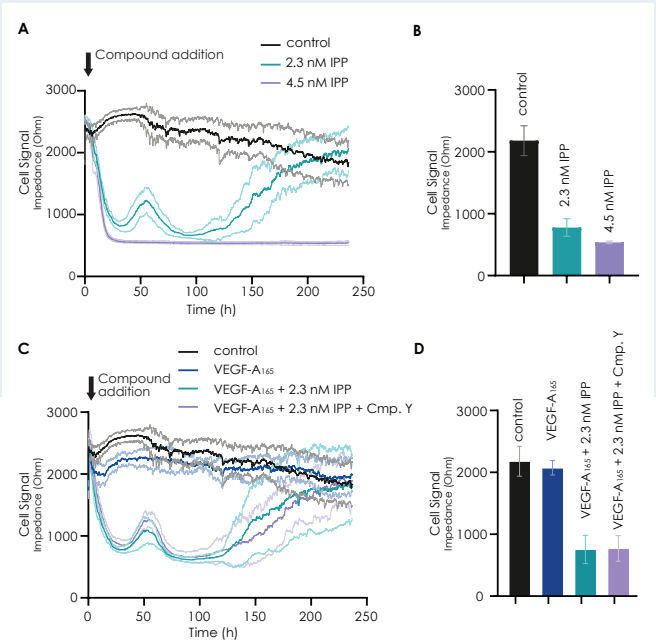


Figure 2: A, *Continued barrier formation that is still seen in vehicle control (black) until approx. t = 130 h. VEGF-A_{165} and VEGF-A_{165}+ANG2 induce a decay/ delay of resistance increase.* ***B,*** *Cell adherence, reflected by capacitance readout, is almost not affected. Arrows: VEGF-A_{165} or VEGF-A_{165}+ANG2 addition. Data shown n = 8 +/- SD.*

Figure 3: *IPP concentration-dependent cell signal change.* ***A,B,*** *IPP at 2.3 nM induced barrier dysfunction, cells recovered after 10 days (signal going back to control values). IPP at 4.5 nM induced cell death.* ***C,D,*** *Compound Y does not rescue the 2.3 nM IPP induced effect. Bar graphs show the effect size at t = 150h. Data shown n = 8 +/- SD.*



suggesting cytotoxicity and cell death. A test compound (Compound Y) did not rescue the IPP (2.3 nM) induced phenotype (Figure 3C,D).

Overall, VEGF-A165, especially in combination with ANG2, induces sustained barrier disruption. IPP induces a persistent barrier dysfunction in iBREC cells at 2.3 nM, while 4.5 nM induced cell death.

In conclusion, the AtlaZ platform is ideally suited for assessing endothelial barrier integrity, as it permits continuous, label-free, and high-throughput analysis of monolayer function by electrical impedance spectroscopy. This approach allows discrimination between alterations in barrier properties and effects on cell viability or cytotoxicity, thereby improving mechanistic interpretation of treatment-induced responses.

Workflow of AtlaZ assay to investigate barrier integrity

Target cells are seeded in AtlaZ sensor plates and they adhere on the surface with embedded gold electrodes (Figure 4). 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 *transepithelial* or *transendothelial electrical resistance* (TEER signal) then is recorded in parallel to the cell attachment, proliferation, cell death or morphology changes in the presence of drugs or stimuli of interest.

Key findings

- AtlaZ Control Software allows to decompose the impedance signal into its resistive and capacitive components, enabling clear discrimination and quantification of cell-cell contacts (barrier function) independently of other morphological changes.
- AtlaZ enables label-free, real-time investigation of cell adhesion, proliferation, and maturation while simultaneously monitoring barrier integrity.
- Recordings can be performed for up to 6 x 96 = 576 samples either simultaneously or independently.

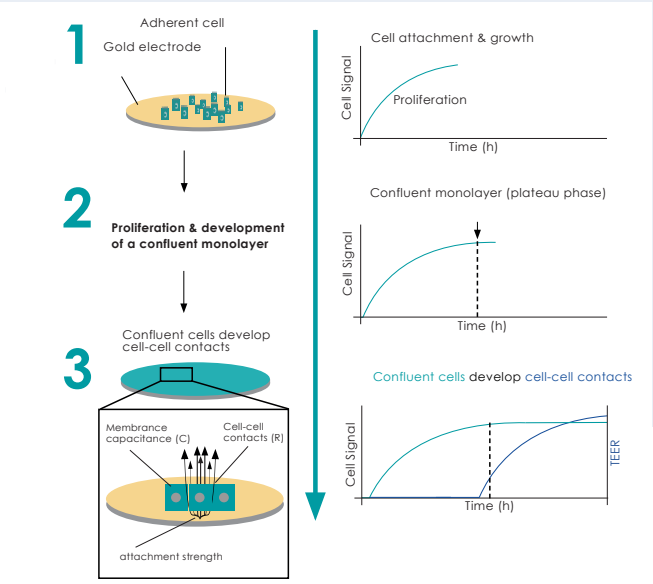


Figure 4: *Workflow for a TEER assay.* *Tight junction forming cells adhere and proliferate after seeding (1). Once a confluency of cells is reached (2), tight junctions seal the paracellular gap increasing the resistance values (TEER). Resistance is recorded in parallel to cell proliferation (3). Cell Signals (e.g. TEER) can be monitored continuously over time, revealing kinetics of stimulus-induced effects.*

References

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