cellZscope® – How It Works

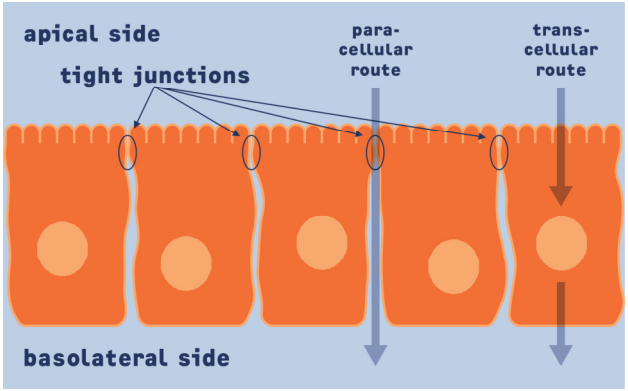
This technical note explains the fundamentals of impedance spectroscopy on barrier-forming cells. In particular, it gives information on the basic technology behind the cellZscope – a device designed for automated measurements of the transepithelial / -endothelial resistance (TER) and capacitance (C_{cl}) of cell layers under physiological conditions. The biophysical origin of the cell layers' impedance and the different contributions to the measured impedance are discussed. Based on these concepts the cellZscope automatically derives the relevant parameters related to the cell layers' properties and provides them as easy readout parameters.

Barrier Forming Tissue

In multicellular organisms epithelial and endothelial cell layers serve as functional barriers and perform very complex and vital activities. While comprising various tissues of the body, these cell layers form selectively permeable interfaces between compartments of different chemical composition. They not only control diffusive permeation of solutes along intercellular clefts between adjacent cells but can also actively transport substances along transcellular paths. Key components of epithelial and endothelial cell barriers are the connecting points between adjacent cells. These tight junctions are of particular relevance for the active barrier functionality of the cell layer. They regulate the passage of molecules across the barrier as they selectively open and close in response to various signals from the inside and outside of the cells.

While the barrier function is responsible for a multitude of physiological activities and is thereby of vital importance for the correct functioning of the organism, it is at the same time also a severe obstacle for specific types of medical treatment, in particular for targeted drug delivery: in order to get a drug to the intended site of action it has somehow to pass through these tissue barriers.

Consequently, in the field of fundamental research, pharmaceutical research, and drug development there is great interest in understanding and controlling the barrier function of epithelial and endothelial tissue.



Epithelial and endothelial cell layers form selectively permeable barriers. Transport of molecules and ions from the apical to the basolateral side and vice versa requires passage either through the cells (transcellular route) or between the cells and thus through tight junctions (paracellular route).

Finding methods to overcome this hurdle is of particular relevance to medication by drug delivery across the

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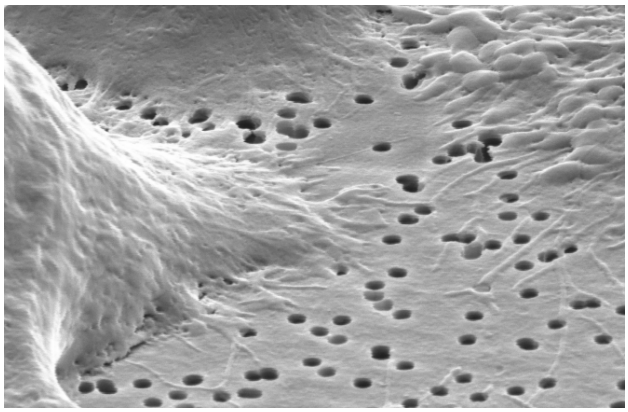
blood-brain barrier, blood vessels, nasal tissue, or gastrointestinal tissue. Therefore adequate in vitro cell models and assays are required - for instance for identifying compounds that reversibly increase drug permeation through tissue barriers.

A direct correlation between the permeability of a cell layer and its electric resistance, i.e. the so called transepithelial / -endothelial electric resistance TER, exists. This fact can be utilized to form the basis for an assay: tight cell layers exhibit high electric resistance and - vice versa - high permeability correlates with low electric resistance. Therefore, the electric resistance measured across a cell layer is a highly qualified parameter for quantifying leak tightness of barrier forming tissue. Consequently, it can be recorded to compare and monitor the establishment or modulation of barrier-forming cell-to-cell contacts.

Analyzing the properties of a cell layer by means of electrical measurements is not limited to measuring the electric resistance, but can be complemented by recording the electric capacitance C_{cl} as well. This parameter provides additional information about the cell layer properties: in particular it is indicative of the expression of microvilli and other membrane extrusions.

The two quantities, resistance TER and capacitance C_{cl}, combine for the complex impedance Z of the cell layer, which can be measured electronically. In contrast to other assay techniques electrical measurements require neither a fluorescent or radioactive marker nor any other type of physiologic modification of the cell system. While providing a wealth of information on the barrier properties electrical measurements can be performed without affecting the native cell culture under investigation.

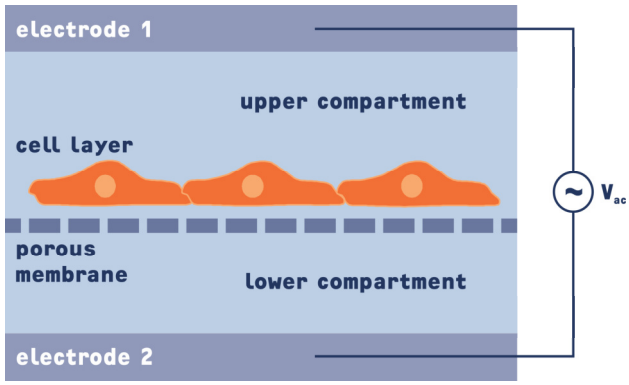


Scanning Electron Microscopy image of a subconfluent cell layer grown on a permeable membrane.

Measuring the Impedance of Cell Layers

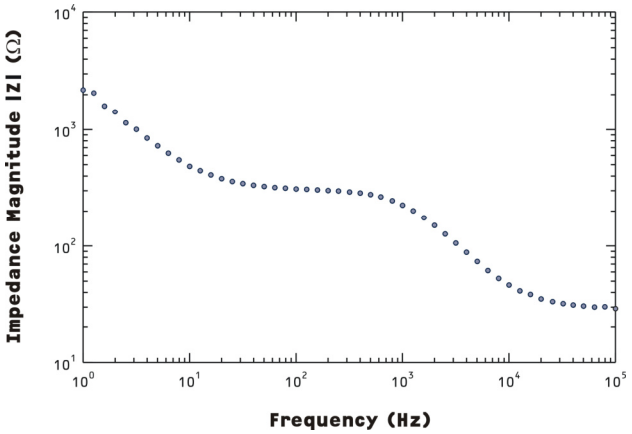
Nowadays a wide variety of well-established in vitro cell models exists. Numerous models are based on cell cultures grown on permeable membranes. The latter are available from different manufacturers as inserts for standard well plates and are routinely used as lab consumables. The inserts mainly differ in their geometric design, the membrane material, as well as the pore density and size. Albeit those technical differences all inserts comprise a porous membrane which separates an upper from a lower medium-filled compartment. These inserts are ideally suited for performing electrical measurements across cell layers cultivated on the permeable membrane.

By placing an electrode on each side of the membrane, i.e. one in the upper compartment and one in the lower, and applying a small AC voltage V_{ac} the electric impedance of the cell system can be measured.



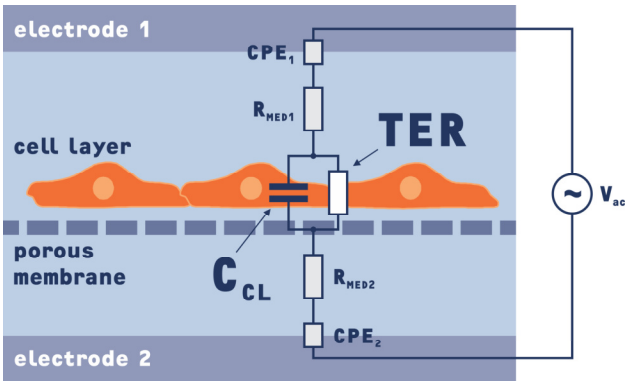
An epithelial or endothelial cell layer cultured on a permeable membrane forms the interface between two medium-filled compartments while an AC voltage is applied across the electrodes.

Such a setup mimics the physiological location of epithelial or endothelial cell layers as interfacial tissue between two fluid compartments. Provided that the permeability of the membrane support is properly selected, the cell layer is the ion current-limiting entity. However, also the cell medium and the interface between the electrodes and the culture medium have to be taken into account. They both contribute to the measured total impedance of the system. In combination with the electric resistance and capacity of the cell layer a non-linear frequency dependence of the total impedance results.



Experimental data showing the typical frequency-dependence of the impedance magnitude for a cell layer cultivated on a porous membrane of a standard insert and with electrodes placed in each of the two medium-filled compartments.

Equivalent circuits and corresponding mathematical models can be applied in order to extract parameters which mirror the barrier properties of the cell layer under examination. They allow separating impedance contributions stemming from the cells from the rest of the impedance spectrum. Although cell layer are rather complex biological systems, their electronic characteristics integrated over a large collective of cells can in good approximation be modeled by basic elements. The following schematic shows an equivalent circuit which is well suited to model the setup described above.

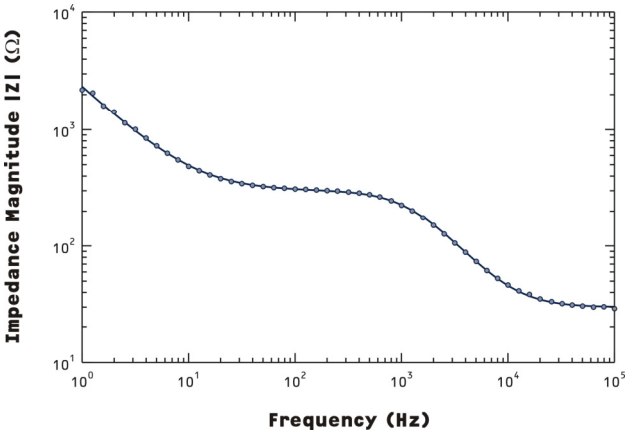


The transepithelial/-endothelial electric resistance (TER) and capacitance (C_{cl}) of the cell layer is measured by recording the frequency-dependent impedance (Z) and using an electric equivalent circuit to analyze the data.

The two main components directly attributable to the cell layer are the resistance TER and capacitance C_{cl}. The ohmic resistance TER describes the parallel connection of the paracellular paths, while the capacitance of both the apical and the basolateral membranes is summarized in C_{cl}. Further possible contributions to the cell layer's total impedance, for instance the ohmic resistance across the cell membranes can well be neglected in first order approximation. Based on these model assumptions the parallel circuit of TER and C_{cl} is well-suited to describe the integrated cell layer's properties.

The culture medium in the upper and lower compartment is modeled in good approximation by a simple ohmic resistance R_{med}. The electrodes and in particular the interface between the electrodes' metal and the culture medium exhibits a more complex impedance behavior. The so-called constant phase element (CPE) is an empiric but well-established model based on two parameters A_{cpe}, n_{cpe}. The CPE model is suited to mathematically describe the characteristic frequency-dependence of the electrode–medium interface's impedance.

The equivalent circuit and the corresponding mathematical models allow deriving an analytical expression for the total impedance of the system. The resulting function depends on five parameters (TER, C_{cl}, R_{med}, A_{cpe}, n_{cpe}). On the basis of this parametric function an algorithm can be applied to fit the experimental data. Finally, a set of best fit parameters, including the two cell layer related parameters TER and C_{cl} is obtained.



Experimental data and resulting fit curve which is based on the following fit parameters:

$TER = 295.5\ \Omega \cdot \mathrm{cm}^2$
$C_{CL} = 0.46\ \mu\mathrm{F}/\mathrm{cm}^2$
$R_{MED} = 30\ \Omega$
$A_{CPE} = 84.7\ \mu\mathrm{F} \cdot \mathrm{s}^{n_{CPE}-1}/\mathrm{cm}^2$
$n_{CPE} = 0.835$

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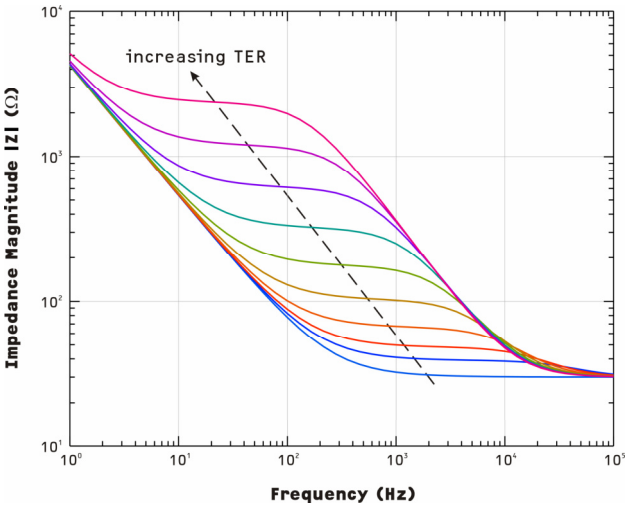
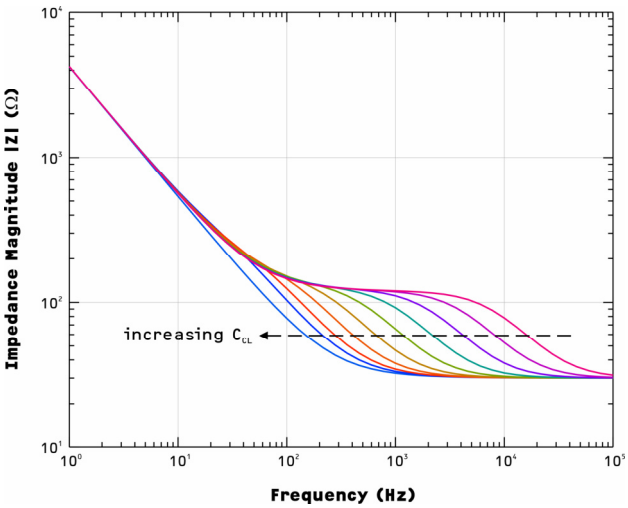
Understanding Impedance Spectra

The cell layer's resistance TER and capacitance C_{cl} contribute predominantly at mid-range frequencies to the total impedance, leading to the formation of a plateau. This characteristic permits to separate these two contributions of interest from the peripheral impedances dominating the low and high frequency end of the spectrum. It is instructive to consider how changes in resistance TER or capacitance C_{cl} affect the measured impedance spectrum. The following diagrams show that - as a rule of thumb - the resistance TER determines the height and the capacitance C_{cl} the width of the plateau.



An increase in the resistance TER shifts the plateau upwards.



An increase in the capacitance C_{cl} narrows the plateau.

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