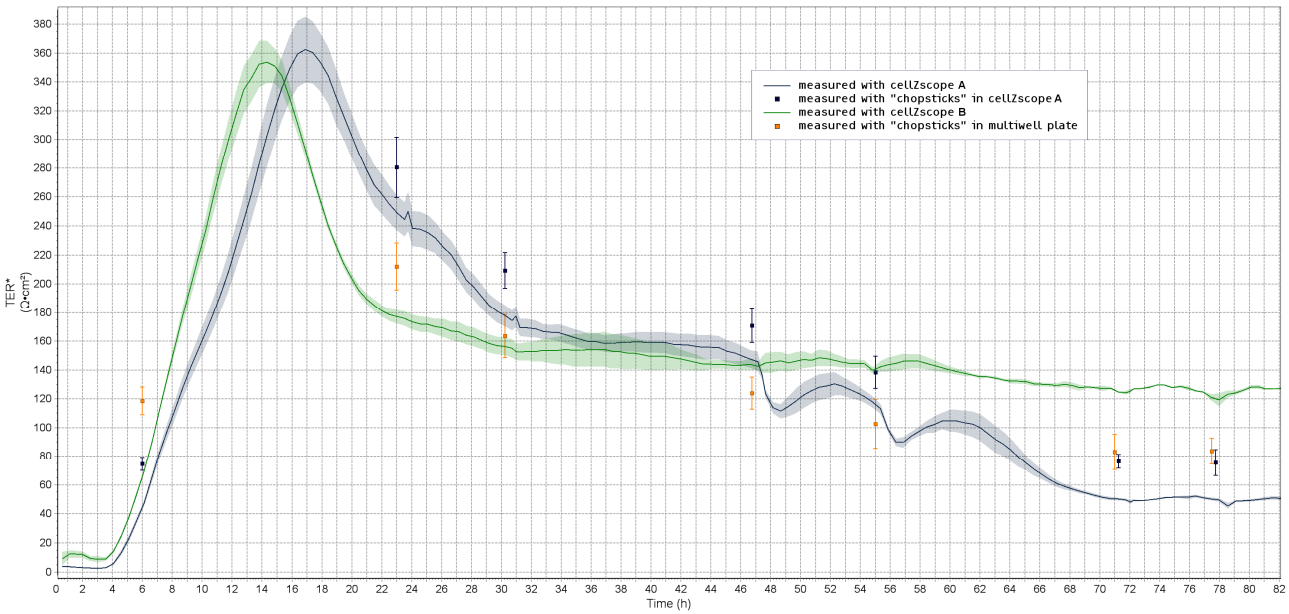
Measuring TER — A Comparative Study: cellZscope® vs. Handheld Devices With "Chopstick" Electrodes

The transepithelial / -endothelial electrical resistance (TER) of cell layers is often measured with simple handheld devices made up of "chopstick"-like electrodes. For lack of alternatives and despite several disadvantages and inherent limitations these devices have found widespread use for studying the barrier properties of epithelial or endothelial cells grown on the permeable membranes of standard cell culture inserts. In this technical note we compare TER values measured with handheld "chopstick" electrodes to results obtained with the automated cellZscope.

One of the defining characteristics of epithelia and endothelia cell layers are the intercellular junctions which form a barrier between the apical and basolateral domain of the cell layer with highly specific permeability. A quantity describing the tightness of this barrier is the electrical resistance measured across the cell layer [1]. A straightforward method for measuring the resistance would be to apply a defined DC voltage and to measure the resulting current. Using Ohm's law (U=R·I) one could then simply calculate the resistance of the cell layer. However, the DC current causes adverse effects on both the cells and the electrodes. In principle, this problem can be avoided by performing AC measurement instead. The most simple approach would be to apply a defined AC voltage with constant frequency, to measure the resulting AC current, and then to calculate TER using Ohm's law while neglecting contributions stemming from the capacitance of the cell layer and the electrodes. However, such a oversimplification can easily lead to erroneous results. A more sophisticated measuring technique is required for obtaining reliable results. Therefore the cellZscope



Time course of the transepithelial electrical resistance of MDCK-II cells as measured with the cellZscope and a handheld device with "chopstick"-type electrodes.

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records full impedance spectra by sweeping the frequency of the applied AC voltage and measuring the amplitude and phase of the resulting AC current. Based on this set of information not only TER but also the capacitance (C_{cl}) of the cell layer is automatically extracted and provided as a readout parameter [2].

Materials and Methods

Experiments were performed with Madin-Darby canine kidney cells (MDCK-II, ECACC). They were grown in Eagle's Minimum Essential Medium (EMEM, Sigma-Aldrich) with Earle's Salts (Sigma-Aldrich) supplemented with 10% Fetal Bovin Serum (FBS superior, Biochrom), 2mM L-Glutamin (Biochrom) and 1% Pen/Strep (Biochrom). The cells were fed twice a week and split once a week at a ratio 1:5 on 25cm^2 flasks (Greiner Bio-One). For the experiments cells were seeded on ThinCert® inserts (part no. 665640, Greiner Bio-One) at a density of $5{\cdot}10^5$ cells/cm^2. Inserts were placed in two separate cellZscopes and one Cellstar® multiwell plate (Greiner Bio-One). With the two cellZscopes TER was measured automatically every 30 minutes. cellZscope B holding inserts remained in the incubator throughout the experiment (except for media exchange) for maintaining optimal physiological conditions. The second cellZscope module (cellZscope A) and the multiwell plate were taken out of the incubator twice a day and transferred to a flow bench. There manual TER measurements were performed in each well of the insert holding cellZscope A and the multiwell plate using a handheld device (EVOM+STX2, World Precision Instruments). The "chopstick"-type electrodes were inserted at three different positions (120 deg turns) in each well and the readings averaged. After completion of the manual measurements the multiwell plate and the cellZscope A were returned to the incubator. Data recording was started right after cell seeding and carried on for a total time span of 3.5 days.

Results and Discussion

The figure above shows both the data recorded automatically with the two cellZscope systems and the readings taken manually with the handheld device. Although the cellZscope software performs automatic data analysis of the measured impedance spectra and thereby provides both cell-related parameters TER and C_{cl}, the latter is not depicted here, because the "chopstick" electrodes based device can only measure TER and permits no comparison of C_{cl} values. The graph shows the typical growth dynamics of MDCK-II cells, which formed a fully differentiated cell layer after approx. 40 hours as indicated by a steady state level of TER readings. The shaded areas plotted along the continuous curves represent the standard error of the averaged cellZscope data. Data obtained with the handheld device are shown as distinct data points with their respective error bars. All data plotted in blue (points and curve) were measured inside the same cellZscope module, whereas the green curve represent data recorded with the cellZscope B. The orange data points were measured with the handheld device in the multiwell plate.

When comparing the two data sets obtained with the "chopstick"-type electrodes (blue and orange data points) only minor differences are observed between cell layers grown inside the cellZscope and inside the multiwell plate. In particular, at later time points, i.e. in the steady state phase after approx. 70h values converge to exactly the same level within error margins. Data recorded with the handheld device (blue data points) and the cellZscope A (blue curve) on the very same inserts exhibit the same overall time course of TER, although the continuously measured data of the cellZscope provides a far more detailed picture of the growth dynamics. While both measurements correlate well, the TER values of the handheld device were found to be slightly higher. This finding is due to the inherent inhomogeneity of the electrical field distribution between the "chopstick"-type electrodes, leading to an overestimation of TER values [3].

Comparing the results obtained with the two cellZscopes, of which one remained in the incubator (green curve) and the other was transferred to a flow bench for additional measurements with "chopstick" electrodes several times (blue curve), reveals that the undisturbed cells exhibit a much smoother time course of TER readings. It is obvious that the manual insertion of the "chopstick" electrodes led to a repeated disturbance of the barrier forming cells, ultimately leading to a significantly lower TER level after long term cultivation. This finding is indicative of the strong interference of the manual measurements with cell culture conditions. Cells should rather be cultivated in a undisturbed manner and culture conditions be kept constant to ensure that cellular parameters can reach a stable state. The cellZscope system supports these requirements with its non-invasive operating principle, thus reducing measurement artifacts to a minimum.

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[1] Powell, D.W., *Barrier function of epithelia*, Am. J. Physiol. Gastrointest. Liver Physiol. **241**, G275 (1981).
[2] for more details see the Technical Note *cellZscope® - How It Works*.
[3] Jovov, B., Wills, N.K., Lewis, S.A., *A spectroscopic method for assessing confluence of epithelial cell cultures*, Am. J. Physiol. Cell Physiol. **261**, C1196 (1991).

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