Using MTECC recording of PD/RT.

MTECC (**M**ulti **T**ransEpithelial **C**urrent **C**lamp) is mainly used for recording of transepthelial resistance (RT) and open circuit potential (PD). Electrical parameters of four epithelia cultured in transwell plates can be recorded simultaneously. MTECC is also designed to do impedance analysis that can be used to record the resistance of the bathing solution in between the voltage sensing electrodes or to determine the contribution of apical and basolateral membrane to RT.

Electric Equivalent electric model for monolayer of cells.

The lipid bilayer of the cell membrane is not permeable for ions. The electrical resistance is infinitely high. The bilayer behaves as an electrical capacitor (Cm) and can accumulate electrical conductive charges. On the other hand, ion channels in the membrane allow ions to cross the membrane and provide an ion conductive pathway that is represented by the electrical resistance RT. When ion channels are activated, the electrical resistance will decrease. In addition, because ion channels are highly selective, they give rise to electrical potential as described by the Nernst or Goldman-Hodgkin-Katz equation. This potential is represented by PD in our model. Finally, the epithelial cell layer is bathed in physiological solution. There is some resistance located in the solution layers at each side of the epithelium. This resistance is represented by Rs. Rs can be recorded by recording the resistance in a reference well containing only the physiological solution. Alternatively, Rs can be recorded by sending high frequency (> 3 kHz) sine wave currents through the epithelium. At high frequencies the capacitance (Cm) behaves as a conductive path and decreases the resistance (impedance) of the membrane. By extrapolation to infinitely high frequencies we can determine Rs.
With the MTECC equipment we want to monitor changes in RT that are caused by the activation of the chloride conductive pathway in the apical membrane of airway cells.

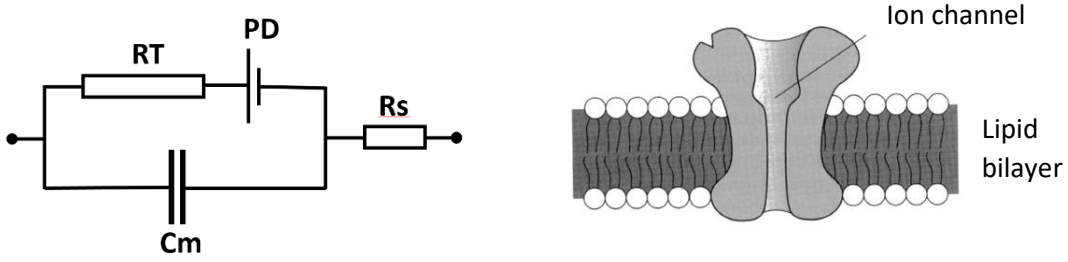

Fig. 1: Schematic diagram of circuit used model electric parameters of monolayers in cell culture well.

For recording of transepithelial parameters we need four electrodes: two voltage sensing electrodes and two current sending electrodes. Electrodes immerged in physiological solutions have an offset potential. For recording transepithelial potential the difference in offset potential between the two electrodes should be as small as possible. Because of limited space in the wells of the cell culture plates Ag/AgCl pellets are used as voltage sensing electrodes. Ag/AgCl electrodes have relative small offset and have been used in electrophysiological research since many decades. Current sending electrodes can be made of Ag foil or, Ag/AgCl pellets can be use as well. Thus, IV units are made of two Ag/AgCl pellets or one Ag/AgCl pellet for voltage sensing and Ag foil for current sending. Figure 2 schematically shows the setup of the electrode units in one well of a cell culture plate and the connection to the electronic circuit. The voltage sensing Ag/AgCl pellets are connected to a differential voltage amplifier (V-Ampli). The apical current sending electrode is connected to a circuit that generates the current

(I-stimulus). The injected current is recorded with an electronic ampere meter (I-recording). The output voltage of the voltage amplifier (V-Ampli) as well as the output of the current recording circuit are acquired by the microcontroller controlled data acquisition system in the MTECC system.

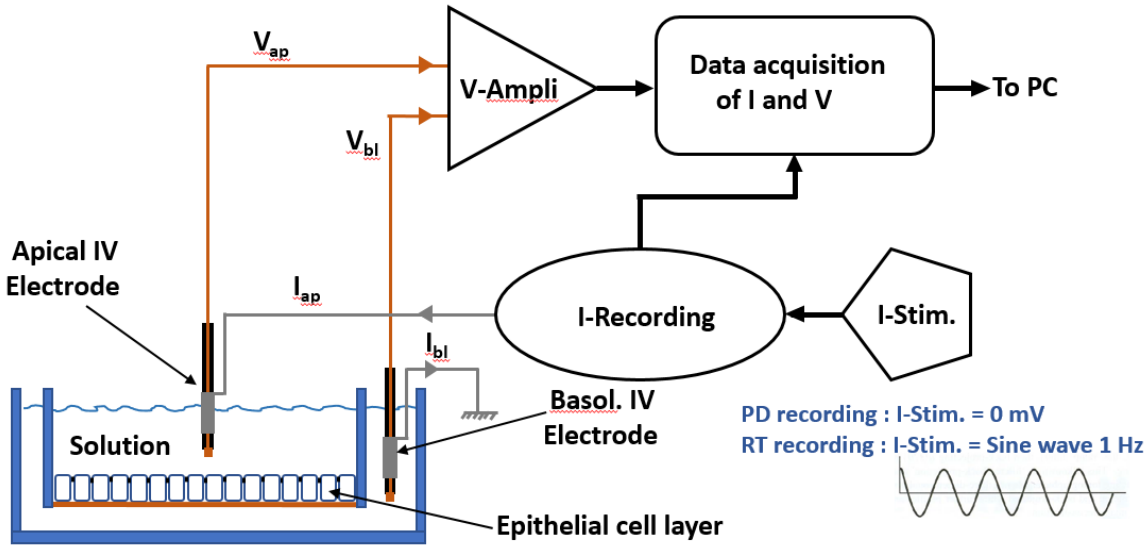

Fig. 2: Diagram of the current clamp circuit for recording RT and PD.

For recording RT and PD, EP Devices has chosen to use Current Clamp instead of Voltage Clamp. In current clamp mode the feedback amplifier controls the current through the membrane: I-stimulus. To record the RT we use a sine wave current. The frequency of the sine wave is 1 Hz. We record the current stimulus as well as the voltage response, which has a sine wave pattern as well. Figure 3 compares the current and voltage sine waves. The microcontroller determines the amplitudes of current and voltage with Fourier analysis. The ratio of voltage amplitude over current amplitude is equal to the transepithelial resistance RT: $$\mathrm{RT} = \frac{\mathrm{V-Amplitude}}{\mathrm{I-Amplitude}}$$

With frequencies as low as 1 Hz, the sine wave analysis gives an accurate estimation of RT, similar to resistances calculated from voltage responses to rectangular, stepwise changes in current. Moreover, the measurements are less sensitive to external noise because signals that do not follow the 1Hz sine wave pattern are rejected by Fourier analysis. For instance, line frequency interferences (50 Hz in Europe) are fully filtered out, or removed from the 1 Hz pattern. Also the DC offsets are fully removed.

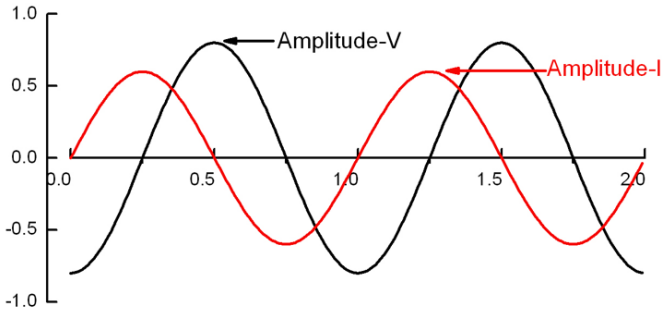

Fig. 3: Sine wave voltage and current signal. Note that the phase shift between the sine waves at 1 Hz is markedly smaller than shown in this figure. At 1 Hz I and V are almost in phase.

Transepithelial PD is recorded twice: before and after the RT recording. During PD recording, the stimulus is stopped and no current is flowing through the membrane. Thus recorded PD is the open circuit voltage across the epithelium.

From the open circuit voltage and recorded RT we calculate the "Equivalent Short Circuit Current": I_{eq}. This is the current that would flow when the transepithelial voltage is clamped to zero. Using a manual short circuit method Prof. H. H. Ussing (Ref. 1) described active transport through frog skin epithelium: the current (I_{sc}) through the epithelium is flowing in the absence of an electrochemical gradient, with equal solutions on both sides and in the absence of a transepithelial voltage. I_{sc} reflects the current carried by and active transport mechanism: de Na/K pump. In many epithelia this current is limited by the uptake of Na through the apical membrane and the short circuit method has been applied in numerous studies of Na uptake through the apical barrier.

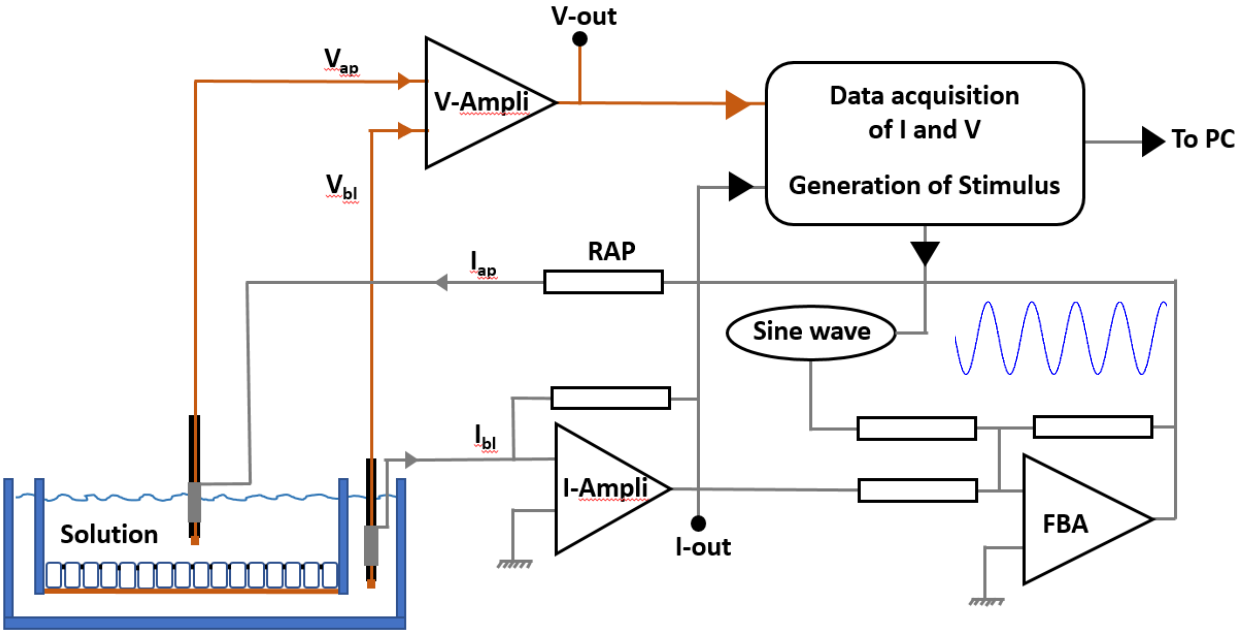

Figure 4: Schematic of current clamp circuit as used in MTECC. Transepithelial voltage is recorded with a differential instrumentation amplifier (V-ampli) with variable gain, set by the microcontroller. The current is recorded with a current to voltage converter (I-Ampli). The output of I-amp (I-out) is connected to a feedback amplifier (FBA). FBA compares I-out with a second input (Sine wave – 1 Hz) to obtain a sine wave stimulus.

References:

KOEFOED-JOHNSEN V, USSING HH.: The nature of the frog skin potential. Acta Physiol Scand. 1958 Jun 2;42(3-4):298-308.

Two membrane model.

The ion permeabilities of the apical and basolateral membranes of epithelia are quite different. The basolateral membrane has the Na/K pump that will keep intracellular Na concentration low and accumulate K from the external bathing solution. The basolateral membranes are highly permeable to K

which is the main cation involved in the generation of the basolateral diffusion potential. Depending on the nature and function of the epithelial layer, other transporters are present in the basolateral membrane, e. g. the Na-K-2Cl cotransporter or the Cl-HCO3 exchanger. In the figure below, the apical membrane has Na selective channels providing the pathway for Na uptake, and Cl channels that make the secretion of Cl possible. The transepithelial resistance (RT) recorded in our setup will be the electrical equivalent resistance of a two membrane structure represented by electrical model below. This model shows that the RT is equal to the serous resistance op R_{ap} and R_{bl} with the paracellular resistance in parallel:

$$RT = \frac{R_{par} \cdot (R_{ap} + R_{bl})}{R_{par} + R_{ap} + R_{bl}}$$

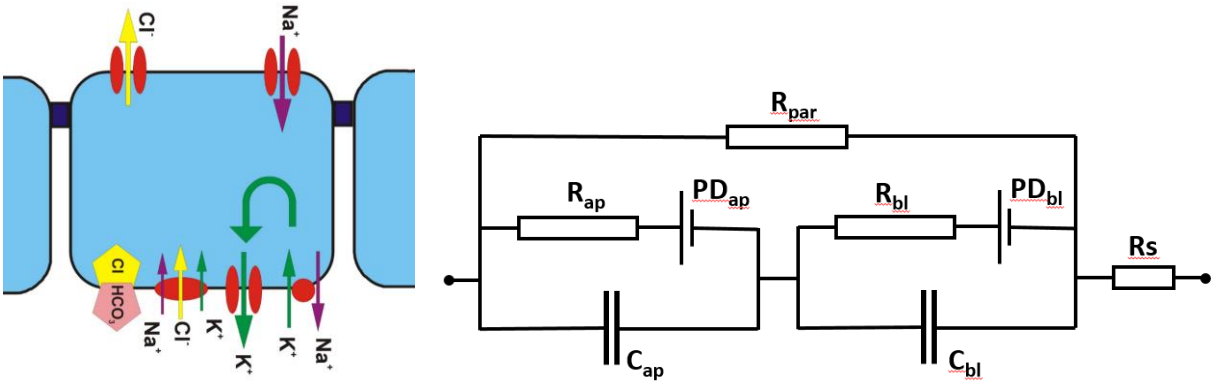

Figure 5: Example of epithelial cell and electric equivalent circuit representing the two membranes and the paracellular pathway.

Under certain conditions Rap and Rbl can be determined with impedance analysis, which is also implemented in the MTECC hardware.

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