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Temperature corrected transepithelial electrical resistance (TEER) measurement to quantify rapid changes in paracellular permeability

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Determining the transepithelial electrical resistance (TEER) is a widely used method to functionally analyze tight junction dynamics in cell culture models of physiological barriers. Changes in temperature are known to have strong effects on TEER and can pose problems during the process of TEER measurements in cell culture vessels, complicating comparisons of TEER data across different experiments and studies. Here, we set out to devise a strategy to obtain temperature-independent TEER values based on the physical correlation between parameters such as TEER, temperature, medium viscosity and pore size of the cell culture inserts. By measuring the impact of temperature and different electrode types on TEER measurements on Caco-2 and HPDE (normal human pancreatic ductal epithelium) monolayers, we were able to derive a mathematical method that is suitable for the correction of TEER values for temperature changes. Applying this method to raw TEER values yields temperature-corrected TEER (tcTEER) values. Validity of tcTEER was demonstrated by showing a direct correlation with permeability of monolayers as determined by flux of RITC dextran. Taken together, the mathematical solution presented here allows for a simple and accurate determination of paracellular permeability independent of temperature variation during the process of TEER recording.

1. Introduction

Transepithelial electrical resistance measurements are routinely used to characterize monolayer integrity in the context of cell monolayer permeability experiments, or to quantify permeability changes, e.g. as a consequence of paracellular permeability enhancers (Masungi et al. 2009; Jonker-Venter et al. 2006). As a functional parameter, TEER measurement of epithelial monolayers is widely used for the functional analysis of tight junctions (Nagumo et al. 2008). Molecularly, the expression and function of occludin is the most decisive structural factor for TEER changes (Hombach et al. 2008).

Previous studies have shown that TEER measurements are temperature-dependent (Matter and Balda 2003; Torres et al. 2007). Thus, unless TEER measurements are conducted in the incubator, it is mandatory to equilibrate temperature prior to TEER measurements in order to overcome temperature-induced TEER changes that result from the difference between the 37 °C in the incubator and room temperature (RT) (Kaitu'u-Lino et al. 2007). If TEER is to be determined at RT, a major problem of this strategy is that it takes more than 20 min to obtain reproducible TEER values. Ideally, TEER measurements should be conducted at a constant temperature of 37 °C to mimic physiological conditions of human cells, which requires that the electrode is fixed in the incubator. Alternatively a constant temperature could be maintained with a slide warmer (Tippin and Thakker 2008). A chopstick electrode which is mostly used for TEER measurements is difficult to use in the incubator without a tripod. Alternatively a more complex equipment, such as an EndOhm electrode (Feighery et al. 2008), or an Ussing cham-

ber (Sawai et al. 2002) can be used. Both experimental setups are feasible, but have significant disadvantages compared to a chopstick electrode such as cell cultures are disturbed by transfer from tissue culture plates and changes in buffer. Additionally, the TEER values of a smaller number of monolayers could be measured at the same time.

In this report, we evaluate the influence of temperature on TEER values, and provide a mathematical solution to calculate temperature independent TEER values under various experimental conditions. The method described may be used for biochemical and pharmacological studies of epithelial cell monolayers and pharmacokinetic studies.

2. Investigations and results

2.1. Physical background

The resistance of a substance is proportional to its thickness l , and inversely proportional to the cross-sectional area A . This is given by

$$R = \frac{l}{\kappa A} \quad (1)$$

where κ is the conductance (Adamson 1979).

The length of the pores of an insert is given by l and the width of the pores by A . In case both variables are constant during measurement, changes in resistance are a consequence of changes of the specific conductivity (κ). The specific conductivity of a liquid is higher when the ionic strength and the agitation of the

Table 1: Specifications of hanging cell culture inserts (without cells) taken from description of products

Well size	pore				TEER			
	diameter (μm)	size (μm ²)	density (pores/cm ²)	total size (cm ² /insert)	change/K (Ω K ⁻¹)	at 295 K (Ω)*	at 301 K (Ω)*	at 308 K (Ω)*
6	0.4	0.1257	2 * 10 ⁶	0.0107	3.13	180	158	140
6	1	0.7854	2 * 10 ⁶	0.0668	2.62	155	138	122
6	3	7.0686	0,6 * 10 ⁶	0.1804	2.46	149	131	118
6	8	50.2656	0,15 * 10 ⁶	0.3207	2.27	144	127	114
12	0.4	0.1257	1 * 10 ⁸	0.1257	2.69	174	157	139

ions is increased. Agitation is a result of the size of the hydrate envelope that encircles the ions, and the viscosity of the fluid (Atkins et al. 2006). The Walden rule states that the product of the viscosity and the molar conductivity is approximately constant (Johansson et al. 2008). The correlation between the viscosity and the temperature is described by the Andrade-equation

$$\ln \eta = a + \frac{b}{T} \quad (2)$$

where η is the viscosity, T is temperature, and a and b are coefficients of unknown size for the media used (Wang et al. 2007). The correlation between temperature and viscosity is demonstrated in Fig. 1. Therefore, under the conditions of a particular measurement, when all of the parameters that affect resistance are constant except temperature, changes in TEER are a consequence of temperature changes. In this case, Eqs. (1) and (2) can be combined to

$$R = e^{a + \frac{b}{T}} \frac{l}{A} \quad (3)$$

The length of the pores of the insert is usually constant during the experiment. Previous studies have shown that the overall barrier thickness does not change even if the monolayers have different resistances (Lu et al. 1996). Therefore, the changes in resistance are reciprocal to the product of temperature and width of the pores.

2.2. Blank corrected unit area resistance

The total resistance measured across a tissue culture membrane is composed of the background resistance of a blank insert and the resistance of the tissue. For a reproducible tissue resistance

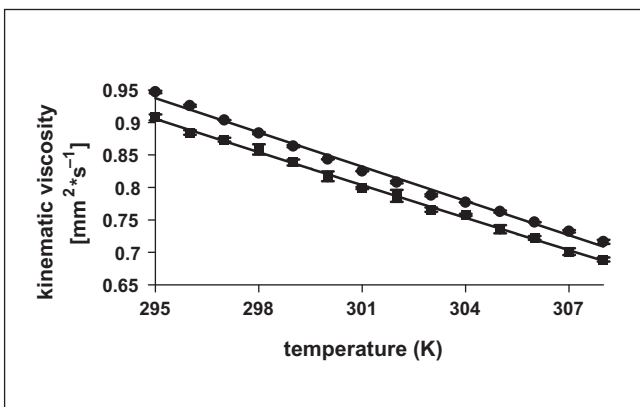


Fig. 1: Temperature-dependency of viscosity of the culture media used for Caco-2 (●) and HPDE cells (■). An Ubbelohde-viscosimeter (Schott capillary viscosimeter CT42) with a 0C-capillary (0.46 mm diameter) was used according to the manufacturer's instructions. The viscosity of the medium was measured at each °C between 295 K and 308 K. All tests were done in triplicate. Error bars are generally too small to extend beyond the symbols for mean values

it is necessary to correct it for the adapted blank resistance which requires that the values for blank and the values for total resistance must be collected at the same temperature (T_α). If e.g. TEER measurement of a 12-well monolayer is conducted at RT (295 K) 174 Ω must be subtracted from the value for total resistance. In case of a measurement at 308 K the blank is smaller (139 Ω) (Table 1). The product of tissue resistance and culture area must be calculated to obtain the unit area resistance, which is defined here as $TEER_{(tissue)}$. This is independent of the insert and may be employed to compare data obtained from inserts of different sizes.

$$TEER_{(tissue)} = (TEER_{(total)T_\alpha} - TEER_{(blank)T_\alpha}) \text{culture area} \quad (4)$$

2.3. Calculated TEER values of inserts

Figures 1A and B demonstrate the correlation between TEER values and temperature shown for four 6-well inserts with different specifications described in Table 1. However, this correlation is biased by the total pore size of the insert. The smaller the total pore size, the higher the TEER value and the greater the changes in TEER/K.

Based on these findings, temperature-corrected TEER (tc TEER) values can be calculated. The TEER change/K could be calculated by

$$\frac{\Delta TEER}{\Delta T} = [\alpha_{295K} + \beta(T_\alpha - 295K)] TEER_{T_\alpha} - \gamma \quad (5)$$

T_α = actual temperature

$TEER_{T_\alpha}$ = measured TEER at actual temperature

$\gamma = 1.0618 \Omega K^{-1}$, which is given by the y-intercept shown in Fig. 2B and $\alpha_{295K} = 0.022 K^{-1}$, which represents the slope of the linear regression at 295 K (●) (Fig. 2B).

$$\beta = \frac{0.032 K^{-1} - 0.022 K^{-1}}{13 K} = 7.692 \times 10^{-4} K^{-2} \quad (6)$$

where $0.032 K^{-1}$ is the slope of the equation at 308 K (◆) (Fig. 2B). Hence, β demonstrates the change of the slopes at every degree between 308 K and 295 K. Therefore, the TEER value at e.g. 300 K ($TEER_{300K}$) is described by Eq. (7):

$$TEER_{300K} = TEER_{T_\alpha} - \frac{\Delta TEER}{\Delta T} (300K - T_\alpha) \quad (7)$$

2.4. Calculated TEER values of Caco-2 monolayers

Figure 2A demonstrates the dependency of TEER-change/K on the TEER value by twenty 12-well Caco-2 monolayers. The higher the density of the monolayer, the higher the TEER value and the greater the changes in TEER/K. TEER values are given

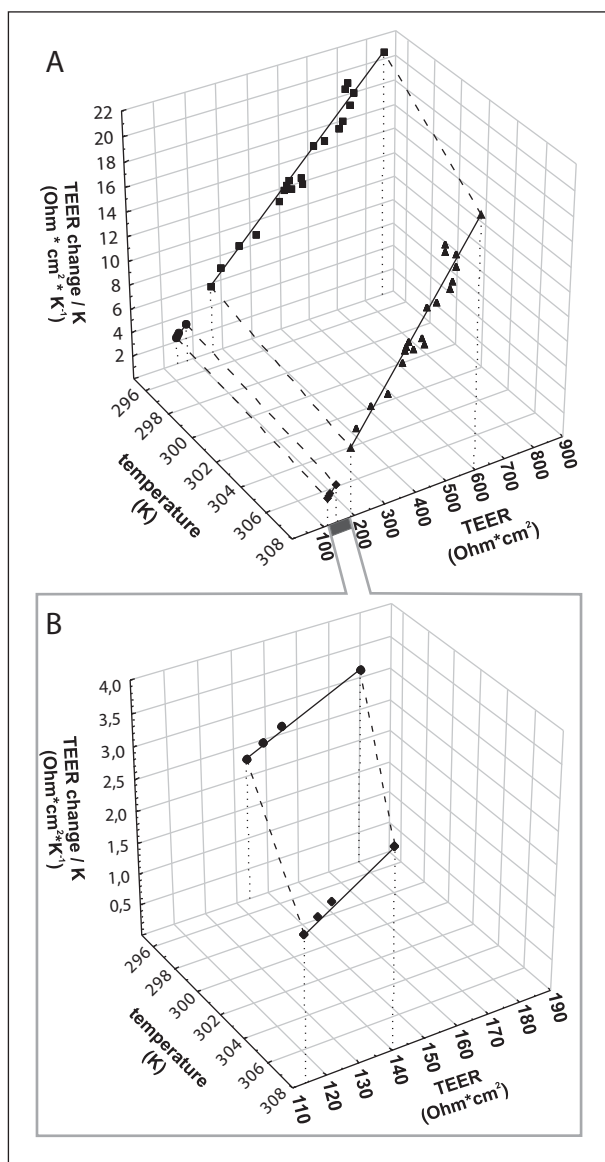


Fig. 2: Dependency of TEER-change/K on TEER values. (A) Linear regression for the TEER change per K of twenty different tight Caco-2 monolayers at 308 K (▲) is $TEER/K = 0.0361 \times (\Omega \text{ cm}^2) - 1.305 (\Omega \text{ K}^{-1})$, ($R^2 = 0.9145$) and at 295 K (■) $TEER/K = 0.0251 \times (\Omega \text{ cm}^2) - 1.175 (\Omega \text{ K}^{-1})$, ($R^2 = 0.9608$). Temperature-dependency of the TEER value is representatively shown for 12-well Caco-2 monolayers with the lowest and the highest TEER value of all twenty monolayers (---). (B) Linear regression for the TEER change per K of four different pore sized inserts (Tab. 1) at 308 K (◆) is $TEER/K = 0.0326 \times (\Omega \text{ cm}^2) - 1.397 (\Omega \text{ K}^{-1})$, ($R^2 = 0.986$) and at 295 K (●) $TEER/K = 0.0225 \times (\Omega \text{ cm}^2) - 0.9129 (\Omega \text{ K}^{-1})$, ($R^2 = 0.985$). Temperature dependency of the TEER value is representatively shown for two 6-well inserts (---)

by $TEER_{(tissue)}$ calculated by Eq. (4).

$$\beta = \frac{0.0361 \text{ K}^{-1} - 0.0251 \text{ K}^{-1}}{13 \text{ K}} = 8,461 \times 10^{-4} \text{ K}^{-2} \quad (8)$$

where 0.0361 K^{-1} represents the slope of the linear regression at 308 K (▲) and 0.0251 K^{-1} the slope at 295 K (■) (Fig. 2A). Hence, β demonstrates the change of the slopes at every degree between 308 K and 295 K. Comparing β given by Eq. (6) and β given by Eq. (8) the apparent constants of monolayers are almost the same as for inserts without cells. Hence large trapezium (TEER values of twenty individual Caco-2 monolayer) and small trapezium (TEER values of four different pore sized inserts) are almost coplanar (Fig. 2A).

Therefore, the calculation of $tcTEER_{(tissue)}$ values is almost identical with those for inserts without cells. In the case of a monolayer it is necessary to calculate $TEER_{(tissue)}$ at the actual temperature (T_α) by Eq. (4). TEER change/K is in that case shown by

$$\frac{\Delta TEER}{\Delta T} = [\alpha_{295 \text{ K}} + \beta(T_\alpha - 295 \text{ K})]TEER_{(tissue)} - \gamma \quad (9)$$

$\alpha_{295 \text{ K}} = 0.0251 \text{ K}^{-1}$, which is the slope of the linear regression at 295 K (■) given in Figure 2A and γ is $0.8445 \Omega \text{ K}^{-1}$.

Table 2A compares the calculated $TEER_{(tissue)}$ values and the corresponding temperature corrected $TEER_{(tissue)}$ values of five 12-well Caco-2 monolayers. The $tcTEER_{(tissue)}$ value at 308 K, which was based on the $TEER_{(tissue)}$ value at 301 K in this examples, is almost equal to the $TEER_{(tissue)}$ value at 308 K. The relative error between the $TEER_{(tissue)}$ values and the $tcTEER_{(tissue)}$ values at 308 K is 2.3%.

The method of calculating $tcTEER_{(tissue)}$ values is exemplarily described by Caco-2 monolayer 1 (Table 2A):

1. Calculation of $TEER_{(tissue)}$ values by Eq. (4)

$$(296 \Omega - 157 \Omega) \times 1.1 \text{ cm}^2 = 153 \Omega$$

2. Calculation of TEER change/K by Eq. (9)

$$[0.025 \text{ K}^{-1} + 8.461 \times 10^{-4} \text{ K}^{-2} \times (301 \text{ K} - 295 \text{ K})] \times 153 \Omega - 0.8445 \Omega \text{ K}^{-1} = 3.757 \Omega \text{ K}^{-1}$$

3. Calculation of temperature corrected TEER values by Eq. (7)

$$153 \Omega - 3.757 \Omega \text{ K}^{-1} \times (308 \text{ K} - 301 \text{ K}) = 127 \Omega$$

TEER measurements of Caco-2 monolayers using the EndOhm electrode do not show any significant difference to those obtained by STX2 (data not shown).

2.5. Calculated TEER values of HPDE monolayers

Human pancreatic duct epithelium (HPDE) cells (Radulovich et al. 2008) were provided by Prof. Dr. Schäfer (Clinic for General Internal Medicine, Laboratory of Molecular Gastroenterology and Hepatology, University of Kiel, Germany). Table 2B demonstrates similar results for HPDE monolayer as for Caco-2 monolayer. The calculation of $tcTEER_{(tissue)}$ values for HPDE monolayers is based on the findings of Caco-2-monolayers. The relative error between $TEER_{(tissue)}$ values and the $tcTEER_{(tissue)}$ values at 308 K is 1.02%.

2.6. Correlation of TEER and RITC- Dextran 70 kDa flux

The amounts of RITC-dextran which permeated through a Caco-2-monolayer during a 2.5 hour period are shown in Figure 3 for ten individual monolayers. A direct correlation between $tcTEER$ values and RITC-dextran permeability is demonstrated.

2.7. Time-temperature correlation

The rates at which the temperature of the medium changes after removal of the culture plate from the incubator are shown in Fig. 4. Nearly constant values are observed after ≥ 20 minutes. Similar results were obtained for the inverse procedure (data not shown).

Table 2A: Comparison of the measured TEER, the calculated $TEER_{(tissue)}$ and the calculated temperature corrected $TEER_{(tissue)}$ of five individual Caco-2-monolayers

Caco-2-monolayer	measured TEER (Ω)		calculated $TEER_{(tissue)}$ ($\Omega\text{ cm}^2$)		
	301 K	308 K	301 K	308 K	temperature corrected
					308 K
1	296	252	153	124	127
2	334	284	195	160	160
3	359	299	222	176	181
4	398	326	265	206	215
5	415	345	284	227	230

Table 2B: Comparison of the measured TEER, the calculated $TEER_{(tissue)}$ and the calculated temperature corrected $TEER_{(tissue)}$ of five individual HPDE-monolayers

HPDE-monolayer	measured TEER (Ω)		calculated $TEER_{(tissue)}$ ($\Omega\text{ cm}^2$)		
	301 K	308 K	301 K	308 K	temperature corrected
					308 K
1	200	179	47	40	43
2	632	531	523	431	418
3	1005	815	933	744	742
4	1345	1059	1307	1012	1037
5	1650	1342	1642	1323	1302

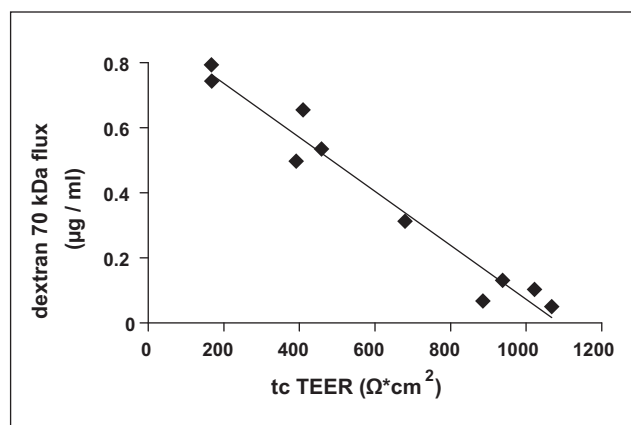


Fig. 3: Amounts of RITC-Dextran permeated through ten individual Caco-2 monolayers with different TEER values during a period of 2.5 hours ($R^2 = 0,9599$). Total dextran concentrations in the samples were determined by fluorimetry as described in Materials and Methods

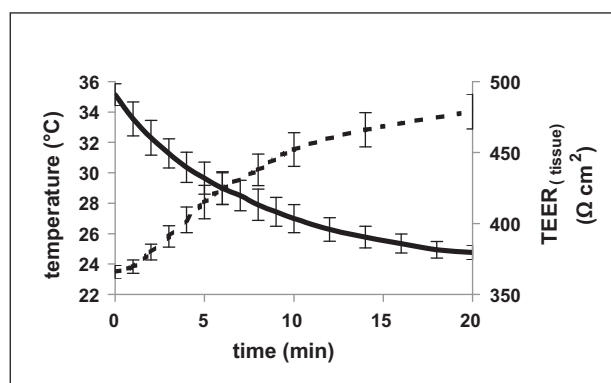


Fig. 4: Temperature profile of the medium (—) and the corresponding $TEER_{(tissue)}$ value of three Caco-2 monolayers (---) following the transfer of a six-well plate from the incubator ($37.2^\circ\text{C} \pm 0.6^\circ\text{C}$) to a laminar flow hood ($24.8^\circ\text{C} \pm 0.28^\circ\text{C}$). Shown are the mean S.D. of three independent experiments

3. Discussion

TEER measurement is a useful method to detect changes in bio-/physiological barrier function induced by agents such as VEGF (Antonov et al. 2008), nicotine McGilligan (McGilligan et al. 2007) or thrombin (Trojanovsky et al. 2008) or to evaluate the paracellular permeability enhancing potency of e.g. chitosan (Thanou et al. 2001) or dodecylmaltoside (Tirumalasetty and Eley 2005). Since TEER is temperature-dependent, TEER measurements are ideally performed at a constant temperature, which, however, poses some practical problems in assembling measuring devices in an 37°C incubator. Performing measurements at constant RT may represent an alternative, but this is time-consuming (equilibration from 37°C to RT usually requires a period of at least 20 min) (Fig. 4). Moreover, measurements carried out before equilibration is complete may be inaccurate and could potentially preclude the detection of effects of agents that act within the first 20 min. In order to circumvent these problems and to allow measurements during cooling or heating, we developed a mathematical method to correct (raw) TEER values for the actual temperature at which they were recorded and named these temperature-corrected TEER (tcTEER).

TEER measurements are often used to routinely determine the tightness and integrity of cell monolayers. From the above it is obvious that precise temperature recording during any stage of the experiment is mandatory to calculate tcTEER values. A more general application of this procedure will ensure that TEER values measured at various temperatures can be faithfully compared across independent experiments and perhaps even different laboratories.

Permeability changes induced by heat stress of a monolayer are often quantified by TEER (Yang et al. 2007). In light of the impact of temperature on TEER measurements, calculating tcTEER($tissue$) values might help to obtain more reliable values. Several studies were performed to evaluate the temperature dependency via a carrier-mediated, active transport through a Caco-2 monolayer. It was observed that the active transport

decreased upon lowering the temperature (Lalloo et al. 2004). Therefore, it is conceivable that these effects are also responsible for temperature caused TEER changes. Further an effect of temperature on paracellular pore size cannot definitely be excluded. If active transport or changes in paracellular pore size are the critical factors for temperature-based TEER changes, the temperature dependency of a monolayer must be higher than that of an empty insert. In this study we demonstrated that monolayers and empty inserts reacted almost in an analogous manner (Fig. 2). Therefore, the $tcTEER_{(tissue)}$ values shown in Table 2 which are obtained by using the equations for monolayers, can also be calculated by the equations for blank inserts. In this case the relative error is 5.4%. Consequently, the impact of temperature on active transport or paracellular pore size did not appear to be critical for temperature dependency of TEER measurement. Researchers often use solute transport for cell monolayer studies. Dextran flux is employed both in *in vitro* (Bonferoni et al. 2008) and in *in vivo* (Yoshida et al. 2008) permeability and absorption studies. Several investigations validated that a decreasing TEER value accompanies an increase in paracellular permeability (Hashimoto et al. 2008; Catalioto et al. 2009). Otherwise permeability of transcellular markers is independent from TEER (Neuhaus et al. 2008). TEER measurements are primarily biased by the resistance of epithelial tissue to paracellular ion flow, hence resistance to passive transcellular ion flow is too high to be significant in TEER measurements (Lu et al. 1996). Caco-2 cells have been extensively validated as a model for intestinal drug transport and permeability studies. This study suggests a direct correlation between $tcTEER_{(tissue)}$ values and dextran 70 kDa flux (Fig. 3). Therefore TEER measurement can be deemed to be an equivalent method for the measurement of solute paracellular transport. This finding matters as TEER measurements are easier and more rapidly to perform than paracellular marker flux experiments.

Performing TEER measurements in cell culture vessels outside the incubator and without a slide warmer is difficult because of rapid changes in temperature and TEER values (Fig. 4), especially if TEER values of more than one monolayer are to be detected at 37 °C. If $tcTEER_{(tissue)}$ values are used, there is no need for temperature equilibration to perform. This is an important advantage because it saves time, and minimizes the thermal fluctuation that the cells are exposed to. Additionally it can be derived from Fig. 4 that also pre-warmed medium cools down rapidly. Therefore the effect of temperature caused TEER changes can only be reduced by pre-warmed medium.

The mathematical solution provided here has been approved by TEER measurement of HPDE monolayers. The calculation of the $tcTEER_{(tissue)}$ value for the HPDE monolayer was done in the same way as established for Caco-2 monolayers. The relative error between $TEER_{(tissue)}$ and $tcTEER_{(tissue)}$ values at 308 K (Table 2) is as small as for Caco-2 monolayers. Therefore, it could be assumed that the equations to obtain temperature independent TEER values are applicable to different cells and media.

The method described here greatly facilitates the generation of reliable TEER values to assess cell monolayer integrity and to detect rapid changes in paracellular permeability.

4. Experimental

4.1. Caco-2 and HPDE cell culture

All cells were grown at 37 °C, in an atmosphere of 5% CO₂ and 90% relative humidity. The Caco-2 cell line was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). After harvesting at 90% confluency the Caco-2 cells were cultured on 0.4 µm 12-well hanging cell culture inserts (Millipore, Billerica, Ma, USA). The culture area was 1.1 cm². A total of 2.5×10^5 cells were seeded in 0.4 ml of medium into the inserts, and

the basolateral chamber was filled with 2 ml medium. The culture medium was Dulbecco's modified Eagle's medium (DMEM) containing 4500 mg/L D-Glucose, GlutaMax and phenol red (Invitrogen, Karlsruhe, Germany), 10% fetal bovine serum (FBS) South American origin (Lonza, Basel, Switzerland) and 1% penicillin-streptomycin-glutamine 100x (PSG) (Invitrogen). In both chambers the medium was changed three times a week. HPDE cells were cultured the same way as Caco-2 cells. The medium used for HPDE cells consists of two culture media in equal parts. One of this media was RPMI with 10% fetal bovine serum and 1% glutamine. The other medium was keratinocyte serum-free complete medium (KSFM) supplemented with bovine pituitary extract (50 µg/ml) and epidermal growth factor (5 ng/ml). Caco-2 cells and HPDE cells were cultured for three to fourteen days to obtain different tight monolayers which were required to investigate the impact of different TEER values on temperature dependency of TEER measurements and on paracellular marker flux.

4.2. TEER measurement of a Caco-2 monolayer with STX2 and EndOhm

The TEER values of twenty 12-well Caco-2 monolayers differing in tightness were measured. A standard chopstick electrode (STX2, World Precision Instruments (WPI), Berlin, Germany) and an EVOM epithelial volttohmmeter (WPI) were used according to the protocol (Tsuzuki 2007). The temperature was taken by a digital thermometer (K102, Voltcraft, Hirschau, Germany) equipped with a Type K thermal element. One thermal element has been positioned in the apical medium of each culture, while ensuring that the monolayer was not disturbed. Another thermal element was inserted in the culture medium of the well. The STX2 electrode was fixed on a tripod to ensure a constant position during measurements, regardless of whether the equipment was positioned in the incubator or on the work desk. The whole apparatus was placed in the incubator. Once the medium reached a temperature of 35 °C the equipment was removed from the incubator and TEER measurements were initiated. The TEER values were measured for every °C between 35 °C and 22 °C. To avoid further potential damage of the monolayer by low temperatures, such as 22 °C, we performed these tests using a descending temperature scale. The resistance measurement of the monolayers was conducted within the following eleven days.

The background resistance was established through multiple measurements of blank inserts by the procedure described for Caco-2 monolayers with STX2.

TEER of six Caco-2 monolayers with different tightness was also measured with an EndOhm-12 tissue resistance measurement chamber (WPI). After the Endohm-12 electrode was assembled, as described in the instruction manual, two thermal elements were added and the experimental set-up was repeated as previously described for Caco-2-monolayers and STX-2.

4.3. TEER measurement of HPDE monolayers

TEER values of ten HPDE monolayers with different tightness were measured with a STX2 as previously described for Caco-2 monolayers. The measurement of ten monolayers was conducted within the nine following days.

4.4. TEER measurement of inserts with different pore sizes

The TEER values of four six-well inserts (Greiner bio-one, Solingen, Germany) with total pore sizes between 0.01 cm² and 0.32 cm² were recorded using electrodes consisting of two silver pins placed in a plastic holder. This holder was fixed with a cover for a six-well plate. The cover contained 12 holes where the electrodes were attached to ensure that they remained in a constant position. The electrode was calibrated and compared to a STX2 before use. Two thermal elements were added and the experimental set-up was repeated as already described for Caco-2 monolayers.

4.5. Time-temperature correlation

Thermal elements were inserted into two wells of a six-well culture plate (Greiner) containing 4 ml of DMEM. The equipment was positioned in the incubator until the temperature of the medium reached 35 °C. Time and temperature measurements were initiated immediately following transfer of the equipment to the laminar flow hood. During the measurements, air temperature changes in the laminar flow hood were detected using another thermometer. Additionally the same experiment was conducted in reversed order. Therefore time-temperature measurements were initiated when a culture plate containing medium at RT was transferred to the incubator.

4.6. Viscosity measurements

The viscosities of both previously described media for Caco-2 and HPDE cells were measured with an Ubbelohde-Viscosimeter (Schott capillary viscosimeter CT42) containing a 0C-capillary (0.46 mm in diameter) according to the manufacturer's instructions. The capillary was carefully filled with the medium to avoid bubbles caused by FBS. The viscosimeter was positioned in a water bath for at least 20 min at the required temperature. The viscosity of the medium was measured for each °C between 22 °C and 35 °C.

4.7. Penetration of RITC-dextran 70 kDa and TEER measurement

The flux of rhodamine B isothiocyanate (RITC)-conjugated dextran 70 kDa (RITC-dextran; Sigma-Aldrich, Steinheim, Germany) across 12-well Caco-2 monolayers of different tightness was determined. A solution containing 0.5 mg/ml RITC-dextran in DMEM without phenol red (Invitrogen) was assembled once again for each experiment. A total of 1200 µl DMEM without phenol red was applied in a 12 well culture plate. An amount of 500 µl RITC-Dextran containing medium was administered in the apical chamber after removal of culture medium. Time measurement was started and the equipment was transferred to the incubator. After 1.5 h samples were withdrawn from the well followed by immediate replacement of the same volume of pre-warmed DMEM without phenol red. Those samples were served as blank values. The equipment was transferred into the incubator for further 2.5 h. Then the samples were withdrawn from the well again. Total dextran concentrations in the samples were determined using a fluorimeter (infinite F200) (Tecan, Männedorf, Switzerland).

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References

- Adamson AW (1979) A textbook of physical chemistry, 2nd ed., New York, pp. 431–433.
- Antonov A, Snead C, Gorshkov B, Antonova GN, Verin AD, Catravas JD (2008) Heat shock protein 90 inhibitors protect and restore pulmonary endothelial barrier function. *Am J Respir Cell Mol Biol* 39: 551–559.
- Atkins PW, De Paula J, Bär M (2006) *Physikalische Chemie*, 4th ed., Weinheim, pp. 845–849.
- Bonferoni MC, Sandri G, Rossi S, Ferrari F, Gibin S, Caramella C (2008) Chitosan citrate as multifunctional polymer for vaginal delivery. Evaluation of penetration enhancement and peptidase inhibition properties. *Eur J Pharm Sci* 33: 166–176.
- Catalioto RM, Festa C, Triolo A, Altamura M, Maggi CA, Giuliani S (2009) Differential effect of ethanol and hydrogen peroxide on barrier function and prostaglandin E2 release in differentiated Caco-2 cells: selective prevention by growth factors. *J Pharm Sci* 98: 713–727.
- Feighery LM, Cochrane SW, Quinn T, Baird AW, O'Toole D, Owens SE, O'Donoghue D, Mrsny RJ, Brayden DJ (2008) Myosin light chain kinase inhibition: correction of increased intestinal epithelial permeability in vitro. *Pharm Res* 25: 1377–1386.
- Hashimoto K, Oshima T, Tomita T, Kim Y, Matsumoto T, Joh T, Miwa H (2008) Oxidative stress induces gastric epithelial permeability through claudin-3. *Biochem Biophys Res Commun* 376: 154–157.
- Hombach J, Hoyer H, Bernkop-Schnürch A (2008) Thiolated chitosans: development and in vitro evaluation of an oral tobramycin sulphate delivery system. *Eur J Pharm Sci* 33: 1–8.
- Johansson KM, Izgorodina EI, Forsyth M, MacFarlane DR, Seddon KR (2008) Protic ionic liquids based on the dimeric and oligomeric anions: [(AcO)xH(x-1)]. *Phys Chem Chem Phys* 10: 2972–2978.
- Jonker-Venter C, Snyman D, Janse van Rensburg C, Jordaan E, Schultz C, Steenekamp JH, Hamman JH, Kotze AF (2006) Low molecular weight quaternised chitosan (11): in vitro assessment of absorption enhancing properties. *Pharmazie* 61: 301–305.
- Kaitu'u-Lino TJ, Sluka P, Foo CF, Stanton PG (2007) Claudin-11 expression and localisation is regulated by androgens in rat Sertoli cells in vitro. *Reproduction* 133: 1169–1179.
- Lalloo AK, Luo FR, Guo A, Paranjpe PV, Lee SH, Vyas V, Rubin E, Sinko PJ (2004) Membrane transport of camptothecin: facilitation by human P-glycoprotein (ABCB1) and multidrug resistance protein 2 (ABCC2). *BMC Med* 2: 16.
- Lu S, Gough AW, Bobrowski WF, Stewart BH (1996) Transport properties are not altered across Caco-2 cells with heightened TEER despite underlying physiological and ultrastructural changes. *J Pharm Sci* 85: 270–273.
- Masungi C, Mensch J, Willems B, Van Dijck A, Borremans C, Noppe M, Brewster ME, Augustijns P (2009) Usefulness of a novel Caco-2 cell perfusion system II. Characterization of monolayer properties and peptidase activity. *Pharmazie* 64: 36–42.
- Matter K, Balda MS (2003) Functional analysis of tight junctions. *Methods* 30: 228–234.
- McGilligan VE, Wallace JM, Heavey PM, Ridley DL, Rowland IR (2007) The effect of nicotine in vitro on the integrity of tight junctions in Caco-2 cell monolayers. *Food Chem Toxicol* 45: 1593–1598.
- Nagumo Y, Han J, Bellila A, Isoda H, Tanaka T (2008) Cofilin mediates tight-junction opening by redistributing actin and tight-junction proteins. *Biochem Biophys Res Commun* 377: 921–925.
- Neuhaus W, Plattner VE, Wirth M, Germann B, Lachmann B, Gabor F, Noe CR (2008) Validation of in vitro cell culture models of the blood-brain barrier: tightness characterization of two promising cell lines. *J Pharm Sci* 97: 5158–5175.
- Radulovich N, Qian JY, Tsao MS (2008) Human pancreatic duct epithelial cell model for KRAS transformation. *Methods Enzymol* 439: 1–13.
- Sawai T, Lampman R, Hua Y, Segura B, Drongowski RA, Coran AG, Harmon CM (2002) Lysophosphatidylcholine alters enterocyte monolayer permeability via a protein kinase C/Ca²⁺ mechanism. *Pediatr Surg Int* 18: 591–594.
- Thanou M, Nihot MT, Jansen M, Verhoef JC, Junginger HE (2001) Mono-N-carboxymethyl chitosan (MCC), a polyampholytic chitosan derivative, enhances the intestinal absorption of low molecular weight heparin across intestinal epithelia in vitro and in vivo. *J Pharm Sci* 90: 38–46.
- Tippin TK, Thakker DR (2008) Biorelevant refinement of the Caco-2 cell culture model to assess efficacy of paracellular permeability enhancers. *J Pharm Sci* 97: 1977–1992.
- Tirumalasetty PP, Eley JG (2005) Evaluation of dodecylmaltoside as a permeability enhancer for insulin using human carcinoma cells. *J Pharm Sci* 94: 246–255.
- Torres R, Pizarro L, Csendes A, Garcia C, Lagos N (2007) GTX 2/3 epimers permeate the intestine through a paracellular pathway. *J Toxicol Sci* 32: 241–248.
- Troyanovsky B, Alvarez DF, King JA, Schaphorst KL (2008) Thrombin enhances the barrier function of rat microvascular endothelium in a PAR-1-dependent manner. *Am J Physiol Lung Cell Mol Physiol* 294: L266–275.
- Tsuzuki W (2007) Absorption properties of micellar lipid metabolites into Caco2 cells. *Lipids* 42: 613–619.
- Wang ZF, Wang LS, Fan TB (2007) Densities and viscosities of ternary mixtures of heptane, octane, nonane, and hexyl benzene from 293.15 K to 313.15 K. *J Chem Engin Data* 52: 1866–1871.
- Yang PC, He SH, Zheng PY (2007) Investigation into the signal transduction pathway via which heat stress impairs intestinal epithelial barrier function. *J Gastroenterol Hepatol* 22: 1823–1831.
- Yoshida D, Todo H, Hasegawa T, Sugibayashi K (2008) Effect of molecular weight on the dermatopharmacokinetics and systemic disposition of drugs after intracutaneous injection. *Eur J Pharm Sci* 35: 5–11.