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Online monitoring of transepithelial electrical resistance (TEER) in an apparatus for combined dissolution and permeation testing

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ABSTRACT

The aim of this study was to evaluate a newly implemented feature for the online monitoring of transepithelial electrical resistance (TEER) in an apparatus for combined *in vitro* dissolution and permeation testing. In a first step, the course of TEER was analyzed simultaneously to the permeability of sodium fluorescein, and a time frame of cell monolayer integrity inside the apparatus of approximately 3 h was found. In successive experiments cell monolayer integrity was challenged by application of EDTA (8, 6, 3 and 2 mM) in the apical compartment. After application of high EDTA concentrations of 8 and 6 mM for 45 min TEER did not recover, and permeability of the monolayer was steadily increasing. For the lower concentrations TEER recovered again while permeability of sodium fluorescein remained at an elevated level. This suggests that the EDTA induced opening of the tight junctions was preserved during the period of TEER recovery and did not change within the lifespan of the cell monolayer inside the apparatus. Online monitoring of TEER appeared to be a suitable method for real-time control of barrier integrity throughout each experiment. Moreover, this feature is intended to be used to analyze formulation approaches aiming for an improved oral drug bioavailability by application of excipients that increase the paracellular permeability of the intestinal epithelial barrier.

1. Introduction

A new apparatus for the combined dissolution and permeability assessment of solid oral dosage forms has recently been developed in our laboratory (Motz et al., 2007b). Similar approaches have already been proposed by other groups (Ginski et al., 1999; Kobayashi et al., 2001; Kataoka et al., 2003). In contrast to those, however, by using a flow through cell, our apparatus allows the analysis of complete oral dosage forms rather than only non-formulated compounds.

The general motivation to develop such a set-up is the intention to better understand the multifactorial interactions between dissolution and absorption processes in order to predict the *in vivo* performance of an oral formulation (Ginski and Polli, 1999). Those apparatus are of special interest when it comes to the analysis of drugs with limited permeability belonging to classes III and IV of the biopharmaceutical classification system (BCS). In that case the influence of excipients or formulation factors that interfere with the intestinal barrier can be evaluated and perhaps be used strategically to improve oral bioavailability. The relevance of a potential food effect with focus on both, dissolution and absorption characteristics, can be evaluated, and the data may be used for establishment of an *in vitro-/in vivo correlation* (IVIVC) (Buch et al., 2009).

The principal constituents of our apparatus are a compendial flow through dissolution cell (apparatus 4, USP) and a custom made flow through permeation cell (FTPC) that allows the mounting of a Transwell® permeable support (Corning Costar, Transwell® type 3460) between the apical and the basolateral side (Fig. 1). The proper calibration of the apparatus and its suitability to estimate the performance of complete oral dosage forms was demonstrated with propranolol HCl tablets of different dosage strengths and varying release profiles (Motz et al., 2007b). In a second step automated sampling and detection by means of Sequential Injection Analysis (SIA) have been implemented (Motz et al., 2007a).

During the continuous optimization of this apparatus, some modifications have been introduced (Fig. 1): The basolateral compartment was revised, allowing a reduction of the acceptor volume from formerly 5.5 to now 3.8 ml. This is favourable for the assessment of low permeable compounds, as higher concentrations will be reached now. Secondly, a magnetic stirrer was integrated into

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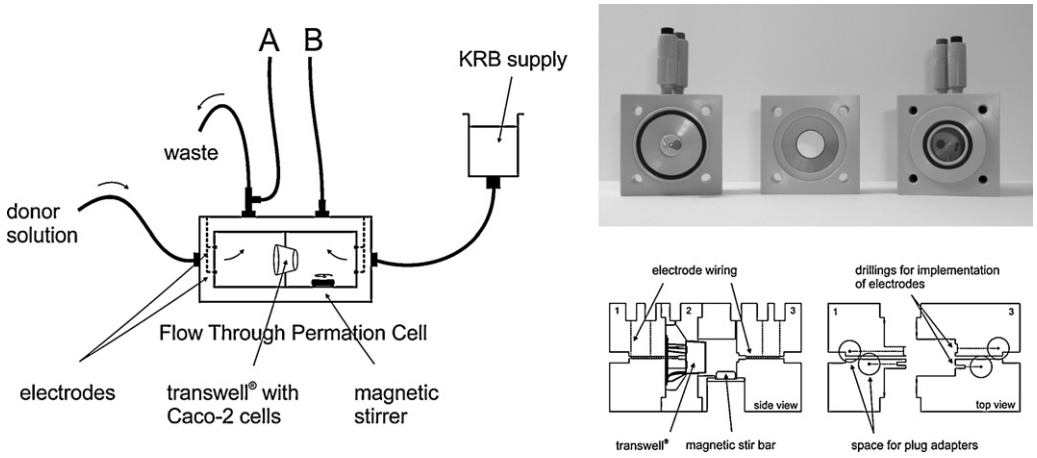

Fig. 1. Left: Schematic depiction of the flow through permeation cell (FTPC). The donor solution enters the apical compartment from the left side and leaves it again on top. During this process a fraction of the drug molecules permeates across the Caco-2 monolayer into the basolateral acceptor compartment. Here a magnetic stirrer provides a homogeneous distribution of the accumulating drug substance. Sampling at the apical (A) and the basolateral (B) outflow is done by means of SIA automation. Replenishment of the sample volumes is provided by the KRB supply. Top right: Photo of the opened FTPC. Bottom right: Technical drawing of the FTPC that shows the implementation of electrodes and wiring for online TEER measurement.

the basolateral cavity of the FTPC. Hydrostatic pressure balance was provided by means of adjustment of the height of the liquid column at the KRB supply. Basolateral sampling was done by means of SIA automation that was extended by a second pump, a valve module and a second autosampler. This additional equipment turned out to be necessary to overcome a carry-over effect in case of larger differences between apical and basolateral concentrations. Thus complete separation of the basolateral sampling route warrants an unbiased measurement also for low permeable compounds.

So far, the integrity of the cell monolayer could only be checked by trans epithelial electrical resistance (TEER) measurements outside the FTPC, before and after an experiment. However, this was considered as insufficient as during an experiment the Caco-2 cells come into contact with the various compounds released from the investigated dosage form, in particular excipients such as non-ionic surfactants with potential effects on epithelial permeability (Dimitrijevic et al., 2000). Thus, it appeared necessary to implement a tool that allows continuous surveillance of the epithelial barrier properties in real time throughout an experiment.

As a surrogate for epithelial integrity, TEER measurement is well established (Karnaky, 1992). This readily measurable bioelectrical parameter reflects the tightness of intercellular junctions between mature epithelial cells (Hilgendorf et al., 2000). Next to the detection of any unpredictable mechanical damage of the monolayer, online TEER measurement should also allow for the quantification of effects caused by the active pharmaceutical ingredient itself or other components of the formulation (e.g. solubilizers).

In order to evaluate the new set-up for continuous TEER measurement we decided to use its known dependency on the extracellular concentration of Ca^{2+}. For tight monolayered epithelia it is known that a switch to Ca^{2+} free conditions causes a drop of TEER, resulting from an opening of the normally tight intercellular junctions. This effect is even stronger if Ca^{2+} is depleted by means of a cation chelator, whereas after switching back to a Ca^{2+} containing medium TEER will recover again (Cereijido et al., 1978). The velocity of resealing of the tight junctions was pointed out to be dependent on the time that the cells were kept in the status of Ca^{2+} depletion before (Martinez-Palomo et al., 1980). In the vast majority of such studies the Ca^{2+} depletion was performed on both sides of the respective cell monolayer. Later on, a study by Noach et al. demonstrated that Caco-2 cells react much more sensitively to the application of EDTA from the basolateral side than from the apical side (Noach et al., 1993). However, in our experiments Ca^{2+} depletion was performed solely from the apical side as this is the only scenario that is of *in vivo* relevance.

This paper reports, as a proof of principle for the functioning of thus modified apparatus, the effects of donor solutions with different EDTA concentrations on the TEER of Caco-2 monolayers. This was achieved by a newly implemented feature for the online monitoring of transepithelial electrical resistance (TEER). Simultaneously, the permeability of the paracellular transport marker sodium fluorescein was measured.

2. Materials and methods

2.1. Buffer solutions

Krebs Ringer Buffer (KRB) pH 7.4 as described by Motz et al. was used as acceptor medium and as a basis for all types of donor solutions (Motz et al., 2007b). The standard donor solution contained a concentration of 5 μg/ml sodium fluorescein in KRB pH 7.4 (Flu-KRB). EDTA solutions contained 2, 3, 6 or 8 mM EDTA next to 5 μg/ml sodium fluorescein and were prepared without addition of Ca^{2+} and Mg^{2+} (Flu-EDTA). Justification for the fairly high EDTA concentrations can be found in the work of Noach et al. who pointed out that application of a 5 mM EDTA solution in the apical compartment for 60 min was followed by a ~45% reduction of the TEER (Noach et al., 1993). The changes in osmolality, which were caused by the addition of EDTA and the omission of the divalent ions, were compensated to the calculative value of KRB using NaCl. Isoosmolality of all solutions of 308 ± 4 mosmol/kg was checked via freezing point depression (Semi-micro Osmometer, Knauer GmbH, Berlin, Germany).

2.2. Cell culture

Caco-2 cells, clone C2BBe1, were purchased at passage 60 from American Tissue Culture Collection (ATCC; Manassas, VA) and used at passages 63–78. Cell culture and seeding on permeable supports (Transwell® type 3460, Corning Inc., Acton, MA) was conducted as described by Motz et al. and cells were used within 21–25 days after seeding (Motz et al., 2007b).

2.3. Sodium fluorescein quantification

For quantification of sodium fluorescein a Cytofluor II fluorescence reader was used (λ_{exc} = 485 nm, λ_{em} = 530 nm), (PerSeptive Biosystems, Wiesbaden-Norderstedt, Germany). Linearity ($R^2 > 0.999$) was ensured between 5 ng/ml and 1 μg/ml.

2.4. TEER measurement

To realize continuous TEER measurement a pair of Ag/AgCl voltage sensor electrodes (In Vivo Metric, Healdsburg, CA) and two pieces of pure silver wire as current electrodes were installed in the apical and the basolateral brick of the FTPC and connected with an EVOMX (World Precision Instruments, Sarasota, FL). After customization with a relay and installation of a multifunction data acquisition device (NI USB-6009, National Instruments, Austin, TX) triggering of TEER measurement, A/D conversion of the signals and data collection could be performed by a PC using LabVIEW software (version 8.5.1, National Instruments, Austin, TX). The scan rate for online TEER measurement was set to 1/min in all experiments.

2.5. Microscopic analysis

The Caco-2 cells were checked routinely during growth using an inverted microscope (Axiovert 25C, Carls Zeiss GmbH, Jena, Germany). For a closer analysis the cells were fixed with 4% formalin in phosphate buffer. Afterwards, the nuclei were stained with hematoxylin and 4 μm cross-sections of the polyester membranes with the Caco-2 cells on top were prepared. Microscopic analysis of the cross-sections was done with a Leica DMRB upright microscope (Leica Microsystems GmbH, Wetzlar, Germany).

2.6. Experimental procedure

In the present study no solid formulations but donor solutions were applied. Therefore, the dissolution equipment remained out of operation and the buffer solutions were pumped through the apical compartment of the FTPC directly. For this purpose a Stepdos 03® membrane dosage pump in combination with a pulsation absorber (KNF Neuberger, Freiburg, Germany) was used. The flow rate of the pump was set to 1.0 ml/min. Before each experiment a Caco-2 monolayer grown on a Transwell® support was inserted into the FTPC, both compartments were filled with preheated KRB and the apical pump was switched on. After 15 min of equilibration the experiments were started by the activation of PC controlled TEER measurement and automated sampling. The first switch of donor solutions was conducted equally in all experiments from blank KRB to Flu-KRB in order to check the tightness of the cell monolayer. This switch did not show any influence on the course of TEER in any of the experiments. In order to analyze the modulation of cell monolayer tightness, the donor medium was switched to a Flu-EDTA solution for a certain period of time to illustrate some permeation enhancing effect on the monolayer and back again to Flu-KRB. All experiments were conducted with a pH of 7.4 on both sides of the cell monolayer. Switching between the different donor solutions was feasible without interruption of the apical flow and without aspiration of any air bubbles due to the discontinuous suction of the membrane pump. The sampling interval in the experiments without EDTA (Fig. 2) was set to 30 min. In case of using EDTA to open up the tight junctions the sampling interval was decreased to 5 min (Fig. 5 and Fig. 6).

2.7. Data treatment

The apparent permeability (P_{app}) of sodium fluorescein across the Caco-2 cell monolayers was calculated according to the following equation:

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{A \times c_d}$$

In this equation A [cm^2] is the surface area of the monolayer, c_d [μg/ml] is the sodium fluorescein concentration applied at the donor side and dQ/dt [μg/s] is the rate of sodium fluorescein appearing in the basolateral compartment.

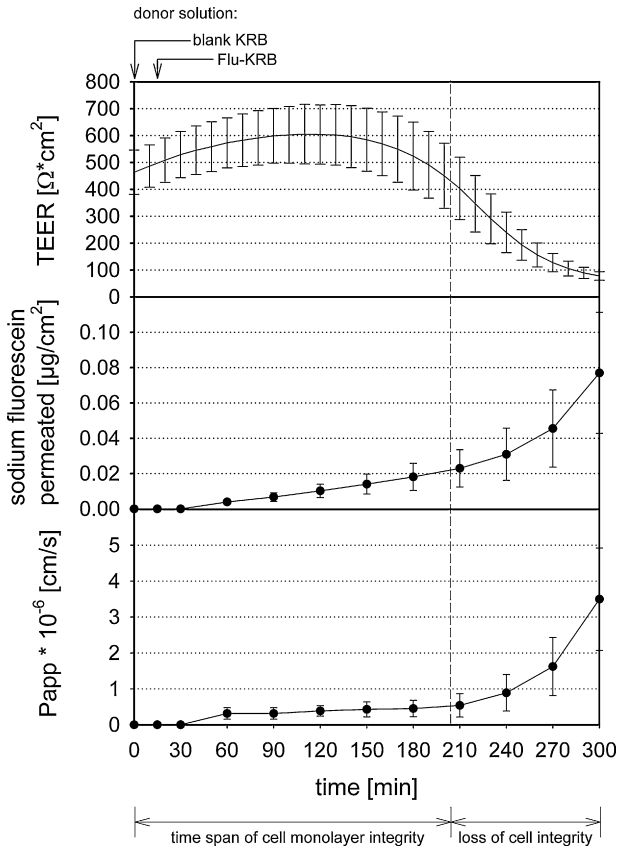

Fig. 2. Time course of TEER, fluorescein transport and apparent permeability (P_{app}) as calculated from the latter. Shown are mean ± SD of $n = 3$ experiments.

3. Results

3.1. Time span of monolayer integrity

Fig. 2 shows the permeation of sodium fluorescein and reveals that the Caco-2 cell monolayers keep up their barrier properties inside the FTPC for nearly 3.5 h after the start of an experiment. The application of the constant donor concentration of 5 μg/ml sodium fluorescein at the apical side led to a linear permeation behaviour of the compound as long as TEER remained on a high level. A mean apparent permeability of $0.4 \pm 0.18 \times 10^{-6}$ cm/s was calculated from the slope of the linear part of the permeated amount between 60 and 180 min. This value lies within the range of reported Caco-2 permeability for sodium fluorescein of $0.21–0.623 \times 10^{-6}$ cm/s (Duizer et al., 1997; Imai et al., 1999) and permeability did not increase until TEER dropped below 300 Ω cm^2. When the time of cell monolayer integrity had expired, TEER dropped to low values and at the same time the permeability increased strongly.

3.2. Microscopic analysis of the Caco-2 cells

The Caco-2 cells were analyzed microscopically during the period of growth to verify confluence before using them in the experiments. To check for possible changes of cellular morphology during the course of the experiments the status of the monolayers after 2 and 5 h inside the FTPC was evaluated. Therefore, TEER was recorded and after removal of the Transwells® from the FTPC cross-sections of the monolayers were prepared. As expected, TEER was still on a high level after 2 h inside the FTPC whereas after 5 h

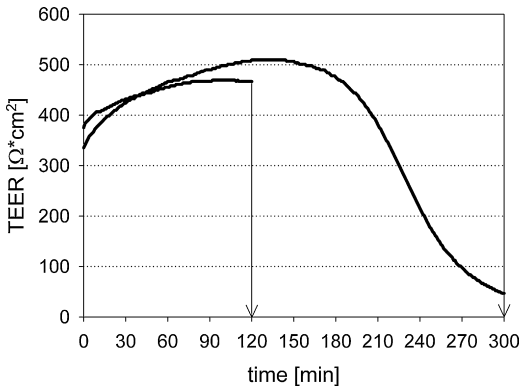

Fig. 3. TEER of two Caco-2 cell monolayers that were inside the FTPC for 2 and 5 h, respectively. At the end of the experiments the Transwells® were taken out of the FTPC and prepared for microscopic analysis.

TEER decreased to a low value beneath 100 Ω cm^2 (Fig. 3). In agreement to the course of TEER there was no morphological difference between the cell monolayer after 2 h inside the FTPC (Fig. 4b) and a monolayer that has been fixed directly after cultivation (Fig. 4a). In contrast to that the cells have shrunk and the interconnections between adjacent cells have loosened after a period of 5 h inside the FTPC (Fig. 4c). Furthermore, small cavities between the cells and the surface of the membrane could be detected at that stage.

3.3. Effect of calcium depletion by EDTA

In preliminary tests a time frame of 45 min for application of Flu-EDTA solutions was found to be suitable for the experiments. Starting with the highest concentration of 8 mM a step by step reduction of the EDTA concentration was conducted in the experiments shown in Fig. 5. The application of high EDTA concentrations of 8 and 6 mM led to a pronounced drop of TEER and after switching back to Ca^{2+}-containing Flu-KRB solution no stabilisation was reached. In agreement with the course of TEER the permeability of sodium fluorescein increased after application of the Flu-EDTA solutions and no reduction or slowing down of this process after Ca^{2+} restoration was observed. In coincidence with the ongoing drop of TEER the cumulative amount of sodium fluorescein showed a parabolic profile, corroborating a continuously increasing permeability of the monolayer.

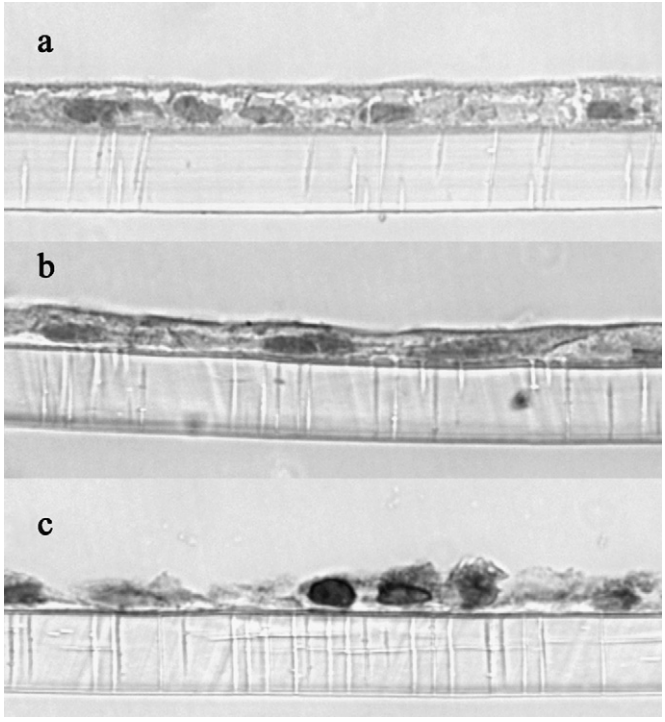

Fig. 4. The figure shows cross-sections of three Caco-2 cell monolayers on top of polyester membranes (10 μm thickness, 0.4 μm pore size, Transwell® type 3460) at a 400-fold magnification, nuclei were stained with hematoxylin. (a) A monolayer that has been fixed directly after cultivation serves as a control. (b) This monolayer has been fixed after it was inside the FTPC for 2 h applying blank buffer with an apical flow rate of 1.0 ml/min. (c) This picture shows the status of a third monolayer that was inside the FTPC for 5 h under the same conditions as described for (b).

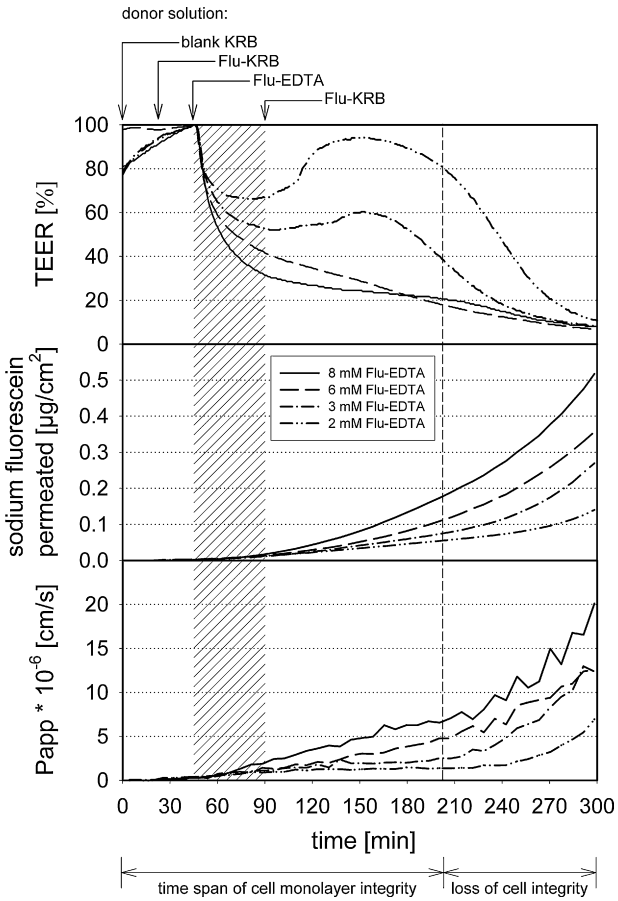

Fig. 5. Application of 8, 6, 3 and 2 mM EDTA containing buffer for a time interval of 45 min and subsequent return to KRB. All solutions applied later than 15 min after the start of an experiment contained 5 μg/ml of sodium fluorescein (Flu-KRB; Flu-EDTA). TEER values were normalized to the maximum value of the respective experiment in order to allow for a better comparability of the EDTA effect. The figure shows single data for each EDTA concentration.

Reduction of the EDTA concentration to 3 and 2 mM led to a different picture. After the EDTA induced drop, TEER recovered as soon as Ca^{2+} was available in the apical medium again. Within the period of TEER recovery the cumulative amount of permeated sodium fluorescein increased linearly and permeability persisted at an almost constant level. Only when the expected time span of cell monolayer integrity ended after about 3.5 h inside the FTPC epithelial permeability increased again.

3.4. Recovery of TEER after repeated Ca^{2+}-switching

Afterwards, a repeated switching between Flu-EDTA and Flu-KRB solution was evaluated. As can be seen in Fig. 6, switching between 8 mM Flu-EDTA and Flu-KRB for four times with an exposure to the Ca^{2+} chelator for only 10 min each time led to a stepwise decrease of TEER and was followed by a decreasing capability of the

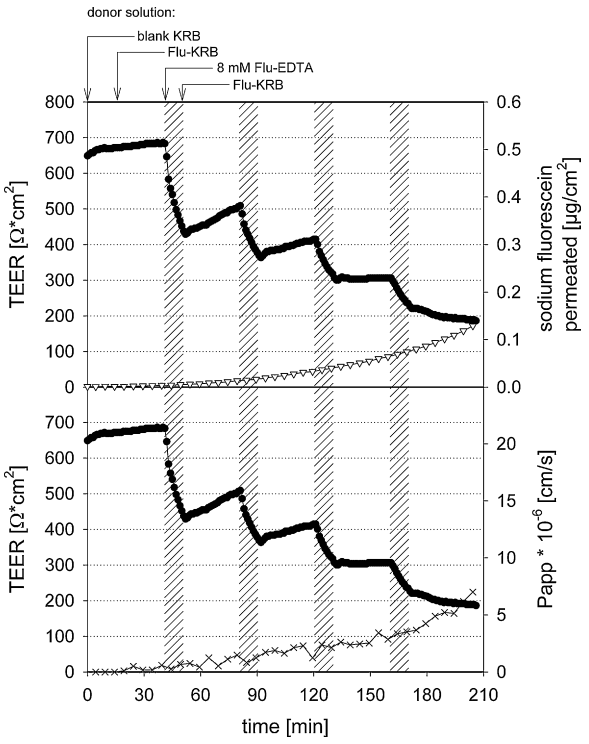

Fig. 6. Change in cell monolayer leakiness following four 10-min applications (hatched areas) of 8 mM EDTA assessed during the course of the same experiment.

cells to recover again. Despite that, the cumulative amount of permeation showed a parabolic shape and the flux over the monolayer increased steadily with a tendency to increase above average after 3 h, as already seen in the previous experiments.

4. Discussion

The physiologic basis for the diffusional barrier properties of a monolayered epithelium is the intactness of the tight junctions between adjacent cells. The microscopic appearance of the so-called "zonula occludens" and some physiological aspects of the paracellular pathway have already been studied decades ago. Electron microscopic studies of the junctional structures have revealed that a net-like meshwork of fibrillar strands, surrounding adjacent epithelial cells at the apical end, builds up the actual permeation fence (Miller, 1960; Staehelin et al., 1969; Friend and Gilula, 1972). The subsequent biophysical characterisation of different epithelia demonstrated that the paracellular pathway acts as a charge and size specific semipermeable diffusion barrier (Reuss, 1992). In the meantime more than 40 proteins that are involved in the formation and regulation of the tight junction complexes have been identified and lately there is good evidence that the family of the claudins plays the major role in gating and restriction of the paracellular pathway (Gonzalez-Mariscal et al., 2003; Schneeberger and Lynch, 2004; Angelow et al., 2008). Evidence that claudins may mediate charge selectivity is based on overexpression and site-specific mutagenesis studies (Schneeberger and Lynch, 2004). In contrast to that, there is only little experimental evidence for a connection between paracellular size specificity and the claudins (Van Itallie et al., 2008). A model has been proposed, claiming that claudins build up tissue specific pores with charged internal linings and thereby create individual effective pore dimensions for charged and uncharged solutes. Thus it would be explainable that TEER can change without affection of the permeation of non-charged molecules (Van Itallie and Anderson, 2004; Van Itallie and Anderson, 2006). In this context the Renkin molecular sieving function proved to be a valuable tool to characterize the paracellular barrier properties in unaffected and perturbed Caco-2 cell monolayers (Adson et al., 1994; Knipp et al., 1997). An older model derived from the microscopic appearance of the junctional network tried to explain a logarithmic relation between magnitude of TEER and number of junctional strands and proposed a dynamic barrier with pores that fluctuate between an open and a closed state (Claude, 1978). A crystal structure that gives hints about the spatial arrangement of the claudins would probably help to explain the detailed assembly of the paracellular pores.

In the first set of our experiments the time frame of cell monolayer integrity in the FTPC has been determined to be roughly 200 min (Fig. 2). By online monitoring both, TEER and sodium fluorescein permeability, it was found that as long as TEER remained above a value of 300 $\Omega\,cm^2$ the permeated amount of sodium fluorescein increased linearly and permeability remained at a constant level. This finding is in good agreement to the literature and seems to be valid also for other epithelial *in vitro* models (Steimer et al., 2005). A linear permeation pattern of sodium fluorescein was obtained although TEER did not remain on a constant level within the first 3 h. This observation that certain differences in TEER are not reflected in the permeation profile is not an uncommon phenomenon for Caco-2 cells (Riley et al., 1991; Artursson et al., 1993; Lu et al., 1996) and might partly be caused by an insufficient resolution of standard marker measurement. Furthermore, it should be considered that mechanistically TEER measurement is a marker for ionic permeability and mainly based upon Na^+ permeability (Reuss, 1992). So, comparing the different methods to check paracellular permeability it has to be considered that TEER may measure different properties of the tight junctions than flux studies do. In fact, the calculation of pore radii from permeability data by means of the Renkin function revealed a nonlinear correlation between pore size and TEER in rat alveolar cell monolayers (Adson et al., 1994). Nevertheless, the concept of establishing a minimum TEER to be maintained throughout an experiment seems to be an adequate tool for in process control, provided that the interrelation between TEER and solute permeability has been elucidated before.

EDTA induced Ca^{2+} depletion from the apical compartment led to a pronounced drop of TEER. Synopsis of the experiments with identical procedure and an application of 8–2 mM EDTA for 45 min revealed a concentration dependent decrease of TEER within the time of exposure to the chelating agent. Comparison of the respective permeability that was reached after 200 min, the time that the cell monolayer integrity was shown to be limited to, also reflected the rank order of EDTA concentrations (Fig. 5). Next to that, a dose dependency was found for the recovery of TEER after switching back to Flu-KRB solution. Whereas after application of higher EDTA concentrations (8 and 6 mM) for 45 min, the decrease of TEER was irreversible, the exposure to lower EDTA concentrations (3 and 2 mM) was followed by a recovery of the TEER (Fig. 5). A closer look upon sodium fluorescein permeation throughout the periods of TEER recovery revealed a linear increase of the permeated amount and a constant permeability on a higher level in comparison to an unaffected cell monolayer. It was concluded that recovery of TEER in this case did not lead to a complete resealing but to a conservation of the current permeability of the Caco-2 monolayer for sodium fluorescein. In this context a study by Watson et al. who have introduced a novel tool that allows detection of graded changes in paracellular permeability provided helpful mechanistic insight (Watson et al., 2001). By means of simultaneous permeability screening of a series of polyethylene glycol oligomers (PEG-profiling) the authors pointed out that upon Ca^{2+} chelation the cell monolayer lost its ability for size discrimination. Functional modelling suggested an increase in pore size as the underlying mechanism. The evaluation of PEG-profiling with different EDTA concentrations conducted by the same authors did

not result in a clear gradation for the effect of Ca^{2+} depletion. This might be caused by the fact that the Ca^{2+} chelator was also added to the highly sensitive basolateral side of the cell monolayer. Higher EDTA concentrations (8 and 6 mM) did not lead to a recovery but showed a continuously decreasing TEER. In agreement to that finding, the permeated amount of sodium fluorescein showed a nonlinear growth and the permeability was increasing steadily. So it was concluded that the process of pore size increase initiated by EDTA in these cases could not be stopped by means of Ca^{2+} reconstitution anymore.

In comparison to flux studies TEER measurement is the more sensitive method with a high temporal resolution (Yap et al., 1998). However, this advantage reveals its full value only in case of continuous recording which could be pointed out by repeated switches to 8 mM Flu-EDTA and back to Ca^{2+} containing buffer (Fig. 6). In contrast to the instantaneous and sensitive response of TEER upon the repeated short term EDTA application the permeability of sodium fluorescein followed with a certain delay and did not show a gradation. It was concluded that the time periods of 30 min in between the single EDTA bolus applications were too short for detection of an equilibration of the flux.

In consequence the different methods to characterize paracellular permeability of an epithelial cell monolayer provide complementary information and have to be interpreted cautiously. In order to get a complete picture of the state of paracellular permeability, combinations of TEER and diffusion measurement would be required (Matter and Balda, 2003). Furthermore, it has to be considered that membrane permeation even of hydrophilic drugs may not solely be based upon one single paracellular mechanism. Sodium fluorescein, which longtime has been regarded as the standard marker dye for paracellular transport, seems to be transported actively in case of application of a pH gradient between the apical and the basolateral compartment and was stated to be substrate of a proton coupled monocarboxylic anion transporter (Konishi et al., 2002; Kuwayama et al., 2002). Therefore, in this study all experiments have been carried out under iso-pH conditions of pH 7.4 on both sides of the cell monolayer which is supposed to limit epithelial transport to the paracellular route. The TEER of a Caco-2 cell monolayer grown on a permeable support is highly sensitive to surrounding conditions like buffer changes and temperature drops. Thus, equilibration periods under controlled conditions for at least 15 min after each manipulation are required in order to obtain reliable TEER values (Matter and Balda, 2003). Here our set-up offers advantages by its design as a flow through system providing a seamless transition of the donor composition inside the apical compartment. Due to the relatively high heat capacity of polyetheretherketone (PEEK) used to manufacture the FTPC and submersion into a preheated water bath, an equilibration is only necessary at the start of an experiment. Finally, the availability of only two single TEER values, one measured before and one after an experiment in absence of constant external conditions, are to be regarded as insufficient. Continuous TEER measurement inside the FTPC is much more reliable and less error prone. These advantages, however, are attained with a reduced potential experimental time-frame of up to 3 h. For Caco-2 long-term experiments in culture plates, which are usually conducted using cell culture medium instead of buffer solutions, experimental times and monolayer integrity of up to 6 h have been reported (Artursson, 1990; Finley and Monroe, 1997). The reason for this difference is attributed to the dynamic flow through character of the set-up that implies stronger mechanical stress on the monolayer. On the other hand 3 h are a sufficiently long period of time to study the performance of oral immediate release (IR) dosage forms of BCS class III and IV compounds which is the main focus of the apparatus. Next to that, this precondition fits well to the reported human small intestinal transit time of 3 ± 1 h (Davis et al., 1986).

5. Conclusions

Cell monolayer integrity and, regarded with a closer focus, the actual status of the paracellular permeability are critical parameters that should be controlled during a transport experiment across Caco-2 cells. This applies especially in case of intentional manipulation of the cell monolayer permeability as purposed with the apparatus. By means of continuous TEER measurement a sensitive tool for online monitoring of the barrier status of Caco-2 cell monolayers has been implemented into the apparatus for combined measurement of dissolution and permeation. The instantaneous availability of the respective TEER profile to each experiment provides an adequate in-line control and points out the validity of the experimental data. Next to that, the informative value of the online and on-site TEER measurement is much higher in comparison to the prior situation, when TEER could only be measured outside the FTPC, before and after an experiment, respectively. However, it has to be considered that TEER measurement cannot provide a complete picture of the paracellular permeability. Therefore, a careful interpretation of the data is essential to obtain a deeper insight into the changes at the cell monolayer and into the complex interplay of drugs and excipients with the intestinal epithelial barrier. Nevertheless, we are confident that the newly implemented feature will be a valuable tool to analyze the impact of various factors upon cell monolayer integrity like administration of excipients, pH, temperature, dissolution medium and other variables in more detail. This also allows investigating advanced formulation approaches for oral drug delivery and aims for the establishment of rationales for the use of permeation enhancers or combinations of excipients with additional physicochemical or biological targets such as efflux pumps and gut wall associated metabolic enzymes.

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