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Research paper

A new Pharmaceutical Aerosol Deposition Device on Cell Cultures (PADDOCC) to evaluate pulmonary drug absorption for metered dose dry powder formulations

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ABSTRACT

Absorption studies with aerosol formulation delivered by metered dose inhalers across cell- and tissue-based *in vitro* models of the pulmonary epithelia are not trivial due to the complexity of the processes involved: (i) aerosol generation and deposition, (ii) drug release from the carrier, and (iii) absorption across the epithelial air–blood barrier. In contrast to the intestinal mucosa, pulmonary epithelia are only covered by a thin film of lining fluid. Submersed cell culture systems would not allow to studying the deposition of aerosol particles and their effects on this delicate epithelial tissue.

We developed a new Pharmaceutical Aerosol Deposition Device on Cell Cultures (PADDOCC) to mimic the inhalation of a single metered aerosol dose and its subsequent deposition on filter-grown pulmonary epithelial cell monolayers exposed to an air–liquid interface. The reproducibility of deposition of these dry powder aerosols and subsequent drug transport across Calu-3 monolayers with commercially available dry powder inhalers containing salbutamol sulphate or budesonide could be demonstrated.

In the context of developing new dry powder aerosol formulations, PADDOCC appears as a useful tool, allowing reducing animal testing and faster translation into clinical trials.

1. Introduction

Dry powder inhalers represent an important platform both for local as well as systemic pulmonary drug delivery. As for all new medicines, aerosol powder formulations need to be tested for safety and efficacy. Mostly these experiments are done *in vivo* by animal experiments or on *ex vivo* lung preparations [1,2]. Based on the 3R principle of animal welfare, there is a demand for *in vitro* test systems to determine the efficacy of these aerosols, but they are rarely available. For the characterisation of aerosol properties and pulmonary deposition, devices like the multi-stage liquid impinger (MSLI) or Andersen impactor are widely established. However, these methods allow no conclusions about the influence of formulation factors on drug permeability and absorption across pulmonary epithelial barriers, nor do they provide any information about the biocompatibility with or possible toxic effects on those cells and tissue. Therefore, an apparatus allowing to simultaneously study both deposition and subsequent drug absorption of pharmaceutical aerosols appears to be highly desirable.

Aerosol effects on lung cells are often addressed in environmental toxicology [3–9]. There are different methods available, such as electrostatic precipitation [10–13] to collect particles from atmospheric aerosols, which are typically having a rather low particle density, onto cell monolayers. The main focus of those investigations is long-term effects of low-dose exposed materials like diesel dust or particulate matter. In contrast, pharmaceutical aerosols are aimed to deliver a specific dose of a given drug with a single bolus or puff. However, there are very few models available to study the effects of aerosolised drug powders on cell culture systems. One approach is the integration of cell monolayer in a liquid impinger system. Cooney et al. [14] used an Andersen viable cascade impactor as a deposition device and delivered aerosolised FITC-dextran solutions onto cell monolayers. Fiegel et al. [15] deposited large porous particles onto Transwell® filters which were placed on the stages of a liquid cascade impinger. In both setups, no particular attention was paid to the turbulences that were caused of the bowel-like form of the Transwell® filters. Therefore, Bur et al. [16] refined this approach by integrating upside down Transwell® filters in the bottom of a multi-stage liquid impinger to minimise turbulences in the air streams and subsequently increase deposition efficiency on the Transwell® grown cell monolayers. Still, these models rely on impaction as the main deposition mechanism. Besides impaction

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and diffusion, the prevailing deposition mechanism in the deep lung is sedimentation. Sedimentation is a predictable process [17,18] and does not cause changes of the aerosol properties like electrostatic precipitation does. Therefore, we decided to develop a system in which the deposition of aerosol particles mainly occurs by sedimentation at the air–liquid interface of filter-grown pulmonary epithelial cells.

Tight cell monolayers are needed to study drug absorption and permeability *in vitro*. The respiratory Calu-3 cell line forms tight junctions and produces mucus, making it suitable for modelling the airway epithelial barrier [19,20]. Another advantage of the Calu-3 cell line is the formation of tight monolayers on permeable filter supports at an air–liquid interface (ALI), yielding closer resemblance to the native epithelium than under liquid covered culture (LCC) [21].

This study describes a further evaluation of the new Pharmaceutical Aerosol Deposition Device on Cell Cultures (PADDOCC) based on filter-grown cell monolayers placed on the bottom of a sedimentation chamber. While the development and the experimental protocol of the apparatus with focus on optimisation of the deposited amount have already been described elsewhere [22], we here report additional validation steps by conducting combined deposition and transport experiments with air–liquid interface grown Calu-3 cells using commercially available dry powder aerosol formulations of salbutamol sulphate and different doses of budesonide. The results were compared with the standard procedure of liquid-interface transport studies.

2. Materials and methods

2.1. Materials

Snapwell® permeable filters (pore size 0.4 μm, 1.13 cm^2, Snapwell® type 3801) and Transwell® permeable filters (pore size 0.4 μm, 1.13 cm^2, Transwell® type 3460) were purchased from Corning Costar (Bodenheim, Germany). RPMI 1640 (without phenol red) and fetal bovine serum (FBS Gold) were obtained from PAA (Pasching, Austria) and sodium pyruvate was obtained from Lonza (Verviers, Belgium). Buffer for transport experiments was KRB. Composition was as follows: 1.41 mM $CaCl_2$, 3.00 mM KCl, 2.56 mM $MgCl_2$, 142.03 mM NaCl, 0.44 mM K_2HPO_4, 4.00 mM D-glucose and 10.0 mM HEPES. All these reagents were obtained from Sigma Aldrich (Deisenhofen, Germany). KRB was adjusted to pH 7.4 by means of NaOH. Budesonide and salbutamol sulphate powders were a gift from Boehringer (Ingelheim, Germany). All other chemicals were of highest available grade.

2.2. Cell culture

Calu-3 cells, clone HTB-55, were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and used at passages 38–52. Calu-3 cells were grown to 90% confluence in 75-cm^2 T-flasks with RPMI 1640 supplemented with 10% FBS Gold and 1 mM sodium pyruvate. Culture medium was changed three times a week. The incubator temperature was set to 37 °C in an atmosphere of 90% relative humidity and 5% CO_2. After trypsinisation, cells were seeded on Snapwells® or Transwells® at a density of 100.000 cells cm^{-2} with 1.5 ml medium in the basolateral compartment and 500 μl in the apical compartment. After 2 days, the cells in the Snapwells® were set to air–liquid interface conditions with 1.0 ml medium in the basolateral compartment. After 10–14 days, the cells were ready for the deposition experiments.

2.3. Bioelectrical measurements

Transepithelial electrical resistance (TEER) was measured to monitor the tightness of the cell monolayer. As a consequence of the air–liquid interface cultivation, the apical compartment has to be filled with 500 μl pre-warmed medium and the basolateral compartment with 1.5 ml before TEER measurement and equilibrate in an incubator for 30 min. TEER was measured with an Electrical Volt-Ohm Meter (EVOM, WPI, Berlin, Germany) equipped with chopstick electrodes. Thereafter the medium in both compartments was removed and the basolateral compartment was filled with 1.0 ml medium again. TEER was measured until 2–3 days before deposition experiments to assure the recovery of the mucus layer on the cells, and after the transport experiments to confirm the integrity of the cell monolayer during the experiments.

2.4. PADDOCC system

The design and experimental protocol of the PADDOCC system has been previously described by Hein et al. [22]. Briefly, the PADDOCC system consists of three components: the air flow control unit, the aerosolisation unit and the deposition unit which are all connected by silicon tubes. The apparatus is schematically depicted in Figs. 1 and 2. For simplification, only one connection between aerosolisation unit and sedimentation chamber is shown.

The aerosolisation unit (Fig. 1II) consists of an inhaler chamber equipped with a commercially dry powder inhaler HandiHaler® (Boehringer, Ingelheim, Germany, "HandiHaler® chamber", Fig. 1b) and an aerosolisation chamber (Fig. 1c). A capsule containing a determined mass of powder is placed in the HandiHaler®.

The air flow control unit (Fig. 1I) is an Akita® system (Activaero, Gemünden/Wohra, Germany, Fig. 1a) for controlling aerosol generation and transport to the deposition unit. By pulling and pushing the plunger of the syringe (Fig. 1e), a temporary low pressure is generated to trigger a dispersion impulse (60 l/min) for 0.2 s to aerosolise the powder into the aerosolisation chamber and a ventilation flow (6 l/min) for 2.0 s to transport the aerosol to the deposition unit.

The deposition unit (Figs. 1III and 2) consists of a block with three sedimentation chambers (Fig. 2a), a sampling unit (Fig. 2b) and a pedestal where discharged air is deflected. The sampling unit contains three sampling wells with Snapwell® inserts covered with cell monolayers alternating with three ventilation holes. It can be adjusted in two different modes. During the dispersion impulse and the ventilation flow, the sampling unit is in ventilation mode

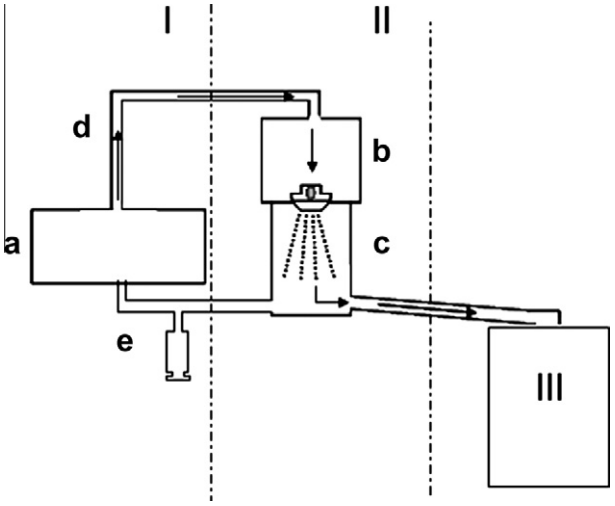

Fig. 1. Schematic view of the PADDOCC system, (I) air flow control unit, (II) aerosolisation unit, (III) deposition unit, (a) Akita® device, (b) HandiHaler® chamber with capsule, (c) aerosolisation chamber, (d) aerosolisation tube, (e) y-shaped tube with syringe.

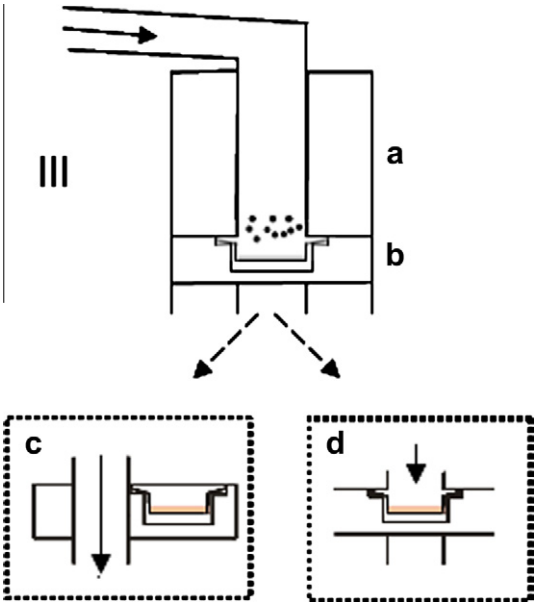

Fig. 2. Schematic view of the deposition unit of the PADDOCC system, (a) sedimentation chamber, (b) sampling unit, (c) sampling unit (with Snapwell®) in ventilation mode, (d) sampling unit (with Snapwell®) in deposition mode.

(Fig. 2c) to deflect the generated air and to prevent the cells from damage caused by pressure of air streams. After the ventilation flow, the sampling unit is put into the deposition mode (Fig. 2d) so that the aerosol particles sediment onto the cells. For all the experiments described here, the PADDOCC system (without the Akita® device) was housed in an incubator at 37 °C and three cycles of dispersion impulses were performed to empty the capsule completely.

2.5. Dose-dependent deposition

Experiments to demonstrate a linear dependence of the deposited amount of drug were performed first by aerosolising a dry powder capsule with different concentrations of budesonide (Cyclocaps® Budesonid 200 μg, 400 μg and 800 μg, PB Pharma, Meerbusch, Germany) and depositing the aerosol in the sampling wells (i.e. without cells). After three aerosolisation–deposition cycles, each well of the sampling unit was washed with 1 ml of HPLC mobile phase and quantified by HPLC analytics.

2.6. Deposition experiments and subsequent transport studies on ALI Calu-3 monolayers

Sixty minutes before the experiments, cell monolayers grown in Snapwells® were transferred to a 6-well culture plate, each containing 750 μl pre-warmed KRB buffer in the basolateral compartment and equilibrated in an incubator. After this pre-equilibration, they were placed into the sampling wells of the PADDOCC, filled with 500 μl pre-warmed KRB buffer, and the apparatus was then assembled to conduct the deposition experiment. To keep the temperature constant, the entire system (without Akita® device) was placed inside an incubator at 37 °C. Commercially available capsules of dry powders were aerosolised and deposited onto ALI grown Calu-3 cells via the deposition system. The studies were performed with Cyclocaps® Salbutamol 400 μg (containing 480 μg salbutamol sulphate, PB Pharma, Meerbusch, Germany) and Cyclocaps® Budesonid 400 μg (PB Pharma, Meerbusch, Germany) and dose dependent studies were performed with Cyclocaps® Budesonid 200 μg and 800 μg (PB Pharma, Meerbusch, Germany).

Sedimentation for respirable particles (i.e. with a MMAD in a range between 2 and 5 μm) takes no longer than 10 min in this chamber (calculated according to Dua et al. [17]) and performing three deposition cycles resulted in a window of 30 min for the aerosolisation and deposition process. After these 30 min, the Snapwells® were then transferred back to the 6-well culture plate filled with 750 μl pre-warmed KRB buffer in the basolateral compartment and rotated gently on a shaker (150 rpm). Samples of 100 μl were taken at different time points from the acceptor compartment and replaced by 100 μl fresh buffer. Samples were quantified by HPLC analysis. After the transport experiments, TEER values were measured to assure the integrity of the monolayer. To determine the total amount of drug deposited at the end of the experiment, the cells were lysed with 200 μl dimethylsulfoxide in case of budesonide as model drug, or with 200 μl isopropanol for salbutamol sulphate, unified with the apical and basolateral compartment (total volume 2.2 ml) and centrifuged for 3 min at 14,000 min^{-1}. The supernatant was then quantified by HPLC.

2.7. Liquid-interface transport experiments of budesonide and salbutamol sulphate

Transport experiments were also carried out with Calu-3 cells after dissolving of the drugs in buffer and pipetting the resulting solution to the apical cell compartment. The donor concentration of budesonide was 30 μM and of salbutamol sulphate was 1000 μM. Samples from the acceptor compartment were taken at different time points and quantified by HPLC analysis. P_{app} values were calculated as follows: $P_{app} = J/A * C$, where J is the flux, A is the area (1.13 cm^2) and C is the initial concentration in the donor compartment.

2.8. HPLC analysis

The deposited and transported amounts of budesonide were analysed by RP-HPLC on a Dionex System comprised of a 690 pump, ASI 100 automated sampler and UVD 340 U UV/VIS detector (Dionex, Idstein, Germany). A LiChrospher® 100 RP-18 column (125 mm × 4.0 mm, Merck, Darmstadt, Germany) was used. Detector was set to 240 nm, and mobile phase was phosphate buffer pH 3.0 (Ph.Eur.) and acetonitrile (60:40, v/v). The flow rate was 1.7 ml/min, and the column temperature was maintained at 30 °C. Injection volume was 80 μl and the lower limit of quantification was 50 ng/ml. Linearity was proven between 50 ng/ml and 250 μg/ml. Chromatograms were analysed by estimating the area under the peak in the curve by employing a computerised data integration program (Chromeleon 6.5, Dionex, Idstein, Germany) and compared to external standards. A similar method was utilised for the salbutamol sulphate quantification. The mobile phase consisted of a triethylamine-phosphate buffer (pH 6) and methanol (90:10, v/v). Composition of triethylamine-phosphate buffer was as follows: 0.03 M triethylamine, 0.03 M NaH_2PO_4 dihydrate in 1000 ml water and pH was adjusted with phosphoric acid 85%. Samples were analysed at 276 nm with a flow rate of 1.0 ml/min and a column temperature of 40 °C. Injection volume was 80 μl, and limit of quantification was 100 ng/ml. Linearity was proven between 100 ng/ml and 250 μg/ml. All these reagents for HPLC were obtained from Sigma Aldrich (Taufkirchen, Germany).

2.9. Data analysis and statistics

The area under the curve ($AUC_{absorption}$) as a parameter for absorption rate after 4 h in the transport experiments was

calculated by SigmaPlot 8.0 software (Systat Software GmbH, Erkrath, Germany).

Data are expressed as mean ± SD. Statistical analysis was carried out using SigmaStat 3.0 software (Systat Software GmbH, Erkrath, Germany). Data were compared by one-way ANOVA followed by the Holm–Sidak method for differences between two or more groups ($n > 6$). Differences were deemed statistically significant if $p < 0.05$.

3. Results

3.1. Deposition experiments

Deposition experiments with salbutamol sulphate (400 μg) and different amounts of budesonide (200, 400 and 800 μg) showed a uniform and reproducible deposition of about 0.5% of the aerosolised dose per well. Aerosolisation of salbutamol sulphate (400 μg) resulted in a deposition of 2.53 ± 0.58 μg per well. There was no significant difference in deposition amount between the three wells. As can be seen in Fig. 3, the different doses of budesonide resulted in a proportional deposition in the sampling wells.

3.2. Deposition experiments and subsequent transport studies

Transport experiments after deposition of budesonide aerosol powder (400 μg) showed a linear increase in the transported amount in the first 60 min, but then the curve flattened, resulting in a total transport of about 71 ± 11% of the deposited amount during 4 h (Fig. 4a). Transport of salbutamol sulphate showed a similar time course, with a flattening of the curve after 90 min and a total transport of 19 ± 6% after 4 h (Fig. 4b).

3.3. Liquid-interface transport studies

Transport studies performed with dissolved budesonide in a conventional Transwell® setup showed a linear transport during the first 4 h (Fig. 4a).

P_{app} was 8.37 ± 0.36 × 10^{-6} cm/s and total transport was about 13.8 ± 0.8% after 4 h. Salbutamol sulphate transport studies also showed a linear transport in the first 4 h (Fig. 4b), but total transport was only 0.11 ± 0.08% and P_{app} was calculated to be 0.126 ± 0.09 × 10^{-6} cm/s.

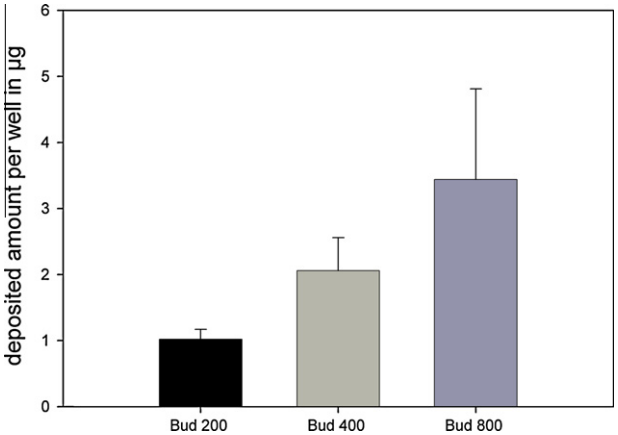

Fig. 3. Deposition behaviour of budesonide in dependency of different doses. The deposited amount of budesonide per sampling well (in μg) is proportional to the original dose (200, 400, 800 μg per capsule) which is equal to about 0.5% of the original dose (mean ± SD).

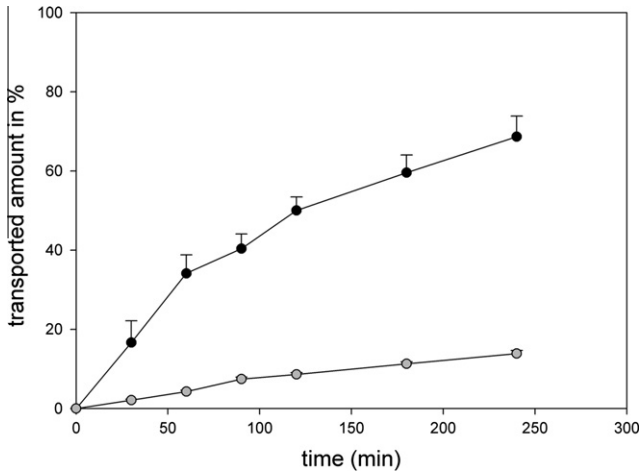

Fig. 4a. Transport of budesonide across filter-grown Calu-3 monolayers: Deposition and subsequent transport experiments for the aerosol powder formulation using the PADDOCC system (full circles, 400 μg capsule, $n = 12$) versus the dissolved drug using a conventional Transwell® setup (open circles, 30 μM solution, $n = 5$).

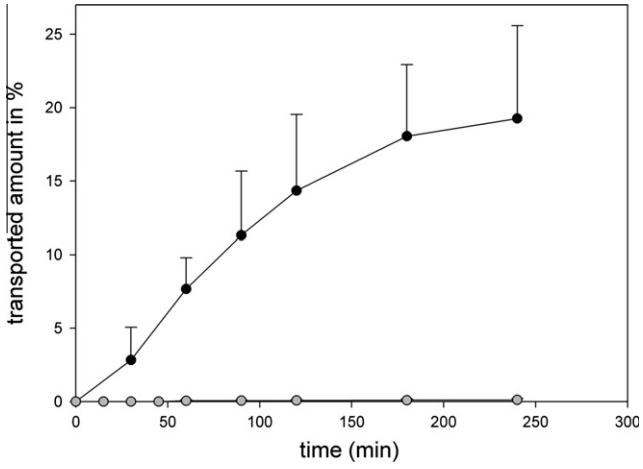

Fig. 4b. Transport of salbutamol sulphate across filter-grown Calu-3 monolayers: Deposition and subsequent transport experiments for the aerosol powder formulation using the PADDOCC system (full circles, 400 μg capsule, $n = 9$) versus the dissolved drug using a conventional Transwell® setup (open circles, 1000 μM solution, $n = 5$).

3.4. Dose-dependent transport studies

After the deposition experiments, the budesonide capsules were aerosolised and deposited with the PADDOCC system, but now equipped with Calu-3 monolayers grown on Snapwell® filter inserts. There is a dose-dependent transport rate of the budesonide molecules through the Calu-3 monolayer. Higher dosing of the drug resulted in an increased transport amount (Fig. 5), while the fraction of the deposited dose transported in% remained constant. After 4 h 62.3 ± 8.5% (200 μg), 65.6 ± 18.8% (800 μg) and 71.4 ± 11.3% (400 μg) of the deposited budesonide amount were transported. The area under the curve ($AUC_{absorption}$) as a parameter for absorption rate of the transport curves after 4 h is proportional to the aerosolised dose of the budesonide (Fig. 6).

4. Discussion

Ideally, an *in vitro* model for testing new aerosols and formulations should be able to mimic the deposition of aerosol particles on

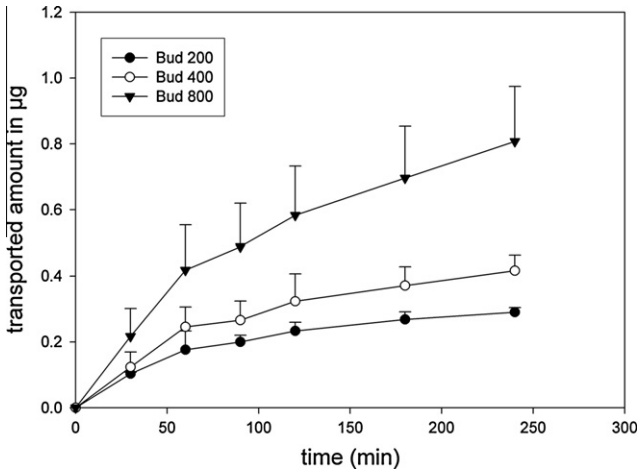

Fig. 5. Transported amount of different masses of deposited budesonide (after aerosolisation in the PADDOCC system from a 200, 400 and 800 µg capsule, $n = 6$) across a Calu-3 monolayer.

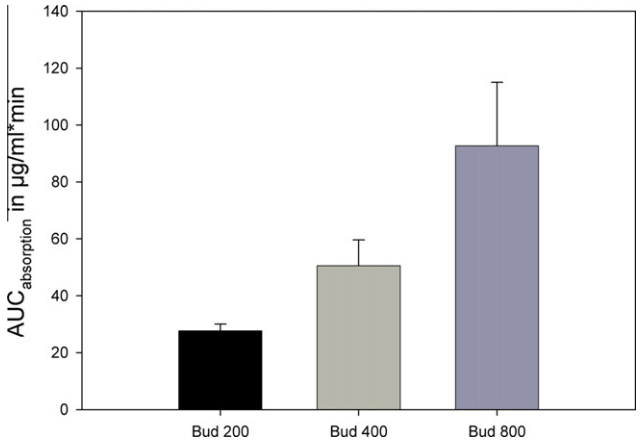

Fig. 6. The absorbed dose ($AUC_{absorption}$ in µg/ml∗min) across the Calu-3 monolayer from capsules of 200, 400 and 800 µg. The transport is proportional to the aerosolised dose (mean ± SD).

epithelial cell cultures by involving the same processes of impaction, sedimentation and diffusion as they occur in the lung *in vivo*. The new PADDOCC system aims to approximate this by the integration of Snapwells® with pulmonary epithelial cells in a sedimentation chamber. In this system, the aerosol particles from dry powders will deposit by sedimentation which is the prevailing deposition mechanism for particles sizes between 1 and 5 µm in the deep lung [23,24]. Before this respirable aerosol fraction reaches the sedimentation chamber, the PADDOCC system separates larger particles, such as lactose carriers, corresponding to the *in vivo* separation of larger particles by the throat and pharynx, allowing only smaller drug particles to reach the deep lung [25]. In the PADDOCC system, larger carrier particles are already deposited in the pedestal, due to higher sedimentation velocities, whereas the drug particles sediment onto the sampling wells which has previously been shown by SEM imaging [22]. Therefore, transport studies are not affected by the carrier particles.

In this study, budesonide and salbutamol sulphate were chosen as model drugs for aerosolisation because they are widely used to treat pulmonary diseases like asthma. Budesonide, as a biopharmaceutics classification system (BCS) class II model drug, is highly permeable and poorly soluble, and therefore, its pulmonary bioavailability is likely to be limited by its solubility in the lining fluid of the cell monolayer. In contrast, pulmonary bioavailability of the highly soluble salbutamol sulphate, a BCS class III drug, may be limited by its low permeability. As for systemic bioavailability after oral administration, solubility and permeability are most likely as important as for pulmonary bioavailability after aerosol delivery to the lung. Apart from effective deposition, adequate absorption across the pulmonary epithelia is pivotal. With the PADDOCC system, the ability of drug to cross the biological absorption barriers of the lung, e.g. by a Calu-3 monolayer, can be determined for dry powder aerosol formulations in a rather early stage of the development process.

Uniform deposition of about 0.5% of the original dose is found in one sampling well of the PADDOCC system with different drugs and different doses. When using budesonide capsules with different drug contents, the deposition is proportional to the original dose (Fig. 3), resulting in a good agreement of the ratio of the amounts deposited in the sampling wells of the PADDOCC system with those of the labelled dose (i.e. 1:2:4). Therefore, it is possible to control the deposited amount by using different doses in the capsule.

The total amount of drug recovered after deposition in the three sampling wells is about 1.5% of the total amount in the capsule. This may appear low compared to electrostatic precipitation methods [10,11] where deposition efficacies up to nearly 100% occur. However, the total surface area of the epithelial cell cultures ($\sim$3 cm^2) in the PADDOCC is even much smaller than the total surface area of the deep lung ($\sim$100 m^2). Assuming homogeneous deposition of a single aerosol bolus in the 0.1–1 mg range would theoretically lead to a density of deposited particles on the alveolar mucosa in the order of 0.1–1.0 ng/cm^2. This is still at least 1000 times lower compared to the actually collected particle concentration on the Snapwell® filter surface in our setup. Further reducing the deposition density would at the one hand demand much more analytical efforts. On the other hand, the $\sim$1000 times higher concentration of particles to which the cells are exposed in this *in vitro* setup appear as a useful "safety margin" to detect any possible adverse effects of a given drug or excipient, e.g. by a change of TEER or other cytotoxicity indicators.

Aerosol generation with a dispersion impulse of 60 l/min for 0.2 s is similar but shorter compared to other studies [14,15] where impactors with flow rates of 28.3 and 60 l/min over 30 s occur. The main advantage is that the sedimentation process of the particles onto the cells happens without any disturbing air streams, so that the cells are not affected. In this study, Calu-3 cells were used which were cultivated at air–liquid interface conditions, reflecting the *in vivo* properties more than liquid covered cultured Calu-3 cells [21]. They are more differentiated than LCC, but their TEER values are much lower than LCC due to the cultivation conditions. Several studies for air–liquid interface grown Calu-3 cells show that TEER of about 450 $\Omega * cm^2$ are needed to obtain a tight monolayer [15,26]; therefore, only results of transport studies with TEER after experiments of more than 450 $\Omega * cm^2$ were used. However, aerosol generation and deposition did obviously not affect the barrier properties of the cell monolayers, as indicated by practically the same TEER values before and after the deposition [22].

Epithelial transport experiments of budesonide after deposition with the PADDOCC system show a linear increase in the first 60 min in the transport rate (Fig. 4a), and then flattens, indicating a depletion in the donor compartment. After deposition of the aerosol on the monolayer, the budesonide particles dissolve only partially, yielding a saturated solution around themselves and some concentration gradient between the particle surface and the lining fluid. Another concentration gradient exists between the lining fluid with dissolved budesonide and the basolateral compartment containing KRB buffer. This concentration gradient

leads to a rapid transport through the monolayer to the basolateral compartment. After 1 h, the transport curve flattens because the concentration gradient between the budesonide particles and the lining fluid decreases. It runs out of sink conditions, and the permeation through the monolayer is limited by the dissolution process of the budesonide particles. As a result, the transport over the monolayer decreases resulting in a slower slope in transport curve. However, the hydrophilic salbutamol sulphate is dissolved in the lining fluid after deposition. In this case, permeation is the limiting factor for transport. Due to the high concentration gradient in the beginning, salbutamol is transported through the monolayer into the basolateral compartment (Fig. 4b). After about 90 min, the transport rate decreases due to depletion in the donor compartment. As expected, the transport rate of the hydrophilic salbutamol sulphate through the Calu-3 monolayer is much lower than of the lipophilic budesonide. The high local drug concentrations on the Calu-3 monolayer due to the landing of dry drug particles onto a cell monolayer with only a thin film of fluid cause the high absorption rates compared to submersed transport studies where budesonide and salbutamol sulphate show much lower absorption rates (Figs. 4a and 4b). The P_{app} values of the submersed transport studies calculated for budesonide and salbutamol sulphate correlate very well with data from Bur et al. [27]. Similar observations of an increased transport when using air–liquid interface cell monolayers were made by Grainger et al. [28] where FITC-dextran particles which were deposited by a twin-stage impinger onto air–liquid interface Calu-3 monolayers showed a 20-fold higher transport rate compared to FITC-dextran solutions.

Deposition and transport of increasing doses of budesonide showed an increase in transported amount in μg (Fig. 5), but the absorption curves measured in% of the deposited dose remained constant.

Different transported amounts (in μg), as seen in Fig. 5, resulted in a proportional increase of area under the curve ($AUC_{absorption}$) values as a parameter for absorption rate (Fig. 6) indicating that the PADDOCC system is able to distinguish between different amounts of the deposited drug. The ratio of the $AUC_{absorption}$ (1:1.8:3.3) is also in good accordance with the ratio of the dosing strength (1:2:4) in the budesonide capsules.

While the processes of aerosol deposition and absorption *in vivo* are much more complex, the PADDOCC system aims to reduce this complexity to a level which at the one hand remains practically feasible, but at the other hand may provide some insight into what happens when a dry powder aerosol formulation reaches the epithelial air–blood barrier of the lungs. Such information goes clearly beyond mere deposition studies of aerosol particles in a conventional impactor device and beyond mere permeability testing of dissolved drug in a conventional Transwell® setup. Alternative to the bronchial epithelial cell line Calu-3, an extension of the current setup towards more sophisticated cell culture systems, like human alveolar epithelial cells [29] or co-cultures with epithelial cells, dendritic cells and macrophages [30], also featuring a more complex apical lining fluid (e.g. to model the surfactant film), appears easily feasible.

5. Conclusion

The PADDOCC system allows combining the aerosolisation and deposition of pharmaceutical aerosol formulations with subsequent absorption studies across filter-grown cell cultures of pulmonary epithelial cells. Sedimentation as the main deposition mechanism is gentle, thereby not affecting cell monolayer integrity. Moreover, it allows separating respirable drug particles from non-respirable carrier particles due to different sedimentation velocities.

Air–liquid interface deposition of drug particles directly on the apical cell membrane, covered by only a minimal amount of lining fluid, makes an important difference to pipetting the drug dissolved in a buffer solution in a conventional Transwell® setup. For the development of pharmaceutical aerosol powder formulations, we trust that this setup could be very useful, replacing animal experiments and facilitating the translation of new concepts for pulmonary drug delivery into the clinic.

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