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Research paper

Permeability of the reconstructed human epidermis model Episkin® in comparison to various human skin preparations ☆

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Abstract

The objective of this work was to compare the barrier function of the small diameter reconstructed human epidermis model Episkin® ($d = 12$ mm) to human skin *in vitro*. For that purpose a modification for the Franz diffusion cell ($d = 15$ mm) had to be developed so as to allow direct comparison with the following human skin preparations: Full thickness skin (FTS), split thickness skin (STS), heat-separated epidermis (HSE), and trypsin isolated stratum corneum (TISC). Among the tested preparations, HSE appeared to be the most preferable due to its clear morphological structure and ease of preparation. The lipid profile of HSE and Episkin® was analyzed and showed significant differences in terms of cholesterol, ceramides and triglycerides contents, whereas cholesterol esters and fatty acids were not different. Permeation data with HSE and Episkin® were then gathered using caffeine and testosterone. Both test compounds permeated much faster through Episkin® than through HSE. Moreover, opposed to Episkin®, HSE differentiated between the two test compounds. In spite of the remarkable progress in developing RHEs in the past years at this time Episkin® can obviously not yet fully replace human skin for *in vitro* permeability experiments.

Keywords: Episkin®; Testosterone; Caffeine; Stratum corneum; Heat-separated human epidermis; Split thickness skin; Full thickness skin; Skin lipids

1. Introduction

Reliable data about permeation of compounds through human skin are necessary for pharmaceutical, cosmetical and toxicological research. However, a formal standardisation of skin absorption experiments is still missing, although documents on conducting skin permeation studies are reported [1–3]. Nevertheless, the differing procedures influence the results and make comparisons difficult. Recently an improvement was the release of the OECD guideline 428 [4] in 2004 and the guidance document 28 [5]. Herein some long sought clarification and standardisation for *in vitro* testing has been provided by setting the regulatory base for conducting *in vitro* skin permeation studies.

The OECD guideline 428 clearly states that human skin preparations – full thickness skin (FTS), split thickness skin (STS), heat-separated human epidermis (HSE) – can be replaced *in vitro* by reconstructed human epidermis (RHE) models. However, the same guideline requests the proof that data gathered with RHE are equivalent to data collected with human skin. Epiderm (MatTek, USA), Skinethic (Skinethik, F) and Episkin® (L'Oreal, F) are so far the most used reconstructed epidermis models for investigative purposes. The areas in which RHE are used are manifold: Phototoxicity, irritancy and corrosivity testing

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are the most thoroughly investigated areas although permeation data are limited (see [6] for a review). For Epiderm and Skinethic a larger body of literature on permeation studies exists [6] but for Episkin® only two publications about permeation can be found [7,8]. The lack of permeation studies with Episkin® is striking. The reason for this might be that the Episkin® model is different from Epiderm and Skinethic in two important points. (i) In contrast to EpiDerm and Skinethic, which are cultivated on inert filter membranes, the Episkin® model is cultured on a layer of collagen. The manufacturer specifies further that as a result of the collagen layer no shrinking of the cell layers occurs and therefore the cell layers are tight. (ii) Episkin® is only delivered in a diameter of 12 mm or smaller whereas Epiderm and Skinethic are also available in a 24 mm format, which fits static Franz diffusion cell (FTDC) with 15 mm orifice which e.g., have been used in a prevalidation study of the German BMBF testing skin permeation (see [9,10] and http://www.foerderkatalog.de; Project Number 0313342). To gather permeation data for the different reconstructed epidermis models with the same diffusion cell an adaptation is necessary.

The aim of this study therefore was (i) to develop an adapter which makes it possible to use Episkin® in static 15 mm FTDC, and (ii) to identify the best suited preparation of native human skin as reference for reconstructed epidermis equivalents in permeation studies under the same experimental conditions. Full thickness skin, dermatomised skin, heat-separated human epidermis and trypsin isolated stratum corneum (the latter not being mentioned in OECD guideline 428) were compared. As relevant criteria for the selection of the reference material were considered the barrier function *in vitro*, ease of preparation, and robustness of the skin preparation.

As skin lipids are widely assumed to play an important role for skin barrier function [11–14], lipid analysis of the most suitable human skin preparation and Episkin® was also performed.

2. Materials and methods

If not further specified, all substances used were of highest analytical degree available and were used without further purification.

Purified water was prepared by means of a Millipore Milli Q Synthesis system (Heidelberg, Germany).

2.1. Test compounds

Both test compounds were obtained from Sigma–Aldrich, Deisenhofen, Germany. All other chemicals were from Merck, Darmstadt, Germany.

4-Androsten-17β-ol-3-on (testosterone); molecular weight [g/mol]: 288.4; $\log K_{ow}$: 3.48 [15].

Caffeine; molecular weight [g/mol]: 194.2 $\log K_{ow}$: −0.08; pK_a: 1.4 [15].

2.2. Buffer solutions

All buffer substances presented here were of analytical grade (Merck, Darmstadt, Germany).

PBS buffer, pH 7.4: 1 L contains KCl 0.2 g; NaCl 8.0 g; KH_2PO_4 0.2 g; $Na_2HPO_4 \times 2H_2O$ 1.44 g; or Na_2HPO_4 1.1486 g; in purified water.

Buffer, pH 2.6: 1 L contains phosphoric acid 1.16 ml; KH_2PO_4 4.08 g; in purified water.

2.3. HPLC analysis of the test compounds

2.3.1. HPLC system

Pump: Dionex P580 Pump; Autosampler: Dionex ASJ 100 automated sample injector; Detector: UVD 170S detector; Column oven: Dionex STH 585 column oven; Software: chromeleon 6.50 SP2 build 9.68.

A LiChrospher® 100/RP-18 (5 μm) column/125 × 4 mm (Merck–Hitachi, Darmstadt, Germany) with a Lichrocart 4 × 4 mm guard column, LiChrospher® 100/RP-18, (5 μm) were used as stationary phase for all substances.

2.3.2. Test conditions

Testosterone: mobile phase: methanol/water 70:30 (v/v); retention time: 4.8 min ± 0.2 min; detection wavelength: 250 nm; flow rate: 1.2 ml/min; injection volume: 50 μl; detection limit 15 ng/ml; quantification limit 50 ng/ml; calibration from 50 to 5000 ng/ml ($r^2 = 0.999$).

Caffeine: mobile phase: buffer, pH 2.6/acetonitrile; 90:10 (v/v); retention time: 5.1 ± 0,2 min; detection wavelength: 262 nm; flow rate: 1.2 ml/min; injection volume: 50 μl; detection limit 15 ng/ml; quantification limit 50 ng/ml; calibration from 50 to 8000 ng/ml ($r^2 = 0.999$).

Methanol and acetonitrile were of HPLC grade (Merck, Darmstadt, Germany).

Drug quantification in samples was done by using the external standard method.

2.4. Lipid separation and quantification

2.4.1. Materials

All chemicals used for lipid separation and quantification were of analytical grade. The following materials and chemicals from Merck/Darmstadt, Germany were used: Diethyl ether, *n*-hexane, glacial acetic acid 100%, methanol (Lichrosolv), chloroform (Lichrosolv), petrolether, isopropanol, HPTLC plates (silicagel 60 non-fluorescent, Merck Nr. 105641) copper sulfate-pentahydrate, phosphoric acid (85%).

The following materials and chemicals from Sigma/Deisenhofen, Germany, were used as standard and reference material: For ceramides: ceramide III, ceramide IV; for triglycerides: triolein; for fatty acids: oleic acid; for cholesterol: cholesterol; for cholesterol esters: cholesteryl oleate.

Fat free cotton was obtained by Soxhlet extraction with chloroform–methanol (2:1; v/v).

2.4.2. Separation and quantification method

The method presented here was based on the method developed by Hauck [16,17] including modifications according to Wertz [18]. Lipids are extracted from freeze-dried samples using a mixture of chloroform and methanol (2V + 1V). The extract is filtered, dried under a stream of nitrogen, and the residue dissolved in a volume of the solvent mixture to obtain about 15 μg total lipids per μl. Depending on the amount of the lipid component indicated by a first overview chromatogram, the amount applied to the HPTLC plates has to be varied from 1 to 5 μl or the sample has to be diluted to be within the range of the corresponding standard solutions.

HPTLC separation was performed on silica gel plates at room temperature using the following solvent systems:

1. For the analysis of ceramides (separation from free fatty acids and sterols): chloroform–methanol–glacial acetic acid (190:9:1, v/v/v). Development 15 cm.
2. For the analysis of cholesterol and fatty acids: *n*-hexane–ether–glacial acetic acid (80:20:10, v/v/v). Development 15 cm.
3. For the analysis of triglycerides, sterol esters, and *n*-alkanes: first development (10 cm) with *n*-hexane–ether (80:20, v/v) and after drying, second development (15 cm) with petroleum ether.

The lipid substances were detected by charring with copper sulfate–phosphoric acid reagent (copper sulfate-pentahydrate 10.0 g; phosphoric acid (85%) 10.0 g, purified water 80.0 g) by heating the plate from 110 to 160 °C (approximately 15 min). After charring plates were scanned using a flatbed scanner (Epson expression 1680 pro) at 150 dpi and quantified with the TNIMAGE program (GNU public license; downloaded at www.icewalkers.com/linux/Software/5250/Tnimage.html) using the method of the external standard. Standard substances were applied in a range from 0.7 to 15 μg (Triolein and oleic acid) or 0.7 to 7 μg (sterols and ceramides).

2.5. Permeation experiments in standard Franz diffusion cells

Permeation experiments were carried out in Permegear static type 6G-01-00-15-12 Franz cells (Perme Gear, Riegelsville, PA); receptor volume: 12.1 ml; donor volume: 0.5 ml; orifice diameter: 15 mm (diffusion area: 1.76 cm^2); acceptor: PBS buffer. At defined time intervals 400 μl samples were drawn and replaced by fresh PBS. For further details, see Wagner [19]. The temperature was 32 ± 1 °C as required by OECD guidelines [4,5]. The total experimental time was set to 24 h for the various skin preparations and reduced to 6 h for Episkin® to guarantee sink conditions due to the higher drug permeation with this model.

2.6. Preparations of human skin

Excised human skin from female Caucasian patients, who had undergone abdominal plastic surgery, was used, approvals from the Ethic Committee of the 'Caritas-Traegergesellschaft Trier e.V.' as well as written consent of every donor and the medical personal in charge according to national regulations being on file.

2.6.1. Preparation of full thickness skin (FTS)

After excision the subcutaneous fatty tissue was removed and the remaining tissue stored at −26 °C. For further details, see Wagner et al. [20].

2.6.2. Preparation of split thickness skin (STS)

Skin sections with a thickness of 500 ± 100 μm were prepared from the thawed (full thickness) skin samples using an Aesculap GA 630 dermatome (Aesculap, Tuttlingen, Germany).

2.6.3. Preparation of heat-separated epidermis (HSE)

Based on a procedure reported by Kligman and Christophers [21] the epidermis was separated by putting thawed and cleaned full thickness skin pieces in water at 60 °C for 90 s. After removing the skin from the water and placing it, stratum corneum side up, on a filter paper, the SC-epidermis layer of the skin was peeled off from the dermis using forceps. The epidermis was put into PBS solution for at least 30 min in order to get a fully hydrated epidermis sheet.

2.6.4. Preparation of trypsin isolated human stratum corneum (TISC)

In order to obtain TISC sheets, skin pieces were punched out of FTS, thawed, and cleaned with isotonic Ringer solution. The skin pieces were transferred, dermis side down, into a Petri dish, which contained a 0.15% trypsin solution in PBS buffer. They were incubated for 24 h at 32 ± 1 °C. This procedure was repeated with fresh trypsin solution until the stratum corneum was fully isolated [22].

2.7. Human skin equivalent Episkin®

The technical data sheet provided by the manufacturer describes the Episkin® model as "type I collagen matrix, representing the dermis, surfaced with a film of type IV collagen, upon which is laid a stratified and differentiated epidermis derived from human keratinocytes".

The Episkin® kit (J13, 1.07 cm^2) was shipped from (L'Oreal, Paris, F) for delivery on Tuesday or Wednesday morning and was used within 24 h post-arrival. After integrity check of the kit (red colour of the agar medium and temperature indicator) the Episkin® models were removed from the nutrient agar and transferred into 12-well plates filled with maintenance media provided by the manufacturer. After storage overnight in an incubator (37 °C, 5.0% CO_2) the Episkin® model was punched out in total from

the plastic insert and using a punch of 11 mm in diameter and afterwards was transferred onto the standard FTDC equipped with a special adapter (see Section 2.8).

The following batches were used for this investigation: 03-EPIS-036(J13); 04-EPIS-014(J13); 04-EPIS-015(J13); 04-EPIS-018(J13); 04-EPIS-021(J13); 04-EPIS-022(J13); 04-EPIS-031(J13).

2.7.1. Separation of the collagen layer

The collagen layer was separated from the epidermal layer by removing the safety ring fixing the collagen layer to the plastic insert. Using forceps the collagen layer could now be pulled free from the plastic insert and transferred to the Franz cell.

2.8. Permeation experiments with Episkin® adapter

To fit the Episkin® model in the standard diffusion cell of 15 mm orifice a reduction of the diffusion area was necessary. The insert made of Teflon (Fig. 1) reduced the diffusion area of the Franz cell from 1.76 cm^2 (15 mm) to 0.385 cm^2 (7 mm) and the receptor compartment volume from 12.1 to 11.4 ml. The upper part was elongated until it surpassed the upper part of the Franz cell. There it could be tightened with Parafilm and aluminium foil. To have the same amount of donor per surface area as in the standard FTDC the donor was reduced to 110 μl.

2.9. Experiments in the plastic filter insert

To obtain hydrostatic equal levels the liquid inside and out the plastic insert the donor volume was adjusted to 150 μl and the receptor volume to 1500 μl, respectively. At predetermined time intervals samples of 200 μl were drawn from the receptor compartment and immediately replenished with preheated receptor medium. During the whole experiment the Episkin® inserts were kept in an incubator at 32 °C without carbon dioxide to have experimental conditions similar to the FTDC experiments. Furthermore, to obtain a proper mixing of the receptor phase and to minimize unstirred water layers the receptor phase was stirred by means of a magnetic bar (4×1.5 mm) at 400 rpm.

2.10. Drug preparation and dosage

For the various human skin preparations caffeine was applied in a concentration of 10,000 μg/ml in PBS whereas for the Episkin® model only a concentration of 1000 μg/ml was used. The higher concentration for the various skin preparation was utilized to reduce the lag-time and the lower concentration with the Episkin® to avoid dilution of the samples for the HPLC analysis. Testosterone was used in a concentration of 40 μg/ml in PBS with 2% Igepal for all experiments. Igepal (Sigma–Aldrich, Deisenhofen, Germany) was added as a non-ionic solubilizer to enhance the solubility of testosterone following the recommendation of the OECD guideline [4]. Schreiber et al. [9] have shown that Igepal in this concentration does not influence the barrier function of the skin and Episkin®. With all concentrations applied 'infinite dose' conditions were realized.

2.11. Permeation experiments with methylene blue for evaluation of the adapter

Transport experiments were carried out analogously in Franz type diffusion cells as described above. Donor concentration 1 mg/ml methylene blue (Sigma–Aldrich, Deisenhofen, Germany); Separation membrane: Dialysis membrane (Methocell, London, UK; 10,000 Da molecular

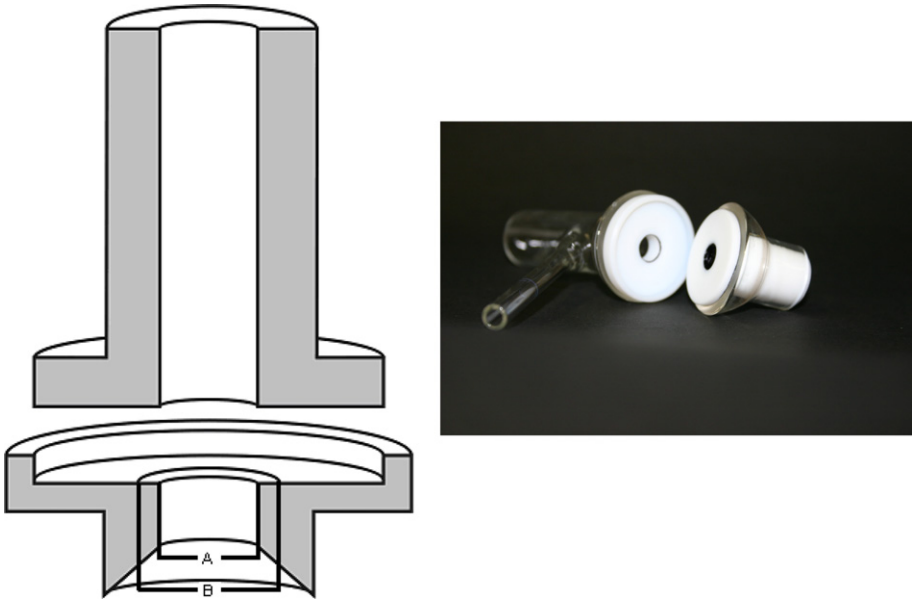

Fig. 1. Schematic representation of the Episkin® adapter. (A=7 mm, B=10.5 mm).

weight cutoff); sampling time points every 2 min. All experiments were repeated six times. The temperature was set to 32 ± 1 °C.

2.12. Data analysis

The apparent permeation coefficient (P_{app}) was extracted from the [μg/cm^2] vs. time curves of the single experiments by fitting a line through the linear range of the curve using a linear regression model. At least 4 data points were used for calculation. Exemplary representative curves for HSE and Episkin® are shown in Fig. 2. The slope of this linear curve yields the flux J of a substance through the membrane, which together with the donor concentration c_d is used to calculate the P_{app} as follows:

$$P_{app} = \frac{J}{c_d}$$

These calculations were done using SigmaPlot 9.0 (SPSS Inc., Chicago, IL 60606, USA).

2.12.1. Statistical analysis

Statistical analysis was performed with Sigmastat 3.1. (SPSS Inc., Chicago, IL 60606, USA).

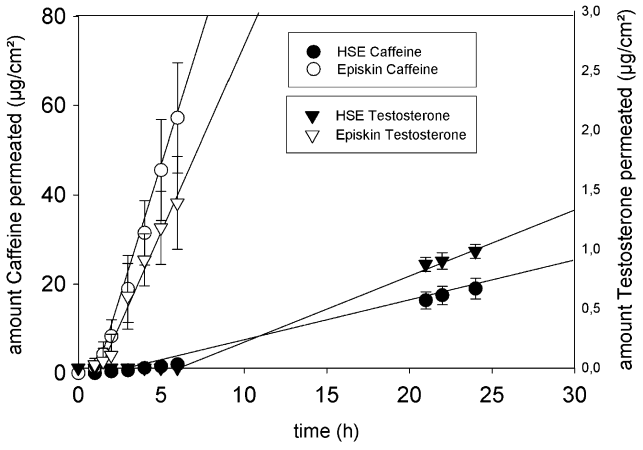

Fig. 2. Representative permeation curves for caffeine and testosterone (means ± SD); right scale testosterone; left scale caffeine.

3. Results

3.1. Evaluation of the Episkin® adapter

To evaluate the Episkin® adapter, experiments were carried out with a well-defined hydrophilic dialysis membrane to reduce the variability of the diffusion barrier first (see Section 2.11). Two groups were compared: The first group consisted of six FDTCs without the Episkin® adapter while in the second group six cells with the Episkin® adapter were used. The resulting P_{app} values from both groups ($P_{app} \times 10^5$ [cm/s] ± SD: with adapter: 6.75 ± 0.52; $n = 6$; without adapter: 6.20 ± 0.33 $n = 6$) showed no statistically significant differences (t-test; $P < 0.05$).

3.2. Barrier properties of the collagen layer

For these experiments the collagen layer was separated from the epidermal model and transport experiments with both test compounds were performed. The test compounds permeated much faster through the collagen layer than through the complete model indicating that the rate-limiting step is the permeation through the stratum corneum and epidermis like layer of the model (Table 1).

3.3. Experiments in the plastic insert of Episkin®

For aqueous solution of caffeine and testosterone, experiments with Episkin® in the plastic insert were performed and compared to the data gathered with Episkin® in the Franz cells with adapter (Table 1). No significant difference was found either for caffeine or for testosterone (t-test; $P > 0.05$).

3.4. Permeation through different human skin preparations

Several types of skin preparation for *in vitro* investigations are specified in the OECD guideline 428 [4] documents: FTS, STS and HSE. In addition, TISC was also included in this investigation. The experiments conducted in this part were all performed using skin from one donor to ensure maximum comparability and avoiding any inter-individual variation. The results are summarized in Table 1. From all *in vitro* human skin preparations FTS showed

Table 1
Results of all permeation experiments

		Caffeine $P_{app} \times 10^7$ ± SD [cm/s]	n	Testosterone $P_{app} \times 10^7$ ± SD [cm/s]	n
In vitro human skin preparations	Full thickness skin (FTS)	0.14 ± 0.014	4	Not detectable	4
	Split thickness skin (STS)	0.36 ± 0.06	4	3.53 ± 0.51	4
	Trypsin isolated stratum corneum (TISC)	0.34 ± 0.026	4	2.72 ± 0.25	4
	Heat-separated epidermis (HSE)	0.26 ± 0.023	4	3.76 ± 0.26	4
Reconstructed human skin equivalent Episkin®	Experiments in Franz diffusion cells with adapter	28.77 ± 9.93	24	22.25 ± 5.57	22
	Experiments in plastic inserts direct	20.1 ± 4.3	3	16.6 ± 1.4	3
Collagen layer	Experiments in Franz diffusion cells with adapter	228 ± 14	6	121 ± 11	6

the lowest permeation for caffeine, while transport of testosterone was not even detectable. For the other skin preparations TISC, HSE and STS permeation data of caffeine and testosterone were each of the same scale. Moreover in the latter three skin preparations testosterone showed a significantly higher permeation (approximately 10×) in comparison to caffeine (*t*-test: $P < 0.05$).

3.5. Comparison of HSE diffusion experiments and Episkin® experiments

The data for HSE (Table 1) show a lower permeation of caffeine compared to testosterone. Notably while HSE is differentiating between both compounds, Episkin® (Table 1) is not differentiating between both compounds (*t*-test; $P > 0.05$). These differences cannot be attributed to the different applied concentrations of the caffeine donor solutions because the presented P_{app} value for the 10,000 μg/ml donor solution is in accordance with the P_{app} value of $0.6 \pm 0.4 \times 10^{-7}$ (cm/s) for 1000 μg/ml donor solution reported in the German prevalidation study [10]. Moreover, the mean P_{app} values for caffeine and testosterone as observed in the Episkin® model are approximately 100 or 10 times higher than for HSE.

3.6. Comparison of the lipid contents of the Episkin® model and HSE

The lipid profile of the Episkin® model and heat-separated epidermis are shown in Fig. 3. Pairwise comparison of lipid classes showed that there are significant differences in cholesterol (*t*-test; $P < 0.05$), ceramide (*t*-test; $P < 0.05$) and triglyceride (*t*-test; $P < 0.05$) amounts. On an average, the Episkin® model contained approximately two times more cholesterol, one-third of triglycerides and two times more ceramides than human skin. No differences were found for cholesterol ester (*t*-test; n.s) and fatty acids (*t*-test; n.s.).

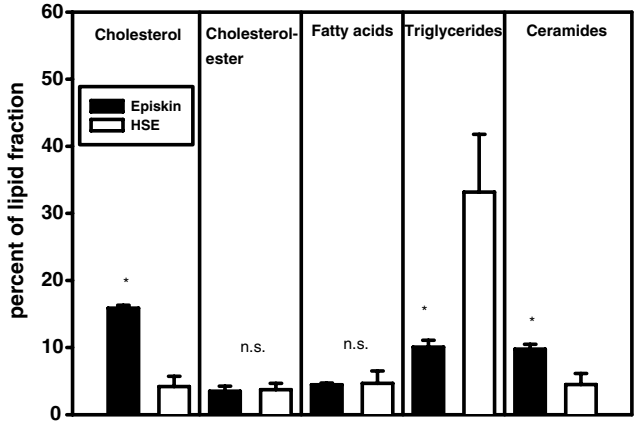

Fig. 3. Results of skin lipid analysis as percent of lipid fraction. * Significant different ($P < 0.05$), n.s. not significant different ($P > 0.05$); HSE $n = 66$; Episkin® $n = 15$.

4. Discussion

One of the major drivers for the development of reconstructed human skin models are to replace human and animal skin in *in vitro* testing. The OECD guideline 428 [4] clearly allows their use for these purposes. Before accepting them as a valid replacement, however, it has to be shown that the permeation data gathered with reconstructed human skin models is equivalent to the data generated with human skin as requested by the OECD guideline. Depending on the preferences of the manufacturer RHEs come in different sizes whereas the Episkin® model being an example for a relative small size epidermal model ($d = 12$ mm). Since a 15 mm Franz diffusion cell has been introduced as standard in a national multicentre prevalidation study of skin penetration in Germany (see [9,10] and http://www.foerderkatalog.de; Project Numbers 0313342 and 0313339) it was a goal of this study to find a possibility of using these FTDC for a smaller RHE such as Episkin® also. As shown by the permeation data with the methylene blue dye the use of the adapter does not influence the permeation data and therefore the use of the same diffusion cell for different sized separation membranes is possible. These results were in accordance with the theory of Fick [23] and data by Chilcott [24] showing that differences of the size of the diffusion area do not have a significant influence on the permeation data as long as a perfect sink is maintained in the acceptor chamber. Furthermore, the experiments performed with the Episkin® model directly in the plastic insert showed similar results as in the Franz cell and therefore this setup might be a reasonable alternative especially for high throughput experiments. However, it must be pointed out that these experiments had to be carried out with stirring of the receptor phase to minimize unstirred water layers, which may be critical concerning rate-limiting of permeation if using low water soluble drugs. Keeping this in mind additional advantage is given by the fact that no damage of the cell layers can occur because no removal of the model from the plastic insert is needed to transfer it onto the FTDC.

Another important point in this investigation was the collagen support layer of the Episkin® model on which keratinocytes are growing to build the epidermis like structure. Since the collagen layer is thicker than the epidermal layer the question arises if this collagen layer represents a barrier for the test compounds. The barrier function of the model should be located in the stratum corneum like epidermis layer as in the case in its human counterpart. The rate-limiting step of the diffusion should thus not be the crossing of the collagen layer. As a consequence, the transport over the collagen layer should be significantly faster than over the whole model. As shown in Table 1 for both test substances the diffusion over the collagen layer was much faster than through the whole model indicating that the barrier function in the Episkin® model is located rather in the cell layers than in the collagen support. In contrast to the experiments with the Episkin®

model by using the collagen support layer alone caffeine permeation is much faster than testosterone permeation. The reason may be that collagen has a high testosterone binding capacity, which is likely due to the high protein binding of testosterone in comparison to caffeine. Therefore, caution should be taken when using a reconstructed epidermis model with collagen support membrane together with drug substances having a high protein binding capacity.

The next point of interest was which type of skin preparation should be used for comparison. FTS did not appear useful because no transport of testosterone into the receptor fluid could be detected during 24 h. Although HSE, TISC and STS performed equally well regarding the permeation of the test substances, HSE was finally selected due to the following reasons. The main advantages of HSE are the well-defined morphological structure, the speed and ease of preparation, and the economical use of human skin. Furthermore, the data set generated with HSE (Table 1) showed P_{app} values consistent with data published by other authors [15].

As shown by Table 1, one clearly notices that Episkin® is more permeable than human heat-separated epidermis. However, the reduced barrier function is not solely a problem of the Episkin® model. Many epidermal models currently on the market are afflicted with the higher permeability [6,10], however, the effects are different. Par example, this is shown by the German prevalidation study [10] where different rankings of the permeability for various commercially available reconstructed epidermis models related to caffeine and testosterone are reported: Caffeine: SkinEthic, Episkin® > EpiDerm. Testosterone: SkinEthik > EpiDerm, Episkin®. Several possible reasons for this have been suggested, including impaired desquamation [25] and the presence of unkeratinized microscopic foci [26]. In any case, the elevated permeability currently limits the value of reconstructed epidermis models for transport studies and may lead to some false positives results in toxicity studies.

Furthermore, in contrast to Episkin®, HSE shows a clear distinction between both test compounds with respect to permeation. The reason for this may be attributed to reduced barrier of the Episkin® model making differences in the permeation behavior of the two test compounds invisible. Moreover, the binding of testosterone to the collagen layer of the Episkin® model may diminish the differences in permeation of caffeine and testosterone by acting as an additional receptor compartment. This will result in a reduced drug amount permeated through the model.

Lipids play an important role in the barrier function of skin. In Episkin® the same major classes of skin lipids were found in comparison to HSE. The high amounts of triglycerides found in human skin were not surprising since skin originated from plastic surgery is always contaminated with triglycerides as shown by Wertz et al. [27]. Thus, the lower amounts of triglycerides in the Episkin® model were not considered as a problem. The differences in the amounts of the different lipid classes between Episkin® and HSE in our investigation are corroborating the findings of Ponec et al. and Boelsma et al. [28–30]. The assumption that the reduced barrier function of Episkin® is somehow linked to the lack of lipids is tempting. Also in our studies there was no obvious correlation between the skin lipid pattern and permeability data obtained for RHE vs HSE. If and how the lack of certain kinds of lipids does affect the barrier function is difficult to answer. Correlation of the amount of certain lipid classes with the transport of drugs has been attempted several times [13,31–33], however, no clear results exist.

5. Conclusion

A standardised experimental setup for small diameter reconstructed human epidermis (RHE), e.g., Episkin® fullfilling the requirements of the OECD guideline 428 has been developed allowing to perform permeation studies in 15 mm static Franz type diffusion cells. Heat-separated human epidermis (HSE) seems to be the best suited human skin preparation for these kinds of experiments. However, regardless if STS, HSE or TISC is used the barrier function of the Episkin® model is still not comparable to human skin preparations. Permeability values for caffeine and testosterone are at least 10 times higher in comparison to HSE. In addition, permeability differences of the two test compounds could only be demonstrated with human skin preparations, but not with Episkin®. We conclude that RHEs like Episkin® represent a remarkable step towards the development of alternative methods, but at present it cannot yet be fully considered as replacement for human skin in *in vitro* permeation studies. Further experiments with a broader panel of substances with different physicochemical characteristics have to show the potential of RHEs for regulatory purposes according to [1,2,4,5].

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