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Review article

The human epidermis models EpiSkin®, SkinEthic® and EpiDerm®: An evaluation of morphology and their suitability for testing phototoxicity, irritancy, corrosivity, and substance transport

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Abstract

The commercially available reconstructed human epidermis models EpiSkin®, SkinEthic® and EpiDerm® demonstrate reasonable similarities to the native human tissue in terms of morphology, lipid composition and biochemical markers. These models have been identified as useful tools for the testing of phototoxicity, corrosivity and irritancy, and test protocols have been developed for such applications. For acceptance of these tests by the authorities, prevalidation or validation studies are currently in progress. Furthermore, first results also indicate their suitability for transport experiments of drugs and other xenobiotics across skin. Still, however, the barrier function of these reconstructed human epidermis models appears to be much less developed compared to native skin. Further adaptation of the models to the human epidermis, especially concerning the barrier function, therefore remains an important challenge in this area of research.

Keywords: Reconstructed human epidermis; Lipid composition; Biochemical markers; Drug delivery; Skin

1. Introduction

In vitro models to study penetration into human skin are important tools for research and development in the pharmaceutical and cosmetic industries. Human skin is the best possible model for such in vitro studies.

Possible sources for human tissue are cadaver skin, biopsy material or cosmetic surgery. However, there are a number of legal and ethical issues concerning the use of human tissues. The demand for human tissue is growing and the available amount is limited by number and by regulations. The European Union prohibits financial gain through the use of human tissue, making a widespread use no matter for which purpose very complicated.

Animal skin is an alternative. However, the relevance of conclusions drawn from animal data for human skin has always been questionable. The ethical problems which arise from use of animal skin for testing purposes finally lead to the EU regulation (76/768/EEC, Feb. 2003) which, beginning in 2009, prohibits use of animals for gathering toxicological data for cosmetic ingredients.

The COLIPA-Guideline [1] from 1995 and OECD-guideline No. 428 [2] describes in vitro methods for testing penetration on human and animal skin. The Draft guidance document for the conduct of skin absorption studies No. 28 clearly mentions commercially available skin models [3]. Nevertheless, it is still required that the comparability of artificial skin models and human skin has to be proven.

In recent years several artificial human skin models have been developed, and some of these are commercially available. These models have undergone various testing in

Abbreviations HPTLC, high performance thin layer chromatography; IL, Interleukin; LDH, lactate dehydrogenase; mRNA, messenger RNA; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; SC, stratum corneum; SG, stratum granulosum; SKALP, skin derived anti-leukoproteinase; SLS, sodium laurylsulfate; SPRR, small proline rich-protein; TLC, thin layer chromatography; UVA, ultraviolett light class A; o/w, oil/water; w/o/w, water/oil/water.

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order to evaluate the possibility of using them to replace animal testing. For this purpose, they must mimic the relevant properties of human skin as closely as possible.

Human skin consists of four layered compartments: stratum corneum, which is nonviable, the viable epidermis, dermis and the subcutaneous tissues. The functions of human skin are summarized in Fig. 1.

Skin has two major functions: prevention of dessication and protection against environmental hazards such as bacteria, chemicals and UV radiation. The consequences for a skin model are—briefly said—that it should have a competent barrier function and show the same reaction to environmental hazards as human skin [4].

The purpose of this review is to give an overview of the most important existing artificial skin models. Existing data has been collected to cover—when possible—aspects of morphology, lipid composition and other biochemical markers, phototoxicity, irritancy testing and transport data. Models lacking published data on two or more of the aforementioned aspects have been left out of this article.

To facilitate understanding of the presented data a short overview of the basic principles of the investigations and tests which have been performed with the models is given here.

1.1. Morphology

Investigations have compared the macroscopic and microscopic appeareance and ultrastructure of the models to human skin.

1.2. Lipid composition

The lipid composition of skin determines its permeability, flexibility, the partitioning of drugs into the skin and many other aspects of skin biology. Therefore, a lipid composition as close as possible to that of the native tissue is required. The methods usually used to determine lipid profiles is analytical TLC or HPTLC [5,6].

1.3. Biochemical markers

Biochemical markers are proteins indicative of the differentiation process. This includes the cornified envelope and its precursor proteins and the enzymes necessary for its

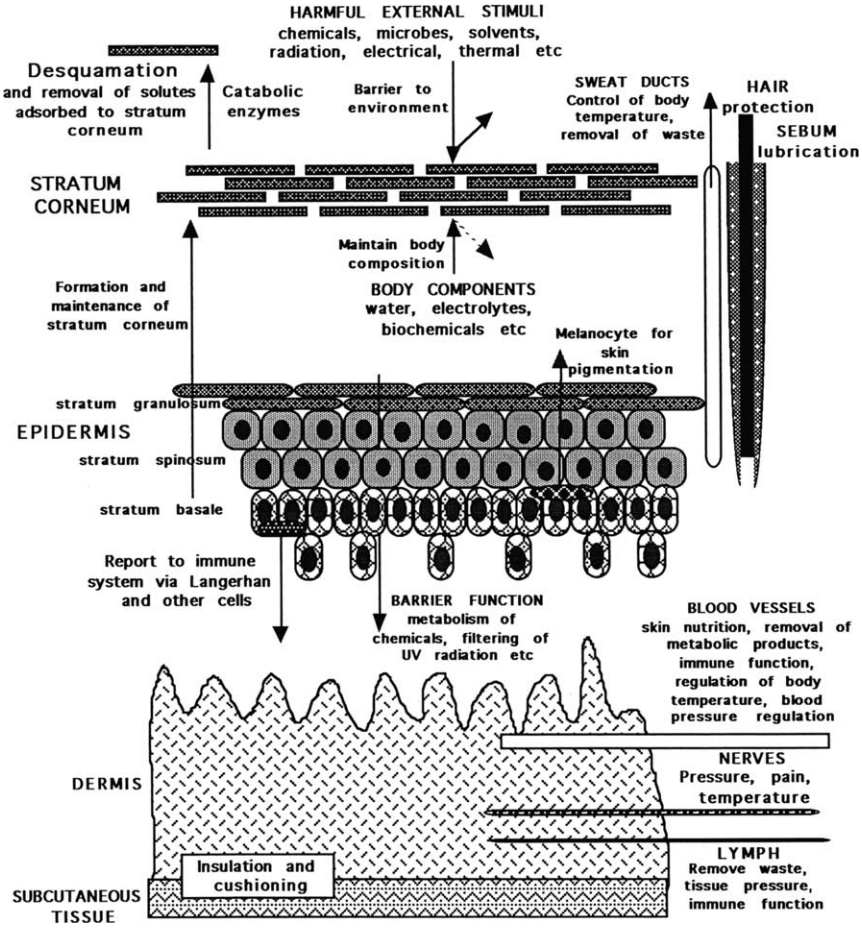

Fig. 1. General functions of the skin reprinted from [4] by courtesy of Marcel Dekker, Inc.

formation. Ideally, these markers should be produced in the models in amounts similar to those found in native tissue.

1.4. Phototoxicity testing

Phototoxic substances are applied to the model and are then irradiated with UV-light of different wavenlengths and intensities to trigger a phototoxic reaction. There are several protocols describing how these tests are performed. Details of individual test protocols will be described in the text.

1.5. Irritancy testing

Irritants are applied to the model in order to see if the model shows the biochemical and histological signs of irritation. There are several protocols that have been used, and again, details will be given in the text.

1.6. Transport data

Drug formulations are applied to the surface of the model and the amount of drug which has been transported through the model is measured as a function of time. Transport data allows evaluation of barrier function.

1.7. Corrosivity testing

The model is exposed to corrosive substance and the reaction is assessed. The exact experimental setup and the protocol which has been followed are detailed in the text.

2. SkinEthic®

2.1. About the company

SkinEthic laboratories (Nice, France) was founded in 1992 by Martin Rosdy in Nice to develop and market the artificial human skin model SkinEthic®.

The company offers a wide range of in vitro test system, not only the epidermal model discussed below. In addition to the epidermal model, SkinEthic® also offers other models, such as, e.g. reconstituted human corneal, oral, gingival, esophageal epithelium, etc.

2.2. General

The technical data and safety sheet from the SkinEthic Laboratories describes the SkinEthic® epidermal model [7] as ‘epidermis reconstituted by air lifted culture of normal human keratinocytes for 17 days in chemically defined medium on inert polycarbonate filters’.

2.3. Morphology

The general epidermal structure of the SkinEthic® model is highly similar to human epidermis. Stratum corneum, stratum granulosum, stratum spinosum which are the major structures of human skin can all be found in the SkinEthic® model [8]. The first description of the model in the literature revealed desmosomes, keratohyalin granules and lamellar granules [7]. Hemidesmosomes with plaques, components of the lamina densa, anchoring filaments and a structure which looks closely like a basement membrane were identified by electron microscopy [9]. However, Ponec et al. could not confirm these findings. This group only encountered hemidesmosomes but no anchoring fibril [10].

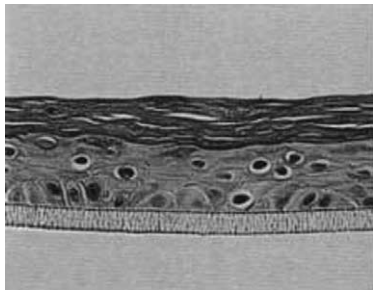

Fig. 2. Morphology of the SkinEthic® model [15], reprinted with permission of S. Karger AG, Basel.

When it comes to the number of viable epidermal and stratum corneum cell layers, the SkinEthic® models seems to differ from native tissue. Lipid droplets have been found through all layers of the SkinEthic® model, which are not found in native tissue. The highest frequency of these droplets was encountered in stratum basale. The extrusion of lamellar bodies was retarded in some SkinEthic® cultures. The research group also discovered characteristic electronmicroscopic structures as alternating electron-dense and electron-lucent lipid lamellar sheets in the intercellular space of the stratum corneum in the SkinEthic® model [6]. (Fig. 2: morphology of the SkinEthic® model).

2.4. Lipid composition

For labeling skin lipids in the part lipid composition we use the nomenclature according to Motta et al. [11] side by side with the nomenclature used by the quoted author. In most cases, the original authors named the ceramides according to the fraction numbers with ceramide 1 corresponding to the least polar fraction.

The major subclasses of ceramides and their precursors, the gucosylceramides, as described in the literature [12–14] are present in the SkinEthic® model (Table 1), although the model seems to have a higher ceramide 2 (Cer[NS]) content than native tissue. Ceramide 7 (Cer[AP]) is missing. The general lipid composition of the SkinEthic® model comes close to that of native tissue [15].

Data are presented as percent of total lipids. In the original publication the last line was by mistake also labeled as cholesterol. This error could be clarified with the help of the author [15].

Table 1
Lipid composition of SkinEthic® model compared with native human tissue [15]

Lipid class	SkinEthic® mean ± SD (n=4)	Native tissue mean ± SD (n=3)
Phospholipids	17.0 ± 10.6	36.5 ± 4.1
Sphingomyelin	2.8 ± 1.3	8.9 ± 1.6
Phosphatidylcholine	6.4 ± 3.8	11.2 ± 0.8
Phosphatidylserine	1.1 ± 0.7	3.9 ± 0.3
Phosphatidylinositol	1.8 ± 1.2	2.2 ± 0.8
Phosphatidylethanolamine	4.9 ± 4.0	10.3 ± 0.8
Cholesterolsulfate	3.8 ± 2.0	5.0 ± 1.6
Glucosphingolipids	3.0 ± 1.7	5.0 ± 0.4
Ceramides	26.5 ± 12.2	12.1 ± 1.8
Free fatty acids	6.9 ± 3.9	7.8 ± 1.2
Cholesterol	19.5 ± 9.5	17.7 ± 3.2
Lanosterol	4.3 ± 3.1	–
Di-/triglycerides	12.6 ± 8.6	8.9 ± 3.7
Cholesterolester	6.5 ± 4.4	7.0 ± 0.4

It should be noted that in the native tissue, the stratum corneum is maintained at a constant thickness, so the ratio of ceramides, which are found mainly in the stratum corneum, to phospholipids from the viable epidermis is constant. In the culture model, there is no desquamation, so the stratum corneum becomes progressively thicker. This could explain why the proportion of phospholipids is lower in the culture model compared to native epidermis and the proportion of ceramides is higher. Also, it should be noted that the origin of the di-/triglycerides in native skin is questionable [5]. This may reflect contamination with sebaceous lipids or subcutaneous fat.

2.5. Biochemical markers

Keratin 1, 10, SPRR, SPRR3, loricrin, involucrin and transglutaminase are all present in the SkinEthic® model. In analogy to human skin loricrin is present in the stratum granulosum. Involucrin and transglutaminase were encountered in suprabasal layers [6].

Keratin 6 and SKALP are not found in human epidermis but are present in the SkinEthic® model [6]. Some words to explain those findings: Yoshida describes SKALP as an epithelial serine protease inhibitor in psoriatic epidermis. It is a heat-stable, cationic protein with an apparent molecular weight of 9–11 kDa. SKALP is not found in normal epidermis but only in differentiating cells in psoriasis and healing wounds. It has an anitinflammatory effect [16].

Loricrin, a small proline rich-protein (SPRR) and involucrin are protein precursors of the cornified envelope, which are cross linked by a transglutaminase in the final stages of keratinization. The proper expression of these enzymes and their substrates is a condition sine qua non for the formation of a competent barrier [17]. Unfortunately Ponec et al. [6,18] did not specify the anatomic site, gender or age of the source of the human reference tissue used. This may have been helpful for comparison with other published lipid compositions.

2.6. Applications

2.6.1. Phototoxicity testing

The relevance of the SkinEthic® model for the assessment of phototoxicity has been shown by comparing experimental results to in vivo data from tolerability studies. Following the protocol described by Liebsch [19], 13 nonphototoxic and phototoxic compounds were applied to the model and exposed to UVA radiation. The model proved capable of discriminating between phototoxic and nonphototoxic compounds [20].

Medina used the leakage of LDH, as a marker for a decrease in cell viability, and increased IL 8 release and expression of IL 8 mRNA as a tools to quantify the phototoxicity of test compounds. It was shown that phototoxic compounds could be correctly identified using the SkinEthic® model [21].

A testing strategy for evaluation of phototoxic hazards using the SkinEthic® model has been described by Jones et al. [22].

2.6.2. Irritancy testing

Sodium lauryl sulfate, calcipotriol and trans-retinoic acid were applied to both human skin and the in vitro model for 24 h. Afterwards the level of cytokine expression and inflammatory skin reaction was measured. Results obtained with the SkinEthic® model correlated with results from intact human skin [23].

In 2002 a study was conducted to determine the reproducibility of data obtained from in vitro irritation testing using the commercially available reconstructed human epidermal models, including the SkinEthic® model. A protocol was established based on the measurement of cytotoxicity using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The release of proinflammatory mediators and enzymes after different times of exposure to sodium lauryl sulfate (SLS) were quantified. The viability results showed that the SkinEthic® model was potentially the most sensitive model to SLS, but also the least reproducible [24].

Another study showed that the release of interleukin-1α and interleukin-8 by the SkinEthic® model correlated with the results from the MTT assay and also could be used to classify sensitizing and irritating compounds [25].

2.6.3. Transport data

The speed of transport across the SkinEthic® model was examined and compared to human skin using lauric acid, caffeine and mannitol as penetrants. It was found that lauric acid was the best permeant of the group followed by caffeine and mannitol. This rank order correlates with findings in human skin. Variations in transport rates were low compared to those seen with human skin [26].

In another investigation the fluxes of terbinafine, clotrimazole, hydrocortisone and salicylic acid through human skin were compared using the SkinEthic® model. The substances were applied at a concentration of 1% in propylene glycol or in propylene glycol/water 9:1 (for salicylic acid). The penetrants were chosen to provide a large range of polarity. Terbinafine showed a high flux through the SkinEthic® model while it showed almost no transport through dermatomed human skin (The authors did not specify the thickness to which the skin was cut.) The drug concentration in the model after the end of the experiment was 55-fold higher than in human skin. Clotrimazole was transported almost nine time faster through the model than through the native tissue. The concentration in the model was up to 200 times higher compared to native skin. Also hydrocortisone penetrated faster through the model than through its native human counterpart, and the concentration was 25 times higher than in human skin. Only with salicylic acid similar concentrations were found in the model and human skin. The transport, however, was 6–12 times higher through the model. It was concluded that the SkinEthic® model does not provide sufficient barrier function to be useful for transport studies [27].

The transport of caffeine from w/o/w multiple and o/w emulsions through the SkinEthic® model and human skin was compared by another group. The cumulative absorbed amount from the o/w emulsion was always higher than from the w/o/w emulsions in human skin and the model. The amount which permeated through the model after 24 h was close to 25 times higher than the amount that permeated through human skin after 6 h [28].

In another study, the effects of albumin on the absorption of drugs and their metabolism in the model was examined and discussed with respect to results from excised pig skin [29]. The drug preparations studied included solid lipid nanoparticles, an ethanolic solution of testosterone and 0.25% cream preparations of prednisolone 17-ethylcarbonate, prednisolone 21-carbonate and prednicarbate. The addition of albumin to the acceptor reduced steroid permeation, especially while testing formulations with low prednicarbate uptake. Furthermore the authors showed that the metabolic pattern of prednicarbate was affected by the presence of BSA when using the reconstructed human epidermis equivalents.

3. EpiSkin®

3.1. About the company

The company known today as L'Oreal was founded in 1909 by Eugène Schueller, a chemist, and has become one of today's biggest cosmetic companies. It is active in the investigation of the skin and artificial skin models. Investigative work with skin models is carried out at the research center of L'Oreal in Aulnay-sous-bois.

3.2. General

The EpiSkin® model was first developed by E. Tinois and was bought by L'Oréal in April 1997. The EpiSkin® kit is currently marketed in the form of 12 well plates.

It consists of a 'type I bovine collagen matrix, representing the dermis, surfaced with a film of type IV human collagen, upon which is laid, after 13 days in culture, stratified differentiated epidermis derived from second passage human keratinocytes' [30]. This model is used for studies of irritation. To produce a model more suitable for drug penetration, the keratinocytes are cultured for 20 days prior to transfer to the collagen substrate [6,31].

3.3. Morphology

The EpiSkin® model shows all of the epidermal layers seen in its native counterpart. The stratum corneum of the EpiSkin® penetration model shows a significantly increased number of cell layers compared to most native stratum corneum samples, and as a consequence, is thicker. The cells of viable portion of the EpiSkin® model are organized somewhat differently than the cells in native epidermis. There are sudden changes in cell shape in the suprabasal compartment. Basal cells tend to be cubical in shape, while the upper cell layers are relatively flat. Extrusion of lamellar body contents is disturbed in EpiSkin® cultures. Granular cells with keratohyalin are present but of irregular shape. Intrabatch variation is low, but there is somewhat greater interbatch variation. As in the SkinEthic® model characteristic electron microscopic structures as alternating electron-dense and electron-lucent lipid lamellar sheets in the intercellular space of the stratum corneum are found [6,10,18] (Fig. 3: morphology of the EpiSkin® irritation

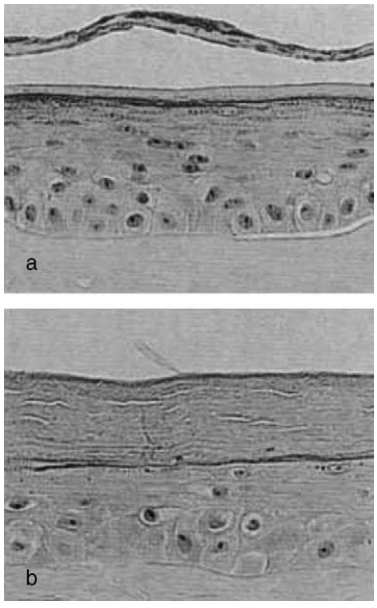

Fig. 3. Morphology of the EpiSkin® irritation model (a) and penetration model (b) [15], reprinted with permission of S. Karger AG, Basel.

Table 2
Lipid composition of the EpiSkin® model compared to native human tissue [15]

Lipid class	EpiSkin® irritation model mean ± SD ($n=5$)	EpiSkin® penetration model m ean ± SD ($n=3$)	Native tissue mean ± SD ($n=3$)
Phospholipids	33.0 ± 12.5	13.7 ± 6.9	36.5 ± 4.1
Sphingomyelin	4.7 ± 1.3	2.2 ± 0.9	8.9 ± 1.6
Phosphatidylcholine	10.8 ± 2.7	5.2 ± 2.5	11.2 ± 0.8
Phosphatidylserine	2.6 ± 1.6	0.9 ± 0.7	3.9 ± 0.3
Phosphatidylinositol	3.6 ± 2.5	1.5 ± 0.6	2.2 ± 0.8
Phosphatidylethanolamine	11.2 ± 5.4	4.0 ± 2.5	10.3 ± 0.8
Cholesterolsulfate	2.0 ± 0.5	1.4 ± 0.5	5.0 ± 1.6
Glucosphingolipids	3.4 ± 1.4	1.2 ± 0.4	5.0 ± 0.4
Ceramides	18.5 ± 6.9	25.1 ± 4.2	12.1 ± 1.8
Free fatty acids	1.8 ± 0.5	2.0 ± 1.3	7.8 ± 1.2
Cholesterol	17.6 ± 4.2	17.5 ± 2.3	17.7 ± 3.2
Lanosterol	1.3 ± 0.5	2.8 ± 1.5	–
Di-/triglycerides	20.9 ± 2.9	34.0 ± 6.4	8.9 ± 3.7
Cholesterolester	1.5 ± 0.5	2.3 ± 0.8	7.0 ± 0.4

model (c) and penetration model (f)). Obviously the penetration model has a thicker and tighter packed stratum corneum than the irritation model.

3.4. Lipid composition

Ponec examined the lipid composition of the EpiSkin® irritation and penetration models (Table 2). All major epidermal classes were found in both models. The phospholipid content found in the EpiSkin® irritation model was very close to that of human epidermis. However, in the EpiSkin® penetration model the amount of phospholipids was low. The precursors of ceramides, glucosphingolipids, were found in comparable amount as in human tissue in both models. Ceramide amounts in the EpiSkin® models differed from those found in native epidermis. While ceramide 7 (Cer[AH]) was missing, ceramide 5 (Cer[AS]) and 6 (Cer[AP]) were unusually low and ceramide 2 (Cer[NS]) was present in a much larger amount than in normal human epidermis. The amounts of free fatty acids and of cholesterol esters were lower than in native tissue. Lanosterol which is not found in significant amounts in human epidermis was found in the EpiSkin® models. Large variations in lipid composition from batch to batch were encountered [10,15].

Table 2 shows a great difference between native tissue and the Episkin models regarding their amounts of di-/triglyceride 8.9 vs 20.9%. The reasons for these variations cannot be explained. The subsequent question which arise from these findings is of course if it does influence the barrier properties of the models.

Correlations of the amount of certain lipid classes with the transport of drugs have been attempted several times [32–34]. Elias et al. [32] outlines that the transport is more closely linked to the total amount of lipids present in skin than to single lipid classes. A prediction of how the higher triglyceride amount affects the barrier function can therefore not be formulated.

An elevated production of triglycerides and retention of lipids within the cornified cell has been associated with hyperproliferation and impaired barrier function. This is seen in a number of hyperproliferative diseases or conditions including atopic dermatitis, psoriasis and essential fatty acid deficiency. This association has been demonstrated in a non-commercial epidermal keratinocyte air–liquid culture system [35].

3.5. Biochemical markers

In the EpiSkin® penetration model Keratin 1 and 10 are present in stratum spinosum and stratum granulosum. Keratin 6 is present in all layers. SKALP is found in the upper stratum spinosum and–as SPRR2 and SPRR3–in the stratum granulosum. Loricrin is absent while involucrin and transglutaminase are present in all suprabasal layers. These results are similar to those from the EpiSkin® irritation model. One difference is that in the EpiSkin® models SPRR3 is missing, and loricrin is present in the EpiSkin® irritation model [15].

3.6. Applications

3.6.1. Phototoxicity testing

In studies to examine the suitability of the model for phototoxicity the effects of several weak phototoxic, 6-methylcoumarin and ofloxacin, were compared to the effect of chlorpromazine, a strongly phototoxic substance. SLS and sulisobenzone served as negative controls. The substances were applied topically, and after 1 h incubation the cultures were exposed to UVA at a non-cytotoxic dose. After incubation for another 18 h the MTT viability test was performed, and the IL1α- released into the culture medium was quantified. The phototoxic compounds combined with UVA lead to an increase in cell mortality and a rise in IL1α-release, and thereby demonstrated the ability of the model to be used for identification of phototoxic substances [36].

Table 3
Surfactants used for irritation testing with EpiSkin®

Nr	Chemical name
1	Polyoxyethylene sorbitan monooleate
2	Polyoxyethylene sorbitan mono monolaureate (Tween 20)
3	Pentadecanol (etherified)
4	Industrial polyoxyethylene sorbitan monolaureate
5	Dodecanol (etherified)
6	1,2-Dodecanediol (etherified)
7	Blend of decanol and dodecanol (both esterified)
8	Octyl phenoxypolyethoxy ethanol
9	Dodecyl mercaptans (etherified 25 r)
10	Dodecyl mercaptans (etherified 15 r)
11	Dodecyl mercaptans (etherified 20 r)
12	Blend of sodium and magnesium laurylethersulfate
13	Triethanolamine acylamidopolyglycolehtersulfate
14	Sodium dodecyl sulfate (SDS)
15	Sodium dodecylether sulfate
16	Ammonium dodecyl sulfate
17	Triethanolamine dodecyl sulfate
18	Sodium lauryl *N*-, ethylglycinate
19	Coprah amphoteric alkylimidazolium dicarboxylate (MIRANOL)
20	Cocobetain derivative
21	Hexadecyltrimethylammonium bromide (CTAB)
22	Tetradecyltrimethylammonium bromide
23	Octodecylmercaptans (etherified)

3.6.2. Irritancy testing

Several studies have been undertaken to investigate if the model responds to irritants in a manner similar to native skin.

In one of these investigations the model was subjected to several surfactants (Table 3).

The release of cytokines (Interleukin 1α) and eventual impairment of the barrier function were measured. The damage of the epithelial barrier was measured using the permeability marker fluorescein. The data obtained was compared with historical data from in vivo ocular irritancy testing and subjected to a statistical analysis. A correlation was shown for the cytotoxic potential ($r=0.93$; $n=23$; $P<0.00001$) and the impairment of the barrier function ($r=0.87$; $n=20$; $P<0.00001$). A correlation was also shown for cutaneous irritation ($r=0.81$; $n=20$; $P<0.0001$) [31].

In another study the concentrations of surfactants necessary to cause irritation were determined and compared with in vivo human skin data from the literature [37]. The results showed that the concentration necessary to trigger a reaction in the model was smaller than required in vivo. The authors suggested that this reflected the impaired barrier function of the model [38].

Another example of the use of the EpiSkin® model in the investigation of irritation in human skin is the work of Cotovio et al. [39]. These investigators exposed the model to 10 ppm of ozone and monitored the formation of protein carbonyls by an ELISA method. Oxidative stress was also assessed using the fluorogenic probe, 2′,7′-dichlorofluorescin diacetate. Comparison with human keratinocytes cultures showed correlating results. The EpiSkin® model was subsequently used to test the potential of compounds for reduction of oxidative stress in human skin. The results showed that the EpiSkin® model was susceptible to oxidative stress induced by air pollutants.

Faller et al. used the EpiSkin® model in a comparison of human in vivo and in vitro skin irritation caused by cosmetic products. Formulations representing 22 different cosmetic products were tested in vivo and in the model. Cell viability as judged by the MTT test and the release of IL-1 α and cytosolic lactate dehydrogenase were measured. There was a good correlation for these measured parameters between skin in vivo and in vitro EpiSkin® model [40].

The European Center for the Validation of Alternative Methods (ECVAM) supported a prevalidation study on in vitro tests for acute skin irritation triggered by chemicals. The performance of the EpiSkin® method for predicting skin irritation was judged as insufficient in phases 1 and 2 of the project during which reproducibility and transferability were examined. On the base of these results, the existing protocol was refined. The new protocol showed good results when it came to sensitivity, specificity and accuracy [41].

3.6.3. Transport data

Several studies have examined transport through the EpiSkin® model. In one case the penetration of caffeine from different vehicles into the EpiSkin® model was examined. The penetration of caffeine and α-tocopherol acetate from a w/o-emulsion, an o/w-emulsion, a liposomal dispersion and a hydrogel were compared with EpiSkin® and human skin. The EpiSkin® model showed the same permeability rank order as human skin [42]. The model was more permeable to both drugs than human skin. Furthermore transport through the model reached its maximum earlier than through human skin. The vehicles affected the skin bioavailability in human skin only slightly. The bioavailability in the EpiSkin® model varied among the formulations. α-Tocopherol acetate absorption from the hydrogel was lower than from the other preparations. After prolonged application of the hydrogel on the skin model, permeation dropped to an insignificant level. The author relates this to an interaction between the lipids in the EpiSkin® model and alcohol and Carbomer 950 in the preparation [42]. Experiments carried out by the same group showed that the model was more permeable than human skin to mannitol [43].

3.6.4. Corrosivity testing

In 1998 the results of an ECVAM supported international validation study on in vitro tests for skin corrosivity using the EpiSkin® model were published. The objective of the study was to find an in vitro test method that could discriminate between corrosive and non-corrosive substances. Testing of 60 different chemicals was conducted in three different laboratories. Intralaboratoy and interlaboratory

reproducibilities were acceptable. The model managed to meet the test criteria and correctly identified corrosive and non-corrosive test chemicals. [44].

4. Epiderm®

4.1. About the company

MatTek Corporation—Ashland, Ma, USA—was founded in 1985. EpiDerm was introduced in 1993 to the market. The company is offering several other types of models, such as, e.g. reconstructed epidermis containing melanocytes, reconstructed corneal epithelium, etc.

Recently a full thickness skin (EpidermFT®) model has been released. Unfortunately, at this time there are essentially no published studies using this model. This article will therefore focus on the EpiDerm® model which has been widely covered in the literature. Two subtypes of the Epiderm® models are covered in the literature: the hydrocortisone-free culture and the percutaneous absorption culture [45].

4.2. General

The Epiderm® model was first described by Cannon et al. in 1994 [45]. The data sheet from the MatTek Corporation describes the EpiDerm® skin model as 'normal, human derived epidermal keratinocytes (NHEK) which have been cultured to form a multilayered, highly differentiated model of the human epidermis'.

4.3. Morphology

The general morphology of the model is comparable to that of normal human epidermis. One difference is that, since the keratinocytes are grown on polycarbonate filters, there are no Rete ridges, which anchor dermis and epidermis in native tissue. All stratas are present in the MatTek penetration model. The number of viable cell layers ranges from 6 to 8, and in the irritation model from 7 to 14, which leads to an epidermal thickness 28–43 μm for the first model and 83–100 μm for the second. Basement membrane is described as patchy for both models and hemidesmosomes are present in 50% of the cultures. The models do not differ in the stratum basale. The cell shape is described as columnar to round. Intracellular lipid droplets, which are absent from native epidermis, are encountered in the MatTek cultures. In the stratum spinosum the cells are flattened, as in native epidermis. Intracellular lipids in the stratum corneum of the culture model are present, but their organization is highly variable. The lamelar bodies in the stratum granulosum appear normal, and the keratohyalin granules appear rounded to stellate in shape. The stratum corneum contains 16–25 loosely packed cell layers with an overall thickness of 12–28 μm. The extrusion of lamellar

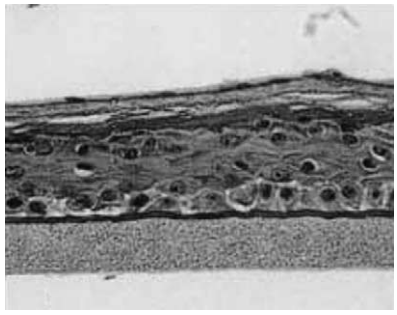

Fig. 4. Morphology of the EpiDerm® penetration model [15], reprinted with permission of S. Karger AG, Basel.

body contents at the SG/SC interface is complete [10,15,18] (Fig. 4: morphology of the EpiDerm® penetration model).

4.4. Lipid composition

The lipid profile of this epidermal model includes all major lipid classes in amounts similar to normal human epidermis (Table 4). The model shows a slightly elevated amount of glucosylceramides. The ceramide profile is overall comparable to human epidermis with all classes of ceramides present but with some deviations in relative proportions. The amount of ceramide 2 (Cer[NS]) was higher than in human epidermis, while ceramides 5 (Cer[AS]) and 6 (Cer[AP]) were present in relatively low amounts. Ceramide 7 (Cer[AH]) was absent. As in other models, contents of cholesterolesters and free fatty acids were lower than in native tissue [15].

4.5. Biochemical markers

Keratin 1, 10 and 6 are present in both the EpiDerm® irritation and penetration models in all suprabasal layers. In the irritation model Keratin 6 is only present intermittently in the lower stratum spinosum. SKALP is present in the stratum spinosum and—as SPRR2—in the stratum granulosum. SPRR3 is present in the stratum granulosum in the irritation model but missing from the penetration model. Loricrin is encountered in the stratum granulosum. Involucrin is present in all suprabasal layers in both models. Transglutaminase is present in all suprabasal layers in the penetration model, but in the irritation model transglutaminase is only present in the upper suprabasal layers [15].

4.6. Applications

4.6.1. Phototoxicity testing

Liebsch et al. transferred a test protocol which was originally applied by the german ZEBET (Centre for Documentation and Evaluation of Alternatives to Animal Experiments) for phototoxicity testing with the Skin2 model to the EpiDerm® model. The principle of the test is to apply test materials topically to the model at five different

Table 4
Lipid composition of EpiDerm® model compared to native human tissue [15]

Lipid class	EpiDerm® irritation model mean ± SD ($n=5$)	EpiDerm® penetration model mean ± SD ($n=3$)	Native tissue mean ± SD ($n=3$)
Phospholipids	36.5 ± 2.7	30.4 ± 1.1	36.5 ± 4.1
Sphingomyelin	8.2 ± 1.5	6.3 ± 0.3	8.9 ± 1.6
Phosphatidylcholine	13.6 ± 2.4	10.7 ± 0.2	11.2 ± 0.8
Phosphatidylserine	3.2 ± 0.7	2.3 ± 0.1	3.9 ± 0.3
Phosphatidylinositol	4.3 ± 0.8	3.7 ± 0.9	2.2 ± 0.8
Phosphatidylethanolamine	7.1 ± 1.6	7.4 ± 0.3	10.3 ± 0.8
Cholesterolsulfate	5.8 ± 1.2	5.7 ± 1.6	5.0 ± 1.6
Glucosphingolipids	9.5 ± 1.3	5.8 ± 0.1	5.0 ± 0.4
Ceramides	18.5 ± 3.5	28.9 ± 0.3	12.1 ± 1.8
Free fatty acids	2.6 ± 0.5	3.1 ± 0.6	7.8 ± 1.2
Cholesterol	14.8 ± 1.3	17.9 ± 0.9	17.7 ± 3.2
Lanosterol	1.2 ± 0.5	1.0 ± 0.1	–
Di-/triglycerides	10.5 ± 2.2	6.9 ± 0.8	8.9 ± 3.7
Cholesterolester	2.7 ± 1.1	2.1 ± 0.4	7.0 ± 0.4

concentrations and then evaluating the reaction with and without a non-cytotoxic dose of UVA and visible light, which simulates sun. One day after irradiation, the cytotoxicity is determined using the MTT assay. The model correctly identified phototoxic compounds [19].

4.6.2. Irritancy testing

In an interlaboratory comparison 16 surfactant containing formulations were tested on different batches of EpiDerm® in three different laboratories. The results from the different labs were then compared mutually and with human in vivo data. The correlation was good and showed that the model can be used to identify irritants [45].

Studies were conducted to identify biochemical markers of skin irritation that are measurable before physiological signs of irritation occur. SLS and tritiated water were applied topically to the EpiDerm® model and to excised human skin. The level of irritation was determined by measuring IL-1α mRNA levels. The response of the EpiDerm® cultures differed significantly from that of human skin. This difference was attributed to the suboptimal barrier function of the model compared to human skin. It was concluded that the model is suitable for screening possibly irritating substances. Because the barrier function is limited, the concentrations necessary to trigger a reaction are lower than they would be in excised human skin [46].

A prevalidation study was conducted to examine the interlaboratory and intralaboratory repeatability of irritancy testing using the EpiDerm® model. The results showed good intralaboratory reproducibility of the assay; however, there were statistically significant differences among the different laboratories [47]. In this study, the substances of Table 5 were used.

A comparison of 22 cosmetic formulations were tested for irritation potential in vivo on human skin and on the model. A good correlation was found, indicating that the model could be useful for assessment of the irritation potential of cosmetic compounds [40].

4.6.3. Transport data

Dreher et al. [42] compared the penetration of caffeine from different vehicles into the EpiDerm® model. The penetration of caffeine and α-tocopherol acetate from the range of formulations described above in Section 3.3 were compared. The results were similar between human skin and the EpiDerm® model.

Also, the rank order of permeability was the same in the EpiDerm® model and human skin. Permeability for both drugs was higher in the model than in human skin. The maximum rate of transport was reached earlier in the model than in human skin. With human skin, bioavailability was only slightly influenced by the different vehicles; however, with the EpiDerm® model bioavailability varied significantly among the different preparations. A lower amount of α-tocopherol acetate was absorbed from the hydrogel than from the other preparations. Prolonged application of the hydrogel on the skin model lead to a drop in permeation. As noted previously, this decrease in apparent permeability was attributed to an interaction between the culture model lipids and the alcohol and Carbomer 950 in the formulation.

Table 5
Irritants used in the EpiDerm® irritation prevalidation study

Nr	Chemical name
1	1-Bromohexane
2	Tetrachloroethylene
3	2-Ethoxyethylmethacrylate
4	*n*-Butyl propionate
5	Alpha-terpineol
6	Heptanal
7	Tallow polypropylene polyamine
8	1,6-Dibromohexane
9	Sodium metasilicate (10%)
10	Sodium bisulphite
11	Methyl palmitate
12	1-Bromopentane
13	3,3′-Dithiodipropionic acid
14	4,4′-Methylene bis (2,6-ditert-butyl)phenol

In another study transport experiments with mannitol through the EpiDerm® model were presented. The results demonstrated that the model is more permeable to mannitol than normal human skin [43].

Further transport experiment were carried out using the lipophilic drug flufenamic acid. The drug was applied to the model in either a wool alcohol ointment (0.1125%) or in Soerensen phosphate buffer pH 7.4 (0.1125% solution). Transport across the model was around forty times higher from the solution than from the ointment. The permeability of the reconstructed skin was five times higher compared to human epidermis [48].

4.6.4. Corrosivity testing

Fentem describes the completion of a successful prevalidation study on the use of the EpiDerm® human skin model for corrosivity testing. Experiments performed to optimize test conditions are presented [49].

5. Conclusion

The SkinEthic®, EpiSkin® and EpiDerm® skin models are well documented in the literature. At the moment they come close to reproducing human skin, but only in certain aspects. Their general structure, composition and aspects of biochemistry bear a close resemblance to human skin. These skin models are useful in toxicity testing, including phototoxicity testing, and to an extent for drug transport studies. Although considerably more permeable than human skin on average, the culture models appear to be more consistant in permeability and responsiveness than human skin, which is highly variable.

The biggest limitation of all three commercially available models is the still relatively weak barrier function. Several possible reasons for this have been suggested, including impaired desquamation [50] and the presence of unkeratinized microscopic foci [51]. In any case, the elevated permeability relative to native human skin obviously limits the value of these skin models for transport studies and may lead to some false positives in toxicity studies.

It is the stated goal of all manufacturer to fit their skin models with a barrier similar to human skin in vivo, but it is not foreseeable if or when they will suceed.

While this review focuses on epidermis-only models one must not forget the role of the dermis in permeability studies. Nakamura et al. [52] outline that full thickness models match more closely the situation in vivo.

The presence of a dermis in vitro solves some problems, but at the same time creates other problems which are adressed by Roberts and Walters [4]. Transport experiments with lipophilic substances through epidermis models might suggest a higher transport through the skin than it is actually the case in vivo because of the lack of a dermis which in vivo is a efficient barrier for lipophilic substances.

The lack of a dermis in vitro could lead to higher transport in vitro which would not correspond to the amounts actually encountered in vivo. But just by adding a dermis to a skin model this problem would only partially be solved. Brain et al. [53] give a more detailed description of the problems encountered here. The dermis in vivo is continously perfused by the subcutaneous vasculature, which penetrates the dermis. This system can rapidly remove permeants reaching the epidermal-dermis junction. In the in vitro situation this system is no longer present. Another problem is that in vitro, the aqueous environment of the dermis will slow the penetration of lipophilic compounds. In vivo this barrier is circumvented by the capillary bed. The authors conclude that the use of dermatomed, epidermal or SC membranes is more appropriate for examining particularly lipophilic permeants.

Summarizing one can say that adding a dermis to an epidermal only model as Apligraf® from Organogenesis (California, USA) or EpiDermFT from Mattek (Ashland, USA) is certainly interesting for investigating highly lipophilic compounds. However, before they can be put to good use the abovementioned problems of the dermis in vitro have to be solved.

Nevertheless, the present commercially available skin models represent a major improvement over what was available just a few years ago. But, it is still not foreseeable if there will be validated models for all necessary testing to completely replace animals by the 2009 ban of animal testing. Because appropriate altenative methods are needed for the development of new products by the pharmaceutical and cosmetics industries, further improvement of reconstructed human epidermis models still represents an important challenge.

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