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Three-dimensional skin models as tools for transdermal drug delivery: challenges and limitations

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Introduction: Transdermal drug delivery has several known advantages over the oral route and hypodermic injections. The number of drugs that can be taken up transdermally is, however, limited owing to the innate barrier function of the skin. New transdermal drug candidates need to be tested extensively before being used on humans. In this regard, *in vitro* permeation methods are highly important to predict *in vivo* permeation of drugs.
Areas covered: This review illustrates how different types of reconstructed skin models are being used as alternatives to human and pig skin for *in vitro* permeation testing of drugs. Insights into how various factors (including the physicochemical nature of molecules and formulations) or skin properties might affect the permeability of drugs in reconstructed skin models are provided. Also, opportunities and pitfalls of reconstructed skin models are highlighted.
Expert opinion: Many studies have revealed that the permeability of reconstructed skin models is much higher compared with human excised skin. This is in accordance with the incomplete barrier found in these models. Nevertheless, the reconstructed skin models available today are useful tools for estimating the rank order of percutaneous absorption of a series of compounds with different physicochemical properties. A major challenge in the further development of reconstructed skin models for drug delivery studies is to obtain a barrier function similar to *in vivo* skin. Whether this goal will be achieved in the near future is uncertain and will be, in the authors' opinion, a very difficult task.

Keywords: partition coefficient, reconstructed human skin model, skin permeability, stratum corneum, transdermal drug delivery

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1. Introduction

The accessibility and large contact surface make the skin an interesting route for drug administration. The application of drugs on the skin can be divided according to their purpose into epidermal, dermal and transdermal delivery. The latter must be differentiated from the others as in transdermal delivery the skin is only a barrier that has to be overcome instead of being the site of local drug activity [1]. Transdermal drug delivery is the non-invasive permeation of drugs from the surface through the various skin layers, into the bloodstream. This route of administration is a convenient alternative to oral medications and hypodermic injections [2]. One of the main advantages of transdermal delivery compared with the oral route is the avoidance of the first-pass effect of the hepatic and gastrointestinal metabolism, allowing the administration of lower daily doses and the application of drugs

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Article highlights.

- Transdermal delivery is an attractive route of drug delivery. In this review, the opportunities and pitfalls of reconstructed skin models in drug permeation studies are discussed.
- Drug penetration across the skin occurs by the transdermal and appendageal route. The transdermal route, which is the main pathway, is more determined by intercellular than by transcellular migration across the stratum corneum.
- Several drug properties influence skin absorption, including molecular mass, chemical structure and the partition coefficient. The stringent criteria of physicochemical drug characteristics that need to be fulfilled to overcome the skin barrier are important limitations of transdermal delivery. The development of appropriate permeation skin models to investigate the transdermal permeation capacity of compounds is therefore very interesting.
- Both epidermal and full-thickness reconstructed skin models are investigated as tools to predict the *in vivo* permeation of drugs. The use of commercial reconstructed skin models (EpiDerm™, SkinEthic™, EpiSkin™) was successfully validated in a large German study. Reconstructed skin models overestimate the penetration of drugs but nonetheless seem appropriate alternatives to human and pig skin. Several factors that influence drug penetration experiments are discussed: log P, molecular mass, lipid composition, dermal compartment, vehicle composition, metabolic activity and reproducibility.
- Reconstructed skin models are elegant tools to investigate the characteristics of transdermal drugs. Future optimizations should focus on improvement of the lipid composition to mimic even more closely the *in vivo* skin absorption.

This box summarizes key points contained in the article.

(e.g., fentanyl, estradiol) that are susceptible to enzymatic degradation when administered orally. Transdermal drug delivery can be particularly useful in drug indications that need steady plasma concentrations [3,4]. It enhances patient compliance and minimizes harmful side effects of a drug caused from temporary overdose. Another advantage is convenience, especially notable in patches that require only once-weekly application. Such a simple dosing regimen can aid in patient adherence to drug therapy [5]. Transdermal delivery also has advantages over hypodermic injections, which induce a minimal pain, generate dangerous medical waste and pose the risk of disease transmission by needle reuse, especially in developing countries [6]. In addition, transdermal devices are non-invasive and can be self-administered. They can provide controlled drug release for long periods of time (up to 1 week), whereas the input of a specific drug can easily be stopped by removal of the patch [7].

Despite the production of commercial transdermal patches for three decades, their use remains limited to a few drugs. Unfortunately, the innate barrier function of the skin allows only permeation of molecules with certain characteristics. With current delivery methods, successful transdermal drugs have molecular masses that are not greater than 500 Da, and have octanol-water partition coefficients that heavily favor lipids and require doses of milligrams a day or less [8]. This limits the number of drugs that can be taken up transdermally. Further drawbacks are the variation in absorption, which is dependent on the site of application, and the lag time between application and onset of action. In addition, the drug or vehicle should not be locally irritating or sensitizing [9]. It is also a big challenge to deliver peptides and macromolecules, including new genetic treatments using DNA or small-interfering RNA intradermally [10,11]. To circumvent the impasse of the stratum corneum (SC) barrier, molecules can be encapsulated into formulations that include a chemical penetration enhancer able to increase skin permeability by reversibly damaging or altering the physicochemical nature of the SC in order to reduce its diffusional resistance. One of the problems associated with many chemical penetration enhancers is that they cause irritation to the skin [12]. Other options consist of exerting a physical action (e.g., iontophoresis) on the drug itself or applying physical and/or mechanical energy to the barrier, such as ultrasound or microneedles.

Transdermal systems, chemical penetration enhancers and topical products are often very difficult to test *in vitro*. Several types of skin model have been utilized for many years. Some examples are: human cadaver skin, (hairless) mouse skin, rat skin, pig skin and cultured skin alternatives or reconstructed skins [13,14]. Freshly excised human skin samples of decent size and quality are preferred over human cadaver skin for permeation experiments but are often not available for research [15,16]. Excised human skin remains viable for only 8 days at 4°C and freezing of the skin for longer storage may introduce variability, especially in studies where skin metabolism has been central [17]. The problems often associated with the non-human models are the variations compared with human skin in terms of permeability, cell type, lipid composition and organization, and other physiological features. The development of an appropriate skin model for *in vitro* testing is challenging. Reconstructed skin models have been under development for the past three decades and have been validated for several *in vitro* and clinical applications, such as permanent skin replacement for burn victims [18]. They have been used to evaluate phototoxicity, corrosivity and skin irritancy. These specific studies are not, however, the main topic of this review and are therefore not discussed. Some excellent papers in the field are referred to [19-22]. At present, reconstructed skins are also being used to study the permeation of new investigational drugs and cosmetics. At the moment, there is no reliable skin model available that matches completely the barrier function and physiology of human skin. The purpose of this review is to describe the hurdles and pitfalls of reconstructed skin models for investigating transdermal drug delivery.

2. Penetration pathways through human skin

Human skin is the body's largest organ, covering the entire outside of the body and weighing ~ 5 kg. It is composed of three layered compartments: the epidermis with the stratum corneum on top, which is non-viable, the dermis and the subcutaneous tissues. The skin protects the body against excessive water loss and prevents the penetration of foreign molecules. The exceptional barrier properties of the skin lie mainly within its uppermost stratum, the stratum corneum. This is a highly hydrophobic layer of 10 – 30 μm, composed of 10 – 20 layers of differentiated anucleated cells [23,24]. These corneocytes are mainly filled with keratin filaments and fillagrin. They are surrounded by a highly permeation-resistant cornified cell envelope and are embedded in a dense structure of multilamellar lipid layers. The intercellular matrix of the SC is produced by keratinocytes of the underlying stratum granulosum that extrude lamellar bodies (carrying high amounts of free sterols, glycosphingolipids and phospholipids) at the inferior border of the SC [12]. The stratum corneum consists of at least nine different ceramides, which are produced in a complex step-wise process. The ceramides in the SC are newly synthesized or are derived from the degeneration of precursors such as glycosphingolipids [25]. Serine palmitoyl transferase is the rate-limiting enzyme in the production of ceramides and converts L-serine and palmitoyl-CoA to dihydrosphingosine, which is processed further by a glucosylceramide- or sphingomyelin-dependent pathway [25,26]. Finally, glucosylceramide and sphingomyelin are released in the intercellular space by fusion of the lamellar bodies with the apical plasma membrane of the granular cells [27]. This is followed by the final hydrolyzing step leading to the formation of ceramides. Glucosylceramide is hydrolyzed by b-glucocerebrosidase and sphingomyelin by acid sphingomyelinase [28].

The viable layers of the epidermis (stratum granulosum, stratum spinosum and stratum basale), which are 50 – 100 μm thick, have a strong metabolic activity but are also avascular. The dermis is the first structure with an extensive vascular network encountered by permeating molecules and allows transdermal drugs to enter the circulation [3,12]. Drugs applied to the surface of the skin can penetrate by two main pathways: the transepidermal route and the appendageal or shunt route (**Figure 1**). The transepidermal route involves the permeation of drug substances directly across the SC and can occur in two ways: transcellular and intercellular. The transcellular route is the direct migration of molecules through all the structures of the SC. In this route molecules permeate through the cytoplasms of corneocytes, which are low in lipids, and also through the intercellular lipid matrix, consisting mainly of fatty acids, ceramides and cholesterol [1]. This route is highly resistant to permeation owing to the variety of lipophilic and hydrophilic structures that have to be overcome. The intercellular route is believed to be the main pathway of epidermal penetration and involves the tortuous diffusion of molecules across the 4 – 20-lipid lamellae around the corneocytes [1,12]. Lipophilic drugs travel through the lipid tails whereas hydrophilic drugs prefer migration along the lipid heads [2]. The appendageal route includes permeation across sweat ducts and hair follicles with their associated sebaceous glands. The functional relevance of this transport route is controversial, although historically considered to have a rather limited capacity as the skin appendages account for only 0.1% of the skin surface. However, more recent research has shown that the follicular surface area is highly dependent on the site of the body, accounting indeed for 0.1% of the forearm but occupying 13.7% of the forehead [29]. Nonetheless, most skin penetration enhancement research considers only the transport across the SC [12].

3. Influence of physicochemical properties of drugs on permeation

A prediction of the essential properties of drugs such as drug absorption, distribution, metabolism and excretion is proposed by the rule of five [30]. The rule of five states that low absorption or permeation can be expected when the molecular mass is > 500 Da, the number of H-bond donors and H-bond acceptors is, respectively, > 5 and 10, and the octanol-water partition coefficient exceeds 5 (log P). More stringent rules need to be applied for transdermal delivery owing to the limited permeability of the SC. Choy and Prausnitz [4] calculated a modified rule for transdermal delivery: molecular mass < 335 Da, number of H donors < 2 and log P < 5. As the SC consists of non-viable, anuclear cells, drug penetration is mainly based on passive diffusion and follows in most cases Fick's first law of diffusion (the flux follows the concentration gradient, with a magnitude proportional to the difference in concentration). In accordance with Fick's law, two main factors influence the flux across the skin: the diffusity and the concentration gradient of the compound [7].

3.1 Diffusity

3.1.1 Molecular mass

The diffusion of compounds across the skin is size-dependent. The upper limit of molecular mass for skin penetration is difficult to determine, however the permeation rate of molecules exceeding 500 Da declines rapidly. In inflammatory skin diseases such as atopic dermatitis, the barrier function of the skin is compromised, allowing compounds of larger molecular size to penetrate. A recent study by Oji *et al.* [31] showed effectively that compounds such as caffeine and testosterone had an increased penetration in skin reconstructs made from fibroblasts and keratinocytes derived from patients with generalized peeling skin disease as compared with models with normal keratinocytes. The epidermal barrier of the disease model was severely compromised owing to the lack of corneodesmosin, an epidermal adhesion molecule. Nonetheless, the development of molecules for transdermal delivery should in general have a maximum mass of 500 Da [32].

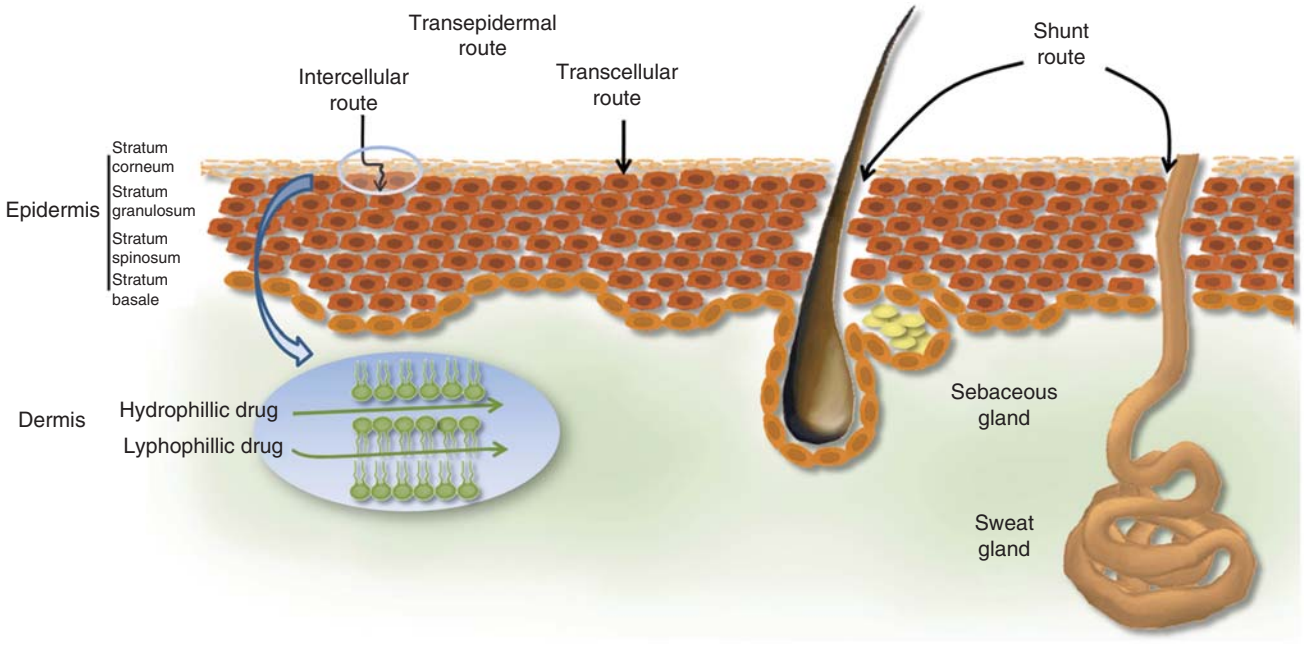

Figure 1. The transdermal permeation pathways: the transepidermal route (including intercellular and transcellular permeation) and the shunt route.

3.1.2 *Chemical structure*

The diffusion of a molecule is also partly determined by the chemical structure, in particular by the dipolar and hydrogen-bonding properties. Hydrogen-bond-forming groups in the drug molecule interact with the polar head groups of the intercellular lipids in the SC. The number of hydrogen-bonding groups is inversely correlated with diffusion rate. This inverse relation is even stronger if the molecule has an asymmetric structure [33].

3.2 Concentration gradient

3.2.1 *Partition coefficient*

The concentration of a drug in the skin is influenced by the octanol-water partition coefficient (log P). A drug has to partition into and out of the skin to reach the underlying vascular network. The lipophilicity of compounds is of crucial importance to diffusing through the lipid lamellae of the SC. By contrast, the underlying layer of the epidermis has a more aqueous composition. Ideally, drugs should have a sufficiently lipophilic and hydrophilic capacity to diffuse both through the SC and the epidermis [34]. The octanol-water partition coefficient has a parabolic association with the diffusion rate. Hydrophilic molecules with low log P values (log $P < 1$) have a decreased partitioning into the skin and will permeate most probably transcellularly. Lipophilic molecules (log $P > 3$) will almost exclusively use the intercellular route but show a low ability to partition out of the skin. In general, log P values for maximum permeation are believed to range between 1 and 3 [7].

4. Reconstructed human skin models

Reliable data about permeation of compounds through human skin are necessary for pharmaceutical, cosmetic and toxicological research. However, for many years skin permeation studies were conducted using different procedures. Animal skin derived from rats or pigs is often used, although the relevance of conclusions drawn from animal data for human skin is often questioned. In addition, there is an inter-laboratory variety in standard operating procedures for application of topical compounds and penetration evaluation techniques. It is obvious that such approaches influence the results and make comparisons difficult. To standardize the predictive testing of chemical compounds for regulatory purposes, an international ring trial was performed by van de Sandt *et al.* [35]. At the same time, the Organization for Economic Cooperation and Development (OECD) adopted guideline 428 [16] and a corresponding technical guidance document 28 [36], which describe methods for assessing absorption by using human and animal skin *ex vivo*. Although excised human skin is preferable to animal skin, it is clearly less available owing to certain regulations and ethical concerns. The European Union prohibits financial gain through the use of human tissue, making widespread use for whatever matter very complicated. Since 2009, the use of animals for gathering toxicological data for cosmetic ingredients has been prohibited in the EU (76/768/EEC, February 2003), making validated *in vitro* skin absorption testing inevitable. During the past few decades several reconstructed skin models

have been developed, and some of them have become commercially available since 1990. The use of these reconstructed skin models was approved by the OECD for skin corrosivity [37] as well as phototoxicity tests [38]. This has not been the case yet for percutaneous drug absorption, although the OECD guidance document 28 [36] states that the use of reconstructed human skin models is acceptable, given that permeation data from reference chemicals are comparable to those when using human skin *ex vivo*. Anyhow, the importance of absorption testing is reflected by the numerous studies that are addressed further below.

4.1 Different types of reconstructed skin

Today, several types of reconstructed skin model exist, which can be categorized as either epidermal models or full-thickness (FT) models. The latter have both epidermal and dermal layers. Epidermal reconstructs were originally used for skin grafting and were made by seeding normal human keratinocytes on the surface of a suitable membrane such as cellulose acetate or polycarbonate [39]. Under submerged conditions, proliferating keratinocytes form a multilayer matrix covering the surface of the membrane. After proliferation, the keratinocytes are exposed to the air (air–liquid interface) in order to induce their differentiation, which then gives rise to a multilayered epidermis (**Figure 2A**).

Full-thickness skin equivalents are mainly established by two different methods. First, a de-epidermized dermis (DED) derived from native human skin may be used. The acellular DED is then populated with fibroblasts using the centrifugal seeding technique and cultured under submerged conditions before seeding of keratinocytes [40]. After seeding of the keratinocytes on top of the DED, differentiation is induced by exposure of the DED to the air. This type of model is often established by academic groups for research purposes [41,42]. Second, human fibroblast cells embedded in a collagen gel may be used as the dermal part of the skin equivalent. The dermal layer will eventually contract into a gel-like structure that possesses characteristics of native human dermal tissue. In a next step, keratinocytes are seeded on top of the collagen matrix and the culture is kept under submerged conditions for a few days in order to induce keratinocyte proliferation. Thereafter, the culture is lifted to the air–liquid interface and nutrients from the medium will diffuse through the underlying collagen layer towards the epidermal compartment [43]. Keratinocytes start to differentiate and stratify in 5 – 10 days into an epidermis, which closely resembles the *in vitro* epidermal layers (**Figure 2B**). Both models described here thus contain an epidermis and dermis, approaching innate full-thickness skin. The limited lifespan of reconstructed skins, being one of the problems in the past, has been improved over the years by modifying culture conditions. Significant changes were the omission of serum, reduction of growth factor concentration (e.g., EGF) and vitamin supplementation [44,45].

4.2 Use of reconstructed skins for transdermal drug delivery

Today, several skin models are commercially available and are being used in the pharmaceutical and cosmetic industry as alternatives to animal skins. Some of these models have been well characterized and compared with human skin *ex vivo* with respect to their morphological characteristics and epidermal lipid composition [46]. Although they are morphologically similar to human native skin, their lipid organization differs slightly from normal skin, resulting in much lower barrier properties. Unlike human skin, these *in vitro* models do not contain structures such as blood vessels, hair follicles or sweat glands. Therefore, with regard to the penetration pathways described above, the only relevant routes of transport will be the inter- and transcellular pathways. **Table 1** gives an overview of three commercially available models (SkinEthic™ (SkinEthic Laboratories, Nice, France), EpiDerm™ (MatTek Corporation, Ashland, MA, USA) and EpiSkin™ (L'Oréal, Paris, France)) that are frequently used in skin absorption studies together with important characteristics influencing permeation. For more details about their morphology, lipid composition, expression of biochemical markers and applications, the authors refer to a comprehensive review written by Netzlaff *et al.* [19].

As stated above, the OECD developed guidelines to conduct *in vivo* and *in vitro* skin absorption studies [16,47]. The need for guidance document 28 [36] became apparent when national experts from several member countries could not agree on the possibilities and limitations of the *in vitro* test. The appearance of commercially reconstructed skin models suggested their use by the OECD for absorption testing as well. However, this requires a thorough validation of their applicability to guarantee equivalency with freshly excised human skin. It is obvious that experimental design and use test conditions can significantly affect the results obtained during permeation studies. In addition, permeation kinetics depends on the type of reconstructed skin used and the physicochemical properties of the test compounds. Schreiber *et al.* [48] conducted a series of experiments in an attempt to set up a standard protocol for the subsequent transfer to partner laboratories and future inter-laboratory validation experiments. Test conditions were optimized, and some important parameters influencing skin penetration are briefly discussed. For skin absorption/penetration testing, two substances (caffeine and testosterone), widely differing in their lipophilicity, were selected. They are recommended as reference substances by the current OECD guidance document 28 for skin absorption studies [36].

In accordance to other groups [35], Schreiber *et al.* [48] stated that the use of static Franz diffusion cells does not differ from dynamic cells for the evaluation of permeation (flux) of caffeine or testosterone. As static diffusion cells are easier to handle and less expensive, they are preferred for experiments. During the course of such experiments the composition of the receptor medium might influence the permeation of compounds. Depending on the solubility of the test compound

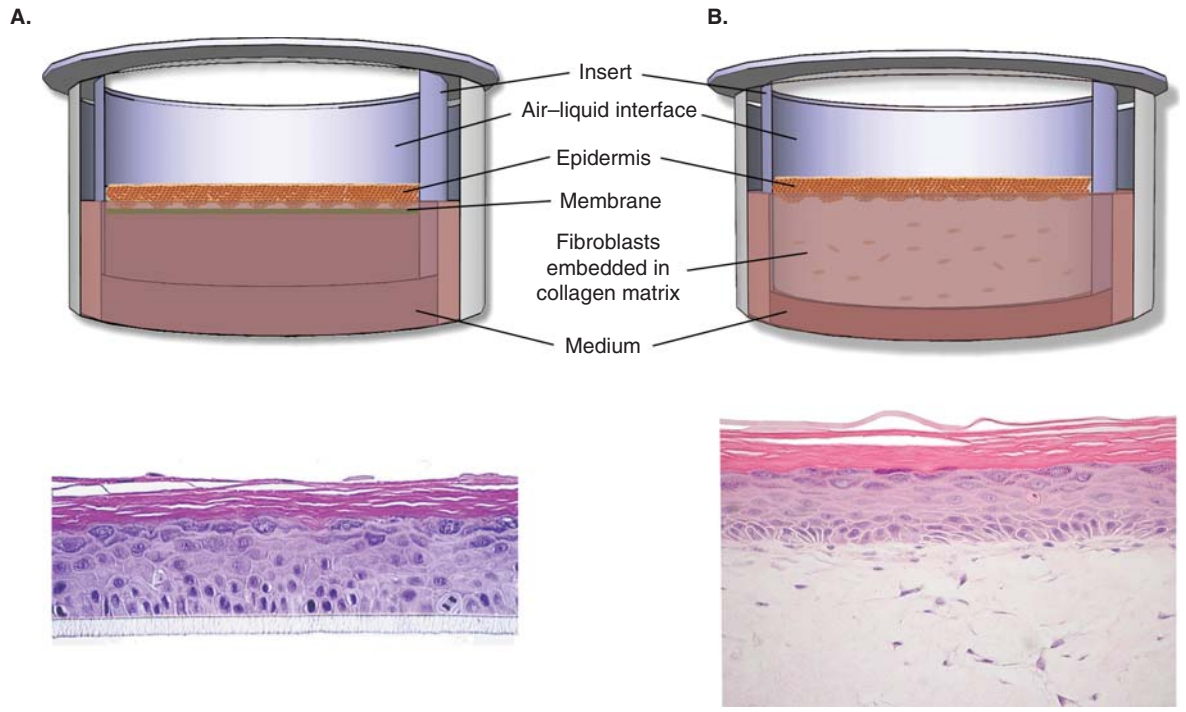

Figure 2. Graphic representation and histologic pictures of (A) epidermal and (B) full-thickness reconstructed skin models.
H&E pictures were kindly provided by (A) SkinEthic Laboratories and (B) MatTek Corp.

Table 1. Characteristics of three reconstructed skin models influencing the permeation properties.

	EpiDerm*	SkinEthic‡	EpiSkin§
Type of model	Epidermal	Epidermal	Full-thickness
Number of viable cell layers	6 – 8	5 – 9	7
Epidermal thickness (μm)	28 – 43	23 – 59	38 – 48
Stratum corneum			
Number of cell layers	16 – 25	14 – 24	60 – 100
Thickness (μm)	12 – 28	15 – 32	73 – 102
Lamellar body extrusion	Complete, rapid	Retarded	Retarded
Arrangement of layers	Loosely packed	Densely packed in some cultures	Densely packed
Lipid composition¶			
Phospholipids	82%	47%	38%
Ceramides	238%	219%	207%
Free fatty acids	40%	88.4%	26%
Di-/triglycerides	78%	142%	382%

Adapted from [46]. Permission granted by S. Karger A6, Basel.
*MatTek Corporation, Ashland, MA, USA.
‡SkinEthic Laboratories, Nice, France.
§L'Oréal, Paris, France.
¶Percentage of normal epidermis.

in this medium and the analytical method used, 5% bovine serum albumin (BSA) or a reduction of applied compound is recommended. A very delicate question is the selection of an appropriate donor solution in case of lipophilic substances. When added to water-based solvents, the inclusion of solubilizers is necessary. High concentrations might interfere with the strictly ordered intercellular lipid structure of the stratum corneum, thereby reducing its barrier function. Therefore, when comparing different matrices for their suitability for permeation experiments, the amount of solvent or solubilizer (e.g., Bovine serum albumin (BSA), Octylphenyl-polyethylene glycol (Igepal® Sigma-Aldrich, Bornem, Belgium)) has to be balanced

carefully versus the toxic effects on the skin. Compromises have to be made according to the unwanted side effects. Also briefly mentioned by Schreiber *et al.* [48] is the importance of a vehicle or formulation in which a drug is applied onto the skin surface. More specifically, vehicle components might alter skin permeability properties or evaporation differences can change concentrations at the skin surface. Therefore, it can greatly influence the *in vitro* penetration profile, as described extensively by Dick *et al.* [49].

Following protocol development, prevalidation studies and validation studies were carried out in a next step. In 2006, the permeation of caffeine and testosterone was determined using three commercially available reconstructed skin models in comparison with human epidermis and animal skin [50]. The permeation of testosterone through the EpiDerm and EpiSkin models was very similar, whereas the SkinEthic model appeared to be more permeable (SkinEthic > EpiDerm, EpiSkin). With regard to the permeation of caffeine, the following rank order of reconstructed skins was found: SkinEthic, EpiSkin > EpiDerm. As expected, the apparent permeation coefficient (P_{app} value) of these two substances was overestimated when comparing reconstructed skin models with human epidermis. These P_{app} values suggested that the barrier functions of the reconstructed skins were clearly less developed.

After a successful prevalidation study [50], a formal validation study was started. This included the scaling up of the numbers of test compounds and laboratories involved. Permeability of the EpiDerm, SkinEthic and EpiSkin models was compared with that of human epidermis and pig skin by using nine compounds widely varying in physicochemical characteristics. These compounds included the OECD standards testosterone, caffeine and benzoic acid. All nine test compounds were tested in infinite-dose experiments. Dependent on their molecular mass and lipophilicity, different permeation results (fluxes) were observed (**Table 2**). In general, permeation of the reconstructed skin models exceeded that of human epidermis and pig skin, yet the ranking of the substance permeation through the three tested models and the pig skin reflected the permeation through human epidermis [51]. It was concluded from this study that EpiDerm, SkinEthic and EpiSkin are appropriate alternatives to human and pig skin for the *in vitro* assessment of the permeation and penetration of substances when topically applied, taking product-specific overpredictability into account.

4.3 Factors influencing permeation studies

It is important to be able to predict skin permeability and to understand the mechanism of penetration for transdermal drug application. Several factors and properties of reconstructed skin models that have to be taken into account in skin permeability studies are described in detail below.

4.3.1 Log P and molecular mass

Lipophilicity and molecular mass of test compounds are considered to be the most important parameters that influence the rate of dermal penetration [52,53]. As mentioned before, human reconstructed skin models have much lower barrier properties as compared with human skin, which results in a much higher permeability and overestimated flux across human skin. Therefore, it is questionable as to whether ideal physicochemical properties of test compounds really contribute to enhanced permeability and whether these properties are still relevant when using models with a reduced SC barrier. This was challenged in a study by Schmook *et al.* [54], who decided to compare the permeability of human, porcine and rat skins with the Graftskin™ (Canton, MA, USA) and SkinEthic reconstructed skin models using four low-molecular-mass dermatological drugs with slightly varying hydrophilicities. All compounds had a molecular mass < 500 Da and a log P that varied between 1 and 3, so relatively good penetration is expected. Experiments with Franz diffusion cells revealed that the transdermal flux of salicylic acid in the reconstructed skin models was sevenfold higher compared with human skin, but flux values were in the same order of magnitude. Permeation of the more hydrophobic compounds clotrimazole and terbinafine resulted in fluxes that were massively higher than those obtained using human skin. The authors ascribed their observations to the weaker epidermal barrier properties of reconstructed skins and stated that the tested models were not very useful for *in vitro* penetration studies of hydrophobic compounds.

In a formal validation study by Schäfer-Korting *et al.* [51], as mentioned previously, the permeability of the EpiDerm, SkinEthic and EpiSkin models was compared with that of human epidermis sheets (HES) and pig skin by using nine compounds widely varying in physicochemical characteristics (**Table 2**). To avoid any influence of the donor vehicle on the barrier functions of the test skin preparations and the permeation properties of the test substances, neither ethanolic nor oily solutions were used for the application of lipophilic test substances. Phosphate-buffered saline (PBS) was used as the donor and receptor medium with the addition of the solubilizer Igepal at the lowest possible concentrations for the dissolution of lipophilic compounds. Igepal had a negligible influence on the viability and barrier function of all the test skins. The OECD reference substances testosterone, caffeine and benzoic acid were included. Permeation was evaluated in the different models (**Table 2**). For benzoic acid permeation data from HES were regarded as not valid for further analysis, but a sufficient number of eligible experiments allowed a ranking of benzoic acid permeation with the other test skin preparations. It was concluded from the permeation data (indicated by Tamhane's test) through the different skin models that the ranking does not differ essentially between three reference samples.

Flufenamic acid, nicotine and clotrimazole were included as test compounds with lipophilic properties (log P varying between 0.72 and 5.76; **Table 2**). Compared with all the test substances, flufenamic acid and nicotine showed the highest permeation with all the test skin preparations, which is in

Table 2. Overview of the physicochemical characteristics of the test substances, the test conditions used and the results of permeation ranking in the different reconstructed skin models.

	Molecular mass	Log P	Donor	Receptor	Permeation ranking
Caffeine	194.2	-0.08	PBS	PBS	SkinEthic > EpiSkin >> Epiderm > pig skin > HES
Benzoic acid	122.1	1.90	PBS	PBS	*SkinEthic > EpiDerm >> Episkin > pig skin
Testosterone	288.4	3.47	PBS + 2% (v/v) Igepal	PBS	SkinEthic >> EpiDerm > EpiSkin >> HES > pig skin
Nicotine	162.2	0.72	PBS	PBS	SkinEthic > EpiSkin > Epiderm >> pig skin > HES
Flufenamic acid	281.2	4.80	Soerensen buffer	Soerensen buffer	SkinEthic > EpiSkin >> Epiderm > HES > pig skin
Clotrimazole	344.8	5.76	PBS + 2% (v/v) Igepal	PBS + 0.05% (v/v) Igepal	Epiderm > SkinEthic > HES > pig skin
Mannitol	182.2	-4.67	PBS	PBS	–
Digoxin	780.9	1.14	PBS	PBS	Very low permeation
Ivermectin B1a	875.1	6.82	PBS + 2% (v/v) Igepal	PBS + 0.02% (v/v) Igepal	No permeation

Adapted from [51].
*Benzoic acid permeation data from HES were regarded as not valid for further analysis, but a sufficient number of eligible experiments allowed a ranking of benzoic acid permeation with the other test skin preparations (pig skin).
HES: Human epidermis sheets.

good accordance with their molecular masses of < 500 Da and moderate lipophilicities (**Table 2**). The very lipophilic clotrimazole permeated through HES and pig skin less than did flufenamic acid and nicotine. Valid permeation values with EpiSkin were not determinable for the latter.

Mannitol was included as a compound with very hydrophilic properties; because the permeation of mannitol through the test skin preparation was very non-homogeneous, no absolute (P_{app}) permeation data could be obtained for HES and EpiDerm. Permeation of mannitol was not observed in the SkinEthic model, in contrast to the high permeability of this model in general. Owing to high variability, comparison of permeation between different models was not feasible so this was not included in **Table 2**.

Substances with supposedly problematic permeation behavior were explicitly chosen, to allow the methods to be challenged under complicated conditions. In the case of ivermectin (molecular mass ~ 875 Da), no permeation was observed at all in any of the skin preparations. The permeation of digoxin was very low. Therefore, it could be concluded that no fundamental differences between the reconstructed skin models and the OECD-approved test skin preparations were detected with respect to the molecular mass cutoff.

In conclusion, it can be stated that differences in physicochemical properties of the test molecules do have an effect on their permeation behavior through reconstructed human epidermis models. For aqueous solutions, the reconstructed skins EpiSkin, EpiDerm and SkinEthic are appropriate alternatives to human skin and pig skin for the assessment of skin penetration *in vitro*. Although, generally, higher fluxes were observed, the ranking of permeation through the models and the pig skin reflected permeation through human epidermal sheets.

4.3.2 *Lipid composition*

Basically, differences in the barrier strength between different skins may be explained by differences in the skin morphology and in the epidermal lipid composition. Considering the latter, the influence of the total epidermal lipid content on the rate of percutaneous permeation has already been established by a strong correlation between the amount of extracted skin lipid and the amount of drug permeation [55,56]. The skin permeability barrier is described to be a function of its lipid composition [57]. In many reports, ceramides (45 – 50%), cholesterol (25%) and free fatty acids (10 – 15%) are described as being the main fractions of epidermal lipids [58-60]. Moreover, other epidermal lipids were found in mammals that potentially influence the percutaneous permeability barrier, although they represent only small lipid classes [61].

Asbill *et al.* [62] developed their own reconstructed skin model and examined its lipid composition and permeability in comparison with human skin and EpiDerm. This new bio-engineered human skin model is a full-thickness *in vitro* model containing a completely differentiated epidermal layer grown on a fibroblast-containing bovine collagen matrix. Analysis of the lipid content of this FT model showed that all the lipid classes found in human skin were also present in the skin model. The lipid composition differed, however, from that of native human skin. The most significance difference was the high amount of triglyceride (55.9 ± 4.65% total lipid weight) compared with human stratum corneum (0 – 3% of total lipid weight), which could possibly be attributed to the culture conditions used during the cultivation of reconstructed skins [63].

With regard to ceramide profiles, polar ceramides such as 6I and 6II were underrepresented whereas the nonpolar ceramides were overrepresented in the FT model. The lack

of polar ceramides has been reported in other skin models as well [46], however the exact mechanism leading to a reduction or inhibition of those ceramides is unclear at present.

To investigate further whether the skin model could be a suitable *in vitro* model for transdermal penetration studies, Asbill *et al.* [62] evaluated the drug permeation of caffeine (log P = -0.07 ± 0.35), tamoxifen (log P = 7.85 ± 0.75) and hydrocortisone (log P = 1.43 ± 0.47) in this model and compared the measured permeation parameters with those obtained in the other skin models. For all three tested drugs, the reconstructed epidermal model EpiDerm had a 10 times higher permeability as compared with human cadaver skin, whereas the permeability of the FT model was only 3 – 4 times higher than that of human cadaver skin. These results suggested that the FT model is a more acceptable model for *in vitro* percutaneous drug permeability testing than EpiDerm. The difference in permeability of this FT model might be attributed to the differences in the lipid profile, leading to increased barrier properties. Of interest is the fact that the FT model was established in culture conditions slightly different (lowering of the relative humidity from 90 to 75% and omission of serum before culturing at the air–liquid interface) from those described previously for the culturing of reconstructed skin models. This suggests that culture conditions could influence to a certain extent the intercellular lipid matrix organization and influence, in part, the degree of barrier function [45].

4.3.3 Dermal compartment and artificial membranes

Permeation studies are often performed by using reconstructed epidermal models only. During the last few years, however, new commercially available FT models were introduced into the market as these models more closely match the *in vivo* situation. Besides EpiSkin and EpiDermFT, the Phenion® Full-Thickness (Henkel, Düsseldorf, Germany) is another example of a skin model containing both a dermis and an epidermis [64]. The barrier function of this skin model was recently compared against previously tested skin models [51] and pig skin by studying the permeation and lag times of four standard compounds. These tests revealed that the Phenion FT model had a slightly weaker barrier against benzoic acid, nicotine and caffeine as compared with EpiDerm, SkinEthic and EpiSkin, whereas the permeation of the highly lipophilic compound testosterone was more efficiently retarded in this specific model.

The lag times of the Phenion FT model were shorter as compared with pig skin, which is in accordance with previous studies using other reconstructed skin models. Interestingly, except for testosterone, lag times of the Phenion FT model were shorter than lag times observed previously with the EpiSkin model. This difference could be explained by the structure of the reconstructed model. The dermis of the Phenion FT model is built up from a bioartificial scaffold that contains fibroblasts embedded into loosely packed newly synthesized extracellular matrix fibers, whereas in EpiSkin the dermal compartment consists of a densely packed collagen layer. Lag times were even shorter in the epidermal models EpiDerm and SkinEthic, where keratinocytes are grown on a synthetic supporting membrane [51]. These data assume that the lack of a dermis *in vitro* might result in a higher transport of lipophilic compounds through the skin than is actually the case *in vivo*. Except for testosterone, the lag times of the compounds studied seemed to depend on the different types of reconstructed skin model used. Similar results were also reported by Schäfer-Korting *et al.* [65], who studied the permeation of steroids in artificial membranes (polycarbonate and cellulose nitrate) and biological ones (human skin and reconstructs). Analysis showed that the steroids were taken up very rapidly by both membranes and no significant lag times were observed. This was in contrast to the other tested skin samples, for which significant lag times were obtained.

In the same study, different hydrocortisone formulations were applied onto artificial membranes in order to study their permeation ranking. Hydrocortisone release was ranked in the following way: w/o emulsion > o/w emulsion > ethanol/miglyol solution, when using the cellulose membrane, whereas differences between the emulsions were less obvious when applied onto the polycarbonate membrane. When compared with human skin, a clearly different hydrocortisone permeation ranking was observed: ethanol/miglyol solution > w/o emulsion > o/w emulsion [65] (see also Section 4.3.4). Other synthetic membranes used as models for human skin are homogeneous permeable polymers such as silicone (polydimethylsiloxane) membranes. Several studies have already described how silicone membranes are useful tools to study the permeation of drugs such as estradiol [66,67] and ibuprofen [68], or to investigate formulation effects provided that permeation enhancers which interact with skin lipids are not present [69,70]. Very few reports, however, have evaluated the permeability of different compounds through silicone membranes. One study by Geinoz *et al.* [71] reported mathematical and experimental data that indicated that the permeability ranking of selected compounds (phenol derivates, lidocaine and nicotine) was comparable through silicone membranes and human epidermis. The authors concluded that silicone membranes may be useful trend-predictive models for skin permeation. In addition, silicone membranes may also be used to predict skin concentration of parabens, active ingredients in cosmetics and topical pharmaceutical formulations [72].

4.3.4 Vehicle composition/formulation effects

In addition to the physicochemical properties of the drug, the vehicle-dependent effects on the penetration behavior of drugs following topical application are well known from the literature. In the past, only a few studies were reported that examined the effect of vehicles on the percutaneous penetration in human skin models. Apart from one study that compared a simple o/w emulsion versus a w/o/w multiple emulsion

containing caffeine applied at an infinite dose on different *in vitro* models [73], most reports relate to the influence on percutaneous absorption of simple vehicles such as water or petrolatum [74,75].

Zghoul *et al.* [76] studied the permeability of EpiDerm by using a non-steroidal anti-inflammatory drug, flufenamic acid. This lipohilic drug was applied in two different formulations (wool alcohol ointment (0.1125%) and Soerensen phosphate buffer pH 7.4 (0.1125% solution)) onto the skin surface and permeability studies were performed by use of Franz diffusion cells. Flux measurements indicated that the penetration rate for the aqueous solution was 40 times higher as compared with the ointment. This means that the skin equivalent can build-up a permeation barrier and can differentiate the permeation of drug preparations such as ointment and solution. This is a prerequisite for using it as an *in vitro* test model. Also, the permeability of the EpiDerm model was compared with human epidermis by applying flufenamic acid in the ointment formulation. The results revealed a five times higher flux value for the reconstructed epidermis. Despite this higher permeability the authors concluded that the EpidDerm model could be useful as a test system to study dermal or transdermal drug transport after topical application of pharmaceutical formulations.

In 2002, the influence of cosmetic vehicles on the bioavailability of caffeine and α-tocopherol was evaluated in two skin models (EpiDerm and EpiSkin) and excised human skin by Dreher *et al.* [77]. The two active compounds were formulated into different cosmetic vehicles such as o/w emulsion, a w/o emulsion, an alcohol-containing hydrogel, or a liposome dispersion and were subsequently applied at finite dose on the surface of the skin. Dreher *et al.* [77] found that, irrespective of the formulation, caffeine permeated human *ex vivo* skin much better than α-tocopherol. In general, caffeine permeated all tested skin samples much better than α-tocopherol, pointing to a similar rank order of solute permeability between the skin models and human skin, at least for compounds with far different physicochemical properties.

For *ex vivo* human skin, no significant differences in skin bioavailability were observed between the preparations for either solute, except for the α-tocopherol hydrogel. *Ex vivo* human skin showed an increased epidermal uptake. This is in contrast to its bioavailability in human skin models. In these models, α-tocopherol absorption from the hydrogel into EpiDerm or EpiSkin was similar or even lower as compared with the other α-tocopherol-containing preparations. An explanation might be related to differences in skin surface properties, such as the elevated hydration of the outermost layers of the stratum corneum in human skin models compared with human skin [73], which might create an artificial penetration barrier for lipophilic compounds. In conclusion, with the exception of hydrogel, similar rank orders of caffeine and α-tocopherol vehicle formulations were observed for *ex vivo* human skin, EpiDerm and EpiSkin. However, it should be noted that the differences in permeation rates for a selected solute are small and that the rank orders only reflect tendencies.

In a relative recent study by Schäfer-Korting *et al.* [65], it was investigated whether the reconstructed epidermal model SkinEthic and the EpiDerm full-thickness model can be used to predict uptake from different vehicle formulations. The permeation of hydrocortisone and testosterone was compared, when both were applied in a w/o or o/w emulsion form or as a solution containing ethanol/miglyol (E/M). Human and pig skin were used as references. In general, there was a more rapid hydrocortisone release from all the preparations as compared with testosterone release, which favors hydrocortisone uptake by the skin. A clearly different formulation dependency of hydrocortisone permeation was observed for all of the models tested, where the permeation was ranked as follows: E/M solution > w/o emulsion > o/w emulsion. Possibly, the well-known penetration-enhancing effect of ethanol caused an increase in permeation values for the hydrocortisone solutions. For testosterone, release data were similar for each of the formulations and ethanol did not enhance testosterone penetration to a significant extent. The authors concluded that for both drugs and all formulations tested, the permeability of the reconstructed tissues and the pig skin was closely correlated with permeation through human skin and that the vehicle formulation used does have an effect on permeation through reconstructed skin models. The rank order was the same in all models tested; only the absolute permeability differed.

4.3.5 *Metabolic activity*

Ideally, skin models should predict not only permeation and penetration but also drug metabolism, which is of particular importance for intensely metabolized drugs. The enzymatic activity in the skin influences the efficiency and safety of transdermal delivery [78]. SkinEthic was one of the first commercial models to be used in drug penetration studies. In 1999, the penetration and metabolic activity of two glucocorticoids, namely prednicarbate (i.e., prednisolone diester) and fluorinated monoester betamethasone 17-valerate, were examined following topical application of these compounds on the SkinEthic model [79]. It was shown that the esterase activity in the reconstructed skin model correlated to that of human skin, indicating that the metabolism of glucocorticoids is well reflected in the SkinEthic model. A similar conclusion was drawn from results of a more recent study in which Lombardi Borgia *et al.* [80] studied different prednicarbate preparations in the EpiDerm model.

Mahmoud *et al.* [81] investigated the metabolism of estradiol in reconstructed epidermis (SkinEthic) and found that the metabolization of 17β-estradiol to estrone by 17β-hydroxysteroid dehydrogenase was similar to human skin. The metabolism of testosterone was also investigated on an epidermal reconstructed model and revealed similar polar and nonpolar metabolites as in normal human skin [82]. Therefore, most authors conclude that reconstructed

skin models are useful and reproductive models representing adequately the contribution of metabolism to drug penetration [82]. Although the drug metabolism in reconstructed skin models seems comparable to the *in vivo* situation, the enzymatic conversion of drugs is highly dependent on the culture conditions. As the use of 5% BSA is already toxic for reconstructed epidermis and inhibits the metabolism of drugs, serum-free media are preferred [83]. An epidermal reconstructed skin is generally an appropriate model for investigating the enzymatic conversion of drugs, although in the case that the metabolization of an applied compound is affected by the dermis or dermal fibroblast/epidermal interactions, full-thickness models may be better [84,85].

4.3.6 Reproducibility

The reproducibility of skin models is a crucial factor for accurate permeation experiments. Lotte *et al.* [86] investigated the reproducibility of EpiDerm, EpiSkin and SkinEthic in a percutaneous permeation/absorption experiment. Therefore, three test compounds with different hydrophilic characteristics were chosen: lauric acid, mannitol and caffeine. As could be expected, inter-batch variation was higher than intra-batch variation. EpiDerm and SkinEthic showed better reproducibility compared with EpiSkin. Mannitol, a highly hydrophilic agent with a limited permeation capacity, showed the most variation [86]. In general, the variability of reconstructed skin permeation experiments seems lower than experiments with pig skin and *ex vivo* human skin. This reduced variability could not be confirmed significantly in the validation study of Schäfer-Korting *et al.* [51], in which nine different substances were taken into account (see **Table 2**). Therefore, there is no evidence at present to reduce the number of experiments needed to adjust for variability in reconstructed skin experiments [50,51]. The results of experiments performed in different laboratories can be compared as the intra-laboratory variation is similar to inter-laboratory differences [50]. In conclusion, reconstructed skin has a similar reproducibility as other penetration models. The permeation characteristics of the applied compound (such as lipophilicity and molecular mass > 500 Da) and inter-batch variation are the two main factors that have to be taken into account.

5. Conclusion

Percutaneous absorption studies are necessary in the development of drugs for dermal or transdermal application. During the last decade a lot of new information has been gathered with regard to the development and use of reconstructed skin models for drug permeability testing. The most frequently used models for this type of study appear to be the commercially available SkinEthic, EpiDerm and EpiSkin models because they are well characterized with regard to morphological appearance, lipid composition and expression of biochemical markers. All reported permeability studies formulated a similar conclusion, namely that the skin models are more permeable than human skin. These results could be explained by the presence of a reduced barrier function in all these models. Despite this disadvantage, reconstructed skins appear to be elegant tools to predict the relative permeability of specific compounds, as illustrated by the fact that the ranking of compounds was similar as compared with human skin. A future challenge lies in the development of models that approach even more the *in vivo* situation and to standardize methodology for pharmaceutical and cosmetic industries.

6. Expert opinion

In vitro methods for measuring skin absorption and (trans) dermal drug delivery have been used intensively during the past decade and have some advantages over the use of live animals (*in vivo* method). Besides the ability to use skin from many mammalian species including humans, several replicate measurements can be made from the same or some different donors, exposure conditions can easily be controlled, and a wider range of physical forms can be applied and studied *in vitro*. Excised human skin is the most relevant and preferable *in vitro* method to evaluate the penetration capacity of applied compounds. Researchers are, however, often confronted with a limited supply of human skin and relatively high donor variability. For regulatory and practical reasons animal skin, usually from rat or pigs, is used instead, although it is well known that animal skin is quite different from human skin with respect to the number of hair follicles, lipid composition, lipid content and morphological appearance. Nevertheless, the use of pig skin is well established in *in vitro* testing because of its similarity to human skin in terms of its morphology and permeability characteristics. However, in the future, animal experiments will be abandoned and new alternatives are needed. Several human epidermal reconstructs or FT skin models have been tested and used to measure percutaneous absorption. As illustrated in this review, several studies examined their potential or suitability for transdermal drug delivery testing. The most frequently used models in these studies were the commercial models, EpiSkin, EpiDerm and SkinEthic. However, different study designs, different panels of test substances, specific vesicle compositions or experimental set ups made comparison of published data very difficult, often leading to contradictory conclusions concerning the suitability of these models for *in vitro* penetration studies. To circumvent these problems a highly standardized protocol was introduced recently by Schreiber *et al.* [48] and used in a formal validation study [51]. From the latter study it was concluded that EpiSkin, EpiDerm and SkinEthic are suitable alternatives to human and pig skin for *in vitro* assessment of the permeation and penetration of substances when applied as aqueous solutions.

Despite this general positive conclusion, the use of reconstructed skin models for *in vitro* penetration studies is associated with specific limitations. All permeation studies reported on the common finding that reconstructed skin models are clearly more permeable than human excised skin or pig skin. This property of reconstructed skins was mainly attributed to the fact that their lipid composition differs slightly from normal skin, resulting in lower barrier properties. It has indeed been reported that skin lipids play an important role in skin barrier function. For the proper formation of the lipid lamellae, the presence of the major stratum corneum lipid classes (cholesterol, ceramides and free fatty acids) is necessary. Lipid analysis revealed that the major barrier lipids are synthesized *in vitro* but not in the same proportions as found in native skin [87]. In the EpiDerm, SkinEthic and EpiSkin models the content of the free fatty acids was low and the ceramide profiles were incomplete as compared with human native skin [46]. It is tempting to speculate that these differences may account for the different lipid lamellar organization in reconstructed skins. The exact mechanism of action is, however, unclear at this moment. Also, the lateral packing in native stratum corneum lipids seems different from that in reconstructed stratum corneum (orthorhombic versus hexagonal), causing an impaired barrier function [88,89]. The lack of desquamation in reconstructed skin models, leading to a lower proportion of phospholipids in the viable epidermis and a higher amount of stratum corneum ceramides, has also been suggested as a possible reason to explain the weak barrier function of reconstructed skin models [90,91]. The take home message is that each reconstructed skin model has a different degree of barrier function, mainly resulting from a different lipid composition, which might influence the permeation behavior of drugs differently [50,51].

The overestimated permeability, as a result of the weaker barrier properties of reconstructed epidermis, does not necessarily have to mean that these *in vitro* models are not useful for studying drug permeability. Most studies that have tested the permeability of compounds with different physicochemical properties report that the rank order of compound permeation through reconstructed skins reflects the permeation of human epidermis. Therefore, these models are, to some extent, useful tools to predict the relative permeability of specific compounds.

Moreover, some trivial advantages are summarized in the following list, pointing out why reconstructed skin models may be good alternatives for viable human skin and animal skin.

- Ready-to-use, allowing a quick and easy testing of a large panel of drugs.
- Data reproducibility.
- Lag time = short and constant, allowing uptake experiments to be performed within 6 – 8 h.
- Testing of infinite as well as finite doses.
- Useful in the evaluation of skin metabolism.
- Useful to study the effect of vesicles on permeation/penetration behavior of drugs.

Despite these advantages and the remarkable step forward in the development of reconstructed skin models, it has to be concluded at this moment that they cannot fully replace human or animal skin for the prediction of skin absorption *in vivo*. The overestimated permeability limits at present the value of these models for transport studies and may lead to some false positive results in toxicity studies, which are often observed when using skin of furry animals as well. In addition, the overall use of reconstructed skins is still limited because of their costs. Permeation studies including commercial reconstructed skin models are more expensive at the moment (at least double in price) than when using hairless SKH-1 mice, for example. Research expenses are also much higher when working with reconstructed skin models instead of pig skin or excised human skin. Porcine skin can be obtained from abattoirs and human skin is also costless but is not always easily available. In addition, ethical committee approvals are required to get the necessary permission to work with human or animal skin. This is not the case when using reconstructed skin models.

A major challenge in the further development of human reconstructed skins for drug delivery studies is to establish models that have similar lipid compositions and permeability characteristics to those of human skin. One way to achieve this could be the modification or improvement of culture conditions. A reduction in humidity, for example, might increase the growth and differentiation of keratinocytes and create a pH gradient within the stratum corneum, which may in its turn modulate the stratum corneum microenvironment. This might lead to the activation of specific enzymes required for desquamation and the catabolism of some lipid classes may be stimulated, thereby approaching native human skin more closely. Whether this goal will be achieved in the near future is unclear at present. In the authors' opinion, this will be extremely difficult and time-consuming because each commercially available reconstructed skin model (and in-house skin models as well) is grown under different culture conditions. The possible effect of each small change to the medium or culturing conditions in general will have to be checked carefully and examined by multiple techniques. A completely standardized protocol for growing reconstructed skins is unfortunately lacking and depends also in great part on the use or not of a dermal matrix. Moreover, the use of primary skin cells induces a donor-to-donor variation, making the culturing process even more complex. Using immortalized cell lines such as the human keratinocyte cell line HaCaT to establish reconstructed skins is not an option yet as this results in the formation of an incompletely differentiated epidermis [92,93]. The presence of an impaired barrier function in normal reconstructed skins could, however, be an advantage when studying the delivery or penetration of drugs involved in psoriasis.

Another point of interest that needs further investigation or clarification is the role of the dermal compartment in drug permeation studies. Some studies reported that the dermis or dermal equivalent of reconstructed skin models can also be considered, similar to native human skin, as an extra barrier for lipophilic compounds [94], whereas others stated that only the stratum corneum is the main barrier for penetration [48,54]. The lack of a dermis *in vitro* could result in a higher transport *in vitro* that could not correspond to the amount actually encountered *in vivo*. On the other hand, the permeation of highly lipophilic compounds (such as testosterone) is more retarded by an artificial dermis *in vitro* as it does not contain subcutaneous vasculatures and its environment is more aqueous as compared with the *in vivo* situation. During the last few years several FT models have become more available on the market. Standardized test protocols are now being transferred to these FT models in order to validate their usefulness for percutaneous absorption testing. It is certainly of interest to compare the permeation of drugs in both epidermal and FT models, as already done in some published reports [62,64].

In conclusion, the development and use of reconstructed skin models has expanded enormously during the last decade, and they are already accepted as valuable alternatives to animal skin for testing skin corrosion and phototoxicity. At present, however, it is still questionable whether reconstructed skin models will ever be able to replace fully animal or human skin in drug permeation studies. Undoubtedly, their barrier function should be much more improved. Ideally, their architecture and lipid composition should mimic completely human native skin. Whether this goal will be achieved in the near future is as yet uncertain. Even tissue-engineered skin produced from autologous keratinocytes, fibroblasts and human acellular donor dermis, which has already passed through clinical evaluation, does not seem to develop a barrier function identical to *in vivo* skin, and probably never will [95]. Nevertheless, reconstructed skin models have been proved to be suitable tools to estimate the rank order of percutaneous absorption of a series of compounds, despite their limitations.

Declaration of interest

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