In vitro skin models as a tool in optimization of drug formulation

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Abstract

(Trans)dermal drug therapy is gaining increasing importance in the modern drug development. To fully utilize the potential of this route, it is important to optimize the delivery of active ingredient/drug into/through the skin. The optimal carrier/vehicle can enhance the desired outcome of the therapy therefore the optimization of skin formulations is often included in the early stages of the product development. A rational approach in designing and optimizing skin formulations requires well-defined skin models, able to identify and evaluate the intrinsic properties of the formulation. Most of the current optimization relies on the use of suitable *ex vivo* animal/human models. However, increasing restrictions in use and handling of animals and human skin stimulated the search for suitable artificial skin models. This review attempts to provide an unbiased overview of the most commonly used models, with emphasis on their limitations and advantages. The choice of the most applicable *in vitro* model for the particular purpose should be based on the interplay between the availability, easiness of the use, cost and the respective limitations.

Key words: *in vitro* permeation models; human skin; animal skin; topical formulations; skin therapy

List of Abbreviations

BUS, perfused bovine udder skin;
D, dermatomed skin (split-thickness skin);
FT, full-thickness skin;
HD, hypodermis;
HSE, heat-separated epidermis;
ISC, bromide solution-isolated *stratum corneum*;
MSE, mechanical separation of epidermis;
NHKs, normal human keratinocytes;
NPs, nanoparticles;
O/W, oil-in-water emulsion;
PAMPA, parallel artificial membrane permeability assay;

PDMS, poly(dimethylsiloxane);

PEG, poly(ethylene glycol);

PVPA, phospholipid vesicle-based permeation assay;

$PVPA_C$, PVPA with egg phosphatidylcholine and cholesterol in the barrier;

$PVPA_S$, PVPA with egg phosphatidylcholine, ceramide, cholesterol, cholesteryl sulfate and palmitic acid in the barrier;

SC, *stratum corneum*;

SCS, *stratum corneum* substitute;

SLNs, solid lipid nanoparticles;

SKALP, skin derived anti-leukoproteinase;

SPRR, small proline rich-protein

TISC, trypsin-isolated *stratum corneum*;

W/O, water-in-oil emulsion.

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1. Introduction

Skin-targeted drug delivery remains challenging despite the fact that skin offers readily accessible surface area for potential drug absorption (Lam and Gambari, 2014).

A rational approach for designing and optimizing skin formulations destined for either topical or systemic drug actions requires well-defined skin models, able to identify and evaluate the intrinsic properties of the formulation. Once the key formulation features contributing to the drug penetration (desired or undesired) are determined, the optimization of the formulation becomes feasible.

The use of animal models has for a long period of time been the main approach in the preclinical development of both the new drugs and optimization of drug formulations destined for administration onto the skin (Semlin et al., 2011). Due to the recent regulatory changes regarding animal testing in the field of cosmetics, the *in vitro* models are receiving more attention as an important tool in the permeation, penetration and skin irritancy studies. Human skin equivalents are increasingly used as an addition, or more commonly, alternative to the extensive use of animals. The original approach was based on the use of normal human keratinocytes (NHKs) proliferating and differentiating on de-epidermized dermis (so-called living skin equivalent) as a model for skin irritancy (Ponec, 1992). The model was improved through using the supporting membranes to grow the NHKs into the reconstructed human epidermis. The full utilization of this approach was achieved when the models become commercially available such as the models representing the human epidermis (EpiSkin®, EpiDerm®, SkinEthic®) or full-thickness skin (Phenion®) (Van Gele et al., 2011). More advanced models represent the full-thickness skin based on the fibroblast populated collagen matrices (dermis equivalent) and an epidermal overlay representing NHKs (Semlin et al., 2011).

Although these skin models frequently exhibit much higher permeability than the human and animal skin (Henning et al., 2009), the models can be applied for the screening purposes in the early phases of drug and drug formulation development (Mathes et al., 2014). To simplify the prediction of skin permeability, several *in vitro* models have been proposed such as the silicone membranes (Oliveira et al., 2010), the ceramide-derived parallel artificial membrane permeability assay (PAMPA) (Sinko et al., 2012) and the phospholipid vesicle-based permeation assay (PVPA) (Engesland et al., 2013).

The objectives of this review are to provide non-biased overview of the currently available *in vitro* models with potential to be used in the topical formulation development. We have avoided the models widely used in the skin irritancy and toxicity studies as the main focus was on the formulation optimization. For more details on those models, the reader is referred to Ponec (1992), Kimura et al. (2012), Netzlaf et al. (2005) and Schaefer et al. (2008). To further narrow the focus of this review we have also excluded the specific skin disease models based on the human skin equivalents and the readers are referred to the extensive overviews by Groeber et al. (2011), Semlin et al. (2011) and Mathes et al. (2014) regarding these models.

2. Skin and skin barrier function

The numerous reviews provide details on the anatomy and physiology of the skin, particularly the barrier properties of the skin (Bouwstra et al., 2003; Bouwstra and Ponec, 2006; Groen et al., 2011; Hadgraft, 2001; Jepps et al., 2013; Mathes et al., 2014; Van Gele at al., 2011;). The *stratum corneum* (SC) is the main contributor to the barrier properties of the skin (Baroni et al., 2012; Menon et al., 2012); however the role of the full epidermis should not be neglected (Andrews et al., 2013). Only the molecule able to deposit onto SC, diffuse through living epidermis and pass through the upper part of the papillary dermis has the potential to reach circulation and exhibit systemic effects. Drug transport through the skin can take place through the epidermal penetration pathway or through skin appendages; for more details the readers are referred to for example Bolzinger and colleagues (2012) and Schaefer et al. (2008). The mode of transport is predominantly by passive diffusion, therefore, the nonviable skin models could mimic this process rather adequately.

One feature that is rather difficult to mimic in any *in vitro* modelling is the metabolism which may occur particularly in the living parts of epidermis and significantly reduce bioavailability of the molecule administered onto the skin and destined for systemic action. From biopharmaceutical perspective, the residence time of a molecule in dermis is rather limited as it is often in the order of a minute (Souto, 2005). Moreover, the dermis includes permeable capillaries driving the molecule to microcirculation upon exiting

epidermis. The deeper layers of the dermis are not significantly influencing possible percutaneous absorption. For a very lipophilic drug, which can overcome the SC barrier, the aqueous interface beneath horny layer will be a serious obstacle to penetration. The rate-determining step will be, in that case, the clearance from the barrier rather than the penetration of the barrier. In dermatopharmacokinetics, the permeation of drugs through the skin is often presented as an infinite sink. The strong skin barrier slows the drug penetration rates, limits drug uptake and contributes to lack of the dosing precision (Lam and Gambari, 2014).The final penetration potential of the drug (penetrant) can be increased (in the case of transdermal therapy) or limited (dermal therapy) by the right choice of a carrier/vehicle. The carrier/vehicle and penetrant interactions are of a great interest in the drug and formulation development. Four physicochemical parameters of the drug, namely molar mass, number of hydrogen-bond donors and the number of hydrogen-bond acceptors, as well as the octanol-water partition coefficient, determine the skin penetration potential of the drug (Bolzinger et al., 2012). Therefore, the characteristics of a penetrant such as its partitioning into skin, diffusivity through the skin and exposure at the skin surface are identified as the major contributors to the permeability of a penetrant. The vehicle can directly affect the mentioned characteristics and improve their limitations (Chittenden, et al., 2014).

3. The effect of carrier/vehicle on the skin penetration

The effectiveness and acceptability of skin formulations are directly related to the properties of the carrier/vehicle used. The permeability of the drug through the various layers of the skin is modulated by the constituents of the carrier/vehicle. The term percutaneous/dermal absorption is often used to describe the passage of various compounds across the skin. The penetration refers to the entry of a substance into a particular skin layer; the permeation can be seen as the penetration through one layer into another, and the uptake into the vascular system is referred to as dermal absorption (Bolzinger et al., 2012). The drug detected in the living parts of the epidermis indicates the percutaneous absorption. Understanding the permeation of hydrophilic molecules is important not only for the potential transdermal delivery, but also for the cosmetics and

occupational exposure. The interactions between the vehicle, skin and drug determine the release of a drug, its penetration through the SC, permeation through the skin layers, followed by the drug deposition inside the skin or the absorption into systemic circulation (Daniels and Knie, 2007). A vehicle can change the physical state and permeability of the skin by the hydration effect or an alteration of the skin temperature. Occlusive and lipophilic vehicles such as paraffin, fats and oils reduce water loss, increase the moisture content in the skin and thus promote the drug penetration. The water-in-oil (W/O) emulsions are less occlusive than the lipid materials, but more occlusive than the oil-in-water (O/W) emulsions. On the contrary, hydrogels do not have occlusive effect but due to the high water content may improve the hydration level of the skin. For example, Stahl et al. (2011) have shown the rapid increase of ibuprofen permeability from the gel formulation within the first 4 hours, followed by a deposition of the drug inside the skin. In comparison, W/O emulsion demonstrated longer lag time than hydrogel, but enabled transdermal delivery of ibuprofen. Penetration enhancers such as ethanol, propylene glycol and oleic acid, which are commonly used as humectants or parts of an oilly or aqueous phase of the formulation, can accelerate the penetration of many substances into/through the skin. Moreover, these substances and the others like phospholipids, terpenes, non-ionic surfactants, are fundamental ingredients of different vesicular drug delivery systems used to increase the skin delivery of the drug such as liposomes, ethosomes, propylene glycol liposomes, invasomes, niosomes, and microemulsions (Dragicevic-Curic et al., 2008; Tavano et al., 2011). Nanoparticles able to pass via hair follicles may enhance drug percutaneous absorption (Raber et al., 2014). Other important parameters affecting the penetration of drug into/across the skin include the size and surface properties (lipophilicity, surface charge) of the drug carriers, which similarly as the physicochemical properties of drug influence the delivery into/through the skin. In the case of vesicle-based nanosystems it has been demonstrated that decreasing the system's size improves the delivery of a drug into/across the skin (Elsayad et al., 2007). On the contrary, microparticles and large vesicles are usually deposited on the surface of the skin acting as the drug reservoirs providing the prolonged drug release. Negative charges on the surface of liposomes have been shown to enhance the skin penetration of a drug to a greater extent than positively charged and neutral liposomes (Gillet et al., 2011a). In

addition, rigidity/elasticity of vesicle's membranes has been proven to influence the deposition of the drug onto/inside the skin. Conventional liposomes and especially polyethylene glycol (PEG)-coated liposomes with rigid membranes have been demonstrated to accumulate drug inside SC (Knudsen et al., 2012). Deformable (elastic) liposomes containing edge activators (single chain surfactants) in the bilayers were proposed as a nanosized delivery system able to squeeze between narrow pores and transport encapsulated drugs deeper into/through the skin (Cevc, 2004; Cevc and Blume 1992; Cevc and Blume, 2001). Although the evidences that the vesicles indeed penetrated in an intact form through the skin remain a topic for discussion, the deformable vesicles have evolved from the first, second (Transferosomes®) into the third generation of hyperadaptable vesicles for improved peripheral skin conditions, inflammation and pain (Cevc, 2012).

The surfactant properties (i.e., charge, carbon chain length and content of surfactant) are known to directly affect the skin permeability of meloxicam when formulated as deformable liposomes (Duangjit et al., 2014). Alomrani et al. (2014) proved the effect of the edge activators and hydroxypropyl-beta-cyclodextrine on the skin disposition of itraconazole. The synergistic effects of the edge activators reducing the vesicle size and increasing the deformability of liposomes together with the increased drug load due to the presence of cyclodextrine were responsible for the increased skin deposition.

Most of the pharmaceutical and cosmeceutical vehicles are the complex mixtures containing more than just one component, therefore the combination of components may have cumulative or synergistic effects on the skin penetration (Chittenden et al., 2014).

Prediction of a penetration of a drug/active ingredient into/through the skin can be modelled by various means. For example the mathematical models of skin permeability offer a potential to determine the key parameters affecting the skin permeability (Mitragotri et al., 2011). The overview of numerical methods for diffusion-based modelling of skin permeation is provided by Frasch and Barbero (2013) and Naegel and co-workers (2013).

The most commonly applied mathematical models are based on the quantitative structure-permeability relationship and mechanistic models (Chen et al., 2013; Lee et al.,

2010). However, to determine the effect of the carrier/vehicle, more complex models, closer to *in vivo* situations, are required.

4. **Artificial *in vitro* skin models**

Most of the current methods used in the investigation of formulations destined for topical treatment, as mentioned earlier, rely on the use of animal models. The use of isolated epidermis or SC sheets from human or animal origin has a number of disadvantages such as high intra- and inter-individual variations, in particular related to diseased skin for which most of the topical drug formulations are developed. The simplified artificial model membranes that could offer a simple and reproducible alternative to study the basic physicochemical mechanisms of drug permeation are preferred to understand the physicochemical determinants of vehicle-membrane interactions crucial for the selection of the optimal penetration enhancers and effective formulation design (Oliveira et al., 2011; Oliveira et al., 2012). The artificial model membranes are thus enabling the testing and evaluation of various drugs and drug formulations as an initial screening approach to narrow the selection of the formulations to be evaluated with a more biologically intact model, thereby improving the efficiency in the optimization of new topical formulations.

The majority of the artificial models are used to mimic the healthy skin with intact barrier properties. Relatively few models offer a potential to mimic the compromised skin. The models are relying either on the various types of the diffusion cells or phospholipid mixture models (de Jagar et al., 2006). In the last several years attempts were made to develop reliable *in vitro* skin models such applying various approaches such as relying on the chromatographic methods (Hidalgo-Rodriguez et al., 2013), non-lipid based models i.e. silicone membranes (Loftsson et al., 2006; Oliveira et al., 2010; Oliveira et al., 2011; Ottaviani et al., 2006), as well as the lipid-based models able to serve as a tool in predicting the desired or undesired dermal absorption.
Among the lipid-based models the focus has been on the:

i) modifications of PAMPA able to mimic the skin (Sinko et al., 2009; Sinko et al., 2012),
ii) PVPA model mimicking the SC barrier of the skin (Engesland et al., 2013; Engesland et al., 2015).
iii) SC substitute (SCS) with synthetic SC lipids (de Jager et al., 2006) and
iv) membranes designed to study the impact of ceramide species on the drug diffusion and permeation (Ochalek et al., 2012a).

The chromatographic systems could, theoretically, also be used in the estimation of the permeability coefficients of different substances to study the main interactions between the substances and biological membranes (Hidalgo-Rodriguez et al., 2013). The systems include the immobilized artificial membrane (Barbato et al., 1998), biopartitioning micellar chromatography (Escuder-Gilabert et al., 2003; Waters et al., 2013), liposome electrokinetic chromatography (Wang et al., 2009), and keratin immobilized on the silica as a stationary phase (Turowski and Kaliszan, 1997). However, in respect to the optimization of the formulation destined for the administration onto the skin, the effects of various additives and carriers/vehicles on the drug penetration, these chromatographic systems exhibit a very limited potential and are therefore not further discussed in this review.

4.1. Non-lipid based model membranes

4.1.1. Silicone model membranes

The poly(dimethylsiloxane) (PDMS) or silicone membranes have been used for decades for the screening of the effects of different vehicles and assessing their impact on the overall mechanisms of drug transport across human skin (Oliveira et al., 2011). Already in 1970 Nakano and Patel used the silicone membranes to study the release of salicylic acid from five ointment bases. The *in vitro* release pattern from various bases was found to be in an agreement with the *in vivo* data reported in the literature, enabling the identification of the most promising ointment bases (Nakano and Patel, 1970). Several studies on the effect of various additives on the skin penetration of the drug have been further performed. Dias et al. (2007) conducted a study on a wide selection of vehicles

(mineral oil, isopropyl myristate, oleic acid, decanol, octanol, butanol, ethanol, propylene glycol, glycerin and water as well as their mixtures) on the permeation of caffeine, salicylic acid and benzoic acid. For example, the effect of both hydrophilic and lipophilic vehicles (water, ethanol, propylene glycol, mineral oil, Miglyol 812) on the penetration of ibuprofen has been studied (Watkinson et al., 2009a; Watkinson et al., 2009b; Watkinson et al., 2011). The studies on the molecular mechanism of interaction between different vehicles (ethanol, isopropyl myristate, dimethyl isosorbide, PEG 200 and PEG 400 and Transcutol P®) with the model membranes, through the thermodynamic and kinetic analyses of the uptake, membrane partitioning and transport studies of a model compound, have also been conducted (Oliveira et al., 2010; Oliveira et al., 2011; Oliveira et al., 2012).

Although these membranes could be used to predict the skin permeability of the lipophilic compounds, it has been suggested that they could not be used for the hydrophilic compounds (Miki et al., 2015). To improve the model the PDMS and PEG 6000 copolymer-impregnated membrane has been developed. The improved model has so far only been tested using the drugs in the aqueous solutions (Miki et al., 2015; its potential in the formulation development still has to be elucidated.

4.1.2. Miscellaneous

Ottaviani et al. (2006) reported on a PAMPA model consisting of a simple pure solvent membrane, composed of 70% silicone oil and 30% isopropyl myristate coated on a hydrophobic polyvinylidene fluoride filter. The model was shown to be able to determine the human skin permeation for a selection of model drugs in accordance to the available literature values. A positive correlation was also established between the membrane retention of the compounds and SC/water partition coefficients (Ottaviani et al., 2006). This model has been further used in the combination with the *in silico* methods in a combined approach to predict the skin penetration and distribution of model substances (Ottaviani et al., 2007). In addition, the model has been proposed as a suitable for the differentiating of the highly permeable compounds, able to distinct between the compounds trapped in the barrier and compounds not retained in the barrier. The capacity of the detection of a substance retained in the barrier mimicking the SC could be useful to

assess the effects of the formulations (Ottaviani et al., 2006). Further, the model has been used in the initial lead compound selection of the synthesized steroids and standard corticosteroids (Dobricic et al., 2014; Markovic et al., 2012), as well as in studying the effect of the vehicles on the permeation of drugs (Karadzovska and Riviere, 2013). Karadzovska et al. (2013) tested a selection of drugs (caffeine, cortisone, diclofenac sodium, mannitol, salicylic acid and testosterone) in three different vehicles (propylene glycol, water and ethanol). The permeability data from this model were compared with the data from the porcine skin in the diffusion experiments. The non-lipid containing PAMPA model showed to perform better than a lipid containing skin-PAMPA model and another synthetic membrane, namely the Strat-M^{TM} membrane. The Strat-M^{TM} is composed of multiple layers of polyether sulfone creating the morphology similar to human skin, including a very tight surface layer, and was recently made commercially available. Its porous structure could be also impregnated with a proprietary blend of the synthetic lipids, imparting the additional skin-like properties to the synthetic membrane (Karadzovska et al., 2013). In the ranking of vehicles according to the compound permeation, the effect of the different vehicles was not found to be consistent between the models; however, when considering only the saturated concentrations of the compounds, four out of six vehicles were correctly ranked by the PAMPA and three out of six by the Strat- M^{TM} membrane. Both the PAMPA and the Strat-M^{TM} thus showed potential in predicting the absorption as well as discriminating between the different vehicles (Karadzovska et al., 2013).

Loftsson et al. (2006) reported on the development of an artificial membrane with the aim to create a model well suited for the evaluation of pharmaceutical formulations as well as the effects of various excipients on drug availability. The unstirred water layer consists of a hydrated semi-permeable cellophane membrane and a lipophilic membrane of pure *n*-octanol in a nitrocellulose matrix. The membrane can be used in the diffusion cells i.e. the Franz diffusion cell and offers the advantage that the two layers can be studied separately. The model was used to study the permeation of drug/model compound from the different cyclodextrin formulations and it was shown that the drug permeation

patterns from the cyclodextrin formulations were similar to those previously observed for biological membranes.

4.2. Lipid based model membranes

4.2.1. PAMPA

The PAMPA was introduced by Kansy and co-workers (1998) as a rapid *in vitro* model for assessment of the transcellular intestinal permeability. The original PAMPA system consists of an artificial membrane containing a hydrophobic filter coated with phosphatidylcholine dissolved in n-dodecane as a membrane barrier, separating the donor and acceptor compartments. The model is focused on a high throughput screening format, and could be modified by adapting the membrane composition. The lipid/solvent mixtures in the membrane are not well characterized and lack the true lipid bilayers found in the biological membranes (Faller, 2008).

Sinko and co-workers developed the skin-PAMPA containing the synthetic certramides, analogues for ceramides, proposed as the replacements for naturally occurring ceramides found in SC (Sinko et al., 2009; Sinko et al., 2012). The certramides are cheaper alternatives to natural ceramides with the potential to prolong the storage time (Tsinam and Sinko, 2013). Although the certramides are structurally different from ceramides, their comparable molecular mass and hydrogen acceptor/donor capacity, enables them to act as the lipid constituents in the PAMPA sandwich membrane together with cholesterol, stearic acid and silicone oil (Sinko et al., 2009; Sinko et al., 2012). The permeability of a selection of the model drugs tested on the skin-PAMPA was correlated with the human skin penetration obtained from the different skin databases. A rather poor correlation between the skin-PAMPA and the epidermis, but good correlation between the skin-PAMPA and the full-thickness skin were obtained (Sinko et al., 2012).

In the previously mentioned study by Karadzovska and Riviere (2013), the skin-PAMPA together with the non-lipid based skin PAMPA and the Strat-M^{TM} membrane were used to evaluate the vehicle effects on the permeation of different drugs. Among the three membranes, the skin-PAMPA was found to be the most representative of SC lipid matrix due to a similar composition of ceramide, cholesterol and free fatty acids; whereas the

correlation with the absorption data from the skin showed the highest association for the non-lipid based PAMPA model. The ranking of vehicles according to the permeation values was not consistent between the different models; however, the two PAMPA models were both able to correctly predict the ranking of the four out of six vehicles. Although further investigations using more complex topical formulations are required, the studied artificial models were all capable to predict the skin penetration, discriminating between the tested vehicles and providing a rapid initial estimation of the amount of the compound remaining in SC (Karadzovska et al., 2013).

Tsinman and Sinko (2013) applied a modified version of the skin-PAMPA to evaluate the different ibuprofen-containing skin formulations; silicone-based gel, silicone and acrylic copolymer and one commercially available formulation. In the simple penetration experiments, the modified skin-PAMPA models were able to distinguish between the different formulations and the ranking of the tested formulations according to the flux was in an agreement with the penetration through human epidermis in the Franz diffusion cells.

4.2.2. PVPA

Recently, the novel PVPA model mimicking the SC barrier of the skin was developed (Engesland et al., 2013). The original PVPA was introduced as a screening model for the intestinal permeability and consists of a tight layer of liposomes on a filter support mimicking the cells of biological barriers (Flaten et al., 2006a,Flaten et al., 2006b) as schematically explained in Figure 1. The structure shown in the Figure is based on the previous structural characterizations performed on the original PVPA (Flaten et al., 2006a). It was shown that after the freeze thaw cycling, both smaller unilamellar and bigger multilamellar structures are found in the barrier as indicated in the Figure 1. The PVPA model has the potential to perform as a high-throughput screening model (Flaten et al., 2009) and the composition of the liposomes used to build the barriers could be adjusted as to mimic the absorption barrier of various absorption sites.

Figure 1

Two modifications of the skin PVPA model able to estimate the skin penetration were developed; the $PVPA_c$ prepared using the liposomes made of cholesterol and egg phosphatidylcholine, and the $PVPA_s$ using the main lipid classes found in the skin (ceramide, cholesterol, free fatty acid and cholesteryl sulphate as well as egg phosphatidylcholine). The permeability of six compounds (flufenamic acid, ibuprofen, indomethacin, salicylic acid, calcein and FITC-dextran) was evaluated in the two PVPA skin models. The results were compared to the literature values for the penetration through animal skin (rat, cattle, dog and pig) as well as calculated *in silico* values. The ranking from the permeability experiments was, except from one outlier, the same as the ranking from the animal skin penetration experiments and the calculated values. Engesland and co-workers (2013) also revealed that the barrier function of the PVPA model could be modified in a controlled manner and demonstrated that the PVPA barriers could mimic different biological barriers by interchanging the lipid constituents in the barriers.

In a subsequent study, the PVPA model mimicking SC was adjusted to evaluate the effect of vesicle carrier on the skin penetration of diclofenac sodium (Palac et al., 2014). Liposomes, including the conventional, deformable and propylene glycol liposomes, containing diclofenac sodium, were examined. The results from the permeability experiments in the $PVPA_s$ demonstrated a clear rank order indicating an increasing permeation of diclofenac sodium from liposomal formulations corresponding to the physicochemical properties of the liposomal carrier. The permeation was shown to be affected by the lipid composition and increased in the presence of the penetration enhancers and edge activators within the liposomes as expected (Palac et al., 2014).

To further establish the potential of the skin PVPA in the formulation development the permeability of three model drugs in the solutions and as liposomal formulations was tested. The permeability results for the PVPA skin models were compared with the results for the reconstructed human skin model, EpiSkin®. The drugs were ranked based on their estimated penetration potentials, and the results were in accordance with the

physicochemical properties of the drugs. The $PVPA_s$ was, in contrast to the EpiSkin®, fully able to distinguish between the drug solutions and liposomal formulations. The PVPA models were, in summary, found to be superior to EpiSkin® regarding their abilities to identify the effects of the formulations on the drug permeability which could be utilized in early drug development, in addition to ease of the use, efficiency, cost-effectiveness and prolonged storage potential (Engesland et al., 2015).

4.2.3. Miscellaneous

Bouwstra and colleagues developed the SC substitute (SCS) consisting of a porous material covered with the synthetic SC lipids, mimicking the lipid organization in the SC closely (de Jager et al., 2006). The steady state flux of the moderately hydrophobic to moderately lipophilic model compounds through the SCS and human SC has been shown to be very similar (de Jager et al., 2006; Groen et al., 2008). The SCS thus may function as a standardized and reliable percutaneous penetration model. Another major advantage of the SCS is that the composition of the synthetic SC lipid mixtures can easily be modified. This allows studying the relationship between the lipid composition, lipid organization and barrier function in one single model.

Ochalek et al. (2012a) reported on the SC lipid model membranes designed to study the impact of ceramide species on the drug diffusion and penetration. These membranes have been used to investigate the impact of the lipophilic penetration enhancer (oleic acid) in the membranes on the penetration of the model drugs covering a broad range of lipophilicity (Ochalek et al., 2012b). The enhancer exhibited a pronounced effect on the barrier properties of SC lipid model membranes; and the effect was dependent on the type of the ceramide present in the barrier. The incorporation of oleic acid into the SC lipid model membranes changed the diffusion and permeation behavior of all model drugs to the certain extent.

5. Reconstructed human skin equivalents

In the past ten years several tissue culture-based human skin models have been developed and become commercially available (Van Gele et al., 2011). They are usually classified as human reconstructed epidermis models (e.g. EpiSkin®, SkinEthic®, EpiDerm®) and living skin equivalent models (GraftSkin®, EpiDermFT®, Pheninon®). The models are composed of the human cells grown as the tissue culture and matrix equivalents normally present in the skin (Godin and Touitou, 2007; Netzlaff et al., 2005).). The comparison of the reconstructed human epidermis models with the human skin is presented by Table 1. The human reconstructed epidermis models are useful tools for the testing of phototoxicity, corrosivity and irritancy, as well as in drug permeability studies. Recently, the models were also utilized in the optimization of vehicle compositions and formulation effects (Van Gele et al., 2011). Compared to the *ex vivo* human skin models, the reconstructed epidermis equivalents are significantly more permeable, but are more consistent in the permeability than the human skin, which is highly variable (Netzlaff et al., 2005; Netzlaff et al., 2006b). For example, the permeation of highly hydrophobic compounds was found to be even 800-folds higher than in the split-thickness human skin. On the contrary, transdermal flux of salicylic acid was found to be similar to that obtained using the human skin (Schmook et al., 2001). Additional disadvantage is their rather high cost.

Zghoul et al. (2001) have shown that the EpiDerm®could be used to differentiate the permeation of drug from ointment and solution. The EpiDerm® models have also been used to study the effect of the liposomal size and bilayer rigidity on the skin diffusion (Babu et al., 2009). However, the authors have not compared the results with the *ex vivo* human skin models. Dreher et al. (2002) have evaluated skin bioavailability of caffeine and alpha-tocopherol from a W/O emulsion, an O/W emulsion, the liposome dispersion and a hydrogel on the EpiDerm® and EpiSkin® membranes in comparison to the human skin *ex vivo*. The rank order of solute permeability could be correctly predicted when the preparations were applied at a finite dose in human skin models. The limited effects of the vehicle on the skin bioavailability were observed only in the human skin *ex vivo* model. The alcohol-containing vehicles seemed to behave differently in the EpiDerm® and EpiSkin® as compared to the human skin *ex vivo.* Labouta et al. (2013) have reported

that *in vitro* skin equivalents could serve as a fast screening in testing the behavior of nanoparticles and extrapolation of their penetration behavior into human skin. Schäfer-Korting (2008) and colleagues used SkinEthic® and the EpiDerm® full thickness model to predict uptake from different vehicle formulation using the pig and human skin and reported that the permeability of the reconstructed tissues, pig skin and human skin was correlating rather well; the ranking was very similar but the absolute permeability differed.

The handling of the models requires the standardized methodology, a fact which might be neglected in some of the reported studies (Van Gele et al., 2011). The 3D constructs mimicking critical features of healthy and diseased skin (Table 1) are a valuable tool in cosmetic and pharmaceutical industry (Mathes et al., 2014).

6. *Ex vivo* skin models

Numerous literature reports on the evaluation of skin formulations are based on the use of *ex vivo* models of either human or animal origin. The choice of an appropriate *ex vivo* model will be influenced by the storage, sample handling, preparation technique and accurate experimental setup for the evaluation of the drug permeability.

6.1. Storage of skin, skin preparation methods and experimental setup

The storage conditions and method of skin preparation might have an effect on the drug permeability. While the rodent skin is most often used fresh, the pig skin is typically used after being stored frozen (at -20 to -30 °C for a period of up to 6 months in a tightly closed container), similarly as the human skin (Barbero and Frasch, 2009). It has been generally accepted that there are no differences between frozen/thawed and fresh pig skin regarding the permeability of the water-soluble compounds. However, after freezing of the skin the permeability of some drugs increased (e.g. water solution of diclofenac) and a lag time decreased in comparison to the fresh skin (Sintov and Botner, 2006). Very recent study by Sintov and Greenberg (2014) has shown that the permeability of caffeine

through frozen/thawed pig skin was higher than in the fresh pig skin and independent on the vehicle properties. The authors used full thickness porcine skin excised from fresh ears of slaughtered white pigs; the skin (without subcutaneous fat) was used either within 5 hours from the pig slaughter (fresh) or was frozen, stored at -20 °C for one week and used thawed. Equally important is the method of the skin preparation. The lipophilic drug will exhibit unrealistically small permeability and long lag times through a skin membrane with excessive watery dermis left in place. Besides, the lack of blood flow in *ex vivo* experiments allows dermis to create a barrier to diffusion which does not exist in the native *in vivo* setting. The most common skin preparation methods used in the drug permeability studies include: (i) full-thickness (FT) skin, (ii) split thickness (dermatomed) skin (D), (iii) heat separated epidermis (HSE), and (iv) trypsin-isolated SC (Henning et al., 2009). FT skin is generally used from the animals with very thin skin such as mouse, rat, rabbit or newborn pig (Barbero and Frasch, 2009). Dermatomed or a split thickness skin is commonly prepared from the thicker animal skin (pig) and human skin. The recommended thickness is 200-400 μm and 400-700 μm for human and pig skin, respectively (Schaefer et al., 2008). The trypsin-isolated SC and HSE are reported to be equivalent regarding the drug penetration rates. The HSE is prepared by placing the thawed and cleaned skin pieces in water (60 °C) for one minute and peeling off the epidermis (Wagner et al., 2001). Using HSE in the permeation studies with a hydrophilic drug may result in a reduced penetration and longer lag times due to the additional partitioning between SC and viable epidermis (Henning et al., 2009). The HSE membranes could be unsuitable for the haired skin such as porcine as the hair shafts remain in the dermis creating holes in the membrane (Barbero and Frasch, 2009). The trypsin-isolated SC is commonly prepared by placing the clean skin samples into 0.15% trypsin buffered solution followed by incubation at 32 °C for 24 hours. This procedure is repeated with fresh trypsin solution until SC is fully isolated (Wagner et al., 2001).

Most of the permeability studies are performed using static Franz-diffusion cell method. It consists of donor and receptor chambers between which the animal model membrane is positioned so that the *stratum corneum* is facing the donor compartment where examined formulation is applied, while dermis (full thickness skin) is touching receptor compartment. For the determination of the skin (membrane) integrity. OECD Guidance

document 28 (2004) recommends measurement of transepidermal water loss (TEWL), electrical resistance or the use of tritiated water as a permeation marker. Although TEWL measurements have advantage that no solutions have to be added to perform the barrier integrity test, Netzlaff et al., (2006a) have proven that TEWL measurement cannot detect small changes in the SC that could influence drug diffusion. Therefore, calculation of the permeability coefficient is still more precise method. The experiments could be done so that the donor chamber is left opened (non-occlusive conditions) or covered (occlusive conditions) to permit or escape drying or hydration of the skin surface (Schaefer et al., 2008). The composition of the receptor medium, which is continuously stirred during the experiment, should be chosen to mimic *in vivo* conditions and to ensure the sufficient solubility of the drug. The experiments are performed at 32 ± 1 °C. More details on the validation of the system are provided by Ng and colleagues (2010). The addition of the solubility-increasing compounds to the receptor fluid (ethanol or PEG) may alter the barrier function of the skin due to a possible back-diffusion to the skin, particularly if ethanol is used at higher concentration (40%). To reduce this risk, PEG-20-oleyl ether (6%) and bovine or porcine serum albumin are recommended as they do not destabilize the integrity of the skin (Moser et al., 2001). When the experiments are lasting for more than one day, possible enzymatic degradation and microbiological contamination of the biological material might occur. Therefore, addition of preservatives such as sodium azide or ethanol could be beneficial, bearing in mind that their presence might have an effect on the barrier properties of skin by changing the lag time and duration of experiment (Henning et al., 2009).

For the quantification of skin penetration and deposition the skin extraction measurements (Rastogi and Singh, 2001), horizontal stripping and sectioning (de Jalon et al., 2001), quantitative autoradiography and spectroscopic methods (Pirot et al., 1997) can be employed. The tape stripping method has been widely used both in *in vivo* (Dick et al., 1997) and *in vitro (ex vivo)* evaluations of topical formulation on human (Cambon et al., 2001; Wagner et al., 2001) and animal (Raber et al., 2014) skin. After topical application of formulation, the cell layers of the *stratum corneum* are sequentially removed from the same skin area by adhesive films. The strips of adhesive tape should be carefully adhered to the stretched skin by applying constant pressure by the suitable

roller that could press a thin and flexible tape strip into the furrows of the skin. Velocity of tape removal is another important parameter influencing the amount of removed *stratum corneum*. A constant velocity should be applied. A slowing down or stopping the procedure could lead to an increase in the *stratum corneum* amount adhered on the tape strip, whereas, an increase in speed could result in a reduced amount of corneocytes. The detached tape strips contain both the amount of corneocytes and the corresponding amount of the penetrated formulation, which can be determined by conventional analytical procedures. It has been proven that different types of formulations can strongly affect the amount of *stratum corneum* removed with every tape strip. For example, after application of an ethanolic solution the adhesion of the horny layer to the tape strips is increased while after application of an oily formulation the adhesion to the tape is decreased. Therefore, for the comparison of the drug penetration from various drug formulations, it is important that the amount of formulation detected on the single tape strip is not related to the tape strip number as a relative measure of the penetration depths but to their standardized real position in the horny layer (Lademann et al., 2009).

6.2. Animal skin models

6.2.1. Porcine skin

Although human skin is considered to be the "gold standard" in *in vitro* drug penetration studies, it is often not feasible. Domestic pig skin is recognized as the most appropriate animal model due to the numerous anatomical, histological and physiological similarities with human skin (Table 2). They include the epidermal thickness, dermal-epidermal thickness ratio, resemblance in hair follicle and blood vessel density in the skin, as well as content of SC glycosphingolipids, ceramides, dermal collagen and elastin (Dick and Scott, 1992; Godin and Touitou, 2007). Even though the hair follicles on pig ear skin are larger than those of humans, the porcine ear skin represents a more suitable *in vitro* model for the analysis of the penetration and storage of topically applied substances in the hair follicles than excised human skin, mainly due to the fact that the human skin contracts after removal. Namely, re-stretching of the skin to its original size mainly stretches the interfollicular fibers, whereas the fibers around the hair follicles remain

contracted. In contrast to excised human skin, pig ear tissue does not contract when the cartilage is not removed (Lademann et al., 2010). The pig skin is readily obtained as a waste from animals slaughtered for food. The comparison of drug permeability using human and pig skin has demonstrated a good correlation particularly for lipophilic substances, while skin from rodents generally exhibited higher permeation rates (Dick and Scott, 1992). In addition, pig skin exhibits less donor variability than the human skin (Barbero and Frasch, 2009). The ear of approximately 6 months old pig is the most similar to the human skin (Table 2). However, most of literature data on the use of pig ears in *ex vivo* permeation studies do not specify the age of animal. It is important that ears had not been scalded or flamed after animal sacrificing because such a pretreatment completely destroys the integrity of epidermis. The central region of the outer side of the porcine ear has been recommended because of the similarity with human skin layers (Meyer et al., 2006).

Various drug formulations including creams, ointments, lotions, (micro)emulsions, microparticles and colloidal drug delivery systems (nanosystems) have been assessed using *ex vivo* pig skin models. Among large number of the available references we have chosen to select and present several examples from each delivery system (Table 3).

Scognamiglio et al. (2013) evaluated the penetration potential of deformable liposomes and ethosomes containing resveratrol using freshly excised pig ear. The deformable liposomes decreased the amount of resveratrol accumulated in dermis as compared to ethosomes. Full-thickness porcine ear skin has been used for optimization of the nanostructured lipid carriers containing minoxidil or finasteride (Gomes et al., 2014), whereas the evaluation of polyamide nanocapsules containing sunscreen filters was carried out on HSE pig ear (Hanno et al., 2012). Senyigit et al. (2010) performed the permeation studies using the FT porcine ear skin to determine the epidermal accumulation of clobetasol from lecithin/chitosan nanoparticles. Nanoparticles assured the increased amount of drug in the epidermis without any significant permeation across the skin.

Semisolid vehicles (O/W, W/O) and amphiphilic bases), often used in dermatotherapy with corticosteroids, were assessed on the dermatomed porcine abdominal skin (0.7 mm

thick). The W/O type vehicle was found to be the superior to other vehicles (Nagelreiter et al., 2013).

Hathout et al. (2010) reported on the optimization of microemulsion composition for transdermal delivery of testosterone using the dermatomed dorsal porcine skin. The skin deposition of quercitin from W/O microemulsion and its percutaneous delivery was examined using the full-thickness porcine ear skin. Improved skin deposition of quercitin without transdermal penetration has been determined 12 hours after microemulsion application (Vicentini et al., 2008).

Newborn pig skin has attracted considerable attention for evaluation of topical drug formulations (Cilurzo et al., 2007). However, the diversity in the thickness of newborn pig skin regarding the age of animal ranging from only one day old pig (~1.2 kg) (Manconi et al., 2011b; Mura et al., 2009) to 40 days old animal (~20 kg) needs to be taken into the consideration (Wang et al., 2014).

6.2.2. Other animal skin models: rodents, snake, bovine udder

Wide range of animal models includes the primates, mouse, rat, guinea pig, rabbit, bovine (udder) and snake models. Due to the fact that primate research is highly restricted and very expensive, rodent skin may also be used as a model in *in vitro* and *in v*ivo (trans)dermal studies. In comparison to pig skin models, use of the rodent skin requires ethical permission. The advantages of rodents are their small size, uncomplicated handling and relatively low cost. In addition, there are a number of hairless species available: nude mice, hairless rats and guinea pigs in which the absence of hair coat mimics the human skin better than hairy skin. The conventional rodents have a disadvantage of an extremely high density of hair follicles, which necessitates hair removal prior to formulation administration (Godin and Touitou, 2007). The differences in the thickness of SC, number of corneocyte layers, hair density, water content, lipid profile and morphology, cause the rodent skin to be more permeable than human skin (Schaefer et al., 2008). Rats and particularly mice show significantly higher permeation rates than human skin (Barbero and Frasch, 2009). Bond and Barry (1988) have proven that hairless mouse skin is completely inadequate model for assessing the effects of the

skin penetration enhancers. On the other hand, the guinea pig skin is considered more appropriate rodent surrogate for human skin studies (Barbero and Frasch, 2009).

Shim et al. (2004) evaluated the effect of the size of self-assembled nanoparticles on the effectiveness of transdermal delivery of minoxidil using several *ex vivo* rodent models, namely hairless mice and both hair and hairless guinea pigs (Table 3). When using hairy guinea pig skin the permeation of the drug in 40 nm-sized nanoparticles was 1.5–fold higher in the epidermis than that of 130 nm-sized nanoparticles. This influence of the nanoparticle size was not observed in hairless guinea pig thus showing that follicular route is the main penetration pathway for the minoxidil loaded nanoparticles, whereas the permeation was promoted with decreasing the size of nanoparticles. For the optimization of microemulsion formulation proposed for the treatment of skin fungal infections, Butani et al. (2014) have performed the permeability studies using the full-thickness abdominal rat skin. Microemulsion consisting of 5% of ispopropyl miristate and 35% of mixture (3:1, Tween 80:propylene glycol) exhibited 2-fold higher drug permeation of amphotericine B into the skin as compared to plain drug solution. The full-thickness skin obtained from the inner side of albino rabbit ear was used for the assessment of the mechanism of enhanced skin drug delivery by deformable liposomes and ethosomes. Penetration-enhancing effect of phospholipids and ethanol, as well as intact vesicle penetration of deformable liposomes and ethosomes into SC has been proven to play a role in improving skin delivery of drugs. Comparison of ketotifen skin permeability from the two types of vesicles has demonstrated ethosomal superiority for skin delivery (Elsayad et al., 2006).

The shed of snake skin has been proposed as an alternative skin model; the snakes molt periodically, a single animal can provide repeated sheds, thus eliminating inter-individual variability seen in other animal models. Moreover, skins can be obtained without injury to animal and do not need to be subjected to chemical or heat procedure prior to use. The shed is a large, intact sheet of epidermis (Itoh et al., 1990). It is not a living tissue and can be therefore stored at room temperature for relatively long period of time (Haigh and Smith, 1994). Similarities with the human SC have been confirmed in terms of the structure, composition, lipid content and water permeability. However, the lack of hair

follicles could influence drug permeability (Godin and Touitou, 2007). Therefore, this model is not appropriate for investigating dermal absorption of drugs that penetrate the skin via follicular route. Rigg and Barry (1990) compared the shed snake membrane with hairless mouse and human skin by evaluating the effect of the different penetrating enhancers on the permeability of 5-fluorouracil and suggested that human skin cannot be replaced by snake skin. In another study by Ngawhirunpat et al. (2008) the skin permeability of the snake and shed snake skin of black rat snake and Burmese python were found to be similar to dermatomed human skin; however the skin metabolisms in snake and shed snake skin were significantly different from the human skin.

Rather unique animal model is based on the udders from slaughtered cows. The perfused Bovine Udder Skin (BUS) model is ranked on the second place immediately after the *in vivo* studies on humans and before the FT or partial-thickness skin testing. Immediately after the slaughter, the udders are isolated and continuously perfused via the left and right external pudendal arteries with an oxygenated nutrient solution (Pittermann et al., 2013). Comparison with *ex vivo* human and porcine skin has demonstrated that bovine udder skin is well-correlated, but also less variable barrier against caffeine, benzoic acid, testosterone and flufenamic acid (Netzlaff et al., 2006b). This model enables the comparison of the dermal penetration, metabolism, and absorption of the substances after topical administration.

7. *Ex vivo* human skin models

Human skin is clearly the most relevant model for the evaluation of (trans)dermal drug delivery from various formulations. Skin obtained from various sources: plastic surgery, amputation and cadaver have been used for *ex vivo* evaluation of drug penetration (Godin and Touitou, 2007). The skin samples are mostly taken from the abdomen, back, leg or breast (Schaefer et al., 2008). However, the use of human skin is very restricted by the ethical permissions and laboratory facilities. Moreover, the skin permeability varies greatly between the specimens taken from the same or different anatomical sites of the

same donor (27% variance *in vivo* and 43% *in vit*ro, respectively), while even greater variations (45% *in vivo* and 66% *in vitro*) are reported between the specimens from different subjects or different age groups (Haigh and Smith, 1994). These variations may be explained by the differences in the lipid composition, skin thickness or hydration, which are determined by the body site, sex, race, age and disease (Barbero and Frasch, 2009). Another limitation of using the human skin is the metabolism and biotransformation of chemicals applied to the skin after excision of the tissue from the donor (Haigh and Smith, 1994).

Various topical formulations have been tested on *ex vivo* human skin (Table 4). For example, Zhao et al. (2009) evaluated nanoparticles incorporated in hydrofluoroalkane foam for enhanced dermal delivery of tocopheryl acetate using the full-thickness human skin. Optimization of a gel vehicle for dermal delivery of epicatechin has been performed on a full-thickness human cadaver skin obtained from the back region of Caucasian subjects. Ultrez® 10 gel was shown to promote the penetration and retention of epicatechin in the upper layers of human cadaver viable skin (Suppasrivasuseth et al., 2006). Dubey and colleagues (2007) compared the penetration potential of ethosomes containing methotrexate with the conventional liposomes using dermatomed (500 µm thickness) human cadaver skin and showed that ethosomes enhanced the transdermal flux of the drug and decreased the lag time across the skin. In another study, the full-thickness breast skin obtained after the cosmetic surgery was used for the estimation of celecoxib skin delivery from different vesicular nanosystems, namely ethosomes, conventional and deformable liposomes. An increased skin accumulation of the drug has been proven by ethosomes and deformable liposomes (Bragagni et al., 2012). The FT human abdominal skin was also used for the optimization of several types of temoporfin-loaded vesicles destined for the photodynamic therapy of cutaneous diseases: invasomes (Dragicevic-Curic et al., 2009a), flexosomes (Dragicevic-Curic et al., 2010) and liposomes-in-gel formulations (Dragicevic-Curic et al., 2009b).

In summary, the advantages and limitations of each of the reviewed *in vitro*/*ex vivo* model are enlisted in Table 5.

8. Conclusions

The restrictions in use of animals in optimization of pharmaceutical and cosmeceutical formulations destined for the administration onto the skin resulted in an increasing number of alternative skin permeation models. The models offer a possibility for rapid screening and faster optimization of skin formulations; however their limitations need to be taken into account. The choice of the most applicable *in vitro* model should be based on the interplay between the availability, easiness of the use, cost and the respective limitations.

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Conflict of interest

The authors report no conflict of interests.

References

Aggarwal, N., Goindi, S., Khurana, R., 2013. Formulation, characterization and evaluation of an optimized microemulsion formulation of griseofulvin for topical application. Colloids Surf. B Biointerfaces 105, 158-166.

Ahmed, T.A., El-Say, K.M., 2014. Development of alginate-reinforced chitosan nanoparticles utilizing W/O nanoemulsification/internal crosslinking technique for transdermal delivery of rabeprazole. Life Sci. 110, 35-43.

Alomrani, A.H., Shazly, G.A., Amara, A.A., Badran, M.M., Itraconazole-ghydroxypropyl-β-cyclodextrin loaded deformable liposomes: in vitro skin penetration studies and antifunhal efficacy using *Candida albicans* as model. Colloids Surf. B: Biointerfaces 121, 74-81.

Andrews, S.N., Jeong, E., Prausnitz, M.R., 2013. Transdermal delivery of molecules is limited by full epidermis, not just stratum corneum. Pharm. Res. 30, 1099-1109.

Babu, S., Fan, C., Stepanskiy, L., Uitto, J., Papazoglou, E., 2009. Effect of size at the nanoscale and bilayer rigidity on skin diffusion of liposomes. J. Biomed. Mater. Res. A 91, 140-148.

Barbato, F., Cappello, B., Miro, A., La Rotonda, M. I., Quaglia, F., 1998. Chromatographic indexes on immobilized artificial membranes for the prediction of transdermal transport of drugs. Farmaco 53, 655-661.

Barbero, A.M., Frasch, H.F., 2009. Pig and guinea pig skin as surrogates for human in vitro penetration studies: a quantitative review. Toxicol. In Vitro 23, 1-13.

Baroni, A., Buommino, E., De Gregorio, V., Ruocco, E., Ruocco, V., Wolf, R., 2012. Structure and function of the epidermis related to varrier properties. Clin. Dermatol. 30, 257-262.
Bolzinger, M.-A., Briançon, S., Pelletier, J., Chevalier, Y., 2012. Penetration of drugs through skin, a complex-rate controlling membrane. Curr. Opin. Colloid Interface Sci. 17, 156-165.
Bolzinger, M.-A., Briançon, S., Pelletier, J., Fessi, H., Chevalier, Y., 2008. Percutaneous release of caffeine from microemulsion, emulsion and gel dosage forms. Eur. J. Pharm. Biopharm. 68, 446-451.
Bond, J.R., Barry, B.W., 1988. Hairless mouse skin is limited as a model for assessing the effects of penetration enhancers in human skin. J. Invest. Dermatol. 90, 810-813.
Bouwstra, J.A., Honey-Nguyen, P.L., Gooris, G.S., Ponec, M., 2003. Structure of the skin barrier and its modulation by vesicular formulations. Progress Lipid Res. 42, 1-36.
Bouwstra, J.A., Ponec, M., 2006. The skin barrier in healthy and diseased state. Bioch. Biophys. Acta 1758, 2080-2095.
Bragagni, M., Mennini, N., Maestrelli, F., Cirri, M., Mura, P., 2012. Comparative study of liposomes, transfersomes and ethosomes as carriers for improving topical delivery of celecoxib. Drug Deliv. 19, 354-361.
Butani, D., Yewale, C., Misra, A., 2014. Amphotericin B topical microemulsion: formulation, characterization and evaluation. Colloids Surf. B: Biointerfaces 116, 351-358.
Cal, K., 2006. How does the type of vehicle influence the in vitro skin absorption and elimination kinetics of terpenes? Arch. Dermatol. Res. 297, 311-315.
Cambon, M., Issachar, N., Castelli, D., Robert, C., 2001. An in vivo method to assess the photostability of UV filters in a sunscreen. J. Cosmet. Sci. 52, 1-11.
Cevc, G., 2004. Lipid vesicles and other colloids as drug carriers on the skin. Adv. Drug Deliv. Rev. 56, 675-711.
Cevc, G., 2012. Rational design of new product candidates: The next generation of highly deformable bilayer vesicles for noninvasive, targeted therapy. J. Control. Release 160, 135-146.
Cevc, G., Blume, G., 1992. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. Biochim Biophys Acta 1104, 226-232.
Cevc, G., Blume, G., 2001. New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultradeformable drug carriers, Transfersomes. Biochim. Biophys. Acta 1514, 191-205.
Chen, L., Han, L., Lian, G., 2013. Recent advances in predicting skin permeability of hydrophilic solutes. Adv. Drug Deliv. Rev. 65, 295-305.

Chessa, M., Caddeo, C., Valenti, D., Manconi, M., Sinico, C., Fadda, A.M., 2011. Effect of penetration enhancer containing vesicles on the percutaneous delivery of quercetin through new born pig skin. Pharmaceutics 3, 497-509.
Chittenden, J.T., Brooksm J.D., Riviere, J.E., 2014. Development of a mixed-effect pharmacokinetic model for vehicle modulated in vitro transdermal flux of topically applied penetrants. J. Pharm. Sci, 103, 1002-1012.
Cilurzo, F., Minghetti, P., Sinico, C., 2007. Newborn pig skin as model membrane in in vitro drug permeation studies: a technical note. AAPS PharmSciTech 8, A94.
Clares, B., Calpena, A.C., Parra, A., Abrego, G., Alvarado, H., Fangueiro, J.F., Souto, E.B., 2014. Nanoemulsions (NEs), liposomes (LPs) and solid lipid nanoparticles (SLNs) for retinyl palmitate: Effect on skin permeation. Int. J. Pharm. 473, 591-598.
Daniels, R., Knie, U., 2007. Galenics of dermal product - vehicles, properties and drug release. JDDG 5, 369-383.
de Jager, M., Groenink, W., Bielsa I Guivernau, R., Andersson, E., Angelova, N., Ponec, M., Bouwstra, J., 2006. A novel in vitro percutaneous penetration model: evaluation of barrier properties with p-aminobenzoic acid and two of its derivatives. Pharm. Res. 23, 951-60.
de Jalón, E.G., Blanco-Príeto, M.J., Ygartua, P., Santoyo, S., 2001. Topical application of acyclovir-loaded microparticles: quantification of the drug in porcine skin layers. J. Control. Release 75, 191-197.
Dias, M., Hadgraft, J., Lane, M. E., 2007. Influence of membrane-solvent-solute interactions on solute permeation in model membranes. Int. J. Pharm. 336, 108-14.
Dick, I.P., Blain, P.G., Williams, F.M., 1997. The percutaneous absorption and skin distribution of lindane in man. I. In vivo studies. Hum. Exp. Toxicol. 16, 645-651.
Dick, I.P., Scott, R.C., 1992. Pig ear skin as an in-vitro model for human skin permeability. J. Pharm. Pharmacol. 44, 640-645.
Dobričić, V., Marković, B., Nikolić, K., Savić, V., Vladimirov, S., Čudina, O., 2014. 17beta-carboxamide steroids--in vitro prediction of human skin permeability and retention using PAMPA technique. Eur. J. Pharm. Sci. 52, 95-108.
Dragicevic-Curic, N., Gräfe, S., Gitter, B., Winter, S., Fahr, A., 2010. Surface charged temoporfin-loaded flexible vesicles: in vitro skin penetration studies and stability. Int. J. Pharm. 384, 100-108.
Dragicevic-Curic, N., Scheglmann, D., Albrecht, V., Fahr, A., 2008. Temoporfin-loaded invasomes: development, characterization and in vitro skin penetration studies. J. Control. Release 127, 59-69.
Dragicevic-Curic, N., Scheglmann, D., Albrecht, V., Fahr, A., 2009a. Development of different temoporfin-loaded invasomes-novel nanocarriers of temoporfin: characterization, stability and in vitro skin penetration studies. Colloids Surf. B Biointerfaces 70, 198-206.

Dragicevic-Curic, N., Winter, S., Stupar, M., Milic, J., Krajišnik, D., Gitter, B., Fahr, A., 2009b. Temoporfin-loaded liposomal gels: viscoelastic properties and in vitro skin penetration. Int. J. Pharm. 373, 77-84.
Dreher, F., Fouchard, F., Patouillet, C., Andrian, M., Simonnet, J-T., Benech-Kieffer, F., 2002. Comparison of cutaneous bioavailability of cosmetic preparations containing caffeine or alpha-tocopherol applied on human skin models or human skin ex vivo at finite doses. Skin Pharmacol. Appl. Skin Physiol. 15 Suppl 1, 40-58.
Dubey, V., Mishra, D., Dutta, T., Nahar, M., Saraf, D.K., Jain, N.K., 2007. Dermal and transdermal delivery of an anti-psoriatic agent via ethanolic liposomes. J. Control. Release 123, 148-154.
Duangjit, S., Pamornpathomkul, B., Opanasopit, P., Rojanarata, T., Obata, Y., Takayama, K., Ngawhirunpat, T., 2014. Role of the charge, carbon chain length, and content of surfactant on the skin penetration of meloxicam-loaded liposomes. Int. J. Nanomedicine 9, 2005-2017.
Egawa, M., Hirao, T., Takahashi, M., 2007. In vivo estimation of stratum corneum thickness from water concentration profiles obtained with Raman spectroscopy. Acta Derm. Venereol. 87, 4-8.
Elmoslemany, R.M., Abdallah, O.Y., El-Khordagui, L.K., Khalafallah, N.M., 2012. Propylene glycol liposomes as a topical delivery system for miconazole nitrate: comparison with conventional liposomes. AAPS PharmSciTech 13, 723-731.
Elsayed, M.M.A., Abdallah, O.Y., Naggar, V.F., Khalafallah, N.M., 2006. Deformable liposomes and ethosomes: mechanism of enhanced skin delivery. Int. J. Pharm. 322, 60-66.
Elsayed, M.M.A., Abdallah, O.Y., Naggar, V.F., Khalafallah, N.M., 2007. Lipid vesicles for skin delivery of drugs: Reviewing three decades of research. Int. J. Pharm. 332, 1-16.
Engesland, A., Škalko-Basnet, N., Flaten, G.E., 2015. PVPA and EpiSkin® in Assessment of Drug Therapies Destined for Skin Administration. J. Pharm. Sci. 104, 1119-1127. Engesland, A., Skar, M., Hansen, T., Škalko-Basnet, N., Flaten, G.E., 2013. New applications of phospholipid vesicle-based permeation assay: permeation model mimicking skin barrier. J. Pharm. Sci. 102: 1588-1600.
Escuder-Gilabert, L., Martínez-Pla, J. J., Sagrado, S., Villanueva-Camańas, R.M., Medina-Hernández, M.J., 2003. Biopartitioning micellar separation methods: modelling drug absorption. J. Chrom. B. 797: 21-35.
Faller, B., 2008. Artificial membrane assays to assess permeability. Curr. Drug Metabol. 9, 886-892.
Flaten, G.E., Awoyemi, O., Luthman, K., Brandl, M., Massing, U., 2009. The phospholipid vesicle-based permeability assay: 5. Development towards an automated procedure for high throughput permeability screening. JALA: 12-21.

Flaten, G.E., Bunjes, H., Luthman, K., Brandl, M., 2006a. Drug permeability across a phospholipid vesicle based barrier 2. Characterization of barrier structure, storage stability and stability towards pH changes. Eur. J. Pharm. Sci. 28; 336-343.
Flaten, G.E., Dhanikula, A.B., Luthman, K., Brandl, M., 2006b. Drug permeability across a phospholipid vesicle based barrier: A novel approach for studying passive diffusion. Eur. J. Pharm. Sci. 27: 80-90.
Frasch, H.F., Barbero, A.M., 2013. Application of numerical methods for diffusion-based modelling of skin permeation. Adv. Drug Deliv. Rev. 65, 208-220.
Gaur, P.K., Mishra, S., Purohit, S., 2013. Solid lipid nanoparticles of guggul lipid as drug carrier for transdermal drug delivery. BioMed Res. Int. Article ID 750690
Ge, S., Lin, Y., Lu, H., Li, Q., He, J., Chen, B., Wu, C., Xu, Y., 2014. Percutaneous delivery of econazole using microemulsion as vehicle: formulation, evaluation and vesicle-skin interaction. Int. J. Pharm. 465, 120-131.
Gillet A., Compere P., Lecomte F., Hubert P., Ducat E., Evrard B., Piel G., 2011a. Liposome surface charge influence on skin penetration behaviour. Int. J. Pharm. 411, 223-231.
Gillet, A., Lecomte, F., Hubert, P., Ducat, E., Evrard, B., Piel, G., 2011b. Skin penetration behaviour of liposomes as a function of their composition. Eur. J. Pharm. Biopharm. 79, 43-53.
Godin, B., Touitou, E., 2007. Transdermal skin delivery: predictions for humans from in vivo, ex vivo and animal models. Adv. Drug Deliv. Rev. 59, 1152-1161.
Gomes, M.J., Martins, S., Ferreira, D., Segundo, M.A., Reis, S., 2014. Lipid nanoparticles for topical and transdermal application for alopecia treatment: development, physicochemical characterization, and in vitro release and penetration studies. Int. J. Nanomedicine 9, 1231-1242.
Groeber, F., Holeiter, M., Hampel, M., Hinderer, S., Schenke-Layland, K., 2011. Skin tissue engineering – in vivo and in vitro applications. Adv. Drug Deliv. Rev. 128, 352-366.
Groen, D., Gooris, G. S., Ponec, M., Bouwstra, J. A., 2008. Two new methods for preparing a unique stratum corneum substitute. Bioch. Biophys. Acta 1778: 2421-9.
Groen, D., Poole, D.S., Gooris, G. S., Bouwstra, J. A., 2011. Investogating the barrier function of skin lipid models with varying compositions. Eur. J. Pharm. Biopharm. 79, 334-342.
Hadgraft, J., 2001. Skin, the final frontier. Int. J. Pharm. 224, 1-18.
Haigh, J.M., Smith, E.W., 1994. The selection and use of natural and synthetic membranes for in vitro diffusion experiments. Eur. J. Pharm. Sci. 2, 311-330.
Hanno, I., Anselmi, C., Bouchemal, K., 2012. Polyamide nanocapsules and nano-emulsions containing Parsol(R) MCX and Parsol(R) 1789: in vitro release, ex vivo skin penetration and photo-stability studies. Pharm. Res. 29, 559-573.

Hathout, R.M., Woodman, T.J., Mansour, S., Mortada, N.D., Geneidi, A.S., Guy, R.H., 2010. Microemulsion formulations for the transdermal delivery of testosterone. Eur. J. Pharm. Sci. 40, 188-196.
Henning, A., Schaefer, U.F., Neumann, D., 2009. Potential pitfalls in skin permeation experiments: influence of experimental factors and subsequent data evaluation. Eur. J. Pharm. Biopharm. 72, 324-331.
Hidalgo-Rodríguez, M., Soriano-Meseguer, S., Fuguet, E., Ráfols, C., Roses, M., 2013. Evaluation of the suitability of chromatographic systems to predict human skin permeation of neutral compounds. Eur. J. Pharm. Sci. 50: 557-568.
Itoh, T., Xia, J., Magavi, R., Nishihata, T., Rytting, J.H., 1990. Use of shed snake skin as a model membrane for in vitro percutaneous penetration studies: comparison with human skin. Pharm. Res. 7, 1042-1047.
Jacobi, U., Kaiser, M., Toll, R., Mangelsdorf, S., Audring, H., Otberg, N., Sterry, W., Lademann, J., 2007. Porcine ear skin: an in vitro model for human skin. Skin Res. Technol. 13, 19-24.
Jepps, O.G., Dancik, Y., Anissimov, Y.G., Roberts, M.S., 2013. Modeling the human skin barrier – Towards a better understanding of dermal absorption. Adv. Drug Deliv. Rev. 65, 152-168.
Jin, S-E., Kim, C-K., 2014. Charge-mediated topical delivery of plasmid DNA with cationic lipid nanoparticles to the skin. Colloids Surf. B Biointerfaces 116, 582-590.
Junyaprasert, V.B., Singhsa, P., Suksiriworapong, J., Chantasart, D., 2012. Physicochemical properties and skin permeation of Span 60/Tween 60 niosomes of ellagic acid. Int. J. Pharm. 423, 303-311.
Kansy, M., Senner, F., Gubernator, K., 1998. Physicochemical high throughput screening: Parallel artificial membrane permeation assay in the description of passive absorption processes. J. Med. Chem. 41: 1007-1010.
Kantarci, G., Ozgüngey, I., Karasulu, H.Y., Güneri, T., Başdemir, G., 2005. In vitro permeation of diclofenac sodium from novel microemulsion formulations through rabbit skin. Drug Develop. Res. 65, 17-25.
Karadzovska, D., Brooks, J.D., Monteiro-Riviere, N.A., Riviere, J.E., 2013. Predicting skin permability from complex vehicles. Adv. drug Deliv. Rev. 65, 265-277.
Karadzovska, D., Riviere, J.E., 2013. Assessing vehicle effects on skin absorption using artificial membrane assays. Eur. J. Pharm. Sci. 50: 569-576.
Khurana, S., Bedi, P.M.S., Jain, N.K., 2013. Preparation and evaluation of solid lipid nanoparticles based nanogel for dermal delivery of meloxicam. Chem. Phys. Lipids 175-176, 65-72.
Kimura, E., Kawano, Y., Todo, H., Ikarashi, Y., Sugibayashi, K., 2012. Measurement of skin permeation/penetration of nanoparticles for their safety evaluation. Biol. Pharm. Bull. 35, 1476-1486.

Knudsen, N.Ø., Rønholt, S., Salte, R.D., Jorgensen, L., Thormann, T., Basse, L.H., Hansen, J., Frokjaer, S., Foged, C., 2012. Calcipotriol delivery into the skin with PEGylated liposomes. Eur. J. Pharm. Biopharm. 81, 532-539.
Kong, M., Chen, X.G., Kweon, D.K., Park, H.J., 2011. Investigations on skin permeation of hyaluronic acid based nanoemulsion as transdermal carrier. Carbohyd. Polym. 86, 837-843.
Labouta, H.I., Thude, S., Schneider, M., 2013. Setup for investigating gold nanoparticle penetration through reconstructed skin and comparison to published human skin data. J. Biomed. Opt. 18, 061218.
Lademann, J., Jacobi, U., Surber, C., Weigmann, H.-J., Fluhr, J.W., 2009. The tape stripping procedure--evaluation of some critical parameters. Eur. J. Pharm. Biopharm. 72, 317-323.
Lademann et al., 2010. Which skin model is the most appropriate for the investigation of topically applied substances into the hair follicles? Skin Pharmacol. Physiol. 23, 47-52.
Lam, P.L., Gambari, R., 2014. Advanced progress of microencapsulation technologies: In vivo and in vitro models for studying oral and transdermal drug deliveries. J. Control. Release 178, 25-45.
Lee, P.H., Conradi, R., Shanmugasundram, V., 2010. Development of an in silico model for human skin permeation based on a Franz cell skin permeability assay. Bioorg. Med. Chem. Lett. 20, 69-73.
Liu, J., Hu, W., Chen, H., Ni, Q., Xu, H., Yang, X., 2007. Isotretinoin-loaded solid lipid nanoparticles with skin targeting for topical delivery. Int. J. Pharm. 328, 191-195.
Loftsson, T., Konradsdottir, F., Masson, M., 2006. Development and evaluation of an artificial membrane for determination of drug availability. Int. J. Pharm. 326: 60-8.
Lv, Q., Yu, A., Xi, Y., Li, H., Song, Z., Cui, J., Cao, F., Zhai, G., 2009. Development and evaluation of penciclovir-loaded solid lipid nanoparticles for topical delivery. Int. J. Pharm. 372, 191-198.
Manca, M.L., Manconi, M., Nacher, A., Carbone, C., Valenti, D., Maccioni, A.M., Sinico, C., Fadda, A.M., 2014. Development of novel diolein-niosomes for cutaneous delivery of tretinoin: Influence of formulation and in vitro assessment. Int. J. Pharm. 477, 176-186.
Manconi, M., Caddeo, C., Sinico, C., Valenti, D., Mostallino, M.C., Biggio, G., Fadda, A.M., 2011a. Ex vivo skin delivery of diclofenac by transcutol containing liposomes and suggested mechanism of vesicle-skin interaction. Eur. J. Pharm. Biopharm. 78, 27-35.
Manconi, M., Sinico, C., Caddeo, C., Vila, A.O., Valenti, D., Fadda, A.M., 2011b. Penetration enhancer containing vesicles as carriers for dermal delivery of tretinoin. Int. J. Pharm. 412, 37-46.
Marepally, S., Boakye, C.H.A., Shah, P.P., Etukala, J.R., Vemuri, A., Singh, M., 2013. Design, synthesis of novel lipids as chemical permeation enhancers and development of nanoparticle system for transdermal drug delivery. PLoS One 8.

Marimuthu, M., Bennet, D., Kim, S., 2013. Self-assembled nanoparticles of PLGA-conjugated glucosamine as a sustained transdermal drug delivery vehicle. Polym. J. 45, 202-209.
Markovic, B. D., Vladimirov, S. M., Cudina, O. A., Odovic, J. V., Karljikovic-Rajic, K. D. 2012. A PAMPA assay as fast predictive model of passive human skin permeability of new synthesized corticosteroid C-21 esters. Molecules, 17, 480-91.
Mathes, S.H., Ruffner, H., Graf-Hausner, U., 2014. The use of skin models in drug development. Adv. Drug Del. Rev. 69-70, 81-102.
Meng, S., Chen, Z., Yang, L., Zhang, W., Liu, D., Guo, J., Guan, Y., Li, J., 2013. Enhanced transdermal bioavailability of testosterone propionate via surfactant-modified ethosomes. Int. J. Nanomedicine 8, 3051-3060.
Menon, G.K., Cleary, G.W., Lane, M.E., 2012. The structure and function of the stratum corneum. Int. J. Pharm. 435, 3-9.
Meyer, W., Schönnagel, B., Fleischer, L.G., 2006. A note on integumental (1-->3)(1-->6)beta-D-glucan permeation, using the porcine ear skin model. J. Cosmet. Dermatol. 5, 130-134.
Miki, R., Ichitsuka, Y., Yamada, T., Kimura, S., Egawa, Y., Seki, T., Juni, K., Ueda, H. Morimoto, Y., 2015. Development of a membrane impregnated with a poly(dimethylsiloxane)/poly(ethylene glycol) copolymer for a high-throughput screening of the permeability of drugs, cosmetics, and other chemicals across the human skin. Eur. J. Pharm. Sci. 66, 41-49.
Mitragotri, S., Anissimov, Y.G., Bunge, A.L., Frasch, H.F., Guy, R.H., Hadgraft, J., Kasting, G.B., Lane, M.E., Roberts, M.S., 2011. Mathematical models of skin permeability: An overview. Int. J. Pharm. 418, 115-129.
Moser, K., Kriwet, K., Naik, A., Kalia, Y.N., Guy, R.H., 2001. Passive skin penetration enhancement and its quantification in vitro. Eur. J. Pharm. Biopharm. 52, 103-112.
Mura, S., Manconi, M., Sinico, C., Valenti, D., Fadda, A.M., 2009. Penetration enhancer-containing vesicles (PEVs) as carriers for cutaneous delivery of minoxidil. Int. J. Pharm. 380, 72-79.
Muzzalupo, R., Tavano, L., Cassano, R., Trombino, S., Ferrarelli, T., Picci, N., 2011. A new approach for the evaluation of niosomes as effective transdermal drug delivery systems. Eur. J. Pharm. Biopharm. 79, 28-35.
Naegel, A., Heisig, M., Wittum, G., 2013. Detailed modeling of skin penetration-An overview. Adv. Drug. Deliver. Rev. 65, 191-207.
Nagelreiter, C., Raffeiner, S., Geyerhofer, C., Klang, V., Valenta, C., 2013. Influence of drug content, type of semi-solid vehicle and rheological properties on the skin penetration of the model drug fludrocortisone acetate. Int. J. Pharm. 448, 305-312.
Nakano, M., Patel, N. K., 1970. Release, uptake, and permeation behavior of salicylic acid in ointment bases. J. Pharm. Sci. 59: 985-988.

Nam, Y.S., Kim, J.-W., Park, JY., Shim, J., Lee, J.S., Han, S.H., 2012. Tocopheryl acetate nanoemulsions stabilized with lipid-polymer hybrid emulsifiers for effective skin delivery. Colloids Surf. B Biointerfaces 94, 51-57.
Netzlaff, F., Lehr, C.-M., Wertz, P.W., Schaefer, U.F., 2005. The human epidermis models EpiSkin, SkinEthic and EpiDerm: an evaluation of morphology and their suitability for testing phototoxicity, irritancy, corrosivity, and substance transport. Eur. J. Pharm. Biopharm. 60, 167-178.
Netzlaff, F., Kostka, K.H., Lehr, C.M., Schaefer, U.F., 2006a. TEWL measurements as a routine for evaluating the integrity of epidermis sheets in static Franz type diffusion cells in vitro. Limitations shown by transport data testing. Eur. J. Pharm. Biopharm. 63, 44-50.
Netzlaff, F., Schaefer, U.F., Lehr, C.-M., Meiers, P., Stahl, J., Kietzmann, M., Niedorf, F., 2006b. Comparison of bovine udder skin with human and porcine skin in percutaneous permeation experiments. Altern. Lab. Anim. 34, 499-513.
Ng, S-F., Rouse, J.J., Sanderson, F.D., Meidan, V., 2 Eccleston, G. M., 2010. Validation of a static Franz diffusion cell system for in vitro permeation studies . AAPS PharmSciTech. 11, 1432-1441.
Ngawhirunpat, T., Opanasopit, P., Rojanarata, T., Panomsuk, S., Chanchome, L., 2008. Evaluation of simultaneous permeation and metabolism of methyl nicotinate in human, snake, and shed snake skin. Pharm. Dev. Technol. 13, 75-83.
Nnamani, P.O., Hansen, S., Windbergs, M., Lehr, C.-M., 2014. Development of artemether-loaded nanostructured lipid carrier (NLC) formulation for topical application. Int. J. Pharm. 477, 208-217.
Ochalek, M., Heissler, S., Wohlrab, J., Neubert, R.H.H., 2012a. Characterization of lipid model membranes designed for studying impact of ceramide species on drug diffusion and penetration. Eur. J. Pharm. Biopharm. 81, 113-120.
Ochalek, M., Podhaisky, H., Ruettinger, H.-H., Neubert, R. H.H., Wohlrab, J. 2012b. SC lipid model membranes designed for studying impact of ceramide species on drug diffusion and permeation, part III: influence of penetration enhancer on diffusion and permeation of model drugs. Int. J. Pharm. 436, 206-213.
OECD, Guidance document for the conduct of skin absorption studies number 28, in: OECD Series on Testing and Assessment, 2004.
Ogunsola, O.A., Kraeling, M.E., Zhong, S., Pochan, D.J., Bronaugh, R.L., Raghavan, S.L., 2012. Structural analysis of ‘‘flexible’’ liposome formulations: new insights into the skin-penetrating ability of soft nanostructures. Soft Matter 8, 10226-10232.
Oliveira, G., Beezer, A. E., Hadgraft, J., Lane, M. E., 2010. Alcohol enhanced permeation in model membranes. Part I. Thermodynamic and kinetic analyses of membrane permeation. Int. J. Pharm. 393, 61-67.
Oliveira, G., Beezer, A. E., Hadgraft, J., Lane, M. E., 2011. Alcohol enhanced permeation in model membranes. Part II. Thermodynamic analysis of membrane partitioning. Int. J. Pharm. 420, 216-222.

Oliveira, G., Hadgraft, J., Lane, M. E., 2012. The role of vehicle interactions on permeation of an active through model membranes and human skin. Int. J. Cosmet. Sci. 34, 536-545.
Ottaviani, G., Martel, S., Carrupt, P. A., 2006. Parallel artificial membrane permeability assay: a new membrane for the fast prediction of passive human skin permeability. J. Med. Chem. 49, 3948-3954.
Ottaviani, G., Martel, S., Carrupt, P.-A., 2007. In silico and in vitro filters for the fast estimation of skin permeation and distribution of new chemical entities. J. Med. Chem. 50, 742-748.
Palac, Z., Engesland, A., Flaten, G. E., Škalko-Basnet, N., Filipović-Grčić, J., Vanić, Ž., 2014. Liposomes for (trans)dermal drug delivery: the skin-PVPA as a novel in vitro stratum corneum model in formulation development. J. Liposome Res. 24, 313-322.
Park, S.N., Lee, M.H., Kim, S.J., Yu, E.R., 2013. Preparation of quercetin and rutin-loaded ceramide liposomes and drug-releasing effect in liposome-in-hydrogel complex system. Biochem. Biophys. Res. Commun. 435, 361-366.
Pathak, P., Nagarsenker, M., 2009. Formulation and evaluation of lidocaine lipid nanosystems for dermal delivery. AAPS PharmSciTech 10, 985-992.
Pirot, F., Kalia, Y.N., Stinchcomb, A.L., Keating, G., Bunge, A., Guy, R.H., 1997. Characterization of the permeability barrier of human skin in vivo. Proc. Natl. Acad. Sci. USA 94, 1562-1567.
Pittermann, W., Hildebrandt, C., Prinz, A., Lemanski, S., Keitzmann, M., 2013. Comparative study of the skin tolerability of hand disinfectants using the BUS model. Hyg. Med. 38, 134-141.
Ponec, M., 1992. In vitro cultured human skin cells as alternatives to animals for skin irritancy screening. Int. J. Cosmet. Sci. 14, 245-264.
Pople, P.V., Singh, K.K., 2011. Development and evaluation of colloidal modified nanolipid carrier: application to topical delivery of tacrolimus. Eur. J. Pharm. Biopharm. 79, 82-94.
Raber, A.S., Mittal, A., Schafer, J., Bakowsky, U., Reichrath, J., Vogt, T., Schaefer, U.F., Hansen, S., Lehr, C.-M., 2014. Quantification of nanoparticle uptake into hair follicles in pig ear and human forearm. J. Control. Release 179, 25-32.
Rastogi, S.K., Singh, J., 2001. Lipid extraction and transport of hydrophilic solutes through porcine epidermis. Int. J. Pharm. 225, 75-82.
Rigg, P.C., Barry, B.W., 1990. Shed snake skin and hairless mouse skin as model membranes for human skin during permeation studies. J. Invest. Dermatol. 94, 235-240.
Sahle, F.F., Wohlrab, J., Neubert, R.H.H., 2014. Controlled penetration of ceramides into and across the stratum corneum using various types of microemulsions and formulation associated toxicity studies. Eur. J. Pharm. Biopharm. 86, 244-250.

Sahoo, S., Pani, N.R., Sahoo, S.K., 2014. Microemulsion based topical hydrogel of sertaconazole: formulation, characterization and evaluation. Colloids Surf. B Biointerfaces 120, 193-199.
Sandby-Møller, J., Poulsen, T., Wulf, H.C., 2003. Epidermal thickness at different body sites: relationship to age, gender, pigmentation, blood content, skin type and smoking habits. Acta Derm. Venereol. 83, 410-413.
Schaefer, U.F., Hansen, S., Schneider, M., Luengo Contreras, J., Lehr, C.M., 2008. Models for skin absorption and skin toxicity testing in: Ehrhardt, K. Kim K-J. (Eds), Drug Absorption Studies. Springer, New York, pp. 3-33.
Schäfer-Korting, M., Mahmoud, A., Lombardi Borgia, S., Brüggener, B., Kleuser, B., Schreiber, S., Mehnert, W., 2008. Reconstructed Epidermis and Full-Thickness Skin for Absorption Testing: Influence of the Vehicles used on Steroid Permeation. ATLA 36, 441–452.
Schmook, F.P., Meingassner, J.G., Billich, A., 2001. Comparison of human skin or epidermis models with human and animal skin in in-vitro percutaneous absorption. Int. J. Pharm. 215, 51-56.
Schwartz, J., Moreno, E., Fernandez, C., Navarro-Blasco, I., Nguewa, P.A., Palop, J.A., Irache, J.M., Sanmartin, C., Espuelas, S., 2014. Topical treatment of L. major infected BALB/c mice with a novel diselenide chitosan hydrogel formulation. Eur. J. Pharm. Sci. 62, 309-316.
Scognamiglio, I., De Stefano, D., Campani, V., Mayol, L., Carnuccio, R., Fabbrocini, G., Ayala, F., La Rotonda, M.I., De Rosa, G., 2013. Nanocarriers for topical administration of resveratrol: a comparative study. Int. J. Pharm. 440, 179-187.
Semlin, L., Schäfer-Korting, M., Borelli, C., Korting, H.C., 2011. In vitro models for human skin disease. Drug Discover. Today 16, 132-139.
Senyigit, T., Sonvico, F., Barbieri, S., Ozer, O., Santi, P., Colombo, P., 2010. Lecithin/chitosan nanoparticles of clobetasol-17-propionate capable of accumulation in pig skin. J. Control. Release 142, 368-373.
Shaikh, K.S., Chellampillai, B., Pawar, A.P., 2010. Studies on nonionic surfactant bilayer vesicles of ciclopirox olamine. Drug Dev. Ind. Pharm. 36, 946–953.
Shim, J., Seok Kang, H., Park, W-S., Han, S-H., Kim, J., Chang, I-S., 2004. Transdermal delivery of mixnoxidil with block copolymer nanoparticles. J. Control. Release 97, 477-484.
Shishu, N.K., 2015. D-optimal experimental approach for designing topical microemulsion of itraconazole: Characterization and evaluation of antifungal efficacy against a standardized Tinea pedis infection model in Wistar rats. Eur. J. Pharm. Sci. 67, 97-112.
Sinko, B., Garrigues, T. M., Balogh, G. T., Nagy, Z. K., Tsinman, O., Avdeef, A., Takacs-Novak, K., 2012. Skin-PAMPA: a new method for fast prediction of skin penetration. Eur. J. Pharm. Sci. 45, 698-707.

Sinko, B., Kokosi, J., Avdeef, A., Takacs-Novak, K., 2009. A PAMPA study of the permeability-enhancing effect of new ceramide analogues. Chem. Biodiversity, 6, 1867-74.
Sintov, A.C., Botner, S., 2006. Transdermal drug delivery using microemulsion and aqueous systems: influence of skin storage conditions on the in vitro permeability of diclofenac from aqueous vehicle systems. Int. J. Pharm. 311, 55-62.
Sintov, A.C., Greenberg, I., 2014. Comparative percutaneous permeation study using caffeine-loaded microemulsion showing low reliability of the frozen/thawed skin models. Int. J. Pharm. 471, 516-524.
Souto, E.B., 2005. SLN and NLC for Topical Delivery of Antifungals. Institut of Pharmacy, Freie Universität, Berlin, p.21.
Srisuk, P., Thongnopnua, P., Raktanonchai, U., Kanokpanont, S., 2012. Physico-chemical characteristics of methotrexate-entrapped oleic acid-containing deformable liposomes for in vitro transepidermal delivery targeting psoriasis treatment. Int. J. Pharm. 427, 426-434.
Stahl, J., Wohlert, M., Kietzmann, M., 2011. The effect of formulation vehicles on the in vitro percutaneous permeation of ibuprofen. BMC Pharmacol. 11, 12.
Suppasrivasuseth, J., Bellantone, R.A., Plakogiannis, F.M., Stagni, G., 2006. Permeability and retention studies of (-)epicatechin gel formulations in human cadaver skin. Drug Dev. Ind. Pharm. 32, 1007-1017.
Tavano, L., Alfano, P., Muzzalupo, R., de Cindio, B., 2011. Niosomes vs microemulsions: new carriers for topical delivery of Capsaicin. Colloids Surf. B Biointerfaces 87, 333-339.
Tsinman, K., Sinko, B., 2013. A high throughput method to predict skin penetration and screen topical formulations. Cosmetics & Toiletries 128, 192-199.
Turowski, M., Kaliszan, R., 1997. Keratin immobilized on silica as a new stationary phase for chromatographic modelling of skin permeation. J. Pharm. Biomed. Analysis 15, 1325-1333.
Van Gele, M., Geusens, B., Brochez, L., Speeckaert, R., Lambert, J., 2011. Three-dimensional skin models as tools for transdermal drug delivery: challenges and limitations. Expert Opin. Drug Deliv. 8, 705-720.
Vicentini, F.T.M.C., Casagrande, R., Verri, W.A., Jr., Georgetti, S.R., Bentley, M.V.L.B., Fonseca, M.J.V., 2008. Quercetin in lyotropic liquid crystalline formulations: physical, chemical and functional stability. AAPS PharmSciTech 9, 591-596.
Wagner, H., Kostka, K-H., Lehr, C-M., Schaefer, U.F., 2001. Interrelation of permeation and penetration parameters obtained from in vitro experiments with human skin and skin equivalents. J. Control. Release 75, 283-295.
Wang, J., Guo, F., Ma, M., Lei, M., Tan, F., Li, N., 2014. Nanovesicular system containing tretinoin for dermal targeting delivery and rosacea treatment: a comparison of hexosomes, glycerosomes and ethosomes. RSC Adv. 4, 45458-45466.

Wang, Y., Sun, J., Liu, H., Liu, J., Zhang, L., Liu, K., He, Z., 2009. Predicting skin permeability using liposome electrokinetic chromatography. Analyst 134: 267-72.
Waters, L.J., Shahzad, Y., Stephenson, J., 2013. Modelling skin permeability with micellar liquid chromatography. Eur. J. Pharm. Sci. 50, 335-340.
Watkinson, R. M., Guy, R. H., Hadgraft, J., Lane, M. E., 2009a. Optimisation of cosolvent concentration for topical drug delivery II: influence of propylene glycol on ibuprofen permeation. Skin Pharmacol. Physiol. 22, 225-230.
Watkinson, R. M., Guy, R. H., Oliveira, G., Hadgraft, J., Lane, M. E., 2011. Optimisation of cosolvent concentration for topical drug delivery III--influence of lipophilic vehicles on ibuprofen permeation. Skin Pharmacol. Physiol. 24, 22-26.
Watkinson, R. M., Herkenne, C., Guy, R. H., Hadgraft, J., Oliveira, G., Lane, M.E., 2009b. Influence of ethanol on the solubility, ionization and permeation characteristics of ibuprofen in silicone and human skin. Skin Pharmacol. Physiol. 22: 15-21.

Zghoul, N., Fuchs, R., Lehr, C-M., Schaefer, U.F., 2001. Reconstructed skin equivalents for assessing percutaneous drug absorption from pharmaceutical formulations. ALTEX 18, 103-106.

Zhao, Y., Moddaresi, M., Jones, S.A., Brown, M.B., 2009. A dynamic topical hydrofluoroalkane foam to induce nanoparticle modification and drug release in situ. Eur. J. Pharm. Biopharm. 72, 521-528.

Zhao, Y-Z., Lu, C-T., Zhang, Y., Xiao, J., Zhao, Y-P., Tian, J-L., Xu, Y-Y., Feng, Z-G., Xu, C-Y., 2013. Selection of high efficient transdermal lipid vesicle for curcumin skin delivery. Int. J. Pharm. 454, 302-309.

Zhou, W., Liu, W., Zou, L., Liu, C., Liu, C., Liang, R., Chen, J., 2014. Storage stability and skin permeation of vitamin C liposomes improved by pectin coating. Colloids Surf. B Biointerfaces 117, 330-337.

Figures and figure legends

Figure 1: The phospholipid vesicle based permeation assay (PVPA) mimicking the *stratum corneum* is consisting of a tight barrier of liposomes deposited on a cellulose ester filter support

Tables and table legends

Table 1. Comparison of the reconstructed human epidermis models with the human skin (adapted from Netzlaff et al., 2005)

Table 2: Comparison between porcine ear and human skin

Table 3: Overview of various animal skin models used for optimization of different skin formulations on the Franz-diffusion cells

Table 4: *Ex vivo* human skin models for optimization of drug/active ingredient formulations

Table 5: Advantages and limitations of different skin models used for optimization of topical formulation

Figure(s)

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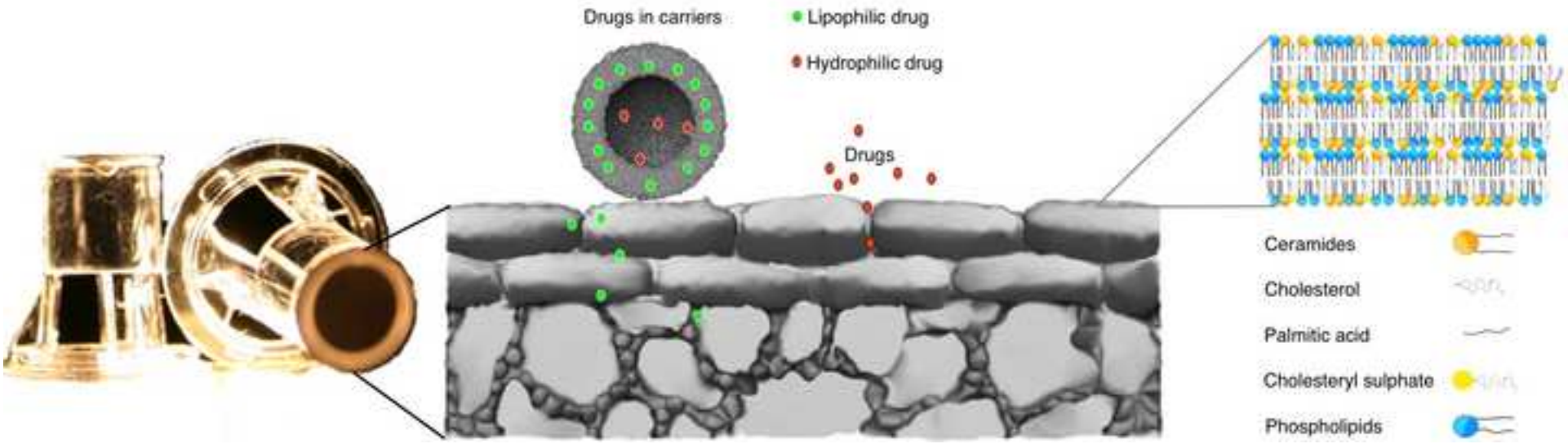

Table 1. Comparison of the reconstructed human epidermis models with the human skin (adapted from Netzlaff et al., 2005)

Parameters	SkinEthic®	EpiSkin® (Penetration and irritation models)	EpiDerm® (Penetration and irritation models)
Morphology	Structure similar to human epidermis; presence of desmosomes, keratohyalin and lamellar granules; lipid droplets present in all layers not found in human skin	Presence of all the epidermal layers; SC thicker than in human skin; different organization of cells in viable layers; basal cells cubic in shape, the upper cells flat; irregular shape of granular cells	Presence of all the epidermal layers; no Rete ridges; penetration model - 6-8 viable cell layers, 28-43 µm thick; irritation model – 7-14 viable cell layers, 83-100 µm thick; columnar to round cell shapes; *stratum spinosum* cells similar to native skin
Lipid composition	Lower amounts of phospholipids and sphingomyelin; higher portion of di/triglycerides, significantly higher ceramides ratio than in human skin	Phospholipids content in irritation model similar to native tissue, but significantly lower in penetration model; higher ceramide level in both models; significantly higher amounts of di/triglycerides (both models)	Phospholipids and di/triglycerides content in both models similar to human skin; higher ceramide amount in penetration model; cholesterolesters and free fatty acids portions in both models lower than in native skin
Biochemical markers	Presence of Keratin 1, 6, 10, SKALP, SPRR, SPRR3, loricrin, involucrin, transglutaminase	Presence of Keratin 1, 6, 10, SKALP, involucrin, transglutaminase; absence of loricrin, SPRR3 (both models)	Presence of Keratin 1, 6, 10, SKALP, involucrin and transglutaminase (both models); SPRR3 only in irritation model

SKALP, skin derived anti-leukoproteinase; SPRR, small proline rich-protein

Table 2. Comparison between porcine ear and human skin

Parameter	Porcine ear skin	Human skin
Stratum corneum thickness	21-28 µm Jacobi et al., 2007	16 µm (cheek), 23 µm (volar forearm), 29 µm (back of the hand) Egawa et al., 2007
Viable epidermis	66-72 µm Jacobi et al., 2007	70 µm (shoulder), 82 µm (buttock) Sandby-Moller et al., 2003
Hair follicle and infundibula	Extending deeply in the dermis Godin and Touitou, 2007	Extending deeply in the dermis Godin and Touitou, 2007
Hair density (hairs/cm^2)	20 Jacobi et al., 2007	14-32 (except for forehead area) Jacobi et al., 2007

Table 3. Overview of various animal skin models used for evaluation of different skin formulations on the Franz-diffusion cells

Skin	Anatomical site/age	Skin	Skin preparation method(s)	Formulation	Drug/active compound	Findings related to drug/active compound	References
Porcine	Ear	Fresh	FT, HSE	*NPs, NPs-in-chitosan gel*	Clobetasol-17-proprionate	Accumulation of drug inside the epidermis, linear to the concentration of nanoparticles	Senyigit et al., 2010
	Ear	Fresh	FT, HSE	*Deformable liposomes, ethosomes*	Resveratrol	Ethosomes more effective in delivery of resveratrol through the skin	Scognamiglio et al., 2013
	Ear	Fresh	FT	*Lipid NPs-in-gel*	Tacrolimus	Increased drug accumulation inside the skin	Pople and Singh, 2011
	Ear	Frozen	FT	*Nanostructured lipid carriers*	Minoxidil, finasteride	Low levels of transdermal drug, appropriate for the treatment of alopecia	Gomes et al., 2014
	Ear	Frozen	FT, TS, HSE	*PEGylated liposomes*	Calcipotriol	Presence of 1 mol% PEG-DSPE in liposome increased deposition in SC	Knudsen et al., 2012
	Ear	Frozen	FT, TS, HSE	*Deformable liposomes*	Bethametasone	Increased permeation into the skin	Gillet et al., 2011b
	Ear	Frozen	FT, D (1 mm), TS	*Chitosan hydrogel*	Bis-4-aminophenyldi-selenide	Better drug retention in the skin	Schwartz et al., 2014
	Abdomen	Frozen	D (0.7 mm), TS	*W/O, O/W and amphiphilic bases*	Fludrocortisone acetate	The vehicles influenced the permeability, aqueous improved delivery	Nagelreiter et al., 2013
	Dorsum	Frozen	D (0.74 mm)	*Microemulsion*	Testosterone	Microemulsion composed of 16% oleic acid, 32% Tween 20, 32% Transcutol and 20% water showed the highest permeation	Hathout et al., 2010
	ND	Frozen	FT+HD (4 mm total)	*Microemulsion, emulsion, gel*	Caffeine	Microemulsion improved delivery into hypodermis	Bolzinger et al., 2008
Newborn pig (1.2 kg)	ND	Frozen	FT, TS, MSE	*Penetration-enhancer-containing vesicles*	Tretinoin, diclofenac, quercitin	Improved accumulation of all the investigated drugs inside the skin	Chessa et al., 2011; Manconi et al., 2011a; Manconi et al., 2011b

Newborn pig	Abdomen	Frozen	FT	*Deformable liposomes*	Metotrexate	Improved skin permeability	Srisuk et al., 2012
Mouse	Abdomen	Fresh	FT	*Microemulsion-based hydrogel*	Sertaconazole	Microemulsion-in-gel increased the drug accumulation within the skin	Sahoo et al., 2014
	Dorsum	Fresh	FT	*Microemulsion*	Griseofulvin	3-7-fold higher permeation	Aggarwal et al., 2013
	Dorsum	Fresh	FT	*Liposomes-in-hydrogel*	Quercitin, rutin	Improved skin permeability	Park et al., 2013
Hairless mouse	Dorsum	Fresh	FT	*Cationic lipid NPs*	Plasmid-DNA	Successful skin delivery of the nanoparticle/DNA complexes	Jin and Kim, 2014
	Abdomen	Frozen	FT	*Pectin-coated liposomes*	Vitamin C	Pectin coating improved skin permeation	Zhou et al., 2014
	Abdomen	4°C, 24h	FT	*Ethosomes, conventional liposomes*	Testosterone propionate	Ethosomes provided an enhanced transdermal flux and a decreased lag time across the skin	Meng et al., 2013
Rat	Abdomen	Fresh	FT	*Niosomes-in-hydrogel*	Ciclopirox olamine	Improved skin deposition	Shaikh et al., 2010
	Abdomen	Fresh	FT	*SLNs*	Isotretinoin	Increased accumulation inside the skin	Liu et al., 2007
	Abdomen	Fresh	FT	*SLNs*	Penciclovir	2-fold enhanced drug uptake in dermis	Lv et al., 2009
	Abdomen	Fresh	FT	*Propylene glycol-, conventional-liposomes, ethosomes*	Curcumin	Penetration across the skin in the order: Propylene glycol liposomes > ethosomes > conventional liposomes	Zhao et al., 2013
	Abdomen	Fresh	FT, TS, MSE	*SLNs-based nanogel*	Meloxicam	Penetration through the skin; higher concentrations in epidermis and dermis	Khurana et al., 2013
	Abdomen	Frozen	FT	*Chitosan NPs in transdermal patch*	Rabeprazole	Substantial permeation through the skin	Ahmed and El-Say, 2014
	Abdomen	Frozen	FT, TS	*Microemulsion*	Econazole nitrate	Accumulation inside the skin	Ge et al., 2014
	Dorsum	Frozen	ISC, FT	*Nanoemulsion*	α-tocopherol	Desirable SC permeability and diffusion into deeper dermis	Kong et al., 2011

	Dorsum	Frozen	FT	*Microemulsion-based hydrogel*	Itraconazole	Enhanced skin retention and permeation	Shishu, 2015
Hairless guinea pig	Abdomen	Fresh	FT, TS	*Nanoemulsions*	Tocopheryl acetate	Increased lipid-to-polymer ratio provided enhanced accumulation in the skin	Nam et al., 2012
	Abdomen	Fresh	FT	*SLNs, nanostructured lipid carriers*	Lidocaine	Better deposition inside the skin by nanostructured lipid carriers than SLNs	Pathak and Nagarsenker, 2009
	ND	ND	D	*Deformable liposomes*	Fluorecent lipid (Dil)	Understanding the mechanisms involved in enhanced skin delivery by flexible liposomes	Ogunsola et al., 2012
Hair and hairless guinea pig	Abdomen	ND		*Self-assembled NPs*	Minoxidil	Permeation from 40-nm NPs was 1.7-fold higher than from 130-nm NPs in hairy animals. No size dependency in hairless animals.	Shim et al., 2004
Rabbit	Ear (inner side)	Fresh	FT	*Deformable liposomes, ethosomes*	Ketotifen	Ethosomes provided better skin delivery than deformable liposomes	Elsayed et al., 2006
	Ear	Frozen	FT	*Niosomes*	Sulfadiazine sodium	Niosomes increased permeation through the skin	Muzzalupo et al., 2011
	Ear	Frozen	FT	*Microemulsion, niosomes*	Capsaicin	Better transdermal delivery with niosomes than microemulsion	Tavano et al., 2011
	Dorsum	Frozen	FT	*Microemulsion*	Diclofenac sodium	Microemulsion with isopropyl alcohol as co-surfactant and propylene glycol as enhancer superior	Kantarci et al., 2005

D, dermatomed skin (split-thickness skin); FT, full-thickness skin; HD, hypodermis; HSE, heat-separated epidermis; ISC, bromide solution-isolated *stratum corneum*; MSE, mechanical separation of epidermis; ND, no data; NPs, nanoparticles; O/W, oil-in-water emulsion; SC, *stratum corneum*; SLNs, solid lipid nanoparticles; TS, tape stripping; W/O, water-in-oil emulsion

In all skin models presented the subcutaneous fat was removed immediately after skin excision.

Table 4. *Ex vivo* human skin models for optimization of drug/active ingredient formulations

Anatomical site/source	Skin storage	Skin preparation method(s)	Formulation	Drug/active compound	Findings related to drug/active compound	Reference
Abdomen, plastic surgery	Frozen	FT, TS, MSE	*Niosomes*	Tretinoin	Improved cutaneous deposition	Manca et al., 2014
Abdomen, plastic surgery	Frozen	FT, HSE	*Niosomes*	Ellagic acid	Efficient delivery of ellagic acid into the deeper layer of the skin. Penetration depended on the vesicle size, amount of ellagic acid and solubilizers	Junyaprasert et al., 2012
Abdomen, plastic surgery	Frozen	FT, TS, D	*Invasomes*	Temoporfin	Enhanced deposition of the drug in SC	Dragicevic-Curic et al., 2008
Abdomen, plastic surgery	Frozen	FT, TS, D	*Flexible liposomes (flexosomes)*	Temoporfin	Cationic flexosomes delivered the highest amount of the drug into SC and deeper skin layers	Dragicevic-Curic et al., 2010
Abdomen, plastic surgery	ND	FT	*Propylene glycol liposomes*	Miconazole	Enhanced skin deposition of the drug in comparison to conventional liposomes	Elmoslemany et al., 2012
Abdomen, cadaver	Frozen	D (0.5 mm)	*Ethosomes*	Methotrexate	Enhanced transdermal flux of drug and decreased lag time	Dubey et al., 2007
Abdomen, plastic surgery	Frozen	FT	*SLNs-in-gel*	Diclofenac sodium	Higher drug permeation through the skin	Gaur et al., 2013
ND, cadaver	Frozen	FT	*Polymeric NPs*	Glucosamine	Improved transdermal permeation, shorter lag time and higher flux	Marimuthu et al., 2013
Abdomen, plastic surgery	ND	D (0.4 mm)	*Nanoemulsion, SLNs, liposomes*	Retinyl palmitate	Higher skin retention of liposomal rethinyl palmitate compared to nanoemulsion and SLN	Clares et al., 2014
Thigh, arms, abdomen of a single donor, cadaver	Frozen	D	*Lipid-based NPs*	Melatonin, ß-estradiol, caffeine, α-MSH, spantide	Cy5 based nanoparticles increased the permeation of all drugs	Marepally et al., 2013
Thigh, post-operatively	ND	FT, TS, MSE	*Microemulsions*	Deuterated ceramide	Enhanced permeability into and across SC	Sahle et al., 2014

Thorax, cadaver	Frozen	FT, TS	*Hydrogel, O/W emulsion, oily solution*	Linalol, terpinen-4-ol	Skin penetration of both terpens in the order: emulsion < oily solution < hydrogel; slower elimination from hydrogel	Cal, 2006
Abdomen, ND (female)	ND	Epidermis	*Nanostructured lipid carriers*	Artemether	Controlled release or the drug over an extended period of time	Nnamani et al., 2014
Brest, plastic surgery (single donor)	Frozen	FT	*Deformable-, conventional-liposomes, ethosomes*	Celecoxib	Penetration into the skin in the order: aqueous solution < conventional liposomes < deformable liposomes < ethosomes	Bragagni et al., 2012

Cy5, 1,1-Di-((Z)-octadec-9-en-1-yl)pyrrolidin-1-ium iodide; D, dermatomed skin (split-thickness skin); ND, no data; FT, full-thickness skin; HSE, heat-separated epidermis; MSE-mechanical separation of epidermis; MSH, melanocyte-stimulating hormones; NPs, nanoparticles; O/W, oil-in-water emulsion; SC, *stratum corneum*; SLNs, solid lipid nanoparticles; TS, tape stripping

In all experiments the subcutaneous fat was removed after the skin excision.

Table 5. Advantages and limitations of different skin models used for optimization of topical formulation

Skin model	Advantages	Limitations
Silicone model membranes	Reproducible Low cost Storage	Non-lipid based Low resemblance to SC Non-biological origin
PAMPA	Reproducible Prolonged storage capabilities Low cost	Synthetic lipids/non-lipid based Lipid organization not characterized/ Low resemblance to SC Non-biological origin
PVPA	Reproducible Lipid composition easily modified Relatively low cost Storage	Lipid organization not characterized Non-biological origin
SCS	Mimicking SC lipid organization Steady state flux similar to human SC Reproducible Lipid composition easily modified Relatively low cost	Not used in formulation optimization yet Non-biological origin
Reconstructed human skin equivalents	Consistence in permeability in comparison to human skin	More permeable than human skin Questionable barrier function High cost
Pig ear	Easily obtained (waste from slaughter) Similarity with human skin	Age of animal influences skin thickness Removal of hairs (skin damage) Storage
Newborn pig	Thickness of the SC is similar to human horny layer	Higher number of hairs than in humans Different anatomical sites: abdomen, back Difference in the skin thickness; newborn and older animals Storage
Mouse	Small size, uncomplicated handling Hairless species available	Ethical permission Very thin skin, highly permeable High density of hair follicles Removal of hairs (skin damage)
Rat	Small size, uncomplicated handling Hairless species available	Ethical permission Thin skin, more permeable than human High density of hair follicles Removal of hairs (skin damage)
Guinea pig	Similar permeability to human and pig ear skin Hairless species available	Ethical permission High density of hair follicles Removal of (skin damage)
Rabbit	Ears as waste from slaughter Similar permeability to guinea pig	Ethical permission High density of hair follicles Removal of hairs (skin damage)
Shed snake	Single animal provides repeated sheds Multiple samples from one shed Storage at room temperature	Absence of hair follicles Differences in skin metabolism, compared to human skin Absence of living epidermis and dermis
Bovine udders	Easily obtained (waste from slaughter)	One donor enables testing of only one sample (BUS)

	BUS-comparable to living skin Multiple samples from one animal	Weaker barrier to some drugs than pig skin Storage
Human	The most relevant model	Ethical permission Higher inter- and intra-variability than with porcine ear skin Different sources: age, sex, race, plastic surgery, amputation, cadaver Different anatomical parts: abdomen, tight, breast, back, etc. Storage

BUS, perfused bovine udder skin; PAMPA, parallel artificial membrane permeability assay; PVPA, phospholipid vesicle-based permeation assay; SC, *stratum corneum*; SCS, *stratum corneum* substitute