Recent developments in skin mimic systems to predict transdermal permeation

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Abstract

In recent years there has been a drive to create experimental techniques that can facilitate the accurate and precise prediction of transdermal permeation without the use of *in vivo* studies. This review considers why permeation data is essential, provides a brief summary as to how skin acts as a natural barrier to permeation and discusses why *in vivo* studies are undesirable. This is followed by an in-depth discussion on the extensive range of alternative methods that have been developed in recent years. All of the major 'skin mimic systems' are considered including: *in vitro models* using synthetic membranes, mathematical models including quantitative structure-permeability relationships (QSPRs), human skin equivalents and chromatographic based methods. All of these model based systems are ideally trying to achieve the same end-point, namely a reliable *in vitro-in vivo* correlation, i.e. matching non-*in vivo* obtained data with that from human clinical trials. It is only by achieving this aim, that any new method of obtaining permeation data can be acknowledged as a potential replacement for animal studies, for the determination of transdermal permeation. In this review the relevance, and potential applicability, of the various model systems will also be discussed.

Introduction

Skin is a natural barrier yet, for many years has been a desirable route of administration for therapeutic drugs. For any cosmetic skincare product, environmental, or pharmaceutical compound, it is vital to know both the rate, and extent, of transdermal permeation to satisfy regulatory authorities. There are several ways the required data can be acquired, broadly categorized into *in vivo, ex vivo* and *in vitro* models (1). For all products that are intended for percutaneous permeation the main intention is optimisation of the drug and formulation to achieve maximum *in vivo* performance. Ideally, human studies would be undertaken to ascertain such information. However, this is not normally feasible during development. Thus, researchers resort to the aforementioned models. Until recent years, the majority of studies in this area utilised a wide variety of animals, mainly rodents (2), to obtain drug permeation data which can then be used as a basis to predict clinical outcomes. Although the volume of data that has arisen from such *in vivo* work has been beneficial in the development of some pharmaceutical products, there is a clear trend to move away from animal studies (3) for three main reasons. Firstly, ethical issues surrounding the use of live animals is a major incentive to consider other testing methods. This is especially true in the EU and several other markets where it is already no longer permissible for cosmetic product testing to involve the use of animals, with further restrictions to follow (4). Although their use in the pharmaceutical industry is still permissible, there is

a clear move towards avoiding animal studies where possible. Secondly, researchers are adopting skin mimic systems because the animal study data that does exist cannot easily be compared and analysed across experimental studies as a result of the diverse range of animals used in research. Even within datasets from similar species the results may vary so considerably that comparative analysis is impossible, even within human studies inter-individual variation can be an issue (5, 6). The third, and possibly the most compelling, reason that transdermal permeation studies may avoid the use of animals is a lack of clear correlation between animal and human clinical trial data. For example, it has been shown that rodent skin generally shows higher permeation rates than human skin, often leading to incorrect conclusions from experimental data (7). Porcine skin, particularly that from the ear, is used in permeation studies as it has been shown to have similar properties to human skin (8) yet full animal studies with pigs are not ideal for the reasons previously outlined.

The outermost layer of human skin is the stratum corneum and is the main barrier for transdermal permeation. In human stratum corneum, the major lipid classes are ceramides, cholesterol and saturated long chain free fatty acids (9). Lipid organisation is fundamental to skin barrier function, for example in diseased or dry skin the lipid composition is different from that in healthy subjects. However, the specific details regarding lipid organisation within the stratum corneum are not fully understood, although it is accepted they will have an influence on transdermal permeation (10). It should be noted that in some cases it is not transdermal permeation that is desired but dermal absorption, i.e. delivery within particular regions of the skin itself – see ref. (11-13) for examples. In such circumstances transdermal permeation must still be measured, and considered, to satisfy regulators regardless of whether permeation is the intended outcome or not. For dermal delivery studies, drug permeation and distribution is not as specifically focused on the stratum corneum permeation as transdermal studies, but will also consider the deeper layers individually, namely viable epidermis and dermis. These are not so relevant to transdermal research as it is often assumed that stratum corneum penetration is the rate limiting step in permeation and must therefore be the focus of such studies.

Skin permeability is closely linked with the hydrophobicity/hydrophilicity of the molecule in question. In general, researchers have shown that skin permeability decreases with increasing hydrophilicity, as expected based on the lipidic structure of the skin (14). Furthermore, the rate and extent of transdermal permeation is dependent upon several other factors including product formulation, location of application, temperature (15, 16), volume applied and skin integrity. Recent studies have begun to substantiate the importance of formulation and the considerable impact it may have on permeation (17, 18) yet much work is still to be done to fully understand the relationships involved.

This article reviews recent developments and current trends in the variety of methods reported to determine transdermal permeation data, avoiding the use of *in vivo* studies. Collectively, all such techniques can be referred to as ‘skin mimic systems’ yet individually they are comparatively diverse, subdivided into:

- *In vitro* models using synthetic membranes
- Mathematical models including quantitative structure-permeability relationships (QSPRs)
- Human skin equivalent models
- Chromatographic models

In vitro models using synthetic membranes

For the past ten years, there has been general acceptance of the Organisation for Economic Cooperation and Developments (OECDs) guidelines for *in vitro* methods in the examination of skin permeation and distribution (No. 428). These guidelines set out a basic study design and requirements to justify certain experimental parameters such as membrane choice, dose concentration and assay

validations. For all major *in vitro* systems, the basic experimental setup includes a phase to replicate the skin surface, a separating membrane barrier and a solution phase to replicate beneath the skin – see reference (19) for a full description. In all studies the sample is applied to the skin surface (donor) phase, given time to pass through the membrane barrier and extracted from the second (receptor) phase at given time intervals to then be analysed to determine concentration. From such data it is possible to calculate a permeability coefficient, assuming 'infinite' doses are considered, most commonly using a Franz-type diffusion cell or flow-through cell design (20). Modifications of this set-up have been published, such as a novel diffusion cell which allows study of membrane diffusion processes without the need for sampling of the receiver compartment (21). The proposed method employs a spectrophotometer quartz cuvette containing the receiver solution with a small PTFE cap containing the membrane and an injection port through which compounds can be applied. The obvious benefit of this more advanced system is the non-invasive nature of obtaining permeation data and its potential for continual and automated permeation measurements. Other researchers have published methods to study membrane transport processes that also avoid the necessity to remove samples such as surface enhanced Raman scattering (SERS) which again provides non-invasive advantages compared with traditional methods (22). More recently, analysis has begun to move away from the standard choice of UV-based techniques to include alternatives, such as attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and target factor analysis where it was found data could be successfully deconvoluted and different components of formulations identified (23).

Drug diffusion studies are often used to measure the movement of selected compounds through a specific membrane, chosen by the researcher to mimic the *in vivo* scenario. Data acquired from such studies can then be correlated with that obtained from human clinical trials. In some cases the membrane selected for use may be of human origin (24, 25), although this is infrequently undertaken for two main reasons, namely economic constraints and data variability. Skin for these studies is often acquired following surgical procedures. Samples can vary significantly in thickness and composition (often frozen for transport), which is not always a problem (26-28), although, it has been reported to be an issue in many studies - as highlighted in the low reproducibility in data obtained (29). Rather than using human skin, some experimental work has employed animal skin as the membrane to separate the two phases. Again, this can lead to significant variability in data depending upon the sample location and the species used. For example, one particular study compared permeability coefficients for several commonly used drugs between hairless mouse skin and human skin and found the human skin values to be far lower than the mouse skin, exemplifying the poor *in vitro-in vivo* correlation (30).

Although the economic costs of using animal skin are far lower than those for human skin, issues of data variability and ethical concerns have led scientists to develop many forms of synthetic membranes to overcome these problems. The vast majority of work in the field of synthetic membranes for transdermal and topical delivery studies has focused on the use of polymeric membranes (31), such as silicone-based membranes (32). Such membranes are ideal for replacing *ex vivo* skin as they can be synthesised to a desired thickness, are easy to handle and store, are comparatively cheap, are inert, and provide reproducible data. For all of these advantages, many studies have considered the suitability of replacing both human and animal skin with silicone membrane for *in vitro* studies, attempting to develop a successful *in vitro – in vivo* relationship to ensure such a membrane can be truly considered as an acceptable model system for the prediction of transdermal permeation in humans. Silicone-based membranes, such as polydimethylsiloxane (PDMS) are generally hydrophobic in nature, and provide a rate limiting step in drug permeation. Many of the topical drug delivery diffusion studies that have employed synthetic membranes are summarised in reference (31), illustrating the vast range available to researchers, from the standard silicone membrane, to polyethylene to cellulose ester. The membranes exhibit variability in their pore size, thickness and permeation resistance, thus affecting the rate and extent of drug penetration, which

in turn leads to variations in calculated permeability coefficients. Some studies involve porous membranes in which case pore size in particular can be an incredibly influential factor, indicated by its alternative name – 'molecular weight cut-off', identifying the relationship between the size of the permeating molecule and the likelihood of permeation occurring. More specifically, porosity and tortuosity are frequently used to define porous synthetic membrane structures, which is vital when considering a porous membrane for *in vitro* studies.

For all synthetic membranes, the particular physicochemical properties displayed by the membrane are mainly derived from the preparation method used, thus it is vital to have a reproducible manufacturing technique to avoid inter-experimental variability. For a non-porous membrane, permeation occurs in three stages – firstly the permeant dissolves in the membrane, secondly it diffuses through the membrane and finally, it emerges from within the membrane. This process follows Fick's First Law of diffusion and permeation depends upon interactions between the compound and the membrane, in a similar manner to that seen between the compound and hydrophobic stratum corneum.

Synthetic membranes are used in transdermal studies for two specific purposes; *in vivo* prediction and qualitative analysis. The latter is routinely measured using Franz-type diffusion cells as part of the quality control process to ensure new products display comparable diffusion profiles to those previously measured. The low cost and simplicity of the technique are ideal to ensure batch to batch consistency in formulations with minimal complications from biological variations that would be encountered with *in vivo* analysis. The former, i.e. the use of synthetic membranes to predict transdermal permeation *in vivo*, is open to more debate as researchers attempt to correlate the experimental data obtained with that expected from clinical studies. Through simplifying the membrane structure to a synthetic material increases the reproducibility of the data yet also in turn, removes the finer detail, such as more complex phenomena including metabolic epidermal activity. For example, it has been found that introducing metabolic inhibitors can have a pronounced effect on transdermal drug delivery (33), i.e. implying synthetic membranes may not be complex enough for such studies. However, many feel the benefits from such a compromise outweigh the costs, for example, even when replicating inflamed skin (34). It has been suggested that artificial membranes can only provide a useful forecast of *in vivo* transdermal delivery when the following criteria are met (35):

- Passive diffusion through the stratum corneum is the major resistance to transport
- The drug under investigation is known to be metabolically inert and not specifically bound in viable skin
- The formulation does not contain a permeability enhancer which can interact with skin but not membrane and
- *In vivo* experiments can be performed and correlated with *in vitro* results.

Investigations into the formulation aspects of the applied product have further confirmed the variability that can occur as a result of modifications in the chemical nature of the excipients selected (36-41). For example, research within our own group has found substantial effects based upon the simple addition of an anionic surfactant to the formulation, yet no effect upon the addition of a cationic surfactant (37). For example, a study into the influence of ethanol on the solubility, ionisation and permeation characteristics of a model drug (ibuprofen) found the flux through silicone membranes increased up to a maximum of 100 % ethanol yet, in human skin, flux was optimal at lower ethanol percentages (42). Following on from this study, researchers investigated the influence of propylene glycol (PG) using binary (PG:water) and ternary (ethanol:PG:water) solvent systems. Fluxes were maximum for 70:30 PG:water systems in silicone membrane; however, for experiments conducted with skin, the flux of ibuprofen systematically increased with increasing amounts of PG. For silicone membrane, the flux values of ibuprofen from ternary systems were higher than the

highest values observed from the binary systems (43). Furthermore, permeation from mineral oil (MO), Miglyol® 812 (MG) and binary mixtures of MO and MG found the solubility of ibuprofen to be higher in MG than in MO. However, the permeation of ibuprofen from the pure vehicles and combinations of both was comparable in silicone membrane. Additionally, when the permeation of various hydrophilic and lipophilic vehicles was considered, a trend between flux values for the model membrane and skin was evident suggesting that silicone membrane may provide information on qualitative trends in skin permeation for vehicles of diverse solubility and partition characteristics (44). Other studies have also demonstrated the effects excipients may have on drug solubility and permeation. One such example highlights this phenomenon where drug-polymer dispersions were clearly shown to improve flux for a poorly soluble drug, namely artemisinin (45). However, not all excipients incorporated within transdermal formulations are capable of influencing drug transport or permeating with the drug. For example, concerns had been raised about the possible implications that dermal exposure to nanoparticles may have for human health. The maximum flux of such systems was calculated, and the results confirmed that they are too large to permeate skin (46). Based on all of these findings it can be said that the permeation of compounds through skin *may* be affected by the additional compounds within a formulation, but determining the specific details of such an influence is not a simple matter.

Some researchers have attempted to expand the applicability of using synthetic membranes to maximise their suitability for permeation studies. Ng et al. (47) investigated diffusion rates of ibuprofen across thirteen membranes finding variable drug fluxes, thus categorising the membranes into high-flux and low-flux membranes. For example, they found that porous membranes derived from various polymers demonstrated different degrees of diffusional resistance to ibuprofen, indicating that there would be wide discrepancies in results obtained from different laboratories using different porous membranes. They suggest that when selecting a membrane for formulation analysis it is best to choose one with high porosity, a tortuosity of one and be relatively thin. Furthermore, the compatibility of the membrane with the donor, and receptor, components must be considered, along with the cost effectiveness of the membrane. In transdermal permeation studies, it is common to measure drug release rates by monitoring the cumulative mass of drug appearing in the receptor solution (M_R). However, if a synthetic membrane is placed between the donor and receptor phases then there is a delay in drug transfer, and M_R is not immediately linear with respect to time. As a result of this non-linearity it is vital that permeation studies are conducted for long enough that the relationship achieves linearity to ensure calculated drug diffusivity values are correct (48).

More complex synthetic membranes have shown promise to provide a better representation of the stratum corneum barrier, for example, using a polydimethylsiloxane-polycarbonate block copolymer membrane (Carbosil) (49). In one such study, permeability was examined as a function of permeant molecular weight, melting point, solubility, partition coefficient and diffusivity for 14 drugs covering a wide range of chemical structures. It was found that Carbosil provided a higher drug solubility, and consequently, higher permeability compared with human skin. However, by varying the block copolymer membrane the diffusivity could be significantly modified, implying a more appropriate *in vivo* model can be created. Further work in this field has led researchers to establish the importance of considering membrane hydration in such studies (50). In another study two synthetic lipid models (designed to replace human stratum corneum) were studied to investigate the impact of volatile organic chemicals on the molecular organization of the skin barrier lipids (51). The models built upon previously developed self-assembled lipid membranes, which have a composition and 3D organisation similar to those of the *in vivo* lipid matrix. In one model the target chemicals were incorporated in the lipids before their self-assembly, and in the other one they were applied on top of a preformed lipid membrane. Encouragingly, the dose-dependent effects of the chemicals on the lateral molecular organization in the models were qualitatively identical to those observed by infrared spectroscopy in human skin. The study concluded that these model systems are suitable for *in vitro* studies in the areas of skin biophysics, dermatology, transdermal drug delivery, and risk assessment.

Natural membranes have also been considered to measure permeation for model drugs, including those of different molecular weights and lipophilicities using Franz-type diffusion cells. For example, membranes can be taken from the outer layers of peach and tomato, the middle layers of onions and with the inner layer of eggs (52). Encouragingly, results showed that the rate and amount of diclofenac permeated through onion skin, metronidazole through tomato skin and erythromycin through egg membrane was not significantly different from that with human skin. From these results it was concluded that natural membranes have pores and channels with hydrophilic properties, permitting permeation of small to middle size hydrophilic drugs to diffuse in a manner similar to human skin. Other natural membranes have also been considered for transdermal studies, and comparisons made with the more standard membrane options (53).

Within the last ten years a parallel artificial membrane permeability assay (PAMPA) has been developed for the rapid determination of passive transport permeability *in vitro*, gaining acceptance in pharmaceutical research. In PAMPA, a 96-well filter plate coated with a liquid artificial membrane is used to separate two compartments: one containing a buffer solution of compounds to be tested (defined as donor compartment) and the other containing an initial fresh buffer solution (defined as acceptor compartment). In one of the earliest studies, isopropyl myristate, silicone oil, and mixtures of the two components were immobilised on filters and tested as liquid supported membranes in PAMPA to evaluate their potential to mimic the human skin barrier (54). Effective permeability coefficients (P_e) were determined for a set of compounds using the PAMPA technique and compared with the corresponding human skin permeability coefficient values (K_p). A good correlation between P_e and K_p was found for compounds tested through a membrane consisting of 70 % silicone and 30 % IPM. Moreover, a positive correlation between the membrane retention of compounds and stratum corneum/water partition coefficients (P_{SC}) was established, implying PAMPA can be used for the prediction of passive human skin permeability coefficients. Further studies in this area have further confirmed the validity of the technique (55) and, along with the low cost, versatility and good reproducibility (56) of the system ensure it is a feasible membrane mimic system. In 2012, Sinko et al. (57) attempted to match the permeability of the rate-limiting barrier in human skin using synthetic analogs of the ceramides present in the stratum corneum. The final skin-PAMPA membrane lipid mixture (certramide, free fatty acid, and cholesterol) was selected and optimized based on data from three different human skin databases and the final model was found to correlate well to all of the databases. The reproducibility of the skin-PAMPA model was investigated and compared to that of other PAMPA models, confirming it to be a quick and cost-effective research tool that can serve as a useful model of skin penetration in pharmaceutical and cosmetic research. More recently, several variations of the artificial membrane employed in the PAMPA study system were analysed (isopropyl myristate (IPM), certramides and Strat-M™) (58). These were evaluated for their ability to predict the skin permeability of caffeine, cortisone, diclofenac sodium, mannitol, salicylic acid and testosterone applied in propylene glycol, water and ethanol as unsaturated and saturated concentrations. Resultant absorption data was compared to skin diffusion cell data. The correlations between membrane and diffusion cell data from saturated and unsaturated concentrations were rather low, although this relationship improved when only saturated concentrations were evaluated. These results suggest the potential of PAMPA as an initial screening approach to assist in narrowing the selection of formulations to be evaluated, thereby assisting in the development of new topical formulations. Based on these findings and others in the field (59), PAMPA has been accepted as a suitable skin mimic system that can provide significant benefits for *in vitro* analysis compared with more simplistic artificial membrane systems. Furthermore, results of PAMPA permeability and retention have been used to create mathematical models that could be employed for the design of novel derivatives with a favorable skin retention/permeability ratio.

In summary, *in vitro* models for predicting transdermal permeation have been thoroughly studied over the years, including the effects of factors such as the formulation and choice of

membrane system. In recent years, researchers have successfully developed more complex artificial membrane systems that are able to provide precise and accurate predictions of transdermal permeation without the need to use human or animal skin. The benefits of such non-*in vivo* systems are significant, for economic, reproducibility and ethical reasons amongst others.

Mathematical models including quantitative structure-permeability relationships (QSPRs)

Using models to predict the permeability of a compound through skin is particularly complex as a result of the intricate nature of the structures and mechanisms that dictate the delivery pathway. While the stratum corneum barrier, which serves as the major rate-limiting component to skin penetration for most drugs, has been the focus of most penetration models, much less is known about transport beyond this stage, and about transport via the appendageal routes. When modelling skin transport processes quantitatively, it is usual to consider the particular physiological regions of interest as compartments (60). Drug levels within the compartments can be modelled as a single time-dependent value, or as function of both position within that compartment (usually skin depth) as well as time. For the latter choice, drug transport within the compartment may be modelled using partial differential equations that describe the effects of drug diffusion, convection, elimination and metabolism (61). Models usually concern the steady-state flux of drug into the skin and related quantities such as the permeability coefficient and the maximum steady-state flux, the lag time between drug application and attainment of the steady state; the clearance of drug through excretion; and drug concentrations in the various skin layers, circulation, or other tissues (62). Theoretical permeation models have become more and more complex over the past fifty years. For example, ranging from simple models that consider the stratum corneum as a single compartment to those more complex that consider the structural characteristics of skin, including contributions from the lipidic components. Complexity may not always be necessary, for example, one particular study in 2011 found that only by using a two-compartment dermal clearance model that includes both diffusion and transport by dermal blood vessels consistency was obtained between observed and previously described *in vivo* literature data (63).

In general, two types of mathematical models have been developed to predict transdermal permeation, i.e. those based on the properties of the compound permeating or those based on the properties of the skin being permeated. Whereas the first is focused on predicting penetration through the skin from a solute's physicochemical properties, the second type models transport processes in skin layers using appropriate equations with the specific aim of predicting the concentration of a given solute in viable skin tissues (64). In general, it has been found that models are an important tool for accurate valuation of skin toxicity experiments, estimation of skin toxicity and for developing new formulations for skin disease therapy. Being aware of toxicological responses is important for a variety of reasons, for example when considering likely exposure of workers to certain compounds (65, 66). However, more comprehensive mathematical models of drug transport in skin, especially those based on more physiologically detailed mechanistic considerations of transport processes, are required to further enhance their role in assessing skin toxicology.

Models of skin absorption have attracted significant interest in the last two decades following the publication of two models for quantitative analysis (67, 68) in which permeability was related to physicochemical properties of the permeant. Prior to that, the majority of studies tended to focus on only small groups of compounds and the relationship with hydrophobicity where, unsurprisingly, it was found an increase in hydrophobicity led to an increase in permeation. Expanding consideration to a wider range of permeant characteristics, for example hydrogen bonding, melting point and ionization properties, led to the development of more ‘refined’ models, summarized in references (35) and (69).

Fitting experimental data to create statistically derived equations creates quantitative structure-permeability relationship (QSPR) models. Most QSPR models consider skin permeation through tortuous lipid pathways that may under-predict skin permeability of hydrophilic solutes, by several orders of magnitude. As a result of this researchers have begun, in recent years, to consider including aqueous pathways to improve the predictive abilities of permeation for hydrophilic solutes (70). As a further complication, some compounds are known to undergo metabolism within the skin thus several models also take into account contributions from metabolite permeation (71). As a variety of physicochemical parameters are known to potentially impact on drug-release profiles, researchers have developed mathematical models that take such parameters into account, for example taking into consideration the effects of dosing level, the type of vehicle (i.e. formulation), concentration profiles (72), solubility in particular solvents (73) and ionisation state of the permeant (74). Although these models tend to be based on several assumptions (for example: that steady-state transport across the skin is achieved even though a finite dose was applied) they have been shown to be successful for predictive ability in certain situations. Combining calculations of skin concentrations within two diffusion layers and results from silicone membrane permeations studies has led to precise predictions of in-skin concentrations (75). A few studies have evaluated the correlation between skin permeability predictive models and human *in vivo* data, with mixed findings, ranging from one study concluding most models correlated well with the *in vivo* data (76), through to another study that declared models were not suitable for accurately predicting permeation but were able to effectively rank the permeants and could help to select candidate molecules for *in vitro* screening (77).

In summary, mathematical models of transdermal permeation play an essential role in the investigation of epidermal and dermal transport of compounds, despite their limitations based on assumptions introduced to simplify the process, they are useful tools for data analysis, and predictions for dermal solute penetration (78).

Human skin equivalent models

As researchers have strived to create an *in vitro* model as close as possible to human skin, many have viewed the development of three dimensional tissue models to be the ultimate goal. A major drive towards this aim has arisen from the cosmetic industry with the necessity to replace animal testing therefore creating a regulatory reason to provide human skin equivalents. This research is now beginning to be adopted by pharmaceutical scientists as applications for measuring transdermal permeation are realised. Although attempts have been made to produce a reliable model for over twenty years, as recently as 2001 there was a belief that such models were not useful for *in vitro* penetration studies compared with other existing methods. For example, one particular study found far higher flux and skin concentration values using a living skin equivalent compared with human skin (79). However, more recently, human skin equivalents have been described as an ‘excellent’ tool, for example to study water distribution following application of moisturiser (80), and many view them as the future of transdermal permeation studies *in vitro* (81-85).

Models formed using only one cell type, known as reconstructed human epidermis (RHE), are used for their high level of reproducibility, yet can be viewed as too simplistic for consideration as a complete human skin equivalent system. The European Centre for the Validation of Alternative Methods has validated several RHE models for skin corrosion and irritancy studies confirming their place as a viable replacement to other methods to obtain such data. To conform to the necessary criteria the test systems must pass minimum standards relating to viability, barrier function, morphology, reproducibility and quality control. Analytical techniques to verify these criteria include infrared and Raman spectroscopy (86) and confocal laser scanning microscopy (87). Researchers have attempted to apply RHE models to determine drug permeation and one such study found values exceeded those for human epidermis yet, showed a tendency towards a lower level of variability (88).

Commercially available examples include SkinEthic®Rhe and Episkin®, both developed by L'Oreal, France and more recently, oral epithelium models such as EpiOral™ (MatTek Corporation, USA).

Full thickness models consist of more complex systems, for example incorporating additional cell types such as melanocytes and stem cells. By increasing the complexity of the models, through incorporation of additional components, researchers believe they more truly replicate *in vivo* scenarios yet it must also be remembered they decrease the reproducibility and can significantly increase experimental costs. A detailed summary of the available state-of-the-art models can be found in reference (89). Full thickness models are composed of both dermal and epidermal layers, i.e. creating a bilayer structure similar to that found in human skin. More and more complex systems are continually being created, such as by the creation of a viable adipose layer (90), allowing researchers to resolve more complex dermatological issues (91). Commercially available examples include RealSkin® (L'Oreal, France) and AST2000 (CellSystems Biotechnologie GmbH, Germany) (92).

Unsurprisingly, there are limitations even with three dimensional skin models which must be addressed when considering the use of such systems for *in vitro* permeation determination. Firstly, most relevant to cosmetic applications, is the inability of the models to generate stratum corneum, as they consist of primary cells with a limited lifespan and, a lack of cells of the immune system. It has been proposed that immortalised cell lines could improve the reproducibility and consistency of skin models reducing intra and inter-laboratory variations (93). Secondly, some studies have reported a dissimilar barrier function of human skin equivalents. For example, one such study found that the models were able to mimic many aspects of human skin but, differed in their barrier properties (94), implying they would not be suitable for permeation studies. Finally, the limited lifespan of these living models (~ eight weeks), reduces their suitability for experimental study, although some work has been conducted in this area to improve this situation, in one case increasing their availability for up to twenty weeks (95).

With such a rapid expansion of work (and commercially available products) in this area it is inevitable that the future of skin mimic systems incorporates aspects of RHE or full thickness models to some extent.

Chromatographic models

High-performance liquid chromatography (HPLC) has been utilised and adapted to predict skin permeability data, mainly in the last twenty years. Recently, the ability of a selection of chromatographic systems to model human skin permeation has been evaluated and tested, by correlating experimental data with *in vivo* data for a representative set of neutral solutes (96). It was reported for the six systems (including the classic octanol-water partition system) that the HPLC systems with C18 columns are the closest to the human skin permeation system whereas the micellar electrokinetic chromatographic (MEKC) systems were most different – based on distance parameters. However, the study also declared that error arising from the original skin permeability data is quite significant and the variance from the C18 systems was possibly too high to provide precise estimations of human skin permeability coefficients. This can be improved by introducing the solute's volume as an additional variable, resulting then in correlation models with good predictive abilities to estimate permeation for untested solutes.

A great deal of interest has been shown in one particular type of column used in HPLC, the so-called immobilised artificial membranes (IAMs). IAMs are synthesised by covalently binding biologically relevant phospholipids to the surface of silica, such as attaching monolayers of phosphatidylcholine to a propylamine silica support. For some years immobilised stationary-phase liquid chromatography has been considered a potential *in vitro* technique (97), including studies to examine its potential for predicting transdermal transport across neutral, basic, acidic and amphoteric

compounds (98). Interestingly, the findings indicated that IAM and partition coefficient values are complementary and not alternative parameters whose combination yields more useful data than either factor alone. Some researchers have taken the use of IAMs towards very specific applications, for example through the physical immobilisation of keratin or collagen on the silica support, permitting a comparison of the keratolytic properties of compounds (99, 100). Other forms of chromatography have also been explored, for example liposome electrokinetic chromatography (LEKC) (101). LEKC has been described as a promising simple method to predict drug penetration based on quantitative retention-activity relationships (QRARs) constructed between skin permeability coefficients and retention values.

Combining the desire to mimic the biological environment with a highly predictable analytical technique has resulted in the development of biopartitioning micellar liquid chromatography (MLC). In MLC, the mobile phase consists of surfactant molecules above the critical micellar concentration, i.e. in micellar form. A compound is then injected into this mobile phase and the retention time recorded over a series of concentrations. For some time, researchers have appreciated the value of biopartitioning micellar separation methods for modelling drug absorption (102), and more specifically, for predicting skin permeability (103). Within the last twelve months our research group has developed, evaluated and published a method to measure the chromatographic retention of drugs which can then be used to predict skin permeability using micellar chromatography, achieving high levels of reliability (104). In our study, we looked at a series of model compounds and found that the replacement of a traditional physicochemical parameter, namely the octanol-water partition coefficient, with a chromatographically determined value resulted in a quantitative value that was robust to variation, i.e. a suitable method to predict transdermal permeation. The advantages of this method are numerous, and of particular benefit is the high level of predictive capability that has not been seen in other studies.

In summary, chromatographic methods appear to show promise using a variety of experimental conditions and, may make a positive contribution to the future prediction of transdermal permeation.

Conclusions

When undertaking a study to investigate transdermal permeability, there are many options to consider, and researchers tend to choose techniques that are most suited to their intended purpose, availability, previous experience or economic constraints. The majority of the techniques currently available have been shown to be suitable for ranking a series of compounds (or formulations), yet do not provide an *in vitro-in vivo* correlation that suggests they can be used as a pure replacement. For many reasons this is the ultimate aim of such studies and work continues to develop systems that can either mimic skin to permit experimental data to be measured (i.e. using synthetic membranes or human skin equivalents), or predict permeation (i.e. using chromatographic methods or mathematical models). Development of the latter will undoubtedly lead to the availability of software that can simulate absorption of dose into the skin, diffusion through the skin and clearance into blood which can be used in the development of pharmaceuticals, cosmetics, household products and agrochemicals. A very limited number of such packages are already available, such as the Skin-in-Silico™ software (Xemet, Finland), yet it is highly anticipated that this is the likely direction of transdermal studies in the future, thus completely replacing the need for animal testing. Clearly, it can be seen that only through understanding the physicochemical properties of the compound under investigation *and* the structure of the skin is it possible to quantify transdermal permeation. For this reason, it will no doubt continue to be a combination of *in vitro* experimental measurement and predictive techniques that yields the most valuable results.

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