Perspectives in Percutaneous Penetration

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Introduction

This year we are celebrating the 21st birthday of the PPP Conference. The origins of the meeting can be traced back to 1989 when Bob Scott of ICI and Jonathan Hadgraft of Cardiff University organized a conference dedicated to 'Prediction of Percutaneous Penetration' in Manchester, UK. The success of this meeting led to a repeat of the format at Southampton University in 1991. As the scientific standards on that occasion far exceeded those of a culinary nature the possibility of moving the meeting outside the UK was explored. The serendipitous friendship between Henri Saunal of Sanofi, Montpellier and Michel Ginestet of AlphaVisa Congres led to the proposal of a new venue in the south of France and, as they say, the rest is history. Responsibility for organisation of the PPP passed to Keith Brain, Ken Walters and Michel Ginestet, with able assistance from the late Valerie James and, more recently, Brenda Williams. A key factor in the success of the meeting has been the input from the Scientific Advisory Board who have steered the programme very effectively to ensure that it continued to reflect items of current interest.

Since 1993 the PPP meeting has taken place at La Grande Motte, near Montpellier, on no less than eight occasions. In 1998 it moved from odd to even years to avoid a clash with the GRC meeting on Barrier Function of Mammalian Skin and the PPP joined with LACDR in Leiden, The Netherlands, in a special meeting celebrating the scientific contributions of the late Harry Bodde. A second exception was a move along the coast to Antibes in 2002, which only confirmed the excellence of our long-term choice venue.

The purpose of the PPP Conference is to provide a forum for the presentation and discussion of all the latest research, development and technology concerning the penetration of exogenous compounds through the skin. The meetings are especially relevant to those involved in dermatological research, the development of formulations, risk assessment and regulatory affairs relating to the dermal effects of molecules within the agrochemical, cosmetic and pharmaceutical fields within industrial, academic and governmental domains. As the meeting expanded its remit, the name associated with the PPP acronym was changed to 'Perspectives in Percutaneous Penetration' (at the suggestion of Professor Brian Barry) to better describe the widening scope of the meeting. An optimised combination of plenary lectures, oral contributions, poster sessions and debates are combined with an appropriate experience of local culture. The continuation of a high level of interest in the area is again reinforced by the large number of contributed presentations, which continue the tradition set at the previous eleven meetings.

Keith Brain
Gary P. Moss
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Permeation studies of ibuprofen from o/w palm kernel oil esters nano-emulsions

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The aim of this work was to study the skin permeation of ibuprofen, solubilised in o/w nano-emulsions formulated with palm kernel oil esters (PKOE).

Nano-emulsions in a water/Cremophor EL/PKOE system, with 80 wt% water and 2 wt% ibuprofen were characterized by DLS at 32°C. Droplet diameters around 17 nm (PI < 0.1) were obtained, similar to the results obtained in the water/Cremophor EL/Miglyol 812 system. Permeation experiments were performed with abdominal human skin and PBS pH 7.4 at 32°C. The median values of the fluxes (6.41 and 6.58 μg/cm^2 per h) from PKOE and Miglyol 812 nano-emulsions were not different ($P = 0.85$). However, the mean profiles of ibuprofen permeated vs time from PKOE were slightly greater than those from Miglyol 812 at the beginning and with statistical differences until the first 6 h. P_2 parameter from PKOE nano-emulsion was higher ($P = 0.03$) than that obtained with Miglyol 812 (78.33/h vs 0.29/h). Differences in the thermodynamic activity due to the slightly lower solubility of ibuprofen in PKOE than in Miglyol 812 could affect the diffusion process.

Flexible nanosomes (SECosomes) enable efficient siRNA delivery in cultured primary skin cells and in viable epidermis of ex-vivo human skin

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The extent to which nano-engineered systems cross intact human skin and can exert pharmacological effects in viable epidermis is controversial. This research sought to develop a new lipid-based nanosome that enables the effective delivery of siRNA into human skin.

Our major finding is that an ultraflexible siRNA-containing nanosome, prepared using DOTAP, cholesterol, sodium cholate and 30% of ethanol, did penetrate into the epidermis of freshly excised intact human skin and was able to enter into the keratinocytes. The nanosomes, called surfactant-ethanol-cholesterol-osomes (SECosomes), show excellent size, surface charge, morphology, deformability, transfection efficiency, stability and skin penetration capacity after complexation with siRNA. Importantly, these nanosomes have ideal characteristics for siRNA encapsulation, in that the siRNA is stable for at least four weeks, they enable highly efficient transfection of in-vitro cultured cells and are shown to transport siRNA through intact human skin where changes in the keratinocyte cell state are demonstrated.

It is concluded that increasing flexibility in nanosomes greatly enhances their ability to cross the intact human epidermal membrane and to unload their payload into targeted epidermal cells.

Impact of microemulsion microstructure on drug absorption into skin

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Microemulsions allow control of drug penetration into the skin and possess excellent solubilizing properties of both hydrophilic and lipophilic drugs. This study aimed to investigate microemulsions for skin delivery related to their nanoscale organization as o/w, w/o droplets or bicontinuous structures at 32°C.

Three such microemulsion types were formulated using appropriate Tween 21/Span 20 surfactant mixtures and fatty esters as oils. The methodology involved 'Kahlweit fish' phase diagrams established as a function of temperature for various Tween 21/Span 20 ratios. The microemulsions' structure was investigated using electrical conductivity scans, PFGSE-NMR diffusion measurements and small-angle neutron scattering experiments. The surfactant demand for bicontinuous microemulsions at 32°C increased according to the size of oil molecules.

Formulations with lowest surfactant demand were selected for assessment of skin penetration *in vitro* on excised pig skin. The percutaneous permeation of caffeine loaded in the three microemulsions was much faster than for the control aqueous solution. The fastest permeation rates were reached for bicontinuous and o/w microemulsions, where water is the continuous phase.

Comparison of two topical o/w emulsions: inner phase droplet size and delivery profile of a model drug through synthetic or natural membranes

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In this work the behaviour of two topical semisolid o/w emulsions (E1 and E2) were compared through three different studies.

Size and distribution size of inner phase droplets. According to Sauter diameters and normal distributions, the droplets (considered to be spherical) of emulsion E1, and their size distribution range, are larger and wider than those of E2.

Delivery profile of active compound through a synthetic membrane. Salicylic acid delivery through cellophane membrane from E2 is much higher than from E1. In both cases, salicylic acid release through synthetic membrane data fit Higuchi kinetics.

Delivery profile of active compound through whole human skin. Salicylic acid release through whole skin is higher from E2 than from E1. These salicylic acid release data fit zero-order kinetic (E1) or Higuchi kinetics (E2).

Comparing both emulsions, E2 is the one that: (1) exhibits the larger and homogeneous inner phase droplet size; (2) shows the higher salicylic acid delivery, both through synthetic membrane and whole skin and (3) fits Higuchi kinetics in any case.

Formulation and transdermal delivery of aciclovir and ketoconazole for HIV/AIDS patients

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Due to their compromised immune systems, HIV/AIDS-infected individuals are more susceptible to skin infections. Since fungal and viral cutaneous manifestations are frequently encountered in combination in HIV/AIDS patients, it is appropriate to formulate a topical product containing both ketoconazole (antifungal) and aciclovir (antiviral).

The efficacy of the novel Pheroid technology system was investigated, for the topical delivery of ketoconazole (2% w/w) and aciclovir (5% w/w). Four formulations containing both ketoconazole and aciclovir were prepared, namely cream and emulgel with and without Pheroid. Full-thickness abdominal skin was used for the in-vitro studies. The donor compartments were each filled with ± 1 ml of the formulation in at least nine vertical Franz diffusion cells. The entire contents of the receptor compartments were withdrawn and replaced with PBS (pH 7.4, 37°C) at predetermined times. It was expected that the Pheroid would promote the permeation of aciclovir and ketoconazole, thus the withdrawal times were started earlier. Each sample was directly assayed by HPLC.

The results demonstrated that the transdermal flux, epidermal and dermal penetration of aciclovir was enhanced by the Pheroid cream formulation. Ketoconazole's transdermal flux, as well as delivery to the epidermal and dermal layers of the skin, was improved by the Pheroid emulgel formula. The topical delivery of ketoconazole and aciclovir was thus enhanced by Pheroid technology.

Topical delivery of selected growth factors

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Growth factors can benefit from controlled release administration technologies (i.e. topical or transdermal delivery). When compared with other epithelial membranes, the skin has less enzymatic activity and so provides a more stable dosage medium and prolongs the delivery. The growth factors that were considered during this study are used for the treatment of alopecia (IGF-1, VEGF and KGF) and pigmentation (TNF-α & TGF-β).

Diffusion studies were done with full-thickness abdominal skin by the use of vertical Franz cells. PBS or Pheroid containing the individual growth factors was placed in the donor compartment of six diffusion cells. The entire receptor compartment was withdrawn and the concentration (μg/cm^2) was measured after 6 h, except for TGF-β which was withdrawn after 12 h. The skin was analysed by tape stripping to observe the concentration (μg/ml) that had penetrated therein. Bestatin hydrochloride was incorporated in the donor phase to avoid degradation. All samples were analysed by ELISA.

TGF-β showed the best diffusion through the skin and the best penetration into the skin of the growth factors used against pigmentation. For the growth factors used against alopecia, IGF-1 showed the best diffusion through the skin and VEGF gave the best penetration into the skin. Pheroid improved the diffusion through the skin and into the skin for all the growth factors except TGF-β.

Cutaneous penetration modulation of a hydrophilic agent by enhancer molecule and colloidal carrier system

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To reach therapeutic drug concentrations, the uppermost skin barrier, the stratum corneum, has to be overcome. Due to the lipophilic properties of the stratum corneum, hydrophilic drugs penetrate especially poorly. Therefore, the topical bioavailability of hydrophilic actives like *N*-acetyl-L-carnosine is highly challenging. Enhancer molecules, like 1,2-pentanediol, or colloidal carrier systems may enhance the dermal penetration of actives.

Ex-vivo studies were conducted to evaluate the penetration profiles of *N*-acetyl-L-carnosine from a topical standard preparation in the presence and absence of 1,2-pentanediol and from a microemulsion system.

Compared with standard preparation and o/w microemulsion, the addition of 1,2-pentanediol increased the skin penetration of *N*-acetyl-L-carnosine. The application of the formulation containing both *N*-acetyl-L-carnosine and 1,2-pentanediol resulted in higher active ingredient amounts at all incubation times in the viable skin layers compared with the preparation without enhancer molecule and with o/w microemulsion.

In conclusion, the use of 1,2-pentanediol in a topical formulation increased the penetration of the hydrophilic active *N*-acetyl-L-carnosine. Furthermore, the bioavailability of the active was improved and high concentrations could be achieved in the target site.

Colloidal carrier systems for enhanced cutaneous delivery of peptides

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The application of peptides and proteins as drugs is a very interesting and growing field in therapeutics. Due to some unfavourable properties, like high molecular weight and polarity, it is necessary to accomplish a modern colloidal vehicle system, such as microemulsions, to enhance their cutaneous penetration. The aim of the present work was to develop and characterise a new microemulsion system, which lends itself to an increased bioavailability of different tetrapeptides in the skin.

A promising colloidal carrier system was established. The isotropic area existed over a wide range in the pseudo-ternary phase. Optically, isotropy, low viscosity and Newtonian behaviour were confirmed for different microemulsions along dilution lines marked in the phase diagram. Additionally, conductivity measurements and DSC investigations were carried out. The results indicated that the designed system possesses the typical features of microemulsions. The incorporation of different tetrapeptides in relevant therapeutic concentrations could be realised. Penetration experiments using diffusion cells according to Franz will be carried out in the future to investigate the penetration enhancement properties of the developed microemulsion system.

The effect of chemical enhancers on the percutaneous absorption of pizotifen

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Diffusion of pizotifen through dermatomed pig ear skin (600 μm) was investigated using Franz diffusion cells (saline-buffered solution 2.4 mg/ml, pH 4.6). Skin was pretreated for 12 h either with saline buffered solution pH 4.6 (control), ethanol (vehicle control) or 5% (w/v) of either Azone (laurocapram), *R*-(+)-limonene, 9-decenoic acid or oleic acid in ethanol, to determine whether ethanol or these enhancers were able to enhance percutaneous permeation of pizotifen respective to the control.

Results showed that with an ethanol skin pretreatment, pizotifen flux values were poorly increased. We found highly increased fluxes for fatty acid enhancers 9-decenoic acid and oleic acid. For Azone and *R*-(+)-limonene we found a moderate increment in the transdermal flux. Among all the enhancers studied, 9-decenoic acid was the best enhancer. However, further work needs to be carried out to ensure that therapeutic blood levels of pizotifen can be achieved by transdermal administration.

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Novel strategy for enhancing transdermal drug delivery

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Increasing fluidity of skin lipids, via heat or chemical agents, is an effective method for improving transdermal drug delivery. Using heat to induce lipid phase changes reduces barrier function of the stratum corneum, although prolonged high temperatures (i.e. > 45°C) can result in burns. As such, the aim of this study was to investigate whether simultaneous

application of certain excipients to perturb the lipids and application of heat would have a synergistic influence on skin barrier function.

Permeation of lidocaine through human epidermis was assessed after application of four formulations. Two generated heat, one with additional excipients and one without; one formulation only contained the additional excipients and one formulation acted as a control. Each formulation was applied above a lidocaine-saturated gel. Samples were taken every 5 min for 30 min for HPLC analysis.

After just 10 min significant increases ($P < 0.05$, t-test) in lidocaine permeation were observed compared with the control (0.16 ± 0.13 $\mu g/cm^2$). A 2.7-fold increase was observed using heat alone (0.43 ± 0.03 $\mu g/cm^2$), a 7.5-fold increase was observed using the additional excipients alone (1.22 ± 0.58 $\mu g/cm^2$), but when the additional excipients were applied with heat a 75-fold increase was observed (11.93 ± 5.79 $\mu g/cm^2$).

Simultaneous application of heat and the additional excipients acted synergistically to enhance the permeation of lidocaine by increasing lipid fluidity. Use of these additional excipients to reduce lipid transition temperatures coupled with moderate heat could significantly reduce the time required to reach a therapeutic drug level while increasing the safety of heat-enhanced transdermal drug delivery.

Long- vs short-chain ceramides: evidence for different penetration into skin and barrier properties

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Stratum corneum ceramides (Cer) are the major determinants of the skin barrier function. We hypothesized that Cer chain length is essential for maintaining their barrier properties.

To confirm this suggestion, a series of short- and long-chain Cer, both natural and fluorescent, was synthesized. First, penetration of exogenous fluorescent NBD-labelled Cer into viable human skin was examined.

Fluorescence microscopy showed that, while the shortest NBD-C6-Cer penetrated into the nucleated epidermis within 12 h, the longer C12- and C24-Cer and pseudoCer 14S24 reached the stratum corneum only. Second, the effect of Cer with chain length of 2–24C on the skin permeability was investigated. The results proved that Cer chain length was crucial for their barrier properties. The shorter-chain analogues increased skin permeability and decreased skin electrical resistance with a maximum for butyryl (4C) derivatives. Moreover, permeability of model long- and short-chain Cer membranes and their thermotropic behaviour studied by FTIR and DSC confirmed these results.

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Effect of dipalmitoyl-phosphatidylcholine/dihexanoyl-phosphatidylcholine (DPPC/DHPC) bicelles on skin lipids molecular organization

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The effect of bicelles formed by dipalmitoyl-phosphatidylcholine (DPPC)/dihexanoyl-phosphatidylcholine (DHPC) on stratum corneum (SC) lipids was studied by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy at different temperatures. In this way, CH_2 stretching vibration gives information about chain conformational order of the SC lipids and CH_2 scissoring vibration provides information about lateral packing of the lipids. To confirm these results, grazing incidence small and wide X-ray scattering (GISAXS and GIWAXS) techniques were used. Analysis of the lipid organization in terms of chain conformational order and lateral packing showed that the use of bicelles hampers the fluidification of SC lipids with temperature and leads to a lateral packing corresponding to a stable hexagonal phase. Moreover, the GISAXS technique showed evidence of higher lamellar order after treatment with bicelles. Additionally, the effect of DPPC/DHPC bicelles was studied at different SC depths. The combination of ATR-FTIR spectroscopy and tape-stripping method was very useful for this purpose. The results suggest an incorporation of the phospholipid from bicelles into the SC, which could contribute to certain reinforcement of the skin barrier function.

Bicelles as preventive agents of thermal damage in skin collagen

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Different factors cause skin collagen denaturation at a rate faster than natural ageing. In this work, native skin and skin treated with bicelles, lipid nanostructures formed by long- and short-chain phospholipids dispersed in aqueous solution, were submitted to UV and thermal treatment. The degree of collagen fibre denaturation in the samples was evaluated by small-angle X-ray scattering using Synchrotron source.

Treatment of data using Fit2D and Fiberfix software allowed us to obtain intensity profiles and to analyse the annular distribution of diffracted intensity. Our results

indicated that skin collagen has high resistance to short UV exposures whereas thermal treatment induces a more drastic effect on the collagen arrangement. Thermal damage in collagen was higher in native skin than in skin treated with bicelles. This fact indicates a protective effect induced by bicelles against collagen degradation promoted by high temperature. Thermal treatments are used to improve some cutaneous disorders in medical and cosmetic protocols. Thus, use of bicelles for preventing possible collagen damage caused by these protocols should be considered as a promising application of these innovative and surprising lipid nanostructures.

In-vitro and in-vivo evaluation of skin barrier creams

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Barrier creams (BCs) were evaluated and compared with a BC reference containing perfluorinated compounds, which are relatively effective but too greasy and expensive. Our BCs were focused on oil in water and Pickering emulsions. They were compared regarding their stability, skin compatibility and cosmetic acceptance. BCs were tested *in vivo* on human skin with a rubefacient (ethyl nicotinate).

To demonstrate their efficacy, ethyl nicotinate was spread on skin after an application of cream. Two responses were analysed: intensity and area of the erythemal zone. Relative to untreated control, the skin with cream should exhibit a smaller erythemal area. It has been shown that application of the cream caused a significant decrease in rubefacient area of ethyl nicotinate for o/w BCs. BCs were then evaluated *in vitro* with caffeic acid on Franz cells and pig skin. After application of BCs and a deposit of caffeic acid for one day, a vertical slice of each sample was analysed by fluorescence microscopy. For one BC the fluorescence was lower than those obtained with other BCs. This emulsion acted as a BC for ethyl nicotinate and caffeic acid; other chemicals with a large range of logP values must be tested.

Skin delivery of a sun filter contained in biofunctional textiles

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The aim of this work was to demonstrate the skin delivery of a sunscreen (ethylhexyl methoxycinnamate, EHMC) encapsulated in two different liposomes, which were embedded in biofunctional textiles following a textile adapted in-vitro methodology. Two types of liposomes (with internal wool lipids (IWL) or phosphatidylcholine (PC)) with EHMC encapsulated were prepared. These liposomes were absorbed by bath exhaustion in two different fabrics (cotton and polyamide).

The percutaneous absorption of the liposomal formulations and the textiles with these embedded liposomal formulations were carried out using Franz type cells and porcine skin. The evaluation of the EHMC in the different skin compartments and in the textile was performed by HPLC.

The results showed that EHMC never reached the receptor fluid. In the evaluation of the percutaneous absorption (epidermis + dermis) of EHMC in PC liposomes, both free and in the textile, we detected more penetration than when EHMC was encapsulated in IWL liposomes. Besides, the liposomes embedded in the textiles always showed higher EHMC skin penetration than the aqueous EHMC liposome, with the polyamide ones showing the higher release properties.

Ex-vivo studies on the effect of formulation and penetration enhancers on the transdermal diffusion of lornoxicam through rabbit skin

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Lornoxicam (LOR) is a potent non-steroidal anti-inflammatory drug with strong analgesic activity, reported to be similar to morphine. It is available commercially as solid oral and parenteral dosage forms. LOR is a lipophilic drug with short half-life and low dose. The objective of the study was to investigate the effect of formulation and penetration enhancers on the transdermal diffusion of LOR through excised rabbit skin.

Using a Franz diffusion cell, 4 mg of LOR was applied to the excised rabbit skin in the form of solution and carbapol gel (CG) in the absence and presence of enhancers – betacyclodextrin (β-CD), dimethyl betacyclodextrin (DM β-CD), hydroxypropyl betacyclodextrin (HP β-CD), Tween 80, oleic acid (OA), urea and citric acid. The cumulative amount of the drug diffused after 24 h was measured and relevant diffusion parameters were calculated.

Results showed that CG formulation in comparison with the solution form resulted in enhanced diffusion due to its bioadhesion properties, which caused retention of the drug on the mucosal surface of the skin. In addition, of all the enhancers tested, HP β-CD resulted in significant increase in drug diffusion (10-fold enhancement), which can be attributed to increased solubility and hydrophilicity of the drug.

Percutaneous absorption of esculetin: effect of chemical enhancers

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Percutaneous absorption of esculetin (6,7-dihydroxy-coumarin), an oxidative damage inhibitor and a cancer chemopreventive, was investigated *in vitro* through dermatomed pig ear skin (600 μm) (saline-buffered solution 210 μg/ml, pH 7.4). The effect of a skin pretreatment with several chemical percutaneous enhancers (Azone, 9-decenoic acid and oleic acid) was also studied. With that purpose 200 μl of enhancer solution in ethanol (5% w/w) was kept in contact with the skin for 12 h before diffusion experiments. The permeation of esculetin under the different conditions assayed was investigated by means of steady-state fluxes. The flux enhancement ratios, calculated for each condition, were compared by means of one-way analysis of variance. Multiple comparison tests of flux enhancement ratio data were carried out with the Dunnet T3 test (heteroscedastic data). Results showed that esculetin permeates through the skin. Among all the chemical enhancers investigated, only Azone and oleic acid significantly increase the flux of esculetin across the skin respective to the control buffer and ethanol groups.

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Suggested mechanism of action of classical and deformable liposomes through pig ear skin

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This work concerns ex-vivo diffusion studies using pig ear skin in order to explain the penetration mechanism of classical and deformable liposomes. Franz diffusion cells were used to study the transport of a model drug, betamethasone (BMS), through pig skin. The method for BMS determination in the different pig skin layers and in receptor medium was successfully validated. Two types of vesicles were studied: the first one encapsulated BMS in the aqueous compartment by the use of BMS–cyclodextrin inclusion complexes while the second one encapsulated BMS in their lipid bilayer. The use of cyclodextrins enhanced the stability of the formulation. BMS diffusion from classical liposomes was reduced from 78.3% to 20.9% by the use of cyclodextrins after one week at 4°C. However, the encapsulation of BMS–cyclodextrin complexes reduced the percentage of BMS in the epidermis from 0.46 μg/cm^2 to 0.23 μg/cm^2 ($P < 0.05$). Surprisingly, deformable liposomes did not improve the penetration of BMS in the skin compared with classical liposomes. These first results seem to show that BMS is released from the vesicles, after which free drug could diffuse through the stratum corneum and partition in the viable skin tissue.

Penetration of gel and liquid state liposomes into barrier-impaired skin

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Many dermal diseases are characterized by changes in skin barrier function, providing a challenge for the delivery of drugs into specific layers of diseased skin. The aim of this study was to elucidate how the fluidity of the liposomal membrane bilayer affects liposomal interaction with intact and diseased skin.

Unilamellar liposomes composed of binary mixtures of dipalmitoyl-phosphatidylcholine (DPPC) and dilauroyl-phosphatidylcholine (DLPC) were prepared by the thin film method, followed by extrusion through 100 nm filters. The thermotropic phase behaviour was determined and two membrane compositions were chosen for skin penetration: a gel state composition (T_m = 39°C) and a liquid state composition (T_m = 26°C). The penetration into pig skin was examined using Franz diffusion cells and ^{14}C-labelled DPPC as a marker.

For both formulations the penetration into barrier-impaired skin was much more efficient than the penetration into intact skin, and a large increase in lipid accumulation in the epidermis was detected in barrier-impaired skin. Accumulation of lipids in the skin was increased from liquid state liposomes, compared with gel state liposomes, suggesting that the membrane fluidity affects the skin penetration of liposomes into both intact and barrier-impaired skin.

Influence of skin barrier on in-vitro penetration properties of solid lipid nanoparticles

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Solid lipid nanoparticles (SLNs) are known to facilitate retention of a drug substance to upper skin layers and increase penetration. This study evaluated the effect of skin barrier impairment and polarity of the lipid on SLN in-vitro penetration properties. SLNs differing in polarity were loaded with betamethasone-17-valerate (BMV). Penetration studies were performed with porcine skin. The influence of

skin structure on SLN penetration properties was examined by tape stripping to simulate diseased skin. SLNs were compared with an ointment.

SLNs stayed mainly on the skin surface. On intact skin, BMV from SLNs was found in higher amounts in the stratum corneum and epidermis. Results indicated that an intact barrier is important for SLN penetration properties. Treatment of diseased skin with SLNs therefore prompts consideration of barrier impairment. The barrier will be low when treatment is initiated and improve upon effect. Initially SLNs may possess penetration properties similar to those of an ointment, but as the barrier improves, SLNs can act more as a depot in the skin. Also, SLNs stayed mainly on the skin surface, which may help to physically restore the skin barrier and reduce dryness.

Influence of cosmetic formulations on the molecular organization of stratum corneum lipids and the barrier efficiency of human skin *in vivo*

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Detailed knowledge of the effects of topical formulations on the properties of human stratum corneum (SC) is of paramount importance for understanding the formulation-mediated interactions between chemicals and human skin.

This work used ATR-FTIR spectroscopic and TEWL measurements taken in the course of tape-stripping to investigate the effects of night cream, Eau de Toilette, and shower gel on the relative amounts and phase content of the SC lipids and on the barrier efficiency of healthy skin.

None of the three formulations affected significantly the overall content of orthorhombic lipid phases in the SC, but the cream penetration in the SC resulted in the formation of a strongly disordered lipidic phase in the topmost SC, and the application of shower gel stabilized the orthorhombic lipid phases at the SC surface. The inside-out barrier efficiency of SC following application of Eau de Toilette and shower gel remained unchanged; because of the occlusive effect of the cream, its effect could not be evaluated.

This study provides reliable baseline values to study the influence of cosmetic or pharmaceutical active ingredients – incorporated in the topical formulations – on the biophysical parameters of healthy human skin.

Human skin penetration of geraniol and citronellol *in vitro*

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Geraniol and citronellol are commonly used fragrance components in consumer products and are listed as alleged fragrance allergens in the European Union. Such allergenic potential is determined by effects on the skin once materials penetrate and elicit an immune response. The goal of this study was to determine skin absorption via evaluating the penetration ability of geraniol and citronellol when used in a typical vehicle for consumer exposure, 3 : 1 diethyl phthalate/ethanol solution. Flow-through diffusion cells with cadaver skin treated with radiolabelled geraniol and citronellol at 2% and 5% were used and scintillation counting was conducted.

Geraniol and citronellol had low skin absorption rates, ranging from 3.5% for 2% geraniol to 7.3% for 5% geraniol. The majority of dose was recovered in the skin washes. These results have important implications for the ability of these compounds to induce allergenicity. To provide a more complete model of skin penetration for fragrances, future studies should be undertaken to examine what remains in the skin or has penetrated through as a metabolized compound since in-vitro flux documents only part of the complexity of penetration. Additional comparisons *in vivo* in humans should clarify the reliability of in-vitro studies for fragrance toxicology.

Development of a rapid acting topical delivery system for menthol as the basis for a new quantitative sensory test

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Our objective was to develop a rapid release topical menthol delivery system and evaluate the sensory response to different concentrations.

Menthol formulations consisting of organic solvent based solutions, gels and sprays were prepared and release determined. Receptor solution samples were collected at 10-min intervals for 60 min and menthol content analysed by GC. In a separate study, menthol gel formulations (10% w/v and 20% w/v) were applied to the volar surface of the forearm in healthy volunteers. Subjects rated the intensity of their sensory responses (cold, heat, unpleasantness and pain) over a 20-min period.

Menthol release from the formulations ranged from 158.9 ± 09.5 mg to 454.7 ± 29.4 mg menthol (0–60 min). Spray and gel formulations containing complex solvent systems released more menthol than simple aqueous ethanol solutions. In the volunteers, both gel formulations produced primarily cold sensory responses. Differences were noted for heat ($P = 0.05$), unpleasantness ($P = 0.010$) and pain ($P = 0.012$) ratings between concentrations.

We concluded that formulation solvent vehicles influence menthol release. Application of menthol to the skin produced a range of sensory responses, including heat and pain, as well as cold. Response intensity was concentration dependent.

Biopharmaceutical evaluation of various dosage forms intended for caffeine topical delivery

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This study aimed to assess the potential of various dosage forms for caffeine delivery in the skin. The behaviour of microspheres (MS), Pickering emulsions (PE) and micro-emulsions (ME) was compared with that of conventional vehicles, such as emulsions (CE), gels and an aqueous solution of caffeine. The amount of caffeine that permeated from MS, PE and ME was significantly higher than from caffeine solution. Caffeine permeation after 24 h exposure was twice as high from MS than from aqueous solution, 2.5 fold for the ME and 1.5 fold for PE.

Permeation from MS was not significantly affected by the presence of hypodermis. On the contrary, ME permeation was significantly slowed down. After 24 h caffeine was equally shared between the hypodermis and the receptor fluid with CE, ME, PE and gels. A 25 : 75 ratio was obtained with MS. This could be due to a different caffeine permeation pathway through the skin for MS. PE, having a solid and dense silica shell, behaved in an intermediate way between MS and ME.

Transiently supersaturated solutions from the dissolution of amorphous powders for topical drug delivery

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The aim of this study was to prepare amorphous forms of clotrimazole to determine whether increasing the rate and extent of dissolution of clotrimazole would result in super-saturated solutions capable of increasing the bioavailability and subsequent therapeutic activity of clotrimazole.

Amorphous clotrimazole was prepared by rapid quench cooling from the melt, solvent evaporation and solid dispersion techniques. Saturated solubility of the prepared particles was measured in 0.1 M citrate buffer pH 5.5 (physiological pH of skin's acid mantle) at 32°C.

Amorphous particles dissolved rapidly to give maximum solubility values of 346.63 ± 8.59 μg/ml, 270.66 ± 8.74 μg/ml and 375.19 ± 7.35 μg/ml for quench, solvent evaporation and solid dispersion (SD), respectively. This represented a super-saturation factor of 6 to 8 times the equilibrium solubility of microcrystallised clotrimazole (43.64 ± 2.35 μg/ml) ($P < 0.05$).

In conclusion, morphisation of clotrimazole resulted in improved dissolution and transient supersaturation for up to 30 min from quench and solvent evaporation particles and up to 50 min for SD particles under conditions mimicking powder deposition onto skin, which could potentially improve the bioavailability and ultimately the efficacy of antifungal therapy.

Novel fast-acting delivery system for lidocaine

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The challenges in reducing lag times for topical delivery are considerable. Formulation thermodynamics can increase driving forces for partitioning into the skin. However, there is a limitation to the maximum flux that can be achieved for any molecule, which may still be below the desired onset of pharmacological activity.

We report a novel system that uses pressure application to reduce blood flow and clearance from the dermal capillaries and formulation thermodynamics to deliver effective doses of lidocaine within 10 min of application, without irritation or discomfort, using a single use and low cost design. The system involves an adhesive-backed cotton pad that makes an impression in the skin, dosed with saturated lidocaine base in water, applied to the skin for 10–15 min. In preliminary studies, the system was applied to human volunteers and either a needle advanced into the skin until pain was felt and scored or standard intradermal injection of 2 ml 1% lidocaine hydrochloride solution administered to assess pain. In all tests volunteers reported either no pain or marginal pain following the short application period.

The use of eutectic/penetration enhancer combinations for percutaneous drug absorption

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Methods of enhancing topical drug delivery include chemical enhancers and eutectic systems. Eutectic systems reportedly increase drug solubility in the skin lipids therefore enhancing topical drug delivery. Chemical enhancers also enhance topical API delivery by temporarily disrupting the barrier properties of the skin. The aim of this study was to investigate the combined effects of eutectic systems and penetration enhancers on drug delivery through the skin barrier using a novel local anaesthetic formulation. Three eutectic (lido-caine : tetracaine) formulations, along with a lidocaine : tetracaine eutectic system in combination with chemical enhancers, were applied to full thickness human skin mounted in Franz diffusion cells maintained at 37°C. Samples were taken at pre-determined time points and analysed via HPLC.

No statistical significance was observed in the permeation of the drugs from the three eutectic systems ($P > 0.05$,

analysis of variance). However, there was a 60% enhancement of drug absorption when one of these systems was combined with penetration enhancers (isopropyl alcohol, transcutol P and benzyl alcohol).

Such results indicate that eutectic/penetration enhancer combinations warrant further investigation into their use in enhancing topical drug absorption.

Novel pH-responsive microparticulate systems to treat atopic dermatitis

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Topical glucocorticosteroids remain the mainstay for managing atopic dermatitis. However, the same mechanisms of action responsible for the positive anti-inflammatory effect of topical steroids can also cause side effects ranging from cutaneous problems, such as atrophy, striae and rosacea, to systemic reactions, such as the suppression of the hypothalamic–pituitary–adrenal axis. The incidence of such side effects can be minimised by drug targeting using a stimuli-responsive drug delivery system that enables controlled drug release according to the disease severity. The pH discrepancy between healthy and atopic dermatitis skin was identified as a trigger for drug release at only the diseased areas of the skin.

The microencapsulation of hydrocortisone into pH-responsive polymers, Eudragit L100 and AQOAT AS-MG, which both have a dissolution threshold around pH 6, was investigated as a possible drug delivery system using both spray-drying and oil-in-oil emulsification methods.

The different production methods resulted in microparticles with differing morphological and release properties. For Eudragit microparticles obtained from the oil-in-oil emulsification method, release studies using nitrocellulose membranes in Franz-type diffusion cells showed that no drug was delivered at normal (intact) skin pH (5.0–5.5) but that delivery could be targeted and controlled to atopic dermatitis skin, where the pH is elevated to around pH 6.0–6.5.

Searching for the optimal methodology for dermal in-vivo sampling

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Only a few methods for minimally invasive dermal drug sampling can be used both *in vitro* and *ex vivo* and in in-vivo studies. In the search for a multi-purpose method we compared two similar dermal sampling methods – dermal microdialysis (DMD) and open-flow microperfusion (OFM). DMD employs a semi-permeable dialysis probe appropriate for sampling of smaller, less lipophilic substances present in the extracellular fluid, whereas membrane-free OFM probes provide unrestricted access to bigger, more lipophilic solutes in the extracellular fluid. Our aim was to evaluate these clinically applicable methods for dermal sampling of the moderately lipophilic drug, fentanyl, *ex vivo* and *in vivo*. Fentanyl is already used in chronic pain treatment, administered by transdermal patch. In-vitro studies have been successful showing relative recoveries of 65% (DMD, 20 kDa), 96% (DMD, 100 kDa) and 98% (OFM). Proceeding to ex-vivo studies, modified sampling conditions (perfusate, flow rate, formulation, perfusion mode) have been necessary to overcome the analytical challenges we have experienced. Results of these ongoing experiments will be presented.

Determination of the skin distribution and metabolism of imipramine using matrix assisted laser desorption ionisation (MALDI)

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The skin plays a vital role in protecting the body from invading pathogens and provides a barrier against the absorption of hazardous chemicals. The degree of protection is dependent upon the nature of the chemical to which the skin is exposed, and for that reason skin studies are used to assess skin absorption and, more recently, distribution and metabolism. The skin contains a number of metabolic enzymes capable of either activating or detoxifying absorbed xenobiotics. Although the metabolic capacity of the skin is considerably lower than that observed in the liver it is still an important factor in determining the nature and extent of compounds entering the systemic circulation. Although it is possible to evaluate metabolism within the skin, the distribution of metabolites can remain unknown.

Matrix assisted laser desorption ionisation imaging (MALDI-MSI) allows spatial resolution of drug and any corresponding metabolites to be obtained. The extent of penetration can be determined and information provided on the nature of compounds likely to enter systemic circulation.

Reporting skin impedance and resistance measurements

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Skin impedance and resistance measurements are used to assess skin integrity and to identify substances that are corrosive to skin. Resistance (R), which has units of ohms, is the ratio of the potential (V) to the current (I) measured using direct current (DC). Resistance depends on the area (A) and thickness (λ) of the sample, as well as the skin resistivity (ρ), which is an intrinsic material property with units of ohms cm (i.e. $R = \rho\lambda/A$).

Because the thickness of a skin sample is not easily measured, it is convenient to report the product of resistivity and skin thickness (i.e. $\rho \cdot \lambda$), which is equivalent to $R \cdot A$ with units of ohms cm^2. Therefore, meaningful comparisons of resistance measurements from different experimental systems are made on the product of resistance and area. Impedance, which has the same units as R, is the ratio V to I measured using alternating current. Skin impedance is largest at low frequency and equal to the resistance that would be determined in a DC measurement. Because skin impedance varies with frequency, impedance criteria for acceptable skin integrity or indicating skin damage also vary with frequency. Measurements made at lower frequencies should better represent skin barrier function.

Single frequency impedance measurements of skin: what frequency should be used?

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Measurements of electrical impedance are commonly used to non-invasively evaluate skin integrity as indicated by the size of the barrier to ion transfer. Because skin's electrical barrier is not just resistive, impedance measurements vary with the frequency of the applied current. At low frequencies, the modulus of the measured impedance approaches the resistance determined in a DC measurement. As frequency increases, the modulus of the impedance decreases. Skin impedance measurements for assessing skin integrity are often determined at only one frequency: 100 Hz and 1000 Hz are common. Impedance measurements that more closely represent the DC resistance value are better predictors of skin integrity. We have examined a large data set of impedance spectra from human cadaver skin to assess the ability of impedance measurements collected at 100 Hz and 1000 Hz to predict DC resistance. As expected, impedance determined at 100 Hz is more predictive of DC resistance than impedance measured at 1000 Hz. As a result, for the purpose of integrity testing, single frequency determinations of impedance measured at 100 Hz are better indicators of skin barrier function than measurements at 1000 Hz and measurements at frequencies lower than 100 Hz would be preferred.

Effect of dimethyl sulfoxide on skin impedance and permeation of 4-chloronitrobenzene

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Impedance spectroscopy is used to assess changes in skin barrier properties arising from chemical or mechanical insults. Permeation of a model compound, 4-chloronitrobenzene (CNB), and impedance through split thickness human cadaver skin were determined before and after exposure to dimethyl sulfoxide (DMSO) or phosphate-buffered saline (PBS) for either 0.25 h or 1 h. Each impedance scan was analysed using an R-CPE circuit model from which the skin resistance (R) and an effective capacitance (C_{eff}) were estimated. Treatment with PBS had no effect. After a 1-h exposure to DMSO, R decreased by a factor of nearly 140. Furthermore, before DMSO treatment R was highly variable, while after the 1-h treatment R was nearly the same for all samples. When treated with DMSO for 0.25 h, R decreased by a factor of only four. DMSO treatment produced a small increase on C_{eff} (4-fold and 2-fold for 0.25 and 1-h treatments, respectively). The increase in CNB flux following DMSO treatment compared with control was small although statistically significant. Apparently, DMSO changed the skin by decreasing the barrier properties of the polar pathway (to allow more ion transport) with little effect on the nonpolar pathway through which transport of a lipophilic chemical like CNB principally occurs.

Potential predictive value of prolonged a.c. impedance profiles in human nails exposed to penetration enhancers

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While a.c. impedance measurements are routinely made on human skin, most such studies on nails have used d.c. In addition, in-vitro measurements are usually made over only a short duration, and under hydrated conditions. As part of a study on nail penetration enhancement we monitored the long term a.c. impedance on pre-wetted nail clippings during the drying out process under ambient conditions using a Tinsley 1604 LCR Databridge. Gravimetric measurement of water loss (c20%) during the drying process showed that this was rapid (c20–30 min) with an exponential profile. In contrast, although the a.c. resistance rose only slowly for a prolonged period, it then unexpectedly showed a rapid rise to a high peak value (Mohm), which then decayed more slowly. Furthermore, the point of onset and profile of this peak was markedly affected by pre-treatment of the nails with a range of solvents and potential enhancers, indicating that it might have a predictive value in enhancer screening. When a.c.

impedance measurements were made on nails that had equilibrated to ambient conditions for an extended time, no temporal change was observed. Similarly, nail impedance measured under a range of constant humidity conditions was constant, demonstrating that the observed phenomenon was related to the drying out process. It is postulated that this phenomenon is a due to the drying nail behaving as a capacitor, as it shows a typical capacitative charge and discharge response to application of a.c. The differences in response observed following enhancer treatment are presumably the result of structural changes in the keratin. Further investigations are in progress to extend the potential of this observation as a predictive tool.

Histochemical visualisation of esterase activity in porcine ear skin

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Skin esterases can catalyse the hydrolysis of topically applied drugs. This can be advantageous to topical ester pro-drugs and co-drugs, which are metabolised to release the active moieties *in situ*. Hence, an understanding of the location of esterase activity in the skin is vital. We have qualitatively determined the presence of porcine cutaneous esterases using a histochemical esterase staining kit.

Our results confirmed that the highest levels of esterases were localised in the epidermis with some activity evident in the stratum corneum. Freshly excised skin showed abundant esterase activity. By using Hanks solution or a growth medium, the enzyme activity could be maintained at a high level, for at least 9 h. Only at 24 h was a slight reduction in esterase activity observed. Skin stored at 20°C retained very little activity, while heat treatment virtually eliminated esterase activity.

It is thus important to use freshly excised, ex-vivo skin when studying the topical delivery of pro-drugs and co-drugs, and in experiments where enzymatic metabolism may potentially modulate drug activity or delivery.

Biodegradable microneedles for intra-epidermal vaccination

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Macromolecular delivery to the epidermis is hindered by the stratum corneum (SC) barrier. We have explored the use of biodegradable microneedle compositions and demonstrated the ability of microneedles to deposit a DNA and protein in the epidermis, circumventing the SC barrier. Topical administration of reporter DNA using silicon microneedles resulted in localised gene expression in the epidermis. HBsAg was similarly delivered and detected in the epidermis by immunohistochemistry. Biodegradable microneedles were micromoulded from a combination of polycaprolactone and poly(lactic-co-glycolic) acid against the silicon microneedle template. The microneedles were administered on to ex-vivo human skin. Microchannels thus created in the skin were examined by scanning electron microscopy (SEM). The degree of SC disruption was determined by measuring changes in transepidermal water loss (TEWL). Topical administration of silicon and polymeric microneedle arrays of 49 microneedles increased TEWL by 1.8 g/m^2 per h and 2.8 g/m^2 per h, respectively. The SEM images verified perforation of the SC by the microneedles.

Painless, bloodless and easy to self-administer, microneedles provide clear advantages in vaccination delivery. Biodegradable polymeric microneedles may further facilitate safe disposal of vaccination devices.

Spray coating of silicon microneedle patches for intradermal drug delivery

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Microneedles facilitate drug delivery through the stratum corneum, creating transient micropores through which macromolecules and nanoparticulates can be passively delivered. Therapeutic agents can be coated onto microneedle arrays. In this study we evaluated the feasibility of coating silicon microneedles using a spray coating process.

The selected film coating agent carboxymethylcellulose (CMC) was spray coated using an atomising nozzle. A Taguchi multi-factorial L8 experimental design model was selected to optimise the film coat sprayed onto silicon wafers. Optimised parameters were then used to spray coat silicon microneedle patches. The coating formed was assessed based on weight, thickness (Zygo white-light interferometer) and appearance (optical light microscopy and scanning electron microscopy (SEM)). The use of optimum parameter settings and polymer solution concentration produced a uniform intact coating on silicon microneedle patches.

The formation of a uniform reproducible film coat is essential to ensure dose standardisation on coated microneedle arrays. The use of the spray coating technology to coat microneedle arrays has potential for industrial scale up. Future work will assess the impact of this coating formulation and coating process on vaccine bioactivity and immunogenicity after percutaneous administration.

Silicon microneedles for painless percutaneous penetration

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The outer layer of the skin, the stratum corneum (SC), is only 10–20 μm thick yet poses a major barrier to the transdermal delivery of drugs, vaccines and other pharmaceutical agents. It is expected that the development of microneedle technology will eventually circumvent these barriers. Microneedles are sharp protrusions, generally ranging in height from 100 μm to 500 μm, that have the potential to increase skin permeability by several orders of magnitude by painlessly creating transient micropores in the skin.

Our wet-etched silicon microneedles have been investigated by a number of groups for applications in drug delivery, DNA delivery, vaccine delivery, optical clearing, EEG measurement, ECG measurement and electroporation therapy. This paper describes the extension of our base microneedle technology to the development and fabrication of hollow microneedle drug delivery systems, based on the convex corner undercutting and dry etching of <100> silicon to form microneedles, and subsequent deep RIE etching of capillaries through these needles.

A microneedle-based syringe is assembled by bonding the microneedle array to a polydimethylsiloxane reservoir and flexible membrane, which is actuated using light finger pressure. Preliminary results have demonstrated successful SC rupture and delivery of a model drug.

Biodegradable sugar glass microneedles for macromolecular drug delivery

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Biodegradable microneedles, where the 'drug' is incorporated within the microneedles and released upon degradation *in situ*, offer advantages over solid microneedles. Sugar glasses are used in nature by anhydrobiotic organisms to stabilise the native structure of cells and cell components during periods of water stress. We explored the suitability of sugar glasses as a substrate for biodegradable microneedle fabrication and stable protein incorporation.

Novel 6×6 sugar glass microneedle arrays were fabricated by injecting a 20% w/v aqueous solution of anhydrous trehalose (T.a.) and sucrose (Suc) (75 : 25 w/w) onto a PDMS micromould held under vacuum. The solution was dehydrated for 24 h in a fume hood at ambient temperature followed by a further 24 h in a heated oven to form a solid array. A model protein, beta-galactosidase, was incorporated into flat sheets of sugar glass by reconstituting the enzyme in sodium phosphate buffer (PB) solution and adding this to a 20% w/v solution of T.a. and Suc., also dissolved in PB, before dehydration.

The sugar glass behaved as an amorphous solid within which enzyme activity was maintained over a period of months following storage at ambient temperature. This was demonstrated by its ability to oxidise X-gal following delivery of the enzyme into incised human skin. Sugar glass microneedles were shown to have sufficient physical strength to create microconduits within the upper layers of human skin upon application and this was confirmed by TEWL.

This study suggests that sugar glasses can effectively and stably encapsulate a range of macromolecules and that sugar glass microneedles have suitable structural rigidity to facilitate effective drug, macromolecule and vaccine delivery into skin.

The effect of occlusion on the percutaneous absorption of aluminium from antiperspirant products

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The percutaneous penetration of aluminium has been the subject of much debate, with aluminium-containing topical products being linked with conditions such as Alzheimer's disease and breast cancer. The aim of this study was to investigate the percutaneous absorption of aluminium from a range of commercially available products, in both occluded and unoccluded experiments.

Franz cell experiments (occluded; 24 h; $n = 6$) using human skin and a citrate buffered receptor phase (pH 5.5) were conducted with and without occlusion for a range of commercial products (0.5 g of either Driclor, Nivea for Men, Sure for Men or Dove roll-ons). Analysis of total aluminium was by ICP-OES (396 nm). Product fluxes were statistically compared between different products and application protocols.

Total amounts of 0.09–0.22 μg of aluminium (occluded) and 0.08–34.4 μg (unoccluded) were found to have permeated the skin. In most cases, no significant differences were observed between occluded and unoccluded experiments for flux or total cumulative absorption of aluminium, except for the Nivea for Men product, where the unoccluded experiment resulted in significantly higher absorption. This may be due to, in some cases, the variance associated with low levels of absorption (often in the ppb range) or the formulation type.

The effect of depilation on the percutaneous absorption of aluminium from antiperspirant products

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The percutaneous penetration of aluminium has been the subject of much debate, with aluminium-containing topical products being linked with conditions such as Alzheimer's disease and breast cancer, including the suggestion that frequency and earlier onset of antiperspirant usage combined with shaving is associated with an earlier onset of breast cancer diagnosis. The aim of this study was to investigate the percutaneous absorption of aluminium from a range of commercially available antiperspirant products.

Franz cell experiments (occluded; 24 h; $n = 6$) using human skin and a citrate buffered receptor phase (pH 5.5) were conducted with and without pre-treatment (depilation) for a range of commercial products (0.5 g of either Driclor, Nivea for Men, Sure for Men or Dove roll-ons). Analysis of total aluminium was by ICP-OES (396 nm). Product fluxes were statistically compared between different products and application protocols.

Very low levels of aluminium were found to permeate the skin from all products. In most cases significant differences were found between pre-treated samples and controls, with the former generally exhibiting greater permeation, although substantial variance was observed, due to either the damage caused to the skin barrier by the pre-treatment regime employed, or the low levels of absorption observed.

Hysteresis of water and xylene sorption in pig epidermis shown by thermogravimetric analysis

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The thermogravimetric analysis (TGA) method (a high-precision scale which measures the weight of a sample, four times/second, as a function of temperature and time) is well suited for analysing dermal absorption and desorption of chemical vapours. A computer-controlled system that generates chemical and water vapour is connected to the TGA apparatus. A piece of epidermis (ø8 mm) is placed inside the TGA apparatus ($T = 35 \pm 0.001^{\circ}C$) and upon vapour exposure the weight of the skin sample increases until equilibrium is reached.

To study sorption behaviour, we exposed skin samples to step-wise increased concentrations of water or xylene vapours, going from $0\% \rightarrow 90\% \rightarrow 0\%$ saturation (6 h, 10%/step). The weight changes were readily recorded and sorption and desorption curves followed different patterns, showing that skin sorption is faster than desorption. More pronounced hysteresis was seen for water than for xylene. In other words, the skin preferably retains water once it is hydrated. The same phenomenon is well known for soils and food stuffs, and several theories exist (e.g. based on capillary condensation), such as the ink-bottle theory.

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Confocal Raman microspectroscopy: a useful method to evaluate the complete removing of stratum corneum by tape stripping

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The use of the well-established tape stripping (TS) method for an in-depth profiling of the stratum corneum (SC) is not sensitive because of its inhomogeneity over the whole surface and not an appropriate method for a complete SC removal. This work sums up existing alternatives, such as trypsinisation and cyanoacrylate skin surface biopsy, and discusses their potential and limits via histological imaging and confocal Raman microspectroscopy (CRM). The trypsinisation was able to remove homogeneously the SC and therefore seemed to be a good alternative. Only the demands of using liquid solvents could disturb several experimental designs. 2-3 Cyanoacrylate adhesive strips were very efficient and provided a more homogeneous removal of the whole SC than the TS method. For the depth-screening application CRM is a great alternative. It is a non-invasive, very exact method that provides small, vertical screening steps. The facility depend on the existence of a characteristic peak in the recorded spectra from the investigated substance.

Tracking of simple pharmaceutical emulsions ingredients in the skin: a confocal Raman microspectroscopy investigation

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Most penetration studies are based on kinetic measurements of the active and on hypothetical arguments for the

reasons for good or bad penetration. In this study we compared the penetration depth via confocal Raman microspectroscopy (CRM) of each emulsion ingredient (retinol, water and dodecane) in epidermis with those of an oily micellar solution (retinol and dodecane) to understand the components' relationship and their different penetration results.

Retinol behaved similarly when applied on skin either in a micellar solution or in an emulsion. On the contrary, dodecane was the continuous phase in the micellar formulation and surrounded by surfactant as oily droplets in the emulsion. It was more bioavailable from emulsion than from the micellar solution, highlighting the influence of emulsion droplets as active vehicles. These results illustrated the ability of CRM to follow all ingredients of a formulation during skin penetration and are a great starting point for more detailed investigations on each emulsion component distribution in the skin.

In-vitro and in-vivo evaluation of penetration enhancer effects of terpene–transcutol mixtures with ATR-FTIR spectroscopy

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Our study aimed to investigate the interaction of various terpenes (3%), nerolidol, *dl*-limonene and eucalyptol, in the presence of either transcutol (TC) or propylene glycol (PG), with the skin barrier. ATR-FTIR spectroscopy was employed to understand the effect of terpene/co-solvent systems on molecular organization of the stratum corneum. In-vitro and in-vivo evaluation of the enhancer effects of terpene/co-solvent mixtures were investigated on excised pig and human skin, respectively.

ATR-FTIR spectra were obtained 3 h after treatment. A non-treated skin site served as control. Attention was focused on characterizing the occurrence of peaks near 2850 and 2920 cm^{-1}, which were due to the symmetric (SSV) and asymmetric (ASSV) C-H stretching vibrations, respectively.

The decrease in C-H stretching peak areas was the highest in in-vitro studies after treatment with PG, TC and terpene/TC mixtures, respectively, whereas terpene/TC mixtures caused a blue shift in in-vivo studies, indicating a chain conformational disorder. In-vitro permeation studies are on-going to investigate the relationship between the observed drug flux enhancements and the effect of terpene/co-solvent systems, particularly TC, on skin barrier properties using hydrophilic and lipophilic model drugs.

Intra- and inter-individual variability in the development of erythema after application of methyl nicotinate evaluated by polarization spectroscopy imaging

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The concept that the time to onset of erythema after the application of the rubefacient and urticant substance methyl nicotinate (MN) indicates skin barrier competence was introduced 30 years ago. MN produces a dose-dependent erythema on topical application to intact skin, the nature of which is known to be fast moving (in the order of minutes) and variable. Using tissue viability imaging (TiVi) the time course and degree of the reaction can be conveniently followed and analysed. Inter-individual variability can be quite marked but intra-individual variability is less pronounced. At the upper end of provocation (higher doses, more sensitive individuals) urtication can occur, which decreases blood flow by increasing pressure on and thus emptying capillaries. The TiVi system can quantitate urtication and inherent (blanched) skin colour. The utility of MN application in the study of individual barrier function and microvascular reactivity is increased by the use of the TiVi system for collection and analysis of data.

Challenges associated with finite dose skin absorption experiments: homogeneous drug distribution of liquid formulations on skin surface

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Per definition, for finite dose experiments a donor volume of 10 $\mu l/cm^2$ or below is applied homogeneously over the skin surface. This, however, can be difficult to achieve, as the skin is not free of wrinkles, even when positioned in a Franz diffusion cell.

To illustrate the drug distribution over the incubation area, dye formulations of different lipophilicities were applied to the skin using different application protocols. For medium-chain triglycerides as a lipophilic compound, uniform distribution was found with all application protocols over the whole incubation area. The formulation spread easily on skin without application of any mechanical stress. However, aqueous formulations presented problems with homogeneous

distribution over the incubation area. These could be solved by applying a Teflon punch and turning it to shear the formulation.

In conclusion, it was shown that drug distribution over the skin surface needs to be assessed before conducting finite dose skin absorption experiments. Dyed formulations can give a hint towards the possible difficulty of distribution of a vehicle over the skin surface.

The correlation between in-vitro and in-vivo skin permeation models of a novel diclofenac formulation

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A novel (NSAID) topical formulation containing diclofenac has been developed based on a novel patented combination of penetration enhancers. The aim of the study was to compare the results obtained in an in-vitro and in-vivo experiment, for the developed formulations (pre-fix DCF100C1) and a topical currently marketed comparator, to determine any correlation observed between the methods.

The formulations were assessed using an in-vitro epidermal membrane skin model and an in-vivo clinical trial. The in-vivo method was a single centre, open label, five treatment, three period crossover (Youden square) design.

Both methods resulted in an increased permeation observed in the DCF100C1 formulations compared with the market comparator (Voltaren Emulgel), ranking the formulations DCF100C1 2.5% > DCF100C1 1.0% > commercial comparator. Within the in-vitro model DCF100C1 2.5% w/w resulted in a greater than 20-fold enhancement through epidermal membrane compared with the commercial comparator. The data demonstrated a correlation between the two permeation assessment models and suggest that such in-vitro models can be used to predict in-vivo data in some circumstances.

Avoidance of dermal exposure to preservatives by packaging

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Dermal exposure to chemicals in cosmetics and hygiene products (e.g. moisturising creams, soaps, shampoos) is increasingly recognized as an important area for risk assessment and regulation. The contents of such products is regulated by classification of exposure types (e.g. stay on/wash off) and regulatory concepts based on toxicological studies and manufacturing or market experience. Positive lists, negative lists or establishment of recommendations on concentration and exposure form a basis for consumer safety. Common problem areas are perfumes, preservatives and the formation of oxidation products after manufacture.

A new patented system, suitable for packages from 100 ml to 5 l, with collapsible plastic bags and unique dosage valves prevents bacteria and air from entering the packaging. Thus the use of preservatives can be avoided.

This may lead to a reduced risk of individual reactions to specific preservatives as well as cross-allergy reactions. The consumer no longer needs to hunt for strange names on small ingredient labels. Also, it could prevent the prospective development of allergy. The avoidance of oxidation products is another advantage.

In-vitro–in-vivo correlation in man for percutaneous absorption

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The literature was reviewed and 58 pharmacokinetic data sets were extracted from 11 in-vitro and 12 in-vivo studies, for in-vitro–in-vivo (IVIV) comparisons. Based upon total % absorption, good overall IVIV correlation was seen. The average IVIV ratio of the data set was 1.17, and the IVIV ratio for any single compound exhibited up to a 3-fold difference (range, 0.30–2.95). Yet, there were often differences between the in-vitro and in-vivo study designs including: (1) concentration of test compound; (2) vehicle dose amount; (3) composition of vehicle; (4) length of dose exposure/wash time; and (5) the in-vitro skin temperature. Of greatest concern, however, was that most of the IVIV comparisons were not run on matched skin body sites. Eleven data sets were identified in which there was full harmonization between the in-vitro and in-vivo study designs. The average IVIV ratio of the harmonized data set was close to one (0.96) with a range of 0.58–1.28. It is exceedingly clear that the data obtained in the excised human skin model closely approximate those obtained in living man, when the respective study designs are precisely harmonized.

Development of a novel phase separating nail lacquer for the treatment on onychomycosis

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Topical treatment of onychomycosis (fungal nail disease) is desirable to avoid the systemic toxicity associated with oral

antifungal treatment. However, treatment is lengthy and the clinical cure rate is low. Hydrophobic polymers are often used to prevent the lacquer being removed during bathing. If the polymer cannot stabilise the drug when the lacquer solvent evaporates, the drug will crystallise, rendering it unavailable for absorption. In this study an amorolfine nail lacquer containing a hydrophilic and a hydrophobic polymer was designed to produce a protective hydrophobic upper layer and a drug stabilising hydrophilic polymeric layer next to the nail.

Polymeric films containing HPMC, Eudragit E100 and amorolfine were prepared. DSC results of combined polymer films indicated the presence of two separate glass transitions corresponding to those of the two polymers. This confirms phase separation in the polymeric film. ATR-FTIR spectroscopy results indicated a higher concentration of the more hydrophobic Eudragit E100 in the upper layer of the film. In addition, when amorolfine was included it appeared at a higher concentration in the lower hydrophilic HPMC-rich layer. These preliminary results indicate the feasibility of developing a phase separating nail lacquer system.

The application of Gaussian processes in the prediction of absorption across mammalian skin and synthetic membranes

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Human skin permeability has been shown to be inherently non-linear when mathematically related to the physico-chemical parameters of penetrants. These studies have also shown that non-linear methods, such as Gaussian processes (GPs), have outperformed other methods, such as quantitative structure–permeability relationships (QSPRs), in terms of predictivity and statistical accuracy. The aim of this study was to apply and validate GP methods to datasets for membranes other than human skin. Two QSPR methods were employed to compare with the GP models. As measures of performance, the correlation coefficient, negative log estimated predictive density and mean squared error were employed. GP models, with different covariance functions, outperformed QSPR models for human, pig and rodent datasets. For the Silastic membrane, GPs performed better in one instance, and gave similar results in other experiments. The GP predictions for some of the Silastic dataset were often poorly correlated, suggesting that the physicochemical parameters employed in this study might not be appropriate for developing models that represent this membrane.

Skin absorption of (volatile) liquids: a skin PBPK model

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Skin exposure to (volatile) liquids occurs by worker exposure and the use of consumer products. A simple skin model has been developed to estimate the extent of evaporation and of dermal absorption dependent on: deposition rate per hour; duration of exposure; maximum volume to be absorbed into the skin; and physicochemical properties of the volatile liquid. The stratum corneum is the critical compartment. Absorption from the skin surface into the stratum corneum and permeation into the viable epidermis are considered as, and modelled by, independent processes. This applies also to the evaporation. Evaporation of the liquid layer on the skin surface is controlled by other parameters than evaporation from the stratum corneum not covered by liquid. Finally, the effect of mixtures and the effect of biotransformation in the stratum corneum are considered.

The model describes, dependent on the time after the start of deposition: the mass upon the skin outside the stratum corneum; the mass in the stratum corneum; the mass absorbed in the blood; the absorption rate; and the mass evaporated from the skin.

Alternative approach to maximum flux for TTC applied to safety evaluation of cosmetic ingredients

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Relevant and accurate prediction of dermal exposure of topically applied chemicals is essential for risk assessment. It can be obtained without recourse to an experimental measurement. Most QSAR models predict permeability coefficients. All of them lead to the same conclusion: small lipophilic chemicals have greatest skin permeability. This analysis often gives rise to confusion. Datasets used to build up this relation concern percutaneous transport from aqueous solution. Flux is then balanced between two competitive factors: permeability and solubility. Concept of maximum flux means that a chemical cannot cross the skin higher than flux measured at steady state with saturated aqueous solution. It allows assessment of the maximum absorbed dose. This concept was recently used in a TTC approach for cosmetic ingredients. However, the default proposed values greatly overestimated the absorption obtained experimentally. A QSAR model recently developed by L'Oréal can be used. It estimates the cumulative mass of a chemical absorbed into and through the skin in typical 'in-use' cosmetic conditions. The model was build up with 101 data. More than 90% were well predicted (i.e. difference between predicted and

experimental values less than a factor of 5). Moreover, some chemical properties, known to affect cutaneous absorption, have been considered in the L'Oréal Model (ionisation and volatility), contrary to the TTC approach. Six data were used to support these benefits of the QSAR model.

Permeability coefficient measurement on episkin reconstructed human skin model

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According to its similarities to native human tissue in terms of histology, metabolic capabilities and biochemical markers, reconstructed human epidermis (RhE) has been identified as a useful tool for in-vitro testing for phototoxicity, corrosivity and irritancy. At the least, RhE models seem to be appropriate alternatives to human skin for the assessment of skin permeation and penetration *in vitro*. Among all RhE models, Episkin is particularly adapted for testing. Indeed, its design allows measurement of penetration directly in the insert without leakage. Permeability coefficient measurement could be done using flow through diffusion cells or using direct inserts with total or partial replacement of a given volume of receptor fluid at a given time interval. Caffeine was used to evaluate both approaches. With sink condition and infinite dose, flux as a function of time should reach a constant value corresponding to the steady state. Results show that steady state is not reached with diffusion cells, contrary to inserts. Variability was evaluated from week to week and month to month and stayed below 15% (CV%). Comparison with human skin data was done with three chemicals. A good correlation was obtained. This reinforced previous studies' conclusions on the RhE model as relevant alternative to human skin for in-vitro penetration studies.

A harmonized study design evaluating in-vitro percutaneous absorption using Franz diffusion cells based upon international guidelines and expert consensus

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There is substantial consensus about appropriate in-vitro percutaneous absorption study design parameters among experts and international guidelines exist (including FDA, COLIPA, SCCP, OECD, ECVAM and EPA). Yet for certain key parameters there remains a lack of consensus among experts, which has precluded standardization on specific protocols. This can lead to a study design that fails to achieve its intended goal of accurately predicting absorption in living humans. Furthermore, this confounds the interpretation and comparison of data between laboratories. As a starting point for harmonization, a study design approach is proposed specifically for finite dose, in-vitro percutaneous absorption studies, utilizing static Franz diffusion cells, for the assessment of dermal absorption of a compound from topical or transdermal formulations. Explanations provided offer rationale for selecting the appropriate choice where a situational range exists for a parameter, or where it is not clearly defined in available guidelines. Recommendations include approaches for skin barrier integrity testing and acceptance criteria, selection of reservoir solutions, study duration, number of donors and replicates, skin preservation and preparation, skin surface temperature, ambient laboratory conditions, mass balance, etc.

The role of method development and validation for in-vitro rate of release studies

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The in-vitro release test (IVRT) method for semi-solid topical products is a valuable tool for comparing product performance. Rate(s) of release of the active ingredient(s) are compared using an established statistical test for equivalence. These studies are often performed as per the FDA SUPACC-SS Guidance, which states 'The *in vitro* release methodology should be appropriately validated', but does not specify how. The Authors propose an IVRT method validation that is compatible with the SUPAC-SS guidance, and incorporates the most critical considerations to ensure that the IVRT is capable of detecting altered product performance, which may arise from changes in manufacturing locations, or changes in sources of excipients or from an altered formulation matrix that may impact product performance. The validation includes the selection of an appropriate membrane and receptor solution, and assesses reproducibility, selectivity and sensitivity of the IVRT method. This validation also specifically evaluates the IVRT method's ability to discriminate rates of release of the compound(s) of interest from formulations with altered concentrations or altered vehicle matrices. As part of the proposed IVRT validation, the HPLC sample analysis method is also validated, per ICH guidelines.

The role of QC, QA and GLP for in-vitro percutaneous absorption studies

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Franz diffusion cells are commonly used for in-vitro studies evaluating topical and transdermal products, to characterize

the permeation of compounds through skin or synthetic membranes. Often, the data from these studies is relied upon as the basis for strategic decisions in drug development and product safety, or is reviewed by regulatory agencies. Yet, in many cases these studies have not been reviewed and verified by quality control (QC) or quality assurance (QA) processes. Furthermore, too few studies utilize documentation practices or control systems that are compatible with Good Laboratory Practices (GLP) or with study conduct guidelines from regulatory bodies and expert groups. The Authors describe how such in-vitro Franz cell studies can be conducted within a quality system that ensures the validity of the results. QC methods provide assurance of the documentation and data integrity. QA methods for auditing and inspecting focus on three main areas: the study, the facilities and the processes. Compliance with GLP, SOPs and Guidelines for in-vitro diffusion cell studies requires a detailed understanding of the relevant texts, and a well-considered implementation in a manner appropriate to the nature of the study.

A phase II study of the efficacy, tolerability and consumer acceptability of a 1% w/w terbinafine topical spray versus a currently marketed terbinafine product in the once only treatment of tinea pedis

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A novel topical metered dose aerosol (MDA) spray that delivers terbinafine hydrochloride to human skin for the treatment of *tinea pedis* (athlete's foot) has been developed. The drug product is manufactured as a solution that, upon actuation, assembles into a microfine occlusive film. The film produces a highly supersaturated system that dramatically increases drug diffusion across the superficial layers of human skin.

The aim of the clinical study was to demonstrate the non-inferiority of the developed MDA (1% w/w terbinafine) spray compared with the marketed leading product (1% w/w terbinafine) after one application determined by the proportion of patients categorised as successfully treated at week 1. In addition, the secondary aims were to assess the tolerability, recurrence/relapse rate and consumer acceptability. The study was a randomised, observer-blind, comparative phase IIa study employing 120 patients with notable or prominent visual signs and symptoms of tinea pedis.

The treatment success rates based on physician's global assessment of signs and symptoms for the novel spray formulation and the marketed product were not significantly different at one week; as such, non-inferiority was confirmed. Statistically comparable results were also observed for most of the other efficacy tests, whereas somewhat better results were seen for the erythema and microscopy (KOH) parameters for the novel MDA spray.

Effect of a novel penetration enhancer system on the ungual permeation of antifungal agents

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A novel penetration enhancer consisting of sequential applications of a reducing agent (thioglycolic acid) and an oxidising agent (urea hydrogen peroxide) has been identified to aid the ungual permeation of drugs applied to the nail surface. The aim of this study was to demonstrate the effect of such an enhancer system on the permeation of three drugs, amorolfine, ciclopirox (presented in marketed nail lacquers) and terbinafine, applied using a spray system developed in house.

Novel diffusion cells were used, where full thickness human nail sections served as the barrier for permeation, the efficacy of the drugs was measured either via the zone of inhibition observed in an agar filled chamber infected with dermatophytes or via ATP levels, which were used as a marker of antimicrobial activity.

The results obtained during this study clearly demonstrated the benefit of a novel permeation enhancing system (thioglycolic acid followed by urea hydrogen peroxide), which reversibly alters the chemical structure of the nail and not only enhances the efficacy of the existing topical formulations (presented in marketed nail lacquers) but increases the delivery and efficacy of terbinafine, when applied ungually.

In-vitro percutaneous absorption of formulated nanosize titanium dioxide on altered irradiated porcine skin

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A sunscreen should remain at the skin surface or impregnate the first outermost layers of the stratum corneum only. Inorganic UV filters, such as TIO_2, have been shown to stay in the upper layers of healthy skin. Possible enhanced uptake in the case of impaired skin should be considered. The goal of this work was to assess *in vitro*, using porcine skin and a compartmental approach, the penetration of a mixture of 20 and 60 nm of dioxide nanoparticles, dispersed in a sunscreen formulation.

In-use conditions were modelled by four experimental groups: normal, solar irradiated, altered by stripping, and altered by stripping followed by irradiation. The skin penetration was investigated by quantitative analysis of mineralized TIO_2 using inductive coupled plasma-atomic emission spectroscopy and transmission electronic microscopy.

More than 96% of the TIO_2 was recovered in the first three tape-strippings. No TIO_2 was detected in the inner stratum corneum and the underlying viable epidermis. We showed that irradiation by itself or alteration of the barrier function did not result in higher skin permeability.

Our data demonstrated that altered irradiated skin is not permeable to the TIO_2 nanoparticles, suggesting that the sunscreen formulation that we used could be considered as safe for impaired skin.

Controlling the disposition of gallium complexes in human skin using iontophoresis

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Topical gallium (Ga(III)) application enhances wound healing. However, the localisation of this metal in the skin is problematic due to metal coordination complex formation. The aim of this study was to determine the effect of Ga coordination with hydroxide and citrate when iontophoresis (ITP) was used to assist topical Ga(III) delivery.

Ga(III) speciation was modelled *in silico* to present Ga(III) as either Ga^{3+} ions, negatively charged citrate complexes (Ga_{neg}) or a combination of positively charged hydroxide complexes (Ga_{pos}). These complexes were applied to full-thickness human skin sections in Franz cells and allowed to permeate for 65 h either passively or after ITP pre-treatment.

Ga(III) skin deposition, determined by tape-stripping, demonstrated that Ga_{pos} gave a 10-fold lower passive permeation rate compared with the Ga^{3+} ions and Ga_{neg}, which gave similar results. The application of ITP increased the flux of Ga(III) 9 and 800 fold from Ga_{neg} and Ga_{pos} solutions, respectively, but Ga^{3+} ion permeation was unaffected ($P > 0.05$, analysis of variance). ITP application increased Ga (III) deposition within the skin four-fold compared with passive when applied as Ga_{pos} and two-fold when applied as Ga_{neg}.

These results demonstrated that using ITP with the correct coordination complex enhanced Ga(III) percutaneous permeation by reducing non-specific binding within the skin.

Transcutaneous immunisation with diphtheria toxoid loaded *N*-trimethyl chitosan formulations and microneedle arrays

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In transcutaneous vaccination the major hurdle is to overcome the skin barrier function, located in the stratum corneum. An attractive approach to reduce the skin barrier is the use of microneedle arrays. In our studies we use solid microneedle arrays to pretreat the skin before formulation application. Vaccination studies using mice were performed to examine the effect of microneedle pre-treatment on the immune response. These studies showed that, when a diphtheria toxoid (DT) solution was used, microneedle pre-treatment effectively increased the IgG titres compared with untreated skin.

In subsequent studies DT-loaded *N*-trimethyl chitosan (TMC) nanoparticles (NP) and TMC/DT mixtures were used in combination with microneedle arrays. Mice were vaccinated by applying the formulations on microneedle pre-treated skin. The formulations were also injected intradermally.

Transcutaneous application of TMC/DT mixtures elicited 15 times higher IgG titres than the TMC NP, while TMC NP did not induce enhanced IgG or neutralising antibody titres compared with a DT solution. Intradermally both TMC-containing formulations induced enhanced titres compared with a DT solution. As differences in efficiency to enhance the IgG titres were observed between TMC nanoparticles and TMC/DT mixtures, in-vivo confocal studies were performed with rhodamine-labelled TMC. These studies revealed that transport of the TMC NP across the conduits was limited compared with a TMC solution.

In conclusion, microneedle pre-treatment drastically increased the IgG titres in transcutaneous immunisation when using a DT solution. TMC/DT mixtures are more efficient for transcutaneous vaccination than DT loaded nanoparticles.

Barrier properties of human skin equivalents

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Human skin equivalents (HSEs) are often used as in-vitro skin models for permeation studies, but the skin barrier of HSE is often impaired. The skin barrier is located in the lipid regions of the stratum corneum. The aim of this study was to elucidate the lipid composition and organization of stratum corneum in three different HSEs. The lipid composition, lamellar phases and lipid packing were determined by means of HPLC, small angle X-ray diffraction and infrared spectroscopy.

Lipid analysis showed that all lipid classes present in stratum corneum of native human skin are also present in stratum corneum of HSEs. The HSEs, however, contained relatively low amounts of free fatty acids. Furthermore, infrared spectroscopy revealed a less dense packing of the lipids within the lipid lamellae. Small angle X-ray diffraction showed that the lipids are organized in lamellar phases, similar to that in native human stratum corneum. Electron microscopy showed that the lamellar stacks appeared to be thicker than those observed in native

human stratum corneum. Diffusion studies with ethyl-PABA showed that the HSEs were more permeable than native human skin.

Previous studies demonstrated that free fatty acids are essential for a dense lipid packing in human stratum corneum. We hypothesize that the reduced free fatty acid level accounts for the predominant absence of the orthorhombic lipid packing in the stratum corneum of HSEs, which may contribute to decreased barrier properties. Furthermore, the presence of thicker lipid lamellae may provide larger channels through which compounds can diffuse.

The detailed lipid composition in human stratum corneum

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In recent years, many studies have reported strong evidence of an impaired barrier function of the skin in skin diseases. However, until now there are not many studies reporting the lipid organization and composition in patients with diseased skin. One of the challenges is sampling of the stratum corneum non-invasively. The main lipid classes in stratum corneum are ceramides, free fatty acids and cholesterol. Analysing the stratum corneum lipid composition and lipid organization of patients may contribute to a better understanding of the impaired skin barrier function. However, current analytical methods for separation and identification of the barrier lipids are scarce and very time consuming.

We present a combined method for quick separation and identification of ceramides, free fatty acids and cholesterol by means of liquid chromatography coupled to mass spectrometry (LC-MS). Lipids from stratum corneum were extracted by a modified method of Bligh and Dyer and analysed by LC-MS. Regarding the subclasses of ceramides, we observed excellent separation of all individual species. This led to the identification of 12 different subclasses, including one not earlier reported in literature. In addition, every single subclass showed a distribution of fatty acid chain lengths. Free fatty acid chain length distribution can be determined as well. In stratum corneum of healthy skin the free fatty acid chain length varies from C14 to C34.

In conclusion we developed an LC-MS method to determine a detailed lipid composition of ceramides, free fatty acids and cholesterol in stratum corneum by LC-MS.