PCCP

Cite this: *Phys. Chem. Chem. Phys.*, 2011, **13**, 5215–5222

www.rsc.org/pccp

PERSPECTIVE

Skin: the ultimate interface†

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Received 22nd December 2010, Accepted 16th February 2011
DOI: 10.1039/c0cp02943b

The outer layer of the skin, the stratum corneum is a unique barrier membrane. On average it is only 20 μm thick (about a quarter the thickness of a normal sheet of paper) but it prevents us from losing excessive water and it protects us from our environment. It forms a special interface between our body, the air, water and various solids. In order to understand the barrier properties of the skin we need to determine its structure at various levels ranging from the macroscopic scale to the molecular level. This has been made easier by the advances that there have been over the recent decade. However, the amount of a material that is capable of penetrating this excellent barrier and reaching the underlying systemic circulation is still only of the order of 1 or 2 per cent of the total applied dose. The purpose of this publication is to explore the strategies currently employed to promote skin permeation and to consider the most exciting approaches currently under investigation. The limitations of current methodology to examine the problem are discussed. New opportunities to fill the gaps in our current knowledge are identified and the importance of interdisciplinary research in the field is emphasised.

The stratum corneum is composed of layers of corneocytes which overlap. In general they are pentagonal or hexagonal in shape with a length of around 30 μm and a thickness of 0.2 to 0.3 μm.[1] They are held together by 'rivets'—the corneodesmosomes. The major constituent of the stratum corneum cells is keratin and they are therefore rather dense in composition. The stratum corneum is dynamic in nature and renews itself every 14 days.[1] The corneocytes slough off from the skin surface. This is a complex process involving various proteases and protease inhibitors. If there is disruption to the delicate balance, which is responsible for desquamation, the skin becomes diseased and conditions such as eczema and psoriasis result.[2]

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† This article is part of the special collection on *Interfacial processes and mechanisms* in celebration of John Albery's 75th birthday

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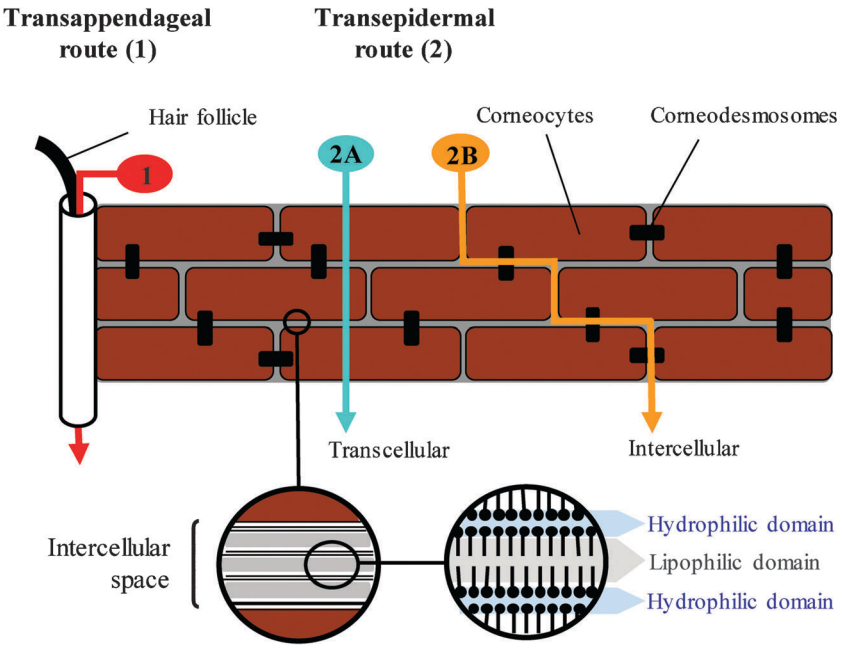

Fig. 1 "Brick and mortar" model of the stratum corneum and routes of penetration.

Michaels *et al.* suggested a 'brick and mortar' structure for the stratum corneum (Fig. 1).[3] To understand the barrier properties of the stratum corneum it is necessary to elucidate the route of permeation through this 'brick and mortar' structure. During the 1970s the route of penetration was unclear but an analysis of the diffusion of methyl nicotinate through skin *in vivo* suggested that the intercellular route was important.[4] This was inferred using complex solutions to Fick's laws of diffusion which also included the possibility of slow interfacial transfer kinetics as the diffusion progressed across the various layers of the stratum corneum. The intercellular route was confirmed by the visualisation of butanol[5] and oestradiol[6] in the 'mortar' using sophisticated microscopic techniques. This means that the effective diffusional pathlength is much larger than the straight thickness of the stratum corneum of 20 μm and may be estimated to be 300 μm from the overlapping brick model.

When analytical techniques developed to a sufficient degree it was possible to determine the constituents of the mortar and a complex mixture of lipids were found including ceramides, free fatty acids and their esters, cholesterol and cholesterol sulfate.[7] Unlike most biological membranes there are no phospholipids in the stratum corneum. However the presence of the ceramides suggested that the lipids would be structured. X-ray studies on the skin demonstrated that bilayers were present and that they could exist in different conformations.[8] Within the mortar there are a number of these layers and therefore a diffusing molecule, such as water, has to cross these sequentially. A number of interfaces are crossed and the transfer involves passage through hydrophilic and lipophilic domains. It then becomes clearer why the skin is a good barrier to the loss of water. A water molecule moving from the inside of the body to the skin surface not only has a tortuous pathway but also has to cross a number of lipophilic regions. The nature of the lipids has also been studied using infra red spectroscopy. The methylene groups have two characteristic bands corresponding to the symmetric and asymmetric stretching frequencies. As the lipids become more fluid these shift towards the blue end of the spectrum. Increased lipid conformational disorder of the lipids also means that the skin should become more permeable; this was seen by Potts and Francoeur when they showed a very

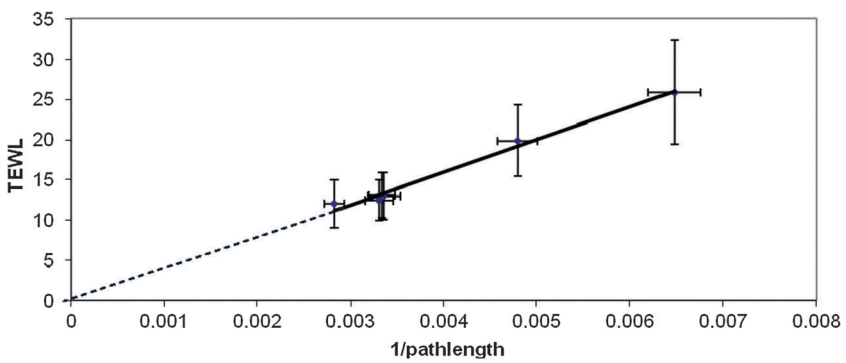

Fig. 2 TEWL ($g/m^2/h$) *vs.* 1/pathlength (μm^{-1}) for different body sites; $y = 3914.1x + 0.1$, $r^2 = 0.99$ (adapted from ref. 12).

good relationship between water permeability and the shift in frequency as the temperature of the skin was altered.[9] Another analysis of this type of data shows that there are various phase changes that take place; the lipids melt (~70 °C), lipids covalently bonded to the corneocytes melt (~80 °C) and at 100 °C the keratin denatures.[10]

Water permeation through the skin has been suggested as an indicator of the barrier properties of the skin. Certainly, as the skin barrier is disrupted (*e.g.* by skin stripping using adhesive tape), the transepidermal water loss (TEWL) increases. Rougier found that as corneocyte area increases the TEWL decreases.[11] A simple explanation for this can be seen if the geometry of the skin is considered. As the corneocytes mature more layers of them are produced and they become larger. The maturity and therefore cell size varies with the site on the body. On the face the cells are smaller and there are fewer cell layers. On the forearm the cells are larger and there are more layers. The tortuosity of the skin will increase both with number of layers and also cell size. A very simple algorithm has been found to relate TEWL with corneocyte size.[12] Interestingly, the data showing how TEWL varies with 1/effective diffusional pathlength fall on a straight line which passes through the origin (Fig. 2) *i.e.* as the corneocytes become infinitely large TEWL decreases to 0.

Voegli *et al.*[13] found that the protease activity was larger on the face than the forearm. Implicit in this is that the higher the protease activity the less mature and smaller the cells. This has been examined in more detail in recent studies by Mohammed *et al.*[14] These workers confirmed differences in cell size, maturity and protease activity at different sites and at different depths into the skin (Fig. 3a and b). The variation in barrier function was also in line with recorded changes in TEWL values.

It is interesting to consider the development of this ultimate interface. Premature babies have virtually no stratum corneum and the barrier is very weak.[15] TEWL is very high and one of the major problems with these infants is excessive water loss. Within days of birth the barrier develops but it is possible to deliver drugs to these babies (*e.g.* theophylline) during their first few days of life. Full term babies have a good barrier function but it is possible that this is broken down by excessive use of soaps which contain surfactants such as sodium lauryl sulfate. If this happens and allergens (such as house dust mite faeces) can enter the barrier, problems such as atopic dermatitis can result. The prevalence of atopic dermatitis has been rising steadily in developed countries since the 1940's.[2] One of the major treatments for this condition in children is aqueous cream. A little knowledge of the interface should immediately reveal why this is an inappropriate choice of topical therapy. The presence of the SLS will have a number of effects. It can affect the pH of the skin, this alters the protease activity and the 'rivets' holding the corneocytes together are broken more readily. This has a detrimental effect on the barrier and exacerbates the problem. It can remove free lipids and decrease barrier function as well as intercalating into the lipids (making them more fluid). Finally the keratin structure can be impaired making it easier for the proteases to break the 'rivets'.[16] It is therefore not surprising that a higher incidence of cutaneous adverse reactions has been observed in children after application of aqueous cream rather than other emollients which do not contain SLS.[17] Mathematical modelling of TEWL values, based on Fick's first law, has recently been used to confirm the thinning effects of aqueous cream on skin barrier thickness in healthy adults.[18]

Notwithstanding the ever-increasing incidence of diseases associated with defects in skin structure the challenge, today, for pharmaceutical and cosmetic scientists is still to overcome its barrier function. The maximum percentage of drug or active that is likely to be delivered through the skin is only of the order of 1–2%.[19] In the context of delivery to or through the skin, current strategies aim at developing formulations or devices which transiently increase the permeability of the skin, or which bypass or even remove the outermost skin layer (Fig. 4). The first approach has largely employed Chemical Permeation Enhancers (CPEs) which interact with skin constituents to promote drug flux. Methods to increase the thermodynamic potential of the drug in formulations have also been employed to a lesser extent.

Both strategies are best understood by considering a modified form of Fick's first law of diffusion (eqn (1)).

$$J = \frac{DKCv}{h} \quad (1)$$

where J is the steady-state flux of permeant through a membrane, D is the diffusion coefficient, K is the vehicle-membrane partition coefficient defined as the ratio between the concentrations of the permeant in the membrane at the donor—membrane interface and the vehicle in which it is applied (C_v), and h is the length of the diffusion path within the membrane.

As described by eqn (1), the potential mechanisms of penetration enhancers comprise changes in two major aspects of the barrier properties of the skin: the solubility/partitioning behaviour of the drug into the stratum corneum or the diffusion properties in this layer. Synergistic effects may also be obtained using CPEs which impact on D and K in the same formulation.[20] Interactions at the molecular level between CPEs and the lipid lamellar structures of the stratum corneum and consequent effects on D or K have been reported by a number of authors.[21–24] As well as its disordering effect on the polar head groups of skin lipids[25] dimethyl sulfoxide (DMSO) may alter the protein domains of the SC by interacting with the intracellular keratin possibly swelling the corneocytes and increasing the uptake of water and other chemicals.[26] Molecular dynamics simulations confirm a weakening effect of DMSO on the lateral forces between ceramides and also suggest that at high levels of DMSO pores are created in the lipid bilayers with consequent enhanced permeability.[27] DMSO, today, is used as a vehicle for idoxurudine in formulations for the treatment of herpes simplex and herpes zoster infections despite the principal side effect of DMSO being a garlic-type taste and odour that emanates from the mouth.[28] Alkanols alter solubility in the skin but may also extract lipids[29,30] and modify the SC hydration[31] hence their inclusion as CPEs in a number of transdermal and topical formulations.

The search for effective chemical penetration enhancers (CPEs) has been the subject of extensive investigation in the

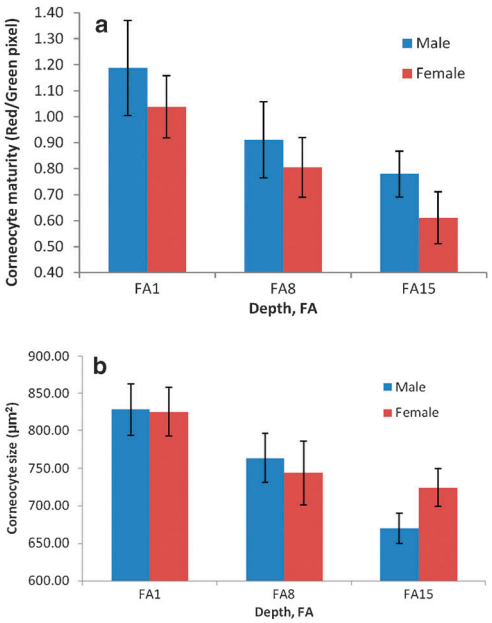

Fig. 3 (a) Corneocyte maturity of the mid ventral forearm (FA) after removal of one tape strip—FA1, eight tape strips—FA8 and 15 tape strips—FA15 (male $n = 13$, female $n = 9$, mean ± SEM.). Mature corneocytes reside in the outer layers and less mature corneocytes are identified in the lower layers. (b) Corneocyte size of the mid ventral forearm (FA) after removal of one tape strip—FA1, eight tape strips—FA8 and 15 tape strips—FA15, (male $n = 13$, female $n = 9$, mean ± SEM). Corneocyte size is clearly seen to be larger in the outer more mature layers.

field of (trans)dermal drug delivery. The ideal candidate should be pharmacologically inert; non-toxic, non-irritant and non-allergenic in the proportions used in the formulation; have a rapid onset and suitable duration of action; reversibly modify the barrier properties of the SC to improve the influx, but not the efflux of materials; be chemically and physically compatible with all components of the formulation or delivery system; and finally, aesthetically acceptable.

Azone® was the first compound specifically developed as a skin penetration enhancer. Its chemical structure includes a lactam group (relatively polar) attached to a long alkyl chain (Fig. 5). Differential thermal analysis studies in human skin have shown the potential of Azone® and analogues with different chain length to incorporate into the stratum corneum, increasing the mobility of the lipid alkyl chains.[32] Similarly, oleic acid (*cis*-unsaturated fatty acid) has been shown to increase the lipid conformational disorder of the hydrocarbon chains of stratum corneum lipids, correlating with enhanced solute permeation. However, oleic acid forms pools in the skin lipids, presumably because of the *cis* double bond whereas Azone® distributes homogeneously.[33] Azone is not used commercially because it never received FDA approval and although oleic acid is found in a number of products its use is still associated with irritation.[34] While the design of new CPE's is the focus of a number of synthetic chemistry groups, there has been limited progress in actual approval of new compounds. This is because the irritant and allergenic potential of emerging chemical enhancers remain the major limitations to their clinical acceptance.

A further hurdle to an exact understanding of CPEs lies in deconvoluting the effects that they have on the rather complex structure of the skin, particularly on K and D, and also on the thermodynamic activity of the drug in the formulation. Recent progress in the field has been achieved with advanced spectroscopic and chemometric approaches. Attenuated total reflectance-Fourier transform infrared (ATR FTIR) spectroscopy and target factor analysis was successfully used to monitor the permeation of multiple components of a commercial ibuprofen formulation in skin and to simultaneously investigate their effects on the skin.[35] ATR FTIR has also confirmed that the effects of DMSO on skin are concentration dependent and that its major effects in the range of 5–10% are to influence partition behaviour.[36] The advent of Confocal Raman Microscopy which has the capability to monitor real team permeation of both drug and CPE as well as other excipients *in vivo* is now ideally positioned to contribute significantly to this area.[37] This should allow a

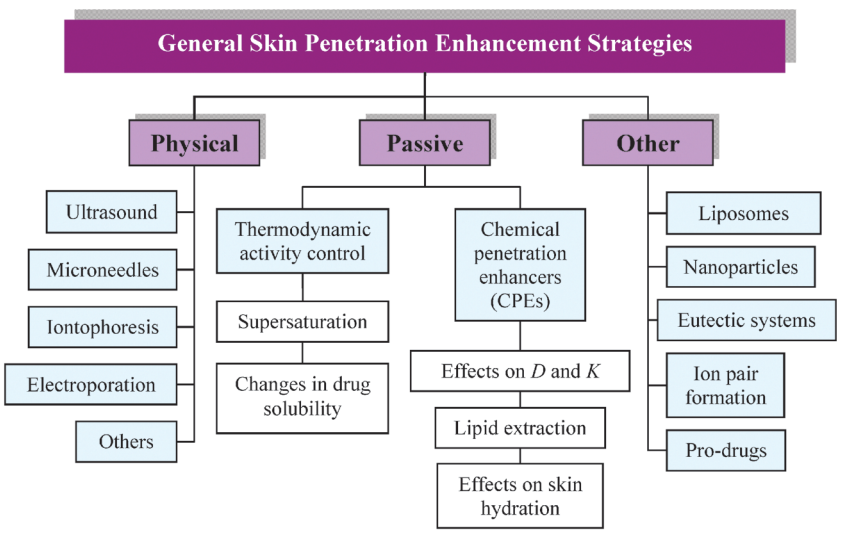

Fig. 4 Possible strategies for skin penetration enhancement.

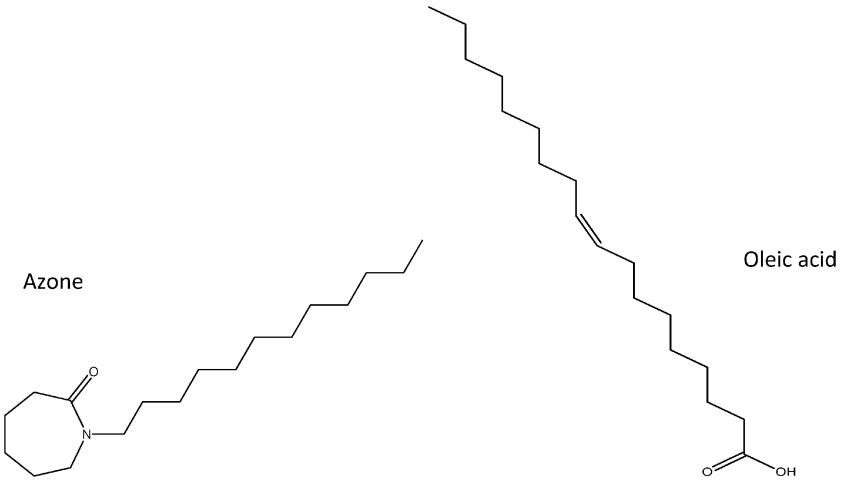

Fig. 5 Chemical structures of Azone® and oleic acid.

move away from empirical testing of CPEs towards the rational design of "smart" topical and transdermal formulations with improved delivery to and through the skin.

From eqn (1) it will be evident that the delivery of actives through the skin also depends on their chemical potential in the formulation. The driving force for diffusion can be artificially increased by creating supersaturation. The penetration into and through the skin has been shown to be proportional to the degree of saturation, up to 4 fold for piroxicam[38] and up to 5 fold for oxybutynin.[39] The stabilisation of the supersaturated states requires the presence of anti-nucleant polymers such as hydroxypropylmethyl cellulose (HPMC). Infra red studies have shown that the HPMC interacts with the small crystallites preventing the diffusion of the active to the interface of the growing crystal surface.[40] Hence the supersaturated state is maintained in this thermodynamically unstable system. Recent studies from small amounts of formulation on the surface of the skin (typical of clinical use) have shown that there are instances where the amount of active penetrating into the skin is in direct proportion to the degree of saturation but the amount penetrating through the skin is not.[39] Experiments following both the transfer of the active and the solvent show that the solvent permeates through the skin faster than the active and that, by inference, the active crystallises in or on the skin. It is therefore not available for permeation into the deeper issues where it is required for pharmacological action. This approach has also been used in the development of transdermal spray technology where supersaturated states are generated because the volatile component evaporates after the spray is applied to skin, with consequent enhancement in skin flux.[41] Because of the dynamic nature of these systems the extent to which the thermodynamic activity of the enhancer as opposed to that of the drug contributes to the overall permeation enhancement process is not yet fully understood but regulatory approval is already in place for a number of these formulations (Fig. 6).

CPEs and supersaturation are passive enhancement strategies in contrast to active approaches which generally involve an energy source to overcome the SC barrier, or require its complete removal or disruption. The principal methods which have been employed to date include laser ablation, ultrasound, iontophoresis, electroporation, jet injection, and microneedles. Iontophoresis is probably the oldest of these techniques and involves the application of an electrical potential gradient to drive solute permeation across the skin. The electrophoretic device, in its simplest form, consists of a

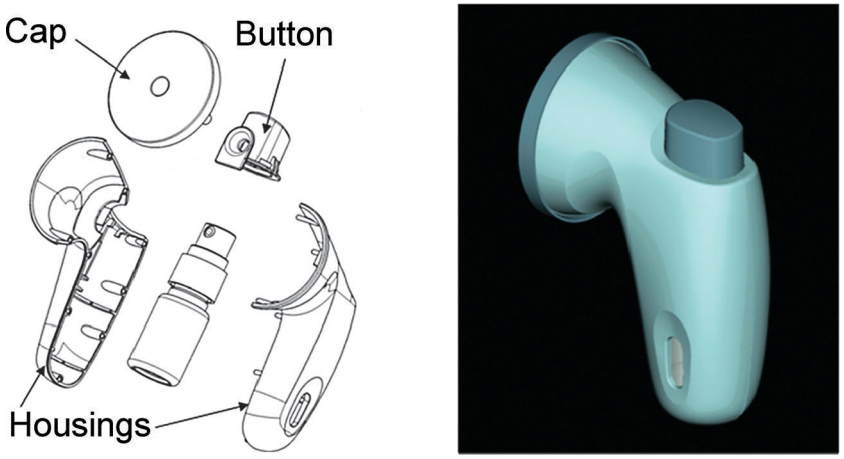

Fig. 6 Metered Dose Transdermal Spray System (Courtesy of Acrux Ltd., Australia).

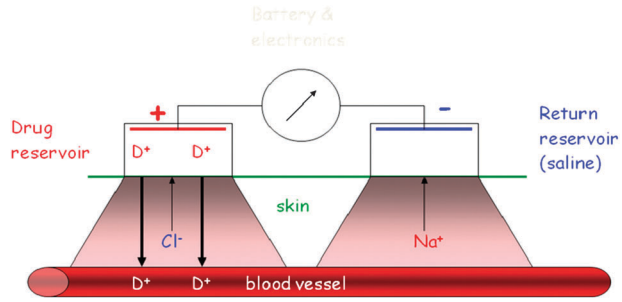

Fig. 7 Schematic representation of transdermal iontophoresis. D^+ are positively charged drug ions.

power source, terminating with an anode and cathode (Fig. 7). Transport across skin is mediated *via* electrorepulsion and electroosmosis. The skin appendages (hair follicles, sebaceous glands) are also believed to contribute to the overall drug flux.[42] Current densities of up to 0.5 mA/cm^2 are considered safe. Iontophoresis has been used in the delivery of both charged and neutral species and a number of devices were approved and launched on the U.S. market between 2000–2006. However, there are currently no iontophoretic devices for drug administration on the market although a number of products are in Phase II and III clinical studies. It is also important to note that these drug delivery systems carried an indication that their use was associated with pain or burning sensations. A more recent application of iontophoresis focuses on extraction of substances from the skin rather than promoting drug permeation through the skin. The Glucowatch® device was designed to extract glucose from the skin and to measure it with an amperometric biosensor. The biosensor chemistry utilized direct detection of H_2O_2 generated from the oxidation of glucose by glucose oxidase. As the transport of glucose in the Glucowatch biographer is in the opposite direction (from the skin outward), this process is termed 'reverse iontophoresis'. Because glucose is uncharged, transport occurs *via* electro-osmosis.[43]

In contrast to iontophoresis which uses small voltages (<10 V), electroporation employs relatively high voltage pulses (10–100 V) for brief periods of time. This is thought to induce aqueous pore formation in the lipid bilayers of the stratum corneum. Drug transport is facilitated by passive diffusion, or can be combined with electroosmosis or iontophoresis. Electroporation has been shown to enhance the transdermal delivery of a diverse range of molecules in the laboratory but has not resulted in a clinical transdermal application to date.[44,45]

Laser ablation, ultrasound and jet injection are also approaches which remain at developmental level and the side-effects associated with iontophoretic devices must inevitably be associated with these techniques. In contrast, thermal ablation which utilizes focused microsecond pulses of thermal energy to remove the stratum corneum has progressed relatively rapidly in the last 5 years. This technique uses microsecond pulses of thermal energy to create multiple microchannels up to 50 μm across the stratum corneum. These microchannels fill with interstitial fluid through which water soluble molecules may permeate to reach the viable epidermis and dermis. As the heat is localized to the stratum corneum for a period of milliseconds or less it is not propagated to the underlying layers. The feasibility of delivering both water soluble small hydrophilic drugs as well as macromolecules has been demonstrated in humans. Patients report a painless application with no sensation of heat.[46]

Of all these "active" approaches the application of microneedles appears to hold considerable potential for the realization of novel dermal and transdermal therapies. Although microneedles were first described in the 1990's this technology has recently experienced somewhat of a renaissance. Microneedles consist of a plurality of micro-projections generally ranging from 25–2000 um in height and which are attached to a base support (Fig. 8). The application of such arrays to biological membranes creates transport pathways with a dimension of the order of microns. Microneedles have been shown to penetrate the skin across the stratum corneum and into the viable epidermis avoiding contact with nerve fibres and blood vessels which are located in the dermal layer. The early microneedles were fabricated with silicon[47] but the major development in recent years has been the design of microneedles using biodegradable and biocompatible materials.[48] Drug can be loaded into the microneedle or coated on to the microneedle and a reservoir patch may also be adhered to the array (Fig. 8). As well as effectively transporting conventional small actives into skin, promising results have been reported for macromolecular targeting *ex vivo* and vaccine delivery in humans and a number of devices are currently in FDA-regulated Phase II clinical trials.[49–51]

In parallel with research in the areas of passive and active permeation enhancement, efforts continue in the more traditional field of formulation technology design to improve dermal and transdermal drug delivery. The use of drug carriers (liposomes and nanoparticles), eutectic mixtures, ion pair formation, and also the selection/development of suitable drug candidates for skin permeation (*e.g.* pro-drugs) currently represent the major activities. Liposomes consist of lipids, typically cholesterol and phospholipids, but other amphiphilic components may also be used in their formulation. Depending on the preparation method these lipid based systems may self-assemble as single bilayer or multiple layer vesicles, Enhanced permeation of insulin and other molecules has been demonstrated but the underlying mechanism for such enhancement have not been elucidated.[52–54] There is no evidence to suggest that they penetrate the skin in intact form but they may alter lipid conformational order *via* intercalation. Despite their cosmetic acceptability, it is doubtful

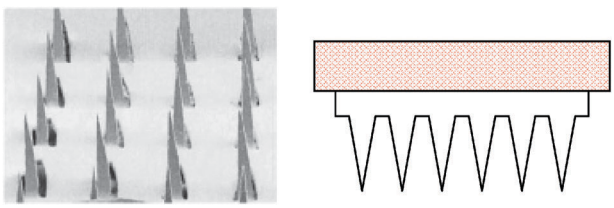

Fig. 8 Microneedle array and schematic of drug loaded patch attached to microneedle array.

that liposomes represent a cost-effective approach for dermal or transdermal drug delivery in the longer term.

Nanotechnology, as in other areas of science is a vibrant area of (trans)dermal skin research but the superior barrier function of the skin clearly throws down the gauntlet to nanoscientists. Evidence for the efficacy of solid lipid nanoparticulates in permeation enhancement has been demonstrated both *in vitro* and *in vivo*.[55,56] The actual permeation of nanosized particles remains a controversial area. The penetration of nanoparticles into the hair follicles has been reported[57] and the possibility of nanoparticles acting as drug depots within the hair follicles has also been proposed. However, the nanoparticles had to be massaged into the skin and the relatively low surface area of the skin occupied by follicles (<0.1%) must temper any expectations of these findings.

Conclusions

The skin is a very complex interface; our knowledge of which increases with the sophistication of the techniques with which we can access its molecular and morphological structure.

New opportunities for interdisciplinary research across the pharmaceutical, chemical and engineering disciplines continue to emerge specifically with the advent of advanced chemometric approaches and Confocal Raman spectroscopy.

Passive permeation enhancement remains the most widely used approach for (trans)dermal drug delivery.

Active permeation strategies focussed around thermal ablation and microneedle technology represent the most promising areas for new drug delivery strategies.

The emergence of nanotechnology has yet to have a major impact on skin permeability. This largely reflects the dearth of fundamental studies into the disposition and fate of the various "nano" systems in the skin.

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