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Biotechnological Aspects of Transport Across Human Skin

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Introduction

The skin provides a fascinating interface between the external environment and our bodies. It has evolved to prevent excessive loss of water and ingress of xenobiotics. This is achieved using a unique structure which forms an impermeable, flexible, and extremely thin barrier (Hadgraft, 2001). The barrier resides in the outermost layer of the skin, the stratum corneum, which has an average thickness of some 20 μm. There are a number of reasons why the skin is so impermeable, not the least of which is its unique architecture, which is shown schematically in *Figure 7.1.*

The major route of penetration is thought to be through the intercellular channels, which are tortuous, and occupy only a small fraction of the total surface area. A diffusing molecule therefore experiences an estimated pathlength of several hundred microns, rather than the 20 μm anticipated from the 'straight-through' thickness. The intercellular channels contain a complex array of lipids, mainly ceramides, cholesterol, and free fatty acids, but, interestingly for a biological membrane, no phospholipids. The lipids are arranged into structured bilayer arrays. The permeant has to cross sequentially these lipid-filled regions, and experiences a series of partition and diffusion steps that add to the barrier properties. It is obviously easy to deliver medicines to the skin's surface but, for the reasons given above, it is difficult for the active to cross the stratum corneum and reach its target site (either deeper structures in the skin or, in the case of transdermal delivery, the blood supply). This provides severe constraints on effective local treatment of dermatological disorders, and on the range of drugs suitable for transdermal delivery.

In the case of local delivery, drug bioavailability is often no more than a few per cent of the applied dose; for transdermal delivery, the maximum daily dose that can

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Biotechnology and Genetic Engineering Reviews – Vol. 21, November 2004
0264–8725/04/21/183–193 $20.00 + $0.00

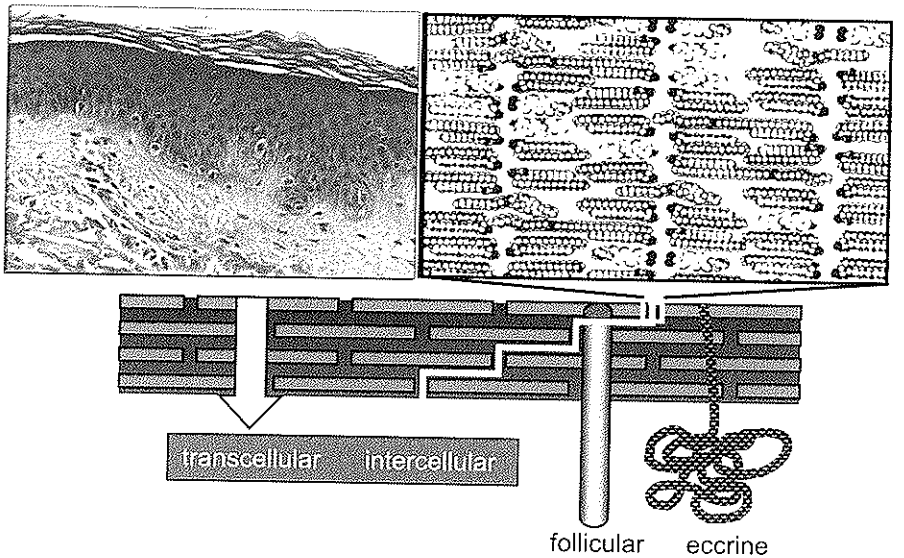

Figure 7.1. A composite picture (adapted from Hadgraft, 2001) showing top left a photomicrograph of the skin with the stratum corneum clearly visible as the topmost layer. Top right shows a molecular graphics representation of the intercellular lipids. The bottom cartoon represents the different routes of permeation that are possible through the stratum corneum.

be delivered is of the order of a few milligrams (Guy and Hadgraft, 2003). An additional constraint on dermal delivery is the potential of dermally applied agents to elicit irritancy and sensitization; such skin toxicity obviously needs to be avoided or minimized if successful therapy is to be achieved. Very few drugs have been specifically designed for dermal–transdermal application, and it is often thought that judicious formulation can compensate for the poor permeability properties. However, this is generally not the case, and there are dermatological products on the market that are far from ideal. This leads to poor therapy in an area that is often neglected by the industry, a surprising observation, given the prevalence of skin disorders and the impact that these problems have on quality of life (Finlay, 1997, 1998).

Physicochemical determinants of dermal delivery

The innate ability of a permeant to cross the skin is a function of its physicochemical properties. Consideration of the bilayer nature of the intercellular channels suggests that the transport of a permeant through the skin is a combination of diffusion and partition. Although the skin is a heterogeneous membrane, Fick's laws of diffusion have been routinely applied to describe drug permeation (Barry, 1999). Most simply, for steady state diffusion, the flux J (amount per unit time) is represented by

$$J = A\,K\,D\,\Delta c/h \tag{7.1}$$

where A is the area, K is the partition coefficient of the permeant between the skin and the applied formulation, D is the diffusion coefficient, Δc is the concentration difference between the formulation and the inner regions of the skin, and h is the

diffusional pathlength. As the concentration in the skin is significantly lower than that applied (c_{app}) *Equation 7.1* can be rewritten:

$$J = A\, k_p\, c_{app} \tag{7.2}$$

where k_p (= K D/h) is the permeability coefficient.

Under normal circumstances, the maximum achievable flux will be when c_{app} equals the solubility of the drug. Hence, the important physicochemical parameters that underpin (trans)dermal delivery are partition, diffusion, *and* solubility. This has been recognized for a surprising number of years (Hadgraft and Somers, 1956). The optimum partitioning properties correspond to drugs with log [octanol–water partition coefficient] (log P) values of between 1 and 3. Diffusion is related to molecular volume, and small molecules move faster, therefore, than large ones (Vecchia and Bunge, 2003). Furthermore, drugs with functional groups that can form hydrogen bonds penetrate the skin slowly (Pugh *et al.*, 1996). The solubility of a compound is a function of its melting point (MP) and, generally, compounds that have low MPs permeate well (Kasting *et al.*, 1987). It is interesting to note that two transdermally delivered drugs, nicotine and nitroglycerin, are good skin permeants, with log P values of 1.2 and 2.2, respectively, and are both liquids at room temperature.

Another important physicochemical parameter to be considered is the pK_a of the permeant (Hadgraft and Valenta, 2000). The skin surface has an estimated pH of around 5, and the ionization state of the active will influence both its solubility and partition behaviour. In general, for transdermal delivery, the free base or acid is preferred over the salt. With the emergence of biotech drugs, peptides, proteins, oligonucleotides, etc., the major problems involved in dermal delivery are the size of the molecule and its polar and/or charged nature, which is often complicated by zwitterionic groups. Without exception, biotech drugs do not diffuse readily through the stratum corneum, and other delivery approaches, rather than simple passive diffusion, have to be considered.

Passive delivery of small molecules (MW <~600)

The formulation of the permeant can have a profound effect on its absorption (Katz and Poulsen, 1971). Typically, the drug is formulated at as high a thermodynamic activity as possible. Enhanced permeation can be achieved using supersaturation, created either by mixing binary solvents, or via solvent evaporation, or by rapid uptake of solvent into the skin (Pellett *et al.*, 2003). It should be remembered that the amount of a topical formulation placed on the skin surface is typically very small (~2 mg/cm^2), and that solvent loss can be rapid.

In the case of biotech agents, enhancement strategies will be required because of their poor permeability characteristics. Simple examples include the use of solvents, which will co-diffuse into the skin and take the agent into the tissue along with the 'solvent' front, as has been observed with oligonucleotides (Nolen *et al.*, 1994). Since many biotech drugs are charged, it is sometimes possible to mask the charge, either by chemically blocking the ionizable group, or by forming an ion pair with an appropriate counter-ion (Green and Hadgraft, 1992). Permeation of ionized species through the skin is small, but can be influenced by substances, such as phloretin, which apparently alter the dipole nature of the stratum corneum lipids (Valenta *et al.*, 2001).

Figure 7.2. A schematic representation of a Transfersome, an ultra-deformable vesicle (www.idea-ag.de).

Passive delivery of large molecules (MW >~600)

There are reports in the literature that vesicles can mediate the skin absorption of large molecules, such as insulin. Several different approaches have been adopted, such as the use of Transfersomes (Cevc, 2003) (www.idea-ag.de). These are ultra-deformable vesicles (*Figure 7.2*), and have been proposed for the delivery of small and large molecules.

The precise mechanism of permeation is still a matter of debate. It has been suggested that the water gradient that exists across the stratum corneum provides the free energy required to 'pull' the vesicle through 'deformations' in the skin lipids (Cevc and Gebauer, 2003). The corollary is that transport will stop if the relative humidity at the skin surface approaches 100%, i.e. these systems do not work under occlusion. Other vesicular systems have been implicated also in the promotion of macromolecules across the skin (e.g. the Biphasix system (www.helixbiopharma.com), Ethosomes (Biana and Touitou, 2003), and Emzaloids (www.mzl.co.za)).

Active delivery of molecules

Even with the use of vesicular systems, or other penetrating-enhancing vehicles, passive delivery rarely allows a sufficient increase in transport to be practically useful. Therefore, considerable effort has been targeted at the delivery of biotech agents using more invasive approaches. These can be broadly divided into those requiring external energy, such as electrical or ultrasonic, and those in which the barrier is physically breached.

ELECTRICAL AND ULTRASONIC TECHNIQUES

The most investigated process is that of iontophoresis in which a small electric current (~mA/cm^2) drives primarily ionized species through the skin (Delgado-

Charro and Guy, 2003a). The amount delivered is proportional to the current, and this provides a mechanism for precisely controlling the dose. Generally, it is easier to deliver cationic species as the skin, under normal physiological conditions, is negatively charged. Also, it must be appreciated that the device will try to deliver all ions that are present with the same charge. Thus, if the positively charged entity is formulated in the presence of sodium ions, for example, both will be delivered, and the relative efficiency of delivery will depend on the transport number of the particular ion. The latter depends on size (it being easier to deliver the smaller sodium ion than a positively charged drug) and the relative concentration of the competing ions.

In addition, the concurrent process of electro-osmosis provides a convective flow of water through the skin, in the anode–cathode direction, which carries along water-soluble solutes that are present. Iontophoresis is therefore a flexible technique, but there are upper constraints on the size of molecule that can be delivered. Further, if a molecule undergoes charge reversal as it passes through the skin from a pH on the skin surface of 4–5 to physiological pH (7.4), delivery is problematic (Sage and Hoke, 1998). This has been seen in the case of insulin.

Iontophoresis has been examined for a range of chemical entities, and both therapeutically active peptides and oligonucleotides have been investigated. Iontophoretic devices have been commercialized, and a system is available for delivery of the local anaesthetic lidocaine (www.iomed.com). A more sophisticated, fully integrated device (www.vyteris.com) is presently under review at the US Food and Drug Administration. Phase III trials have also been conducted using the potent narcotic analgesic, fentanyl, using the E-TRANS system (Phipps *et al.*, 2003), demonstrating the possibility of on-demand dosing (www.alza.com).

Electroporation, which has been evolved from the gene delivery area, is another approach to promote skin penetration (www.genetronics.com). In this process, a short-duration pulse (or sequence of pulses) of high voltage produces transient pathways across the stratum corneum barrier, and allows significantly elevated penetration of even very large molecules (Preat and Vanbever, 2003).

Other mechanisms for producing channels through the stratum corneum include the ViaDerm (*Figure 7.3*) and MicroDerm technologies that make use of radio-frequency energy (www.transpharma-medical.com).

The approach has been investigated *in vivo* (Sintov *et al.*, 2003) in animals, for the transdermal delivery of small and large drugs, including insulin. A related technology, the PassPort system, is also under development for the dermal delivery of biotech drugs. This device also relies on electrical energy to create microchannels through the stratum corneum (www.alteatherapeutics.com).

Ultrasound has been studied in some depth for the delivery of drugs into and through the skin. While variable results have been obtained for the delivery of small molecules using high- (MHz) frequency ultrasound, it has been shown that low- (kHz) frequencies are much more effective in increasing the transport of larger chemical entities, e.g. insulin and erythropoietin (Merino *et al.*, 2003; Mitragotri and Kost, 2003). It is thought that cavitation within the stratum corneum is the major mechanism of action. Commercial interest in this approach is apparent (Kost, 2002; Meiden, 2003).

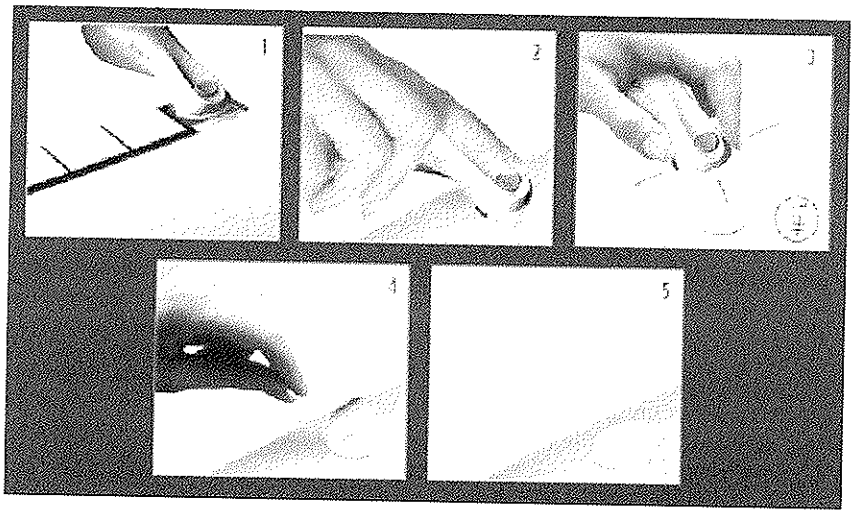

Figure 7.3. The ViaDerm system showing application of a medicated pad and the electronic control unit.

Breaching the barrier

A number of concepts have been developed or proposed in which the stratum corneum is physically breached (Down and Harvey, 2003). Intradermal delivery can be achieved using technologies such as that developed by PowderJect (www.chiron.com/pjct.html) in which a very high velocity helium stream forces particulates through the stratum corneum. The depth of penetration depends on the particle momentum, which is a function of velocity, particle size and density (Sarphie *et al.*, 1993; Bellhouse and Kendall, 2003). Related technologies use high velocity liquids (Levy, 2003; Pass and Hayes, 2003) to breach the barrier (www.antarespharma.com and www.weston-medical.com). Also under development are solid and hollow microneedles (Prausnitz *et al.*, 2003) (www.micronjet.com) that can be designed to penetrate to fixed depths. Similarly, microprojections (Cormier and Daddona, 2003) have been used in the Macroflux (www.alza.com) system for the delivery of oligonucleotides and vaccines (Lin *et al.*, 2001; Matriano *et al.*, 2002).

These systems have the potential to deliver active materials by passive diffusion through the porated skin, or to be coupled to iontophoretic modules that can deliver the active in proportion to an applied current, for example. They also have the potential to be used as a means for analysing interstitial fluid, and therefore monitor active levels in the body or biochemical markers. In this way, the electronics could be used in a feedback approach to titrate input of the active as required. This concept becomes increasingly important as: 1) our understanding of chronopharmacology increases (Gries *et al.*, 1998); and 2) as the era of individualized therapy approaches (Brouwer and Pollack, 2002).

Skin barrier function can also be impaired in a very controlled way using laser ablation, where the strength of the laser pulses used will dictate the depth of penetration, and the number of pulses will control the area that has been compromised (Nelson *et al.*, 1991; Lee *et al.*, 2001).

The skin has evolved to prevent the ingress of xenobiotics, including viruses and bacteria. In any of the approaches that use physical damage, there is always a concern that the 'hole' generated has also the potential to allow passage of unwanted species. The degree of damage, and the speed with which the barrier function regenerates, need to be examined and, as with any of these techniques, there are obvious safety issues that will need to be addressed.

Non-invasive monitoring

The skin is a large interface between the body and the general environment, and the idea that drug levels in the body, for example, may be interrogated transdermally, and non-invasively, is very attractive. Some years ago, it was shown that 'reverse' diffusion of drug from the systemic circulation to the skin surface could be detected. The active, in the blood, diffused across the various strata of the skin to the stratum corneum surface. The diffused drug was collected in an activated charcoal pad, and the amount therein was demonstrated to be proportional to the systemic level (Peck *et al.*, 1988). The process, however, was extremely inefficient and slow. 'Active' processes, like those described above, significantly facilitate extraction. The most widely examined to date is 'reverse' iontophoresis. In preliminary, *in vitro*, experiments, glucose was extracted through the skin by electro-osmosis (Glikfeld *et al.*, 1989). The iontophoretic flux of glucose was then correlated with its concentration in the interstitial fluid, suggesting the method's use, therefore, for the non-invasive measurement of glycaemia (Rao *et al.*, 1993).

The technique has now been developed commercially, and the FDA recently approved a device (The GlucoWatch, *Figure 7.4*) that can be strapped to the arm (for example) and which monitors glucose levels reliably and non-invasively over a 12 hour period (Tamada *et al.*, 1999; Pitzer *et al.*, 2001) (www.glucowatch.com).

Other analytes have been identified that can also be monitored, e.g. phenylalanine (Merino *et al.*, 1999), theophylline (Sekkat *et al.*, 2002), and valproate (Delgado-Charro and Guy, 2003b).

Undoubtedly, in the future there will be other non-invasive techniques that can be usefully employed to examine biotechnological aspects of the skin. NMR imaging

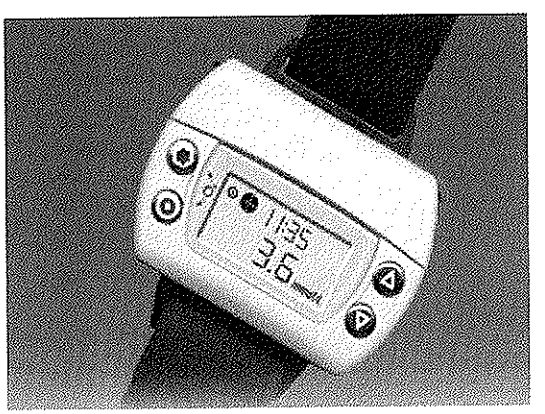

Figure 7.4. The GlucoWatch.

can be used to follow proton distribution in the skin (Dias *et al.*, 2003), and other nuclei might be monitored too. As spectroscopic techniques advance, more information becomes accessible. For example, confocal Raman microscopy can determine concentration profiles (Caspers *et al.*, 2001), and near infrared spectroscopy has been used to examine water profiles across the skin (Wiechers *et al.*, 2003).

Tissue culture

Another biotechnological aspect of topical and transdermal delivery of agents involves the development and application of cultured skin systems (Ponec *et al.*, 2000). Human skin is not readily available, and its permeability characteristics are not well simulated by animal tissue. For this reason, considerable research has been dedicated to the generation of skin from cultured cells. This tissue can be used in permeation studies and in experiments designed to examine cutaneous toxicity (Boelsma *et al.*, 1997). Since the cultured skin is viable, irritancy markers and the release of cytokines can be studied. Initial studies are promising, but the barrier properties of cultured skin remain poor relative to the real tissue (despite its remarkable histological similarity). While the general composition of the stratum corneum lipids is close to that in human skin, their organization, clearly, is not yet as successful in providing an effective barrier. Nevertheless, these systems have been successfully commercialized (e.g. www.skinethic.com, www.mattek.com).

Future considerations

Over the past 10–15 years, there has been a considerable development in the sophistication of biophysical techniques and their application to the understanding of skin barrier function; in particular, we have a far better understanding, at a molecular level, of the processes involved in skin permeation. As the techniques become ever more sensitive, an even greater insight should be possible. In terms of transport-enhancing technologies, there are several which are maturing. However, commercialization takes time! Witness the fact that iontophoresis has been studied for over a century, yet it is only now that products are reaching the market. More aggressive skin permeabilization techniques hold much promise for the delivery of biotech drugs, where the need for precise control of typically low doses is the key. Ultimately, we may anticipate the evolution of truly 'smart' skin technologies, via which the body is first interrogated non-invasively and then drug is delivered in an amount, and at a rate that is optimal for every patient treated.

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