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Introduction

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The skin is the most physiologically complex and diverse organ of the human body. It has many roles, including the regulation of temperature, mechanical and protective functions. This latter function includes the regulation of water ingress and egress, as well as the prevention of entry into the body of exogenous chemical and biological entities.

The skin is the largest organ of the body, accounting on average for approximately 10% of body mass. It receives approximately one-third of the blood circulating throughout the body and has a surface area of approximately 2–3 m^2 [1]. It provides a robust, flexible and self-repairing barrier to the external environment and protects internal body organs and fluids from external influences, harmful molecules and micro-organisms. Its permeability limits excessive water loss and exercises temperature regulation over the body. The skin forms an extensive sensory surface, transmitting sensations such as heat, cold, touch, pressure and pain to the central nervous system. The skin is a multi-layered organ consisting of three main histological layers: the epidermis, the dermis and the subcutis. Mammalian skin is a stratified epithelium, and each layer will be considered individually, below, progressing from the innermost tissues to the outermost.

1.1 The Subcutis (Subcutaneous Fat Layer)

Immediately beneath the epidermis and dermis lies the subcutaneous fatty tissue layer (or subcutis or hypodermis). This layer provides support and cushioning for the overlying skin, as well as attachment to deeper tissues. It acts as a depository for fat and contains

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blood vessels that supply the skin. It also acts as a heat insulator and a shock absorber. The subcutis is variable in thickness, ranging from a few centimetres thick in some regions, such as the abdominal wall, to areas where there is little or no fat, and the subcutis may be difficult to distinguish, such as the eyelid or scrotum. It is often difficult to distinguish the subcutis from the dermis, as both are irregular connective tissues, but the subcutis is generally looser and contains a higher proportion of adipose cells. The deeper layers of the subcutis are fully continuous, with layers of deep fascia surrounding muscles and periosteum.

1.2 The Dermis

The dermis, or corium, lies immediately below the dermo-epidermal junction. It is 10–20 times thicker than the epidermis and ranges from 0.1 to 0.5 cm in thickness, depending on its location in the body. It is a robust and durable tissue that provides flexibility and tensile strength. It protects the body from injury and infection and provides nutrition for the epidermis and acts as a water storage organ. The main feature of the dermis is a matrix of mechanically strong fibrous proteins, consisting mainly of collagen, but with elastin embedded in a gel-like mix of mucopolysaccharides [2]. Embedded within this matrix are various structures, including nerve tissues, vascular and lymphatic systems and the base of various skin appendages. The upper section of the dermis consists of loose connective tissue and a superficial, finely structured papillary layer which progresses upwards into the epidermis. The lower dermis is a coarse, fibrous layer which is the main supporting structural layer of the skin. The transition between epidermal and dermal structures occurs at the dermo-epidermal junction. Both the epidermis and dermis vary greatly in structure, with the former being mostly cellular in construction, whereas the latter contains few cells, other than mast cells. The dermis is the locus of the blood vessels in the skin, extending to within 0.2 mm of the skin surface and derived from the arterial and venous systems in the subcutaneous tissue. The blood vessels supply the hair follicles, glandular skin appendages and subcutaneous fat, as well as the dermis itself [1].

The vasculature of the skin is responsible for regulating the skin temperature, supplying nutrients and oxygen to the skin, removing toxins and waste products and for assisting in wound repair. Clearly, the vasculature also plays an important role in the removal of locally absorbed chemicals, carrying them into the systemic circulation. The blood supply to the skin can sit relatively close to the skin surface, meaning that exogenous penetrants are removed into the circulation from around the dermo-epidermal junction. Thus, for percutaneous absorption into the systemic circulation, including transdermal drug delivery, the blood supply to the skin facilitates the maintenance of a concentration gradient between the material applied to the external skin surface and the vasculature, across the skin barrier. Such clearance may also be facilitated by the lymphatic system, which is similarly located at a comparable distance from the exterior of the skin to the blood supply [3, 4].

1.3 Skin Appendages

Associated with the skin are several types of appendages, including hair follicles and their associated sebaceous glands (Figure 1.1) and eccrine and apocrine sweat glands.

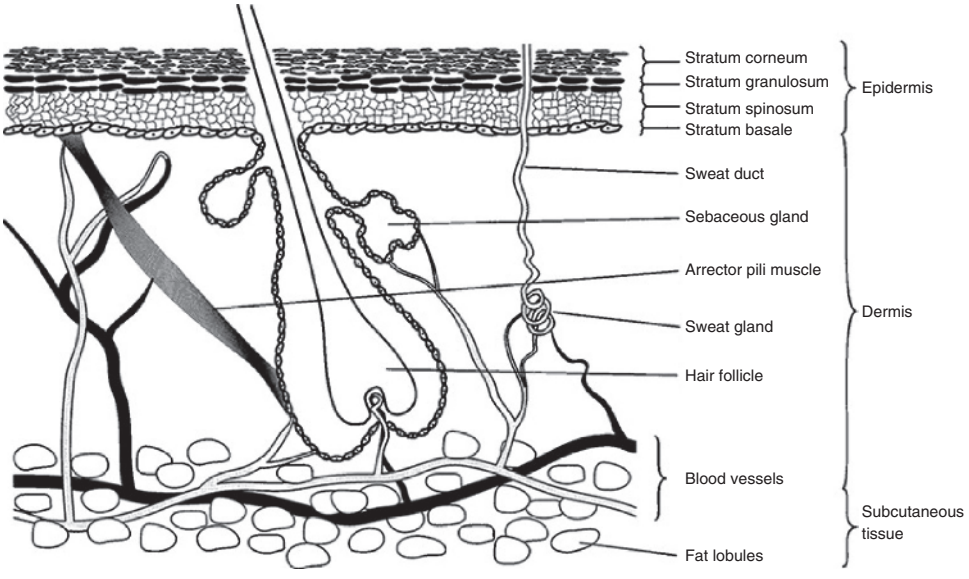


Figure 1.1 *Schematic diagram of the skin. Reproduced with permission from Ref. [5].*

On average, human skin contains 40–70 hair follicles and 200–250 sweat ducts/cm^2 of skin. The skin appendages occupy approximately 0.1% of the total human skin surface [4, 6], although this varies from region to region. Hairs are formed from compacted plates of keratinocytes and reside in hair follicles formed as an epidermal invagination. The associated sebaceous glands (Figure 1.1) are formed as outgrowths of the follicle and secrete an oily material – sebum – onto the skin surface. Sebum is a combination of various lipids and acts as a plasticiser for the *stratum corneum*, maintaining an acidic mantle of approximately pH 5 [6]. The eccrine glands are principally concerned with temperature control and are responsible for secretion and evaporation of sweat when stimulated by an increase in external temperature or emotional factors. These glands commonly occupy only 10^{-4} of the total skin area, and extend well into the dermis. Whereas eccrine glands are found throughout the body, apocrine glands are located in specific regions, such as the axillae and anogenital regions. Similar to eccrine glands, they descend into the dermis.

1.4 The Subcutaneous Sensory Mechanism

The extensive size of the skin lends itself to act as a major source of sensory input for the sensory nervous system. It provides information about the environment from both direct contact and from more remote sources, such as the effect of radiation on skin temperature. Cutaneous fibres within the dermis form a plexus lying parallel to the surface of the skin. This plexus is composed of unmyelinated and myelinated fibres, organised in the same manner as the parent nerve trunks. The dermal networks send twisted extensions into the

papillae, where they form loops which return to the superficial part of the plexus. From the plexus some individual fibres extend to supply particular locations. The terminal branches of each fibre interconnect with and superimpose themselves on each other [7] in such a way that every area in the skin is supplied by several different fibres. Each of these fibres ends in at least one particular receptor. Most of the cutaneous receptors can be excited by various stimuli, but it is the different thresholds of the stimuli required to provoke responses that yields specifically to these receptors [8].

There are three main categories of cutaneous receptor which are distinguished by their different sensitivities to stimuli: mechanoreceptors, thermoreceptors and nociceptors. Mechanoreceptors have high sensitivities to indentation or pressure on the skin, or to movement of the hairs. This group may be further subdivided into the rapidly adapting (RA) and slowly adapting (SA) receptor types. The RA mechanoreceptors include Pacinian corpuscles, found in both hairy and glabrous skin, and Meissner's corpuscles, located in the glabrous skin of primates and hair follicle afferent units found only in hairy skin. Pacinian corpuscles are small pearl-shaped structures found in the deeper layers of the skin. They are 0.5–2 mm long and are composed of an 'onion-like' lamellar structure which is formed from non-nervous tissue. They contain an elongated nerve ending at its core which is not derived from the dermal plexus. The most important characteristic of the Pacinian corpuscle is its ability to detect mechanical vibrations at high frequencies, which may be relayed at greater than 100 Hz/s. Such frequencies are often sensed in traumatised or unanaesthesised skin [9, 10]. The Meissner's corpuscle is an encapsulated myelinated receptor which resides in the dermis of the human glabrous skin. It is tucked into the dermal papillae that fill the grooves formed by epidermal ridges. The entire corpuscle is surrounded by connective tissue, continuous with the perineurium, which is attached to the basal projections of the epidermal cells by elastin fibrils. The Meissner's corpuscle discriminates highly localised sensations of touch, especially in the palmar regions where they are found in their highest density [11].

Hair follicle receptors are myelinated fibres, circumferentially arranged around the hair root sheath below the sebaceous gland which innervate hair follicles. Large hair follicles can be supplied by up to 28 fibres. The hair is well placed in its follicle to stimulate the nerve collar and is primarily associated with tactile sensations [12]. SA mechanoreceptors respond during skin displacement. They also maintain a discharge of impulses when the skin is held in a new position [8]. These receptors include the Ruffini endings and the C-mechanoreceptors. The Ruffini endings are encapsulated receptors which are found in the dermis of both hairy and glabrous skin and provide a continuous indication of the intensity of the steady pressure or tension within the skin [9]. C-mechanoreceptors have small receptive fields (about 6 mm^2) in hairy skin and may emit a slowly adapting discharge when the skin is indented or when hairs are moved. Repetitive stimulation will, however, produce a rapid fall in excitability, and the receptors will fail to respond after 20–30 s because the receptor terminals have become inexcitable [8].

Thermoreceptors are characterised by a continuous discharge of impulses at a given constant skin temperature which increases or decreases when temperature is raised or lowered. The receptive fields of the thermoreceptor are spot-like and cover an area of no more than 1 mm^2. Thermoreceptors are classed as either 'cold' or 'warm' receptors, with 'cold' receptors lying more superficially in the skin than 'warm' receptors. The depth of 'cold' and 'warm' receptors was estimated at about 0.15 and 0.6 mm, respectively, below the surface. The firing frequency accelerates in 'cold' receptors when the temperature is falling – and vice versa for the warm receptors. Such dynamic sensitivity

is high and permits the receptors' response to relatively slow (<1°C in 30 s) and small changes in skin temperature [8].

Damaging or potentially damaging excitation of thermo- and mechanoreceptors is not necessary for such receptors to reach maximum activation, indicating their inability to control pain. They do, however, contribute to the sensory quality of perceived pain. The receptor systems that detect and signal high intensities of stimulation form a distinct class of sense peripheral organs called 'nociceptors'. They have unencapsulated nerve endings and exhibit the smallest identified structures [9–15]. Nociceptors generally reside at the dermo-epidermal junction, and are either *mechanical nociceptors*, which respond to pin-pricks, squeezing and to crushing of the skin, or *thermal* (or mechanothermal) *nociceptors* which respond to severe mechanical stimuli and to a wide range of skin temperatures.

1.5 The Epidermis

The epidermis is the outermost layer of the skin. It is the thinnest part of the skin, with its thickness varying around the body – for example, the thickest skin is commonly found on the weight-bearing planter surfaces (feet and hands, ~0.8 mm) and the thinnest skin is normally found on the eyelids and scrotum (0.06 mm) [5]. Despite the extensive vasculature present in deeper tissues such as the dermis, the epidermis has no blood supply and passage of materials into or out of it is usually by a process of diffusion across the dermo-epidermal layer. It is essentially a stratified epithelium, consisting of four, or often five, distinct layers.

1.6 The *stratum germinativum*

The deepest layer of the epidermis is the *stratum germinativum*, or basal layer. This metabolically active layer contains cells that are similar to those found in other tissues in the body, as they contain organelles such as mitochondria and ribosomes. It is often single celled in thickness and contains cuboid or columnar-to-oval-shaped cells which rest upon the basal lamina. The basal cells are continually undergoing mitosis, as they provide replacement cells for the higher (outer) epidermis. Basal keratinocytes are connected to the dermo-epidermal membrane by hemidesmosomes, which connect the basal cells to the basement membrane. Throughout the basal layer and higher layers of the epidermis, such as the *stratum spinosum*, keratinocyte cells are connected together by desmosomes. The basal layer is also the location of other cells, including melanocytes, Langerhans cells and Merkel cells. The basal cells become flatter and more granular as they move up through the epidermis.

1.7 The *stratum spinosum*

Immediately above the *stratum germinativum* is the *stratum spinosum*, or prickle cell layer. It is often described, in conjunction with the basal layer, as the Malpighian layer. It is several (usually between two and six) layers thick and forged from cells of irregular morphology, varying from columnar to polyhedral in structure as this layer progresses outward. Each cell possesses distinct tonofilamental desmosomes, characterised as prickles or spines,

which extend from the surface of the cell in all directions and which help to maintain a distance of approximately 20 nm between cells. The prickles of adjacent cells link via intercellular bridges, providing improved structural rigidity and increasing the resistance of the skin to abrasion. Though lacking in mitosis, the prickle cell layer is metabolically active.

1.8 The *stratum granulosum*

The next epidermal tier is the *stratum granulosum*, or granular layer. It usually one to three layers deep and consists of several layers of flattened, granular cells whose cytoplasm contains characteristic granules of keratohyalin, which is responsible for their appearance. It is produced by the actively metabolising cells and is believed to be a precursor of keratin. The *stratum granulosum* is the skin layer where degradation of cell components becomes significant, resulting in a decrease in metabolic activity which eventually ceases towards the top of this layer due to the degeneration of cell nuclei, leaving them unable to carry out important metabolic reactions.

1.9 The *stratum lucidum*

The layer above the *stratum granulosum*, the *stratum lucidum*, is easily observed on thick skin, but may be missing from thinner skin, hence the often differing descriptions of the epidermis as having four or five layers. It is often considered that the *stratum lucidum* is functionally indistinct from the *stratum corneum* and that it may be an artefact of tissue preparation and cell differentiation, rather than a morphologically distinct layer. The cells are elongated, translucent and mostly lack either nuclei or cytoplasmic organelles. The *stratum lucidum* exhibits an increase in keratinisation consistent with the progression of cell flattening from the bottom to the top of the epidermis.

1.10 The *stratum corneum*

The outermost layer of the skin is the *stratum corneum*, often called the horny layer. It is the final result of cell differentiation and compaction prior to desquamation and removal from the body. While it is an epidermal layer it is often considered a separate layer of the skin and is often described as such. It consists of a compacted, dehydrated and keratinised multilayer, which is, on average, 15–20 cells thick; that is, around 10 μm in thickness when dry, although it can swell to many times its thickness when wet. The formation of keratin and the resultant death of these cells are part of the process of keratinisation, or cornification. The *stratum corneum* is, in effect, the outer envelope of the body. In areas where the *stratum lucidum* is apparent, the *stratum corneum* is much thicker, being designed to cope with the effects of weight support and pressure. Its thickness also mirrors that of the viable epidermis around the body. Thus, the epidermis in those regions, such as the palms and soles, can be up to 800 μm in thickness, compared to 75–150 μm in other areas. Cells of the *stratum corneum* are physiologically inactive, continually shedding and replenishing themselves from the upward migration of cells from the underlying epidermal layers [1].

The *stratum corneum* is predominant rate-limiting membrane of the skin, and is responsible for regulation of water loss from the body as well as limiting the ingress of harmful materials from the external environment.

The *stratum corneum* is described as the main rate-limiting barrier of the skin with regard to the viable epidermis and dermis [16]. It consists of two alternating amorphous lipophilic and hydrophilic layers, and is comparatively – with regard to the rest of the epidermal layers – more lipophilic. The hydrophilic cells of the *stratum corneum* consist mainly of corneocytes, natural moisturising agents. The water content of the *stratum corneum* is highly variable, depending on both moisture content of the external environment of the body and the location on the body from where the skin is obtained. It varies with the position of the tissue, with the water content generally decreasing as the external interface is approached. The *stratum corneum* has been shown to possess 40% water by weight in an environment where the relative humidity is between 33 and 50%. It has also been estimated that, by weight, the *stratum corneum* is further composed of 40% protein, mostly keratin, and 15–20% lipid, predominately triglycerides, cholesterol, fatty acids and phospholipids [17]. These lipids occupy the intercellular space in the *stratum corneum* and originate from several sources, including the discharged lamellae of membrane-coated granules, intercellular cement and the keratinocyte cell envelope.

The *stratum corneum* is an exceedingly dense tissue and may swell to many times its own thickness in water. Its elongated cells, approximately 1 μm in thickness, form a close-packed array of interdigitated cells stacked in vertical columns [18]. Interdigitation between adjacent cells allows the formation of cohesive laminae. Each cell is contained by a largely proteinaceous envelope rather than the conventional lipid bilayer cell membrane. An individual horny cell is approximately 1 μm thick and occupies an area of 700–1200 μm^2. There are approximately 10^5 cells/cm^2. The mechanical strength of the *stratum corneum* is mostly due to the nature of the proteinaceous envelope, the disulphide bonds of the intracellular keratin and the bridges linking cells that are embedded in an intercellular lipid matrix [19].

The *stratum corneum* constantly sheds its outermost layer in a process called desquamation. The daily loss of flakes from the horny layer of the skin is typically not more than 1 g. The desquamation process involves the cleavage of the intercellular bridges, suggesting that there is a certain degree of metabolic activity and regulatory control occurring in what is often considered to be a dead layer. In normal human skin the rate of *stratum corneum* shedding is generally equal to the rate of epidermal cell regeneration, thus maintaining an epidermis of approximately constant thickness. The *stratum germinativum* and *stratum spinosum* generate one new cell layer per day. Typically, differentiation from *stratum basale* to *stratum corneum* takes an average of 14 days. Cell regeneration is a more complex process in the epidermis, including dehydration and polymerisation of the intracellular material, that ultimately produced the cells found in the *stratum corneum* [1].

Classically, the *stratum corneum* skin barrier has classically been described using a 'bricks and mortar' model [20, 21], with the bricks representing the tightly packed corneocytes which are embedded in a 'mortar' of lipid bilayers. These flattened, often hexagonal – but more accurately described as polygonal – highly proteinaceous cells are the final point of keratinocyte differentiation and are interconnected by structures termed 'corneodesmosomes' (Figure 1.2). The 'bricks' are enclosed within a continuous and highly ordered lipid phase, which is lamellar in structure and often described as a lipid bilayer. It is generally understood that the ceramides are the most important component of this phase. Ceramides are polar

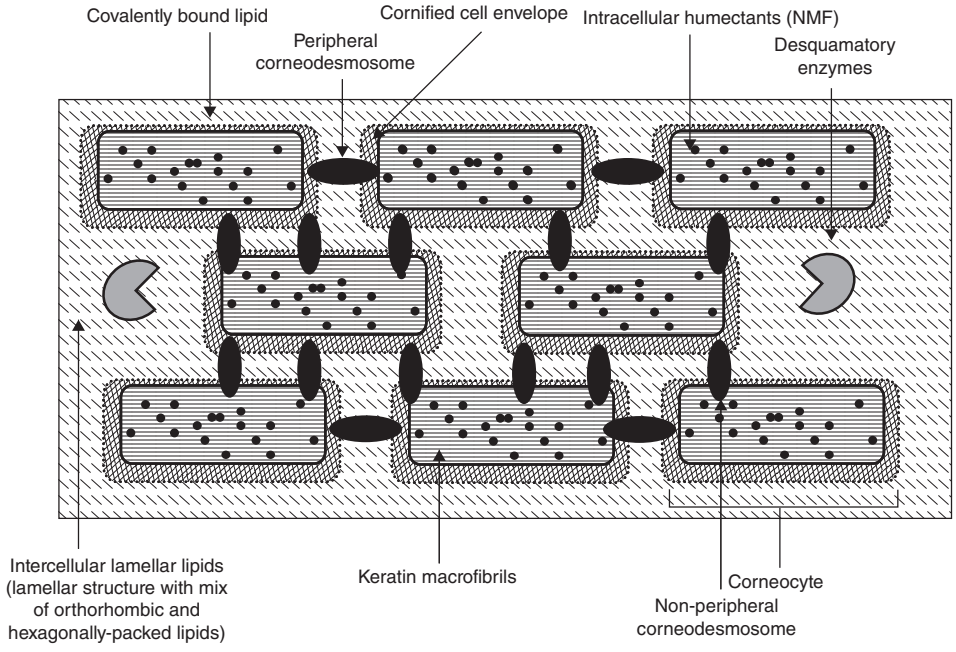


Figure 1.2 *Detailed schematic structure of the stratum corneum. Reproduced with permission from Ref. [23]. © 2010, John Wiley and Sons, Ltd.*

lipids which contain hydroxylated alkyl side chains that, under normal conditions, are packed both hexagonally and orthorhombically. This barrier forms a continuous poly-proteinaceous structure whose thickness and exact composition vary across different body sites. The 'bricks' of the skin barrier may hydrate extensively and cause significant changes in the packing and structure – as well as the permeability – of the *stratum corneum* [22, 23, 25–32]. Thus, it is now understood that the *stratum corneum* does not simply form a homogenous bricks and mortar structure. The corneocytes change in their morphological and biochemical functions as they progress from the lower to higher levels of the *stratum corneum*. Associated with this transition are increases in transglutaminase-mediated protein crosslinking and increased levels of inter-corneocyte ceramides and fatty acids. This results in a progression from fragile to rigid structures (described as the transition from '*stratum compactum*' to '*stratum disjunctum*' [23]) where non-peripheral corneodesmosomes exhibit a reduction in interdigitation towards the outer layers of the barrier. This is concomitant with an increase in the occurrence of (pro)filaggrin – a protein thought to play a role in the aggregation of keratin filaments within corneocytes [23].

Significant advancements have been made in the characterisation and understanding of the *stratum corneum* structure and barrier function in the past 10 years. For example, new species of ceramides, and the synthetic pathways that generate them, are still being identified and their synthetic pathways are still being characterised [23]. The lamellar arrangement of the *stratum corneum* lipids was characterised by electron microscopy and X-ray diffraction and, more recently, by cryoelectron microscopy [27, 33–39]. This latter

technique has proposed the existence of a single-gel phase model for the stratum corneum lipids. The further suggested that cryoelectron microscopy failed to show the expected presence of the trilamellar-conformation long periodicity phase (LPP). Bouwstra and colleagues [40] suggested that the *stratum corneum* lipid phase could be represented by a 'sandwich model'. This model accounts for differences in *stratum corneum* lipid packing – particularly with regard to differing periodicity phases reported in the barrier lipids, highlights the importance of a fluid phase within the *stratum corneum* which may be dictated by the presence of ω–esterified long-chain acylceramides.

However, it is known that the lamellar phase is often missing from the outer layers of the stratum corneum, even in healthy skin. It is now known that other changes also occur [41, 42]. In the most tightly packed lipid barrier – the orthorhombically packed state – the presence of long-chain fatty acids is required to induce the formation of the orthorhombic lattice in ceramide and cholesterol mixtures. Ultimately, the presence of the LPP with orthorhombic packing defines ultimate lipid barrier functionality [43, 44]. The *stratum corneum* is not a homogenous tissue and exhibits characteristic changes as it progresses outwards from the body – often described as the transition from '*stratum compactum*' at the inner base of this layer, to '*stratum disjunctum*' at its outermost layer. Such a transition may be exemplified by a transition in the packing of ceramide sides chains from a transition from a more tightly packed to a less tightly packed hexagonal phase which occurs closer to the skin surface. Further, at the skin surface the lamellar phase is normally missing, becoming amorphous in nature [23, 45, 46].

1.10.1 Routes of Absorption

One of the classic characteristics of the *stratum corneum* barrier function is that the predominant route of absorption is through the lipid layers of this part of the skin. While it is a longer and more tortuous route across the *stratum corneum* compared to the transcellular pathway, it does not require the potential partitioning between the *stratum corneum* lipids and corneocytes, but it relies on partitioning into the stratum corneum lipids from the formulation vehicle and subsequent diffusion across the *stratum corneum*, predominately in a single phase. The other proposed route across the skin, that of permeation via skin appendages such as hair follicles and sweat glands, is limited by the occurrence of such structures as they occupy, on average, approximately 0.1% of the total skin surface and in some cases, such as sweat glands are often morphologically similar to the remainder of the skin surface. This latter point both limits absorption through targeting this route and, in the case of sweat glands in particular, absorption must compete with an opposing outward current. Thus, to understand the absorption process in the context of skin physiology, the *stratum corneum* lipids appear to govern the percutaneous absorption of exogenous chemicals. However, it should be noted that the other potential routes can also play an important part in the overall process of percutaneous absorption [24].

1.10.2 Transdermal Permeation – Mechanisms of Absorption

A chemical must undergo a series of steps if it is to pass across the skin and become systemically available. For example, it will usually be presented to the skin surface in some sort of vehicle, or formulation. This may be a simple aqueous solution, or a complex,

multi-phase pharmaceutical or cosmetic formulation, and it may not remain in the same state during the absorption process (e.g. due to volatility of any of its ingredients). The first step in absorption is therefore the partition of the penetrant from the formulation onto the skin surface, where those molecules in contact with the *stratum corneum* will begin to partition. The rate and extent of this process will depend on the physicochemical properties of each penetrant. The tendency for a chemical to penetrate into the skin may be influenced significantly by the nature of the formulation – if the penetrant has a high affinity for the formulation, then it may remain there, whereas if it has a low affinity for the formulation (or a higher affinity for the *stratum corneum*), it may partition into the skin more readily.

Thus, those molecules adjacent to the skin surface will permeate into the *stratum corneum*. Maintenance of permeation is dependent on the random movement of the penetrant from the bulk of the vehicle to the surface of the skin, which again may be influenced by the nature of the formulation. In the case of suspensions, where the penetrant may be presented to the skin surface as a combination of a saturated solution and as an undissolved solid, partitioning into the *stratum corneum* may be further influenced by dissolution within the formulation to maintain a saturated solution.

Once the penetrant has diffused into the *stratum corneum,* it will diffuse through this layer. This may occur via any of the three main routes mentioned earlier (intracellular, intercellular and transappendageal; Figure 1.3), either individually by combined permeation via more than one of these routes. The next significant permeation step will be at the junction of the *stratum corneum* and the viable epidermis, due to the substantial change in the characteristics of the tissue, which is increasingly hydrophobic in nature. This results in a further partitioning step which is followed by further diffusion into the viable epidermis and partitioning between the viable epidermis and the dermis. Finally, partitioning from the dermis to the capillary system will see the penetrant removed into the blood vessels and the systemic circulation.

The transepidermal route, via the intact *stratum corneum*, is the main route through which penetrants may enter, as it provides the major area available to a potential penetrant. The *stratum corneum* has been morphologically and functionally represented by the ‘bricks and mortar’ model [47]. The ‘bricks’ of this model, the corneocytes, are fibrous protein networks, whereas the ‘mortar’ is an intercellular network predominately consisting of

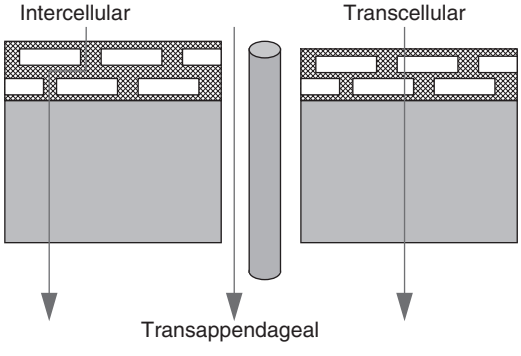


Figure 1.3 *Pathways of drug penetration through skin.*

neutral lipids, as described earlier. Figure 1.2 shows that penetrants may diffuse via a combination of intercellular, transcellular or transappendageal routes.

Successful permeability of the intact *stratum corneum* has been shown to relate predominately to lipophilic materials and depends on the oil/water partitioning property of a particular penetrant. Relationships between permeability and partition coefficients have classically been demonstrated by various investigations which have indicated that the existence of separate lipophilic and hydrophilic pathways is supported by histological and cytochemical studies [48–53].

The other potential route for transdermal penetration is the transappendageal (or 'shunt') route, through hair follicles and sweat ducts. These appendages lack a horny layer and in theory offer low resistance rapid diffusion shunts, allowing penetrants to by-pass the horny layer [54, 55]. By consideration of bulk diffusion relative to shunt diffusion, Scheuplein concluded that transappandageal absorption may be important in the early 'lag' period of the penetration process [51]. Diffusion through glands is generally considered negligible due to the small area they occupy on the surface of the skin, and the current of secretions passing to the outer surface. Valve mechanisms at the openings of glands further lessen their ability as potential routes of access for penetrants. However, whereas Barr demonstrated that areas of the skin rich in eccrine glands do not show significantly greater permeability to chemical penetration [56], more recent studies [55, 57] indicate that glands could contribute to the penetration of hydrocortisone and testosterone. The shunt routes have been shown to potentially play an important route in the delivery of vesicles onto the skin surface [58], although more recent studies, described in the following, suggest that this may be due to deposition of intact particles in skin furrows, or dermatoglyphs. Ultimately, however, as with transepidermal penetration, successful penetration via the shunt route depends upon the physicochemical properties of the penetrant as well as the nature of the *stratum corneum* and may be more successful for some penetrants than for others.

Other factors may additionally influence the penetration process. These include the potential for protein binding, which may occur in the *stratum corneum*, contributing to the reservoir effect associated with that layer. Metabolic activity may see some, or potentially all, of the permeant degraded before it reaches the blood vessels. Further, there is potential for permeants to pass into deeper layers of the skin, including the subcutaneous fatty layer, or even into muscle tissues underlying the skin.

1.11 Theoretical Considerations

Diffusion is 'a process of mass transfer of individual molecules of a substance, brought about by random molecular motion and associated with a concentration gradient' [59]. Passage of a diffusant through a barrier, such as skin, occurs by simple molecular permeation or by movement through pores and channels, as discussed earlier. Diffusion through a non-porous membrane occurs when the penetrant dissolves in the bulk membrane or solvent-filled pores of the membrane. Such diffusion is influenced by the size and physicochemical properties of the penetrant and the nature of the membrane. The three layers of the skin, the epidermis, the dermis and the subcutis, each has their own diffusion coefficient. The diffusion coefficients of all layers other than the *stratum corneum* are

generally considered to be negligible so they are normally treated together and represented by a single diffusion coefficient.

The ability of a compound to pass through the skin has been reported in various manners. Total diffusional resistance of the skin is generally attributed to the *stratum corneum* under passive diffusion and, therefore, Fick's first law of diffusion may be applied [60]:

$$J = -D\frac{\partial C}{\partial x}$$

where:

J is the rate of transfer per unit area of the surface (i.e. the flux).
C is the concentration of the diffusing substance.
x is the spatial co-ordinate measured normal to the section.
D is the diffusion coefficient, or diffusivity.

The dermal permeability coefficient, K_p, is defined by the equations:

$$J_{ss} = K_p C_v \tag{1.1}$$

or

$$K_p = \frac{J_{ss}}{C_v} \tag{1.2}$$

Combination of Equations 1.1 and 1.2 gives:

$$K_p = K_m \cdot \frac{D}{h} \tag{1.3}$$

where

K_p is the permeability coefficient (cm/s or cm/h).
C_v represents the concentration of penetrant in the vehicle when sink conditions apply.
J_{ss} is the steady-state flux of the solute.
D is the average diffusion coefficient (cm^2/s or cm^2/h).
K_m represents the partition, or distribution, coefficient between the stratum corneum and the vehicle.
h is the thickness of the skin.

Thickness of the membrane has generally been recognised as being inversely proportional to flux, although it has been suggested that lipid content, and not thickness, was more relevant [21]. Further, the aforementioned model is more appropriate for *in vitro* systems, as it is unlikely to hold for *in vivo* situations due to the low permeability of the *stratum corneum* which ensures steady-state conditions would take a significant time to become established. However, *in vitro* diffusion is still a highly important area of research, particularly in the development of models for percutaneous absorption, providing excellent theoretical and preliminary investigative models of *in vivo* drug permeation.

Standard methods for membrane studies were derived which stated that, in an *in vitro* diffusion experiment, the concentration of the penetrant should be maintained at a constant

level in the donor phase and conditions in the receptor phase should be invariable [61–63]. Further, he stated that the composition of the donor phase should be kept steady, avoiding losses in evaporation or diffusion, and that both the donor and receptor phases should be continually stirred throughout the course of an experiment. Variations on such methods have been widely employed since.

Thus, from the viewpoint of the percutaneous absorption of exogenous chemical – into and across – the skin, the *stratum corneum* is essentially a lipidic layer, which interfaces with a predominately aqueous medium sitting beneath it. The transport of lipophilic chemicals predominately occurs via the *stratum corneum*, and as these compounds must transfer directly from this comparatively lipid-rich environment into an aqueous medium, compounds that are highly lipophilic will remain largely in the *stratum corneum*. There have, therefore, been considered to be a number of pathways by which compounds with different physicochemical natures may permeate the skin – the so-called polar and lipophilic pathways [6].

1.12 Physicochemical Properties of the Penetrant

Whether through empirical observation or quantitative modelling, the physicochemical properties of a penetrant are known to significantly influence its ability to penetrate into and across the skin. Such properties should be considered in the context of skin permeability and, initially, partitioning.

1.12.1 Partition Coefficient

The Meyer–Overton theory of absorption states that lipid-soluble molecules will pass through the cell membrane due to its lipid content, whereas water-soluble substances pass after the hydration of the protein portion of the cell wall, leaving it permeable to water-soluble substances. The partition coefficient is the ability of a substance to partition between two immiscible phases, usually octanol/water or heptane/buffer. Somewhat simplistically, a higher partition coefficient represents a more lipophilic molecule, and is usually associated experimentally with an increase in permeation via the lipid domains of the *stratum corneum*. For a chemical to cross the *stratum corneum* it must first partition into this membrane, and this may be the rate-limiting step in the permeation process. Barry [64] determined that the partition coefficient, usually described as log P or log K_{OW}, of a penetrant will influence the path it takes in traversing the skin. In practice, the ideal transdermal penetrant should possess both lipophilic and hydrophilic properties [1, 64–66].

It was determined by Bronaugh and Congdon that, for a series of hair dyes, increasing the lipophilicity of a molecule increased the rate of penetration [67]. Le and Lippold indicated that the maximum flux may be estimated from the penetrant's physicochemical properties, including the partition coefficient [68]. Further, Higo *et al*. demonstrated that skin penetration was dependant on the partition coefficient for a series of salicylic acid derivatives [69].

Generally, the lipid bilayers of the *stratum corneum* provide a rate-limiting barrier to the permeation of predominately lipophilic permeants. However, as predominately hydrophilic permeants will have a comparatively higher tendency to permeate via hydrophilic pathways,

such as hydrated keratin-filled keratinoctyes, the effect of partition coefficient for such penetrants is not as clear. For example, the lipid bilayer contains hydrophilic elements (e.g. polar head groups), suggesting that hydrophilic permeants may traverse the skin barrier by a number of different routes. Williams suggested that those permeants with intermediate properties – defined as having a log P of between 1 and 3 – will traverse the skin barrier via both lipid and aqueous pathways but the intercellular route will probably dominate [5]. For lipophilic molecules – those with a log P of greater than 3 – the intercellular pathway will be the predominant route for permeation. Finally, a major consideration for the skin permeation of highly lipophilic molecules is their ability to partition from the lipid domains of the *stratum corneum* and into the predominately hydrophilic tissues of the underlying viable epidermis.

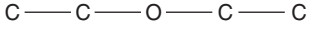
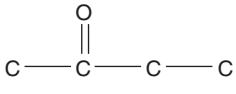
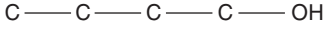
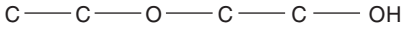
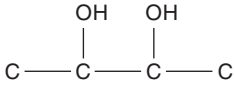
1.12.2 Molecular Size and Shape

Consideration of the size and shape of a molecule are important factors in determining its suitability as a percutaneous penetrant. While molecular volume is the most appropriate term to consider, molecular weight is more frequently used due to convenience and practicality, and assumes that molecules are essentially spherical [5, 70]. An inverse relationship exists between the diffusivity of a molecule and its molecular weight, and as such small molecules may diffuse comparatively faster within a particular medium [71, 72] with a cut-off limit to absorption being generally associated with a molecular weight of 500 Da.

Chemical modifications made to a penetrant molecule can result in substantial changes in its ability to penetrate the skin barrier. For example, Scheuplein and Blank compared the rates of penetration of a series of related compounds, all consisting of four carbon atoms, and varying in the position of either one or two added oxygen atoms, which were present as various functional groups [52].

Table 1.1 illustrates that permeability varies greatly when the functional groups are changed, and the permeability coefficients – determined experimentally – indicate that

Table 1.1 *Effect of molecular structure and functional group on* in vitro *permeability*[a]

Solute	Molecular Structure	Permeability constant, k_p (cm/h)
Ethyl ether		15–17
2-Butanone		4–5
1-Butanol		2–4
2-Ethoxyethanol		0.2–0.3
2,3-Butanediol		0.05

[a] From Ref. [16].

the least permeable molecules are those which are the most polar. It has also been demonstrated that the permeability of steroids decreases when they are modified to incorporate more polar functionalities, such as hydroxyl groups [52].

1.12.3 Applied Concentration/Dose

It is generally recognised that increasing the drug loading of a vehicle increases the amount of drug absorbed across the skin [5, 54, 73, 74]. Further, increasing the surface area available for permeation increases the potential for a topically applied molecule to be absorbed across the skin [72, 75, 76]. Frequency of application will also affect the delivered dose. Although one large application usually results in a higher dose absorbed, a single application may also have a greater toxicological potential compared to frequent, smaller doses [75, 77, 78]. Occlusion and duration of contact can also increase the dose absorbed percutaneously [79, 80].

1.12.4 Solubility and Melting Point

In general, references to the solubility in the context of skin permeability refer to *aqueous solubility*.

The percutaneous penetration of a molecule is greatly influenced by its aqueous solubility and partition coefficient [71, 81, 82]. Generally, lipophilic molecules will penetrate into the *stratum corneum* more rapidly than hydrophilic molecules. However, this needs to be balanced with preferential solubility in deeper layers of the viable epidermis and dermis, as well as the effects of the depletion of the concentration gradient in the vehicle. For example, if a penetrant is relatively lipophilic and is delivered from an aqueous vehicle at either saturated or sub-saturated concentrations, then it may be present in a low concentration in the vehicle, resulting in a diminished concentration gradient as diffusion progresses, and a reduction in the rate of permeation due to donor phase depletion. The partition of the penetrant between the *stratum corneum* and the vehicle is of great importance in transdermal drug delivery. If the drug is more soluble in the *stratum corneum* than the vehicle, then the concentration of that drug in the *stratum corneum* may be greater than in the vehicle at equilibrium. Complete solubilisation in the vehicle has been shown to increase the flux of a penetrant [52, 62, 63, 83–85], although other formulations, including suspensions of largely insoluble drugs, have also been shown to exhibit enhanced permeation [1]. Where drugs are fully solubilised in the formulation the rate of penetration is generally increased by complete diffusion in the vehicle, and may be due to improved diffusion through the vehicle, which replenishes the vehicle/skin interface. Further, melting point is well correlated to aqueous solubility, to the extent that predictive models often employ melting point to determine solubility.

1.12.5 Ionisation

The predominately lipophilic nature of the *stratum corneum* and the largely lipophilic pathway therein infer that the ionised form of a molecule is less likely to permeate the skin than the unionised form. Thus, the degree of penetrant ionisation is essential in determining the successful delivery of drugs by both passive and assisted delivery mechanisms. According to the pH partition theory, if a molecule is unionised, then it may readily penetrate the

stratum corneum via the intercellular pathway. The basis of this theory is that lipophilic regions of the skin act as barriers to ionised species, and that ionised species may pass through pores [5, 86, 87]. Parry and co-workers [88] showed theoretically, by employing a mathematical model, and experimentally that only unionised species enter and traverse the skin. Roy and Flynn [89] demonstrated that the unionised, free base forms of fentanyl and sufentanil are, respectively, 218 and 100 times more permeable than their ionised counterparts. They concluded that the contribution to the process of passive diffusion by ionised species is negligible.

Nevertheless, such comments should be taken in the wider context of a penetrant's physicochemical properties relative to the available diffusive pathways across the skin. Despite the partition theory, several studies [87, 90–93] have shown that both ionised and unionised molecules can penetrate a lipophilic membrane, although the rates and routes of transport are radically different for both species. Classically, it was suggested that ions, ion pairs and electrolytes, such as sodium and potassium salts, can readily traverse the skin [94]. Larger, ionised compounds may penetrate by mechanisms of either ion-pairing [90, 91, 95–97] or ion-exchange [91, 98, 99]. Thus, the ionisation state of a potential penetrant, in the context of its pK_a and the vehicle pH, will significantly affect the permeability of a molecule into and across the skin [100, 101]. Thus, it follows that the different aqueous solubilities of ionised and unionised species will influence permeability as drug flux is the product of the permeability coefficient, K_p, and the effective drug concentration in its vehicle [5]. Adjustment of the pH will therefore alter the amounts of penetrant available in the free base (unionised) or charged (ionised) forms, consequently affecting concentration, solubility and ultimately the rate of penetration across the skin [5, 93, 100].

1.12.6 Physiological Factors Affecting Percutaneous Absorption

The skin is a diverse tissue whose physiology varies considerably around the body. This inherent variation and a range of physiological factors influence the rate of drug delivery into and across healthy, intact skin.

1.13 Physiological Properties of the Skin

1.13.1 Skin Condition

In general, reference to the skin barrier and skin permeability relates to the ingress of chemicals into and across intact, healthy skin. Such skin, particularly the *stratum corneum*, provides a formidable barrier to the passage of substances applied through the skin. However, various disease states may interrupt the continuity of the *stratum corneum* barrier and may result in a number of physiological changes, such as occasionally increasing vasodilation which may result in an increased permeability. Even if the skin is not broken, irritation and mild trauma may reduce the barrier to absorption. Mechanical damage, such as cuts and abrasions, or chemical burns, from acids, alkalis and aqueous phenols may decrease the barrier properties of the skin and increase the rate of absorption. For example, Barry [102] demonstrated that soaking excised *stratum corneum* in chloroform/methanol mixtures dramatically increased skin permeation due to delipidation and the creation of gaps, or artificial

shunts, in the barrier layer. Where the skin is disrupted, it has been shown that absorption of hydrophilic solutes increases significantly more than hydrophobic molecules [103].

1.13.2 Skin Hydration and Occlusion

It has been demonstrated that an increase in the hydration of the skin increases the rate of penetration of most molecules. The exact nature and magnitude of such changes have been attributed to the physicochemical nature of the penetrant and the manner in which excess hydration is induced. Imokawa, for example, concluded that the *stratum corneum* lipids were important in holding water in the skin through the formation of lamellar structures within the *stratum corneum* [104]. Wiedmann suggested that the effective diffusion coefficient across the *stratum corneum* increases with an increase in water content, proposing that the water content of the *stratum corneum* heightens the dynamic motion of epidermal tissue, thereby increasing the effective diffusion coefficient [105]. Diffusion coefficients of skin are also altered by a change in the mobility of skin constituents. The barrier presented by the skin has been shown to decrease rapidly over a short space of time – as little as 10 min – with an increase in hydration [106]. Increased hydration of the skin modifies its rheological properties, altering skin elasticity and increasing its suppleness [106, 107]. Hydration of the skin may also be influenced by the relative humidity of its external environment. Changes in relative humidity have been shown to increase hydration and elevate the rate of diffusion. Fritsch and Stoughton reported increases in acetylsalicylic acid penetration when the *stratum corneum* was fully hydrated, compared to conditions of much lower humidity at the same temperature [108].

Occlusion involves entrapment of water which would normally be lost to the surrounding environment, resulting in a rise in temperature and increased hydration of the skin site [109]. It is normally achieved by placement of a water-impervious dressing on the skin. Increased permeability is also associated with occlusion, where the use of a dressing or formulation which is intrinsically occlusive (i.e. an ointment) is commonly observed to increase permeation as occlusion increases the hydration of the *stratum corneum* [110, 111]. Certain topical formulations may induce occlusion, and increase permeation, by virtue of their high viscosities. The occlusive effect of certain formulations has resulted in an increase of therapeutic activity for a range of drugs, including hydrocortisone [112–114], steroids [115, 116] and citropten [117]. However, Treffel demonstrated that while the rate of penetration of lipophilic citrophen increased under occlusion, amphiphilic caffeine exhibited no such increase when occluded [117]. It has also been shown that volatility of the vehicle in which the penetrant is applied and the physical nature of the penetrant can influence permeation and may not be associated with an increase in permeation under occlusive conditions [118, 119].

1.13.3 Skin Age

The structure and appearance of skin changes significantly with age, but it is often unclear if such changes are as a result of inherent ageing or influenced by environmental factors. With regard specifically to the skin barrier, skin permeability to the ingress of exogenous chemicals is often considered to be lower for infants, and it is often perceived to lessen with age.

The skin of an infant has, compared to adult skin, a higher water content and the *stratum corneum* is not fully developed, leaving it more permeable than fully developed adult skin [120]. The surface-area-to-volume ratio and metabolic activity of an infant or child's skin are much greater than that of an adult, and as such a larger absorption of drug per kilogram of body weight may occur, influencing dosing [121]. For example, Christophers and Kligman [122] and Idson [71] have shown that absorption of topical steroids is greater in children than in adults. This was explained by comparing the decreasing moisture content and increased transepidermal water loss experienced by adult skin at an average age of 40. Further, it was shown that, due to alterations in keratinisation and epidermal cell production which lead to an increase in corneocyte surface area and a decrease in the size of intercellular spaces, the moisture content of human skin decreased with age [123–125]. However, the implications of such a reduction are not clearly decoupled from other effects associated with ageing. It should also be noted that Roy and Flynn concluded that age was not a significant factor in the penetration of fentanyl and sufentanil, suggesting that any age-related permeability effects may not uniformly apply to all penetrants and that, once the *stratum corneum* is fully formed it maintains its barrier function [89]. Nevertheless, other factors may influence the change in permeability. For example, changes to the underlying vasculature may reduce blood flow and thus dermal clearance of topically applied drugs, reducing transdermal flux. However, the main factor in considering skin permeability is the barrier function of the *stratum corneum*.

1.13.4 Regional Variation (Body Site)

Wide variations in absorption rates have been found across different skin sites in the same individual. Additionally, the inherent interpersonal variation of skin means that the most permeable skin sites on one person's body may have the same absorbance as the least permeable site on another person. Although conflicting results have been reported, the permeability rates of molecules can be related to the thickness of the skin at particular points on the body. Wester and Maibach reported that this regional variation in absorption was not necessarily due to *stratum corneum* thickness, and that areas with the same thickness of *stratum corneum* demonstrated different permeability, and areas with different thicknesses of *stratum corneum* demonstrated similar permeability [126]. Thus, while the inherent biological variation of skin ensures that the overall process of skin permeability is multifactorial; trends in the wider literature are apparent and suggest that skin permeability may be ranked by decreasing rates of absorption as follows [21, 50, 89, 114, 127]:

> posterior aricular skin > scrotum > head and neck > abdomen > forearm > thigh > instep > heel > planter

The regional variability of skin permeability may influence absorption and, thus, site of application. Poorly penetrating molecules, such as scopolamine, have been applied postauricularly where permeability is comparatively high. Thus, site-to-site variation of skin permeability should always be considered in the wider context of the other factors discussed herein, including the variation in permeability within a particular body site and the same body site on different individuals [128, 129].

1.13.5 Race

The few studies that have examined how race may affect skin absorption have shown that there are no substantial differences between the permeability across African, Asian or European skin types [130]. It has been suggested that greater skin pigmentation has been shown to present a more resilient barrier and one that recovers after perturbation more rapidly than more lightly pigmented skin [130, 131]. Significant differences have been observed in *stratum corneum* water content between different races [132]. However, while the latter might expect to manifest itself through different drug absorption profiles the limited amount of research carried out in this field, coupled with the inherent variation in skin permeability described earlier, make it difficult to draw definite conclusions on this subject.

1.13.6 Skin Temperature

The effect of temperature on the physiological structure and activity of the skin is complex, affecting both blood flow and metabolism. It is generally accepted that an increase in temperature will increase the rate of absorption and that a decrease in temperature may lower the rate of absorption by up to one order of magnitude [108, 133–135]. Percutaneous penetration usually occurs within a narrow temperature range, although this may be raised from 32 to 37°C by occlusion [5]. However, it has been suggested that there is no change in the rate of absorption when temperature is increased up to 60°C, beyond which point it has been demonstrated that irreversible structural changes take place in the *stratum corneum*, where lipids may solubilise to an extent, resulting in a decrease in skin impedance and resistance [136]. Further, it has been indicated that changes in temperature will mostly assist molecules that normally penetrate the skin readily [16]. Increased vasodilation is a common phenomenon associated with absorption at higher temperatures.

Passive transport through the skin is temperature dependant, as it is initially a diffusion process. The diffusion constant of a penetrant may be related to the temperature of the environment by the Stokes–Einstein equation:

$$D = \frac{kT}{(6\pi r\eta)} \tag{1.4}$$

where D represents the diffusional constant, k represents the Boltzmann constant, T is the absolute temperature, r represents the hydrodynamic radius of the diffusing drug molecule and η represents viscosity.

1.14 Vehicle Effects

Ultimately, rates of percutaneous penetration rely on the effects that the skin, penetrant and vehicle collectively exert on the diffusion process. The vehicle allows optimisation and control of release at a rate adequate to provide a sufficient therapeutic dose of drug. The physical and chemical nature of the vehicle will influence the extent of drug

migration to the skin, and may exacerbate this by exerting changes on the skin physiology in general, and barrier function in particular. The driving force for a drug to diffuse from the vehicle, into and through the skin surface is its thermodynamic activity in the vehicle. As discussed previously, the physicochemical properties of the drug will influence its rate of diffusion. The vehicle must therefore present the drug in a manner which will facilitate its rapid and controlled exit from the vehicle to the skin. The pH of a vehicle will affect the activity coefficient of weakly acidic and basic molecules. For example, it was found that the activity coefficients of weakly acidic compounds were reduced in alkali vehicles [133]. Further, vehicles may affect the skin by hydration and occlusion. Waxes and oil-based vehicles, commonly found, for example, in ointments, increase hydration through occlusion. Aqueous vehicles will occlude the skin less than non-aqueous systems, being generally less occlusive than non-aqueous vehicles, but their aqueous nature may increases hydration at the site of application. Choice of solvent will also affect the drug release from a vehicle; Bronaugh and Franz demonstrated that the release through human skin of caffeine, benzoic acid and testosterone formulated in three vehicles (petroleum, ethylene glycol gel and an aqueous gel) was significantly different [137]. Ethanol has been widely used as a solvent or co-solvent to increase the flux of molecules through the skin [138, 139].

Optimum transdermal delivery is very generally associated with a high concentration of drug in the vehicle, in order to provide a high concentration gradient across the skin. Nevertheless, Idson [140] determined that the ideal vehicle should contain the *lowest possible* concentration of drug, and that all of the drug should be released from the vehicle. Significant success has been observed in the formulation of supersaturated formulations [141–145]. However, no universal vehicle exists for transdermal drug delivery, and the drug carrier must be formulated to consider the physicochemical properties of a particular drug and to maximise its release into and across the skin.

1.15 Modulation and Enhancement of Topical and Transdermal Drug Delivery

Compared to other routes of administration – most notably the oral and parenteral pathways – the transdermal route is limited; this is reflected in the comparative number of drugs available for administration across each route. This is due to the highly efficient manner in which the *stratum corneum* provides a barrier between the body and its external environment. It may also reflect, particularly in transdermal drug delivery, the nature and origin of molecules which were principally designed with other routes of administration in mind.

Much research has therefore been undertaken in improving the transdermal delivery and subsequent bioavailability of numerous drugs for which the transdermal pathway offers many advantages, described above. This work is reviewed excellently elsewhere and is summarised further. The main strategies for enhancing transdermal delivery can be generally divided into two types: chemical and physical enhancement. They are described in the following text.

1.15.1 Chemical Modulation of Permeation

Chemicals incorporated into topical formulations with the express aim of enhancing drug release have been variously labelled penetration enhancers, accelerants or sorption promoters. Katz and Poulsen [83] proposed that the ideal penetration enhancer should be:

- pharmacologically inert,
- non-toxic, irritating or allergenic,
- able to provide immediate onset of penetration enhancement following application,
- able to allow the barrier function of the skin to recover immediately and fully upon removal,
- compatible with a wide range of drugs and excipients,
- able to solubilise drugs,
- clinically compliant and well tolerated by patients,
- inexpensive,
- organoleptically acceptable.

Clearly this is a somewhat idealised list as very few chemicals could fulfil all the aforementioned criteria. Nevertheless, certain molecules do possess several of them and have been incorporated into formulations in order to enhance drug delivery. Such materials usually alter the physiological nature of the *stratum corneum*, either reversibly or irreversibly. Penetration enhancers may promote drug diffusion by either interacting with the skin or promoting release of the drug from the vehicle, or by a combination of these methods [54].

1.15.1.1 Water

The use of water is one of the most widespread and safest methods to enhance skin permeability. This may be achieved by increasing the water content of the *stratum corneum*, either directly or indirectly – for example, by occlusion and reduction in transepidermal water loss. Water exists in a range of states in the skin and may be freely available, bound to skin structural elements or present in skin secretions, such as sebum and the natural moisturising factor (NMF) of the skin [5]. Therefore, the manner in which water increases skin permeability may vary both in mechanism and magnitude depending on the state in which water is present on or in the skin surface. The use of occlusive formulations or dressings, as described above, may reduce transepidermal water loss and increase hydration of the skin, thereby reducing diffusional resistance and increasing permeability.

1.15.1.2 Chemical Penetration Enhancers

Chemical penetration enhancers were classified by Hori and co-workers [146] into three distinct groups depending on their physicochemical characteristics (Figure 1.3). Classical penetration enhancers were placed in group one and were usually aprotic solvents such as dimethylsulfoxide and propylene glycol. Group two consisted of oleic acid and newer and more effective enhancers, such as Azone® (1-dodecylazacycloheptan-2-one). Group three enhancers were mostly organic molecules and were mainly designed for enhancement of specific drug molecules [147].

It has been demonstrated that incorporation of propylene glycol or ethanol, simply as solvents or co-solvents, may enhance the permeability of drugs into and across the skin. Such an increase in flux occurs when presenting saturated solutions of the drug, maximising thermodynamic activity [93, 148]. However, their method of action is unlike that of aprotic solvents, and they do not induce the same changes on the physiology of the skin.

The aprotic solvents employed as penetration enhancers (Figure 1.3) include dimethylsulfoxide (DMSO), dimethylacetamide (DMAC) and dimethylformamide (DMF). They were found to enhance permeation more effectively than other vehicles such as propylene glycol, polyethylene glycol and ethanol [66]. DMSO has been one of the most widely used chemical enhancers in transdermal drug delivery, providing substantially greater enhancement of permeation than either DMAC or DMF. It was first used to increase the rate of transdermal penetration in the 1960s for a wide range of compounds with substantially different physicochemical properties, including water, antibiotics and local anaesthetics [149–152]. Proposed mechanisms of its action include its ability to associate and solubilise skin lipids and proteins, altering the conformation and barrier function of the skin [137, 153]. The high osmotic potential of aprotic solvents allows association and, ultimately, replacement of water in the *stratum corneum*. This distorts the lamellar structure of the sin barrier, resulting in swelling which opens channels in the skin, increasing permeability [154, 155]. Aprotic solvents are, however, irritant to the skin in high concentrations. Topical application of DMSO has been shown to cause halitosis and to leave a bad taste in the mouth of the patient due to the metabolic formation of dimethylsulphide [1].

2-Pyrrolidone and a number of its derivatives have been shown to promote penetration and assist in the establishment of a drug reservoir in skin as they partition into the lipids of the *stratum corneum*. Stoughton demonstrated increased retention of griseofulvin in the *stratum corneum* when presented in a vehicle containing 2-pyrrolidone [156]. 2-Pyrrolidone has also been used to promote the penetration of theophylline, aspirin, ibuprofen, flurbiprofen and caffeine [157–159]. Pyrrolidones, however, produce skin irritation at the high concentrations needed to promote penetration.

Oleic acid (Figure 1.3) is one of a number of long-chain fatty acids that has been used in a range of pharmaceutical applications, including as a skin penetration enhancer. It is classified by Hori *et al*. as a group 2 enhancer, facilitating the permeation of a number of drugs, particularly hydrophilic potential penetrants [5, 146]. It acts in a manner similar to DMSO by mechanisms of lipid fluidisation and, more importantly, phase separation and the formation of novel lipid domains within the skin barrier [160, 161].

Urea, and in some cases its synthetic analogues, acts as both a hydrotrope and a keratolyte [162]. This ability to both hydrate the skin and structurally modify *stratum corneum* lipids has seen urea used as a skin penetration enhancer. For example, formulations containing at least 10% urea have been shown to promote drug penetration by lowering the phase transition temperature of the *stratum corneum*, resulting in fluidisation of the skin lipids at ambient temperatures. Urea has also been shown to increase the hydration of the *stratum corneum* and increase the onset of erythema [163, 164].

Surfactants are found widely in topical many formulations, including pharmaceutical and cosmetic preparations. Anionic surfactants appear to have the greatest activity in terms of increasing skin permeability, followed by cationic and then nonionic surfactants; such comparative activities may also be related to the conformation of surfactants. The anionic

laurate moiety has been found to exhibit the greatest promotion effect [165]. Anionic surfactants have high protein binding affinity, leading to gross swelling of the *stratum corneum*. This results in the uncurling of the protein filaments as they bind to the surfactant [166]. While surfactants are widely considered to cause significant localised irritation (erythema and oedema) they exhibit low chronic toxicity. Non-ionic surfactants have little influence on transdermal penetration and are generally well tolerated by the skin. Skin permeation is not promoted with surfactants containing several long chains instead of a single chain or several short chains, suggesting the enhancement ability of a particular surfactant depends on the ability of the molecules to penetrate the lipid membranes of the *stratum corneum* [167]. The tendency of ionic surfactants to cause irritation to the skin has recently seen their use questioned in pharmaceutical applications, particularly in patients with atopic eczema [168–170].

Azone, shown in Figure 1.4, is a novel, non-polar penetration enhancer widely shown to effectively promote the absorption of certain drugs. Azone has exhibited low toxicity and low irritancy to the skin, and has been shown to be effective in low concentrations [171–175]. However, Baker and Hadgraft showed that Azone had no enhancement effect on the delivery of the antiviral drug arildone [176]. Azone's mechanism of action is thought to be due to fluidisation of the intercellular lipid bilayers of the *stratum corneum*. This reduces diffusional resistance of the skin barrier to the permeation of exogenous chemicals with a wide range of physicochemical characteristics [164, 172].

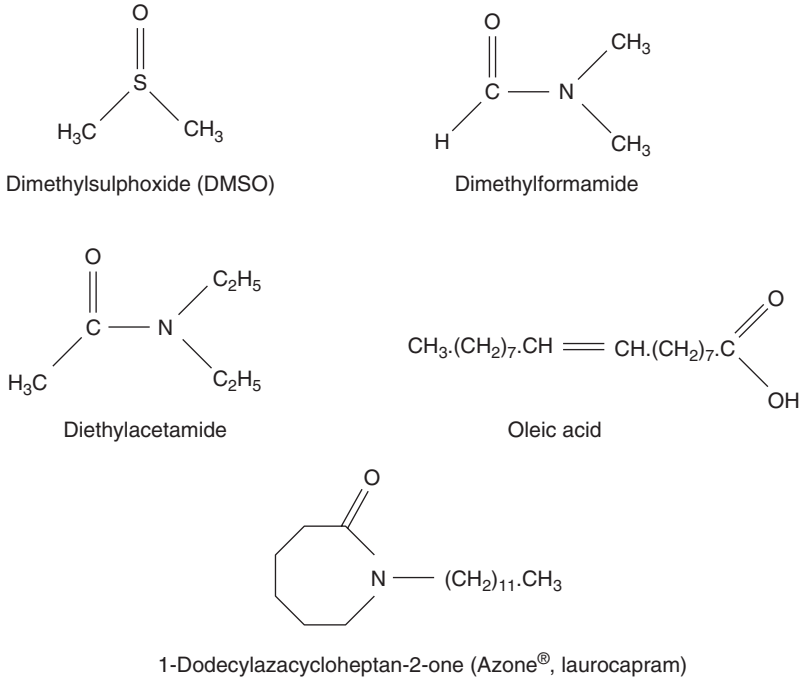


Figure 1.4 *Chemical structures of some penetration enhancers employed for transdermal drug delivery.*

More recently, other chemical classes have been explored for their potential to act as skin penetration enhancers. Terpenes are derived from a number of essential oils. They are based on the isoprene unit (C_5H_8) but are structurally diverse, containing a range of aromatic and aliphatic structures. Williams and Barry examined 17 terpenes for their permeability enhancing effects and found that oxide terpenes and terpenoids had the greatest enhancement effects [177, 178]. Other researchers (i.e. Monti *et al*. [175]) have explored the use of terpenes as enhancers but, so far, they have found few applications in medicinal products [175]. Phospholipids have also found use as enhancers, being formulated as vesicles (liposomes) in order to carry drugs into and across skin. In non-liposomal preparations phospholipids have shown little ability to enhance permeation, although, again, their application in medicinal products has been limited.

A range of other chemicals have found use as enhancers. For example, various phospholipids – often as liposomal preparations [178], HPE-101 (1-[2-(decylthio)ethyl] azacyclopentane-2-one) [179], SEPA™ (2-*N*-nonyl-1,3-dioxolone) [174, 180, 181], *N*-pentyl-*N*-acetylprolinate [182] – have all been investigated for their potential as enhancers. Such compounds have not, as yet, found widespread use with a variety of drugs and vehicles.

1.15.1.3 Prodrugs

Enhancement of permeation has also been achieved by the use of formulation strategies which aim to maximise drug delivery capability of a dosage form. This, in the case of prodrug strategies, may also include the chemical modification of the drug into a form which is more physicochemically amenable to percutaneous absorption. This allows its partitioning, diffusivity and solubility profile to align more fully with the needs of an effective transdermal permeant.

The general strategy for prodrug design is to increase the lipophilicity of the drug by the addition of lipophilic moieties. This is seen, for example, in the use of the lipophilic valerate form of betamethasone-17-valerate which results in improved skin permeation. This increases the permeability of the drug into the *stratum corneum* whereafter it may permeate further into the skin. Further permeation may be accompanied by enzymatic degradation – returning the prodrug back to its parent therapeutic agent – either in the viable tissues of the skin or in the systemic circulation. Despite considerable promise and substantial research [183–185], including the use of co-enhancement strategies (where, e.g., prodrugs are formulated with permeation-enhancing solvents), the clinical use of transdermal prodrugs is limited as such entities are considered to be new chemical entities, thus limiting development opportunities.

1.15.1.4 Ion Pairing

Ionised species seldom penetrate into the relatively lipid *stratum corneum*. The majority of successful transdermal candidates are therefore formulated and delivered as the free acid or base form. However, the use of ion pairing forms a complex in which the charges of each moiety are neutralised. This has been achieved successfully by a number of researchers. For example, the use of simple salts of has been shown to enhance permeation of different drugs [186, 187]. Further, Takahashi and Rytting employed a counter ion with known permeation-enhancing properties, oleic acid [188]. Stott *et al*. used coacervates to enhance absorption, resulting in small increases in permeation [189].

1.15.1.5 Eutectic Mixtures

Modification of physical properties by mixing materials in such a way as to modify their melting point has been a comparatively successful formulation strategy. This has been achieved by the formulation of eutectic mixtures, where the mixing of, in the case of binary systems, two chemicals may inhibit each other's crystal growth, resulting in a reduction of melting point. This has seen significant practical application in EMLA® cream, where a eutectic mixture in a 1 : 1 ratio of lidocaine and prilocaine reduce their melting points, facilitating an increase in skin permeation. After approximately 60 min this results in clinically relevant, transient anaesthesia. Such strategies have also been more broadly employed in other local anaesthetic systems, such as AmetopTM Gel, where water decreases the melting point of tetracaine to 29°C, enhancing permeation and providing a rapid onset of long-lasting (4–8 h) clinically relevant anaesthesia [1, 74, 100, 135, 190–194].

1.15.1.6 Supersaturation

While the optimum transdermal drug delivery is commonly understood to occur from a saturated solution of the permeant, increasing the concentration of the drug above its saturated solution in a particular solvent further increases the thermodynamic activity of the drug in its formulation. However, the formation of supersaturated states is difficult, as most exhibit poor physical stability and usually result in precipitation and crystallisation of the drug from its vehicle. Addition of a range of excipients – such as those used to stabilise suspensions – may improve stability. These include antinucleating agents and materials to reduce or eliminate aggregation of particles. Despite issues of instability supersaturated formulations have shown great promise in significantly enhancing the skin permeability of a range of drugs [141, 195–197].

1.15.1.7 Vesicles

Encapsulation of active ingredients, in both pharmaceutical and cosmetic applications, has been a popular strategy to enhance deposition and delivery onto and into the skin. Various materials, including drugs, humectants and enzymes have all been delivered in this manner. A wide range of vesicles have been used for topical and transdermal drug delivery systems. These include liposomes [198, 199], non-ionic surfactants, or noisomes, elastic or deformable liposomes [200] and ethosomes. This subject is reviewed in detail elsewhere [5].

Despite a wide range of vesicle types and some clinical success – as observed in the literature – the widespread use of such technology to enhancing skin drug delivery has not yet been achieved.

More recently, nanotechnological approaches have been employed to improve percutaneous absorption. For example, the penetration of zinc oxide nanoparticles has been investigated. Multi-photon imaging using time-correlated single photon counting showed that nanoparticles aggregated in the furrows of the skin and that they penetrated laterally from the furrows into the *stratum corneum*, but remained outside of the viable epidermis in non-lesional and lesional tissue [201–203]. Nanoparticulate accumulation in hair follicle following massage has also been observed [204–206].

Luengo *et al*. used atomic force microscopy to describe topical delivery with 328 nm poly-lactic-*co*-glycolic acid (PLGA) drug-loaded particles [207]. Drug release experiments

showed that 100% release was observed after 6 h using human skin *in vitro*; after 24 h the particulate group showed significantly greater delivery in the deep skin layers than the non-particulate control – a 1.8-fold increase.

Other recent studies have shown, for example, the use of capsaicin-loaded nanoparticles in the treatment of pain associated with diabetic neuropathy. Advancements in particle engineering, formulation science and an improved understanding of nanoparticle–skin interactions will undoubtedly lead to important clinically relevant improvements in topical drug delivery [208–210]. In addition, biomimetic nanoparticle engineering has applied principles associated with permeation of nanoscale viruses across compromised skin, such as the human papilloma virus, to designing drug delivery systems applies these 'natural' principles in improving nanoparticle design [211–215].

1.15.2 Physical Methods of Enhancement

As well as formulation-focused chemical methods of enhancement physical methods have also been employed to enhance skin permeation. These methods usually employ a novel device to facilitate delivery and include the use of an electrical current, ultrasound, high-velocity particulate delivery or arrays of microneedles. In general, they may be defined as 'needleless' methods of drug delivery.

1.15.2.1 Ablation

One of the simplest methods of overcoming the challenge to successful drug delivery presented by the *stratum corneum* is to simply remove it. This has been achieved by a number of methods, most notably by Wolf's method of skin tripping with adhesive cellophane [216], so-called 'chemical peels' which may remove the superficial layers of the epidermis, dermabrasion – where the skin is physically abraded to remove the *stratum corneum* – and laser ablation. This last method employs an excimer laser to remove the stratum corneum, resulting in a similar increase in permeability to that observed following tape stripping [217]. However, while subsequent methods have shown similar outcomes, given the overall nature of the methods described they have found little significant use in clinically relevant scenarios.

1.15.2.2 Iontophoresis

The migration of ions or charged drug molecules under an electrical potential gradient allows the transdermal delivery of the salt forms of a drug. Drug delivery assisted in this manner is termed iontophoresis [218]. Iontophoresis is achieved by passing an electric current of the appropriate amplitude through an electrolyte solution containing the charged drug species. Positively charged species, usually salts of weak organic bases, are driven into the skin at the positive electrode, or anode, whereas negatively charged drugs, such as the salts of organic acids, are delivered from the negatively charged cathode. Figure 1.5 shows a typical anodic iontophoresis setup.

Permeation of an ionised species under an applied electric field is due to the electrochemical potential gradient applied across the skin, increased skin permeability under the applied electric field and a current-induced water transport effect, related to electro-osmotic, convective or iontohydrokinesis effects [219, 220].

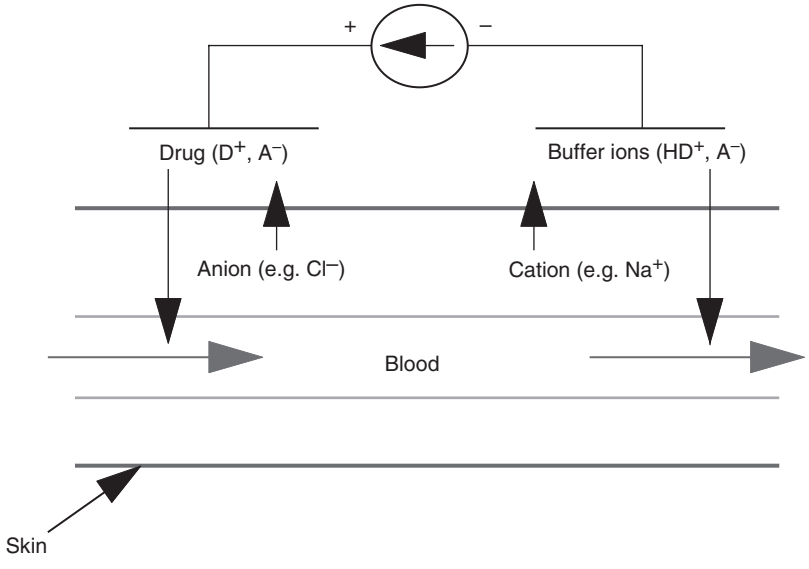


Figure 1.5 *A typical anodic iontophoretic setup, where D^+ represents the positively charged drug, and A^- its counter ion. H^+ and A^- are charged species, usually Na^+ and Cl^-, associated with the extracellular fluid beneath the skin.*

Flux under an applied current may be represented by

$$J_{app} = J_p + J_e + J_c \tag{1.5}$$

where J_p is the flux due to passive delivery, and is given by Equation 1.5. J_e, the flux due to electric current facilitation, is given by

$$J_e = \frac{Z_i D_i F}{RT} \cdot C_i \cdot \frac{dE}{h_s} \tag{1.6}$$

where

Z_i is the electric valence of the ionic species i.
D_i is the diffusivity of the ionic species i in the skin.
F is the Faraday constant.
R is the gas constant.
T is the absolute temperature.
C_i is the donor concentration of the ionic species i.
h_s is the concentration gradient across the skin.
dE/h_s is the electric potential gradient across the skin.

J_c, the flux due to convective transport, may be represented by

$$J_c = kC_s I_d \tag{1.7}$$

where

K is the proportionality constant.
C_s is the concentration in the skin tissue.
I_d is the current density.

The *stratum corneum* has a relatively high electrical resistance, but the underlying layers of the skin are relatively conductive. The applied voltage across the skin thus provides the driving force under which a charged drug may penetrate the skin. The main route of penetration is not through the poorly conductive *stratum corneum* lipids, but through the sweat glands, and, to a lesser extent, through the hair follicles and sebaceous glands covering the surface of the skin. Hydration of the *stratum corneum* may allow diffusion via the intercellular route [221–223]. The isoelectric point of skin is between pH 3 and 4. Thus, above pH 4 the skin has a net negative charge due to the carboxylic acid functionalities of skin proteins. This is also the case at physiological pH. Positively charged drugs, therefore, penetrate the skin across the shunt route more rapidly than negatively charged ions, which have to overcome electrostatic repulsion from the charged groups on the skin surface [222].

A number of factors influence successful iontophoretic transport. They include ionisation, pH related to the pK of the penetrant and associated competitive ion effects, electrolysis associated with the passage of an electric current through the body, the electro-osmotic effect, where polarisation and 'solvent drag' can facilitate the delivery of uncharged species into and across the skin [224, 225], the nature of the applied current (being either a direct or alternating current), and permselectivity, where the valence of a penetrant will influence its permeation. Finally, unlike passive diffusion-facilitated percutaneous penetration molecular size appears to not limit iontophoretic delivery.

The use of iontophoresis has fluctuated over the years, its failure to establish itself as a major method for percutaneous delivery of drugs being mainly due to the potential hazards from burns and mild shocks associated with the applied current, which could be reduced by the use of an alternating current. However, resurgence in the 1990s saw iontophoresis applied to the delivery of anti-inflammatory, local anaesthetic and ophthalmic drugs and for the diagnosis of cystic fibrosis [220] and blood sugar levels – the reverse iontophoresis of the GlucoWatchTM device, associated with the electro-osmotic effect. To date, however, relatively few products of significant clinical relevance have found their way onto the marketplace, despite the publication of a substantial body of research in this field.

1.15.2.3 Phonophoresis (Sonophoresis)

Phonophoresis, or sonophoresis, entails the use of ultrasound to promote enhanced percutaneous penetration by the direct transfer of ultrasonic energy through the drug-carrying vehicle. Phonophoretic delivery may be accompanied by rubbing the skin site (innuction). Such an action by itself may promote drug diffusion in the absence of phonophoresis, and suitable controls are thus needed to ensure enhancement of penetration is due solely to the applied ultrasound. McElnay *et al*. investigated the use of phonophoresis for enhanced absorption of local anaesthetics, and found that phonophoresis did not significantly increase the onset of anaesthesia [226]. Recent studies, however, have indicated that sonophoretic treatment significantly increased drug permeation, compared to control [227, 228].

1.15.2.4 Particulate Delivery

The PowderJectTM system – and related technologies – employed a gas 'gun' to fire solid particle into the skin, thus avoiding the need for painful injections. The advantages of such delivery systems include the ability to deliver fine particles in a pain-free manner (thus having concomitant improvements in compliance and a reduction in patient discomfort or distress due to 'needle phobia'), to avoid needle-stick injuries and the ability to provide solid particles to specific regions of the skin, improving formulation stability and drug targeting.

However, the clinical development of such technologies has been blighted by a number of issues, including consistency of dosing, which may be less accurate than from a needle and which might be tissue-dependant (i.e. dosing into muscle tissue), and which may also be influenced by physiological issues, such as regional changes in *stratum corneum* thickness, technique-dependant issues including 'bounce-off' if the device is incorrectly positioned during use, and environmental factors, including skin hydration.

Despite substantial investment and promising results in early stage clinical studies as recently as 2007 for a range of drugs, no products of this type have as yet been released onto the market.

1.15.2.5 Microneedles

Probably the most significant advancement in dermal and transdermal drug delivery in recent years has been the development of microneedle technology. Microneedles were developed from silicone microfabrication technologies [229] and offer the potential of pain-free delivery of a wide range of drugs into and across the skin. The aim of microneedle devices is to pierce the superficial tissues of the skin, including the *stratum corneum*, in order to facilitate the ingress of therapeutic agents from the delivery device. The depth of delivery is important as, in piercing only the superficial layers of the skin the needles should avoid contact with the pain receptors, which are located at or just below the dermo-epidermal junction [1, 230]. A subsequent study indicated that microneedle delivery was not painless and required vibratory actuation to minimise pain on insertion [231]. Davis *et al*. reported that needle geometry was essential in minimising insertion pain and optimising efficacy while Sivamani *et al*. reported that volunteers felt pressure, not pain, upon microneedle insertion [232, 233].

Needles vary considerably in their design. Initial microneedle systems contain solid needles which, once they had pierced the skin, were removed and a topical formulation applied over the same site. More recent designs have included porous needles though which the drug can diffuse once the skin is pierced or needles which are constructed from composite materials, including drugs, and which dissolve *in situ*, releasing the drug in a controlled manner.

Silicone microneedles were employed by Coulman *et al*. for non-viral gene delivery [234]. Scanning electron microscopy was used to visualise the microconduits created in human epidermal tissue following the administration of microfabricated silicon microneedles. Diffusion of fluorescent polystyrene nanospheres and lipid:polycation:pDNA (LPD) nonviral gene therapy vectors was determined *in vitro* via Franz-type diffusion cells employing human epidermal sheets. It was observed that the diffusion of 100 nm diameter fluorescent polystyrene nanospheres and LPD complexes was significantly enhanced following membrane treatment with microneedles. Cell culture studies confirmed that LPD

complexes mediated efficient reporter gene expression in human keratinocytes. Most recently, Martin *et al*. reported the fabrication of biodegradable sugar glass microneedles for the transdermal drug delivery. Solid amorphous biodegradable sugar glasses containing low residual quantities of water were created by dehydration of trehalose and sucrose sugar combination solutions. These microneedles demonstrated that they were able to facilitate transdermal delivery of a wide range of molecules [235].

Donnelly *et al*. manufactured microneedle devices using hydrogel-forming microarrays [236]. Further, they demonstrated the ability of microneedle pre-treatment to facilitate the delivery of a polylactic-*co*-glycolic acid nanoencapsulated dye across porcine skin, observing a significant improvement in microneedle pre-treated skin [237]. They have also demonstrated microneedle-mediated transdermal bacteriophage delivery [238] and, more recently, the development of hydrogel-forming and dissolving microneedles [239–241].

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