Efficacy, Safety and Targets in Topical and Transdermal Active and Excipient Delivery

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23.1 Role of Efficacy/Active Potency in Topical and Transdermal Delivery

Formulating a suitable topical or transdermal product can often be challenging because the product must be optimized for an appropriate combination of product efficacy, consumer aesthetic acceptance, safety, stability, ease of production and cost. Much of the focus on overcoming the skin barrier to achieve measurable skin penetration therefore has an implicit assumption that the resulting active or excipient will be efficacious or safe, respectively (Roberts 2013). However, it is possible for the barrier
properties of the skin to be completely destroyed, allowing most actives to be delivered in high concentrations. This, in turn, may allow the ingress of previously innocuous excipients and external agents which can then lead to skin irritation and allergic responses. Accordingly, the key question that should be asked is: “Is the transdermal route suitable and required for the proposed therapeutic agent?” Obviously, an active such as acetaminophen, with an oral dose in the hundreds of milligrams, can never be delivered at a sufficient percutaneous flux to achieve efficacy. Alternatively, the skin provides for a regulated, constant delivery rate and much lower first pass metabolism than oral delivery and so may be the best route of delivery for certain actives. However, many actives for which transdermal delivery could be the only viable option are yet to be formulated effectively. This is largely because their physicochemical properties make them unsuitable candidates for skin permeation (Barry 2001).

Having an appropriate efficacy and potency for a therapeutic agent is a key pre-formulation consideration and will govern the selection of a transdermal delivery technology strategy. Such a strategy must also take into account the various factors affecting skin penetration, such as the condition of the skin to be treated (for example, whether it is healthy or diseased), the physicochemical properties of the penetrating molecule, the formulation in which the penetrating molecule is applied and the required dosing conditions (Wiechers 1989). An overriding consideration is also the aesthetic or sensorial properties of the product, as perceived by the user – its appearance, feel, odour, residue, stability, etc. Accordingly, we support Johann Wiechers’ proposed percutaneous product goal: “Both dermal and transdermal delivery aim to achieve one goal summarized in the four R’s of delivery: to deliver the right chemical at the right concentration to the right site in (dermal delivery) or beyond (transdermal delivery) the skin for the correct period of time” (Wiechers et al. 2004). However, we also suggest the inclusion of a fifth R: “…with the right sensorial feel to the consumer”.

23.1.1 Safety and Efficacy-Based Selection of Medication

The formulation of a topical product should start with the choice of an appropriate active. The two considerations here are safety and efficacy. Hayden et al. (2005) assessed the safety of five sunscreens by determining their in vitro toxicity to human keratinocytes in culture then estimated the equivalent concentration to inhibit 50% of cells in viable epidermis, after adjustment for the differences in protein binding in the two media (Fig. 23.1). It is evident that the observed viable epidermal concentrations after topical application are somewhat less than either the observed IC50 in cultured keratinocytes or the equivalent estimated IC50 for viable epidermis, based on adjustments for differences in sunscreen binding in culture and in the viable epidermis. Although oxybenzone has an estimated unbound viable epidermal concentration of >50 times or more than those of the other sunscreens, this is still only 20% of the IC50 determined in keratinocyte culture.

These findings can be summarized by defining a cutaneous margin of safety after topical application as the ratio of concentration of active and excipient associated with toxicity (e.g. IC50) to the viable epidermal concentration of the active or excipient. A desirable margin of safety based on human models should be at least 10 to account for inter-subject variability. Toxicities, such as irritancy, photosensitization and corrosivity, are likely to be better defined by the probability of an event occurring in a given patient after a particular exposure and are dependent on patient susceptibility and the nature and conditions of the product used.

The second safety measure of interest is the systemic margin of safety which arises when an active applied to the skin for local cutaneous effect enters the systemic circulation and causes systemic side effects. This concept is most highly developed for topically applied corticosteroids and, in this case, is quantified by the extent of suppression of the adrenal gland in the hypothalamic–pituitary–adrenal axis. A systematic literature review of the risk of adrenal axis suppression
and skin atrophy after application of topical corticosteroids in plaque psoriasis for the period 1980 to January 2011 found morning cortisol was reduced in 0–48% of patients in 11 short-term studies (Castela et al. 2012). Thong et al. (2007) have listed a range of topical drugs and chemicals known to cause systemic effects. The agents include anti-infectives, antihistamines, minoxidil, insecticides, solvents and steroids.

The corollary measure to safety is cutaneous efficacy and, in general, the more potent the topical product used, the less skin flux required to achieve a desired local cutaneous effect after topical application. A commonly used approach, analogous to that described earlier for toxicity, is to estimate the free drug concentration at the local target site ($C^*$), which, for antivirals, is assumed to be the epidermal basal cell layer (Patel et al. 1996). Afouna et al. (1998) analysed the receptor solutions for full thickness hairless mouse skin mounted in Franz diffusion cells. Assuming that receptor sink conditions applied, $C^*$ was estimated as the ratio of the steady state penetration flux ($J_s$) to in vivo dermis permeability coefficient ($k_{p,d}$):

$$C^* = \frac{J_s}{k_{p,d}}$$ (23.1)

These $C^*$ values derived for various formulations of bromovinyldeoxyuridine (BVDU) and acyclovir (ACV) were then compared with the in vivo efficacy of the formulations against cutaneous herpes simplex virus type 1 infections in hairless mice. Figure 23.2 shows a composite graph of their findings. It is evident that a similar concentration response relationship exists for the 95% dimethyl sulfoxide (DMSO)+5% Azone for both BVDU and ACV and for the 95% DMSO only formulation for ACV, whereas much higher BVDU $C^*$ values are needed for the BVDU in 95% DMSO only formulation. They surmise that Azone could have two effects. Firstly, it may either enhance penetration of the active. This is evident from the 6.5 times higher $C^*$ for 5% ACV in 95% DMSO+5% Azone than in the 95% DMSO only formulation. The effect is less for BVDU, where the $C^*$ for 5% BVDU in 95% DMSO+5% Azone is 1.65 times that of 95% DMSO only formulation. Secondly, Azone may have a synergistic effect with the antiviral agents on viral kill, evident in the shift towards higher potencies of $C^*$ for BVDU but not for ACV (Fig. 23.2).

A key assumption made in this analysis is that the dermal permeability coefficient (which is normally expressed in the pharmacokinetic literature as a dermal clearance ($Cl_d$) (Siddiqui et al. 1989) (i.e. $k_{p,d} = Cl_d / A$, where $A$ is the surface area of application) can be estimated by the ACV skin penetration flux that yields 50% topical efficacy ($J_{50}$) divided by the free plasma levels of ACV that inhibit 50% of cutaneous lesions treated systemically with ACV ($C^*_{50}$), i.e. $k_{p,d} = J_{50} / C^*_{50}$. It is also evident that systemic efficacy of various formulations is much less than
when applied topically, showing the enhanced response that can be achieved by targeted topical application.

Davis has recast the free drug concentration approach described above in Eq. (23.2), which describes the efficacy of the active in terms of both potency of the active and how much penetrates to a target site (Davis 2008; Jepps et al. 2013):

Efficacy = Potency × Delivery  (23.2)

A major issue not fully recognized in the work of Cordero and Davis (Cordero et al. 2001; Davis 2008) is that the fraction of active bound in the in vitro system used to determine potency may differ from that present in skin penetration studies and in vivo. In order to simplify the present description and be consistent with the work reported by Cordero and Davis, this important correction will not be included in the following discussion. Hence, if a cutaneous concentration for a desired effect is defined as $C_{eff}$ and that observed after topical application as $C_{ve}$, the efficacy is given by the ratio $C_{ve}/C_{eff}$. $C_{ve}$ is defined by the penetration flux divided by the clearance from the viable epidermis. Cordero et al. assumed that this clearance, in turn, was related to the in vitro dermis diffusion and thickness. In reality, as discussed earlier in referring to the work of Afouna et al (1998), this should be in vivo clearance. Our own work supports this, suggesting that, firstly, uptake by the blood in the dermal capillaries located just below the viable epidermis is likely to be the rate limiting determinant of clearance in vivo (Cross and Roberts 2006) and secondly, carriage of topical non-steroidal anti-inflammatory drugs (NSAIDS) to deeper tissues below that application site is also dependent on blood flow for highly plasma protein-bound drugs (Dancik et al. 2013a, b). Accordingly, we have, for illustrative purposes, assumed both constant binding and clearance and expressed efficacy simply as a ratio of flux divided by the in vitro minimum inhibitory concentration (MIC), as originally proposed by Mertin and Lippold (Mertin and Lippold 1997).

Thus, a greater efficacy is provided by an active with either a higher flux or a greater potency (a
These concepts are illustrated in Fig. 23.3, where data from Cordero et al. and others for saturated skin flux (Fig. 23.3a), anti-inflammatory activity (Fig. 23.3b) and efficacy (Fig. 23.3c) for a range of non-steroidal anti-inflammatory drugs are shown (Mertin and Lippold 1997; Wenkers and Lippold 2000; Cordero et al. 2001; Jepps et al. 2013).

In Fig. 23.3a, the shaded area indicates the region of optimal skin penetration occurring at a log P of around 3 (Yano et al. 1986; Zhang et al. 2009). Ketorolac and ketoprofen, in this range, consequently have the greatest saturated flux. The more lipophilic actives also have a greater in vitro performance index of anti-inflammatory activity (i.e. lower MIC), and so these two compounds also have high efficacy coefficients. Diclofenac, although having a less favourable log P of >4, still has the highest predicted efficacy coefficient as a consequence of having the lowest MIC. On the other hand, piroxicam has the least saturated flux of all these actives, as well as a relatively high MIC, and consequently has the lowest efficacy coefficient of these non-steroidal anti-inflammatory drugs (NSAIDs). Apart from a comparatively higher molecular weight and melting point, hydrogen bonding seems to be playing a pivotal role in the lower flux of piroxicam (Anrade and Costa 1999).

Adrian Davis (Davis 2007) also presented efficacy and systemic toxicity indices for topical immunosuppressive agents used in treating atopic dermatitis, extracted from Trottet’s PhD thesis (Trottet 2004) at the SCI meeting in 2007 (see Fig. 23.4). The corresponding margins of safety for cyclosporin A, tacrolimus and pimecrolimus, calculated as the ratio of the systemic safety indices and the efficacy indices, were 2400, 47 and 457, respectively.

### 23.1.2 Systemic Concentration of Solutes and Their Effect on Active Performance

The systemic efficacy of many actives, such as anticonvulsants, anti-infective and cardiac drugs, can be defined by plasma concentrations after dosing by a particular route of administration. As shown in Fig. 23.5, in transdermal delivery occurring from a constant rate transdermal passive delivery patch, there is a lag prior to reaching lower dose for the same effect). These concepts are illustrated in Fig. 23.3, where data from Cordero et al. and others for saturated skin flux (Fig. 23.3a),
maximal levels and in returning to baseline on patch removal (Roberts and Walters 2007). Further, after reaching a maximum, the serum levels slowly decline with time, consistent with a reduction in flux due to a gradual depletion in the amount of active in the patch.

Topical products, especially transdermal patches, are often required to provide constant therapeutically effective plasma concentrations, $C_{ss}$. The penetration flux $J_s$ ideally needed to reach such concentrations is given by Eq. (23.3), where $C_{\text{body}}$ is the body clearance and $F_s$ is the availability.

\[
J_s = \frac{C_{\text{sat}} C_{\text{body}}}{F_s} \tag{23.3}
\]

Table 23.1 shows some estimated transdermal fluxes required for the topical administration of a number of pharmaceuticals used in transdermal systems, by substituting desired plasma concentrations and body clearances into Eq. (23.3).

### 23.1.3 Enhancing Efficacy by Increasing Fractional Solubility of an Active in the Formulation

A key goal in formulating an active in a transdermal product is to obtain a desired steady state skin penetration flux (amount penetrated over a period of time) of the active. $J_s$ is defined by Eq. (23.4) (Reeve et al. 2013), where $J_{\text{sat}}$ is the flux from a saturated solution (often referred as maximum flux (Magnusson et al. 2004)), $C_v$ is concentration of the active in a given formulation and $S_v$ is the solubility of the active in the formulation. $f_v$ represents the factional solubility of the active in that formulation, $\frac{C_v}{S_v}$.

\[
J_s = J_{\text{sat}} \frac{C_v}{S_v} = J_{\text{sat}} f_v \tag{23.4}
\]

As is evident from Eq. (23.4), one way to maximize skin penetration is to use a fractional solubility approaching unity. Poulsen led a range of studies that showed maximal skin penetration will occur when an active is at saturation in a formulation (Poulsen et al. 1978).

As discussed in depth by Davis (2008), if an active is used at a concentration below its solubility in the vehicle, the product will normally have a lower efficacy than if the product was used at saturation. Consequently, products may be formulated to contain more active than will actually be needed for an adequate effect, as is the case with transdermal fentanyl patches, which have led to numerous deaths. The danger inherent to fentanyl is its potency (greater than 50–100 times that of morphine) and rapidity of action (Jumbelic 2010). An optimal dosing strategy demands that the rate of active penetration into the skin will achieve and sustain biologically active free levels at the target site within the specified limits. Also, ideally, these conditions should be independent of skin site and skin condition. This is a considerable challenge, given that there is wide variability in skin permeation, depending on the anatomical site (Rougier et al. 1988) and the condition of the skin, such as in particular disease states (Elias and Feingold 1992) or levels of hydration (Roberts and Walker 1993).

As discussed in other chapters in this book, another strategy to increase efficacy is to use a saturated active solution in the vehicle and to further increase either the stratum corneum solubility $S_{sc}$ and/or its diffusivity $D_{sc}$ using co-solvents and
enhancers, in accordance with Eq. (23.5), where \( h_{sc} \) is the stratum corneum thickness:

\[
J_{sat} = S_{sat} \cdot D_{sat} / h_{sc} \tag{23.5}
\]

However, care has to be applied in representing such saturated fluxes as maximum fluxes because increasingly, formulations are being designed to include a volatile component that may result in enhanced skin penetration due to the generation of a transient supersaturated state. Many researchers have now promoted the use of supersaturated solutions (Coldman et al. 1969), with several showing that these can lead to even higher fluxes than those achieved with saturated solutions (Lippold 1992; Morgan et al. 1998; Iervolino et al. 2001; Timothy et al. 2002; Santos et al. 2012). More recently, there is an increasing tendency to either stabilize supersaturated products by using polymers (Raghavan et al. 2003) or to generate supersaturation on application to the skin as a consequence of the evaporation of the volatile components in the product (Hadgraft and Lane 2011). Supersaturated systems may be obtained either by design or via solvent evaporation or by mixing of co-solvents. The stability of supersaturated solutions is an important issue, and it would be useful to develop supersaturated systems that are stable throughout the product shelf life. Santos et al. (2011) investigated the permeation of fentanyl from supersaturated formulations across silicone membranes. Supersaturated formulations containing either propylene glycol (PG)/water or PG/ethanol were prepared with varying degrees of saturation (DS) of fentanyl. Both PG/water and PG/ethanol formulations showed good correlation between the flux and DS. The enhancement observed for PG/ethanol formulations confirmed that enhanced active thermodynamic activity was induced due to ethanol evaporation. In further studies, tape-stripping was used to show that supersaturation of the active is maintained in the outer layers.

However, if the active lacks potency, a penetrating active will still prove to be inefficacious.

Obviously, an improved evaluation of product efficacy and safety will contribute to better therapeutic outcomes. Whereas the determination of bioequivalence for two solid oral dosage forms is based on a comparison of active and/or its metabolite concentrations in blood or urine after dosing from a generic product or that of an innovator in healthy volunteers, such considerations are really only appropriate for transdermal products meant for systemic effect. For most topically acting actives, such an approach is not appropriate as the site of action is local, not systemic. Hence, generic dermatological products are only required to demonstrate bioequivalence in clinical trials, including the vasoconstrictor test used to assess the efficacy of topical corticosteroids (Shah et al. 2011).
Table 23.1  Transdermal delivery

| Solute                  | Indication                  | Molecular weight | Melting point (°C) | log $K_{oc}$ | $t_{1/2}$ | Plasma level (μg/h) | $F_s$ | $CL_{body}$ L/h/70 kg | Estimated $J_{so}$ required (μg/h) |
|-------------------------|-----------------------------|------------------|-------------------|-------------|----------|---------------------|-------|----------------------|-----------------------------------|
| Buprenorphine           | Pain relief                 | 468              | 209               | 3.44        | 4        | 0.1–0.52            | 0.5   | 76                   | 15–80                             |
| Clonidine               | Hypertension                | 230              | 140               | 1.77        | 6–20     | 0.2–2.0             | 1.0   | 13                   | 2.7–28                            |
| Estradiol               | Female hormone replacement  | 272              | 176               | 2.69        | 1        | 0.04–0.15           | 0.16  | 4–7 (CL/F)           | 1–6                               |
| Ethinyl estradiol       | Female contraception        | 296              | 143               | 4.52        | 17       | 0.011–0.137         | 1     | 28                   | 0.31–3.8                          |
| Fentanyl                | Chronic pain                | 337              | 83                | 4.37        | 17       | 1                   | 1     | 25–75                | 25–75                             |
| Isosorbide dinitrate    | Angina                      | 236              | 68                | 1.31        | 105      | 22                  | 1     | 1.22                 | 28                                |
| Methyl phenidate        | ADHD                        | 233              | 74.5              | 2.55        | 3–5      | 20–46               | 1     | 60 L/h/30 kg in children | 720–2160                        |
| Nicotine                | Smoking cessation           | 162              | –80               | 1.17        | 2        | 10–30               | 1     | 72                   | 900–2630                          |
| Nitroglycerin           | Angina                      | 227              | 13.5              | 1.62        | 0.04     | 1.2–11              | 1     | 13.5                 | 16.2–148.5                        |
| Norelgestromin          | Contraception               | 327              | 131               | 4.40        | 28       |                     |       |                      | 56–420                            |
| Norethindrone acetate   | Contraception               | 340              | 162               | 3.99        | 9        | 2–15                | 1     | 28 (CL/F = 25 L/h)   |                                  |
| Oxybutynin              | Enuresis                    | 357              | 130               | 5.19        | 2        | 3–4                 | 1     | 25–34                | 75–135                            |
| Rivastigmine            | Alzheimer’s                 | 250              | 124               | 2.14        | 1–4      | 2–10                | 1     | 130                  | 260–1300                          |
| Rotigotine              | Parkinson’s disease         | 315              | 141               | 4.97        | 5–7      | 0.2–0.6             | 1     | 630                  | 126–378                           |
| Scopolamine             | Motion sickness             | 303              | 59                | 1.23        | 1.2–2.9  | 0.04                | 1     | 67–205               | 2.6–8.1                           |
| Selegiline              | Depression                  | 187              | 138               | 2.95        | 2        | 2                   | 1     |                      |                                  |
| Testosterone            | Hypogonadism                | 288              | 153               | 3.31        | 2.3      | 60–100              | 1     | 3–5                  | 180–500                           |
| Timolol                 | Hypertension                | 316              | 72                | 2.46        | 4.1      | 5–15                | 0.76  | 38                   | 250–750                           |
| Triprolidine            | Antihistamine               | 278              | 60                | 4.22        | 2–6      | 5–15                | 1     | 43.7                 | 218–655                           |

Adapted from Roberts and Walters (2008)
Examples of solutes on the market and their indications, effective plasma levels, body clearances ($CL_{body}$), availability ($F_s$), elimination half-life ($t_{1/2}$) and physicochemical data used to estimate required solute transdermal Flux ($J_s$) [from Eq. (23.3)] for passive topical delivery systems.
Franz et al. (2009) suggested that a substitute for clinical bioequivalence testing is to define the penetration fluxes of an active through excised human skin. Their evaluation, based on glucocorticoids and generic tretinoin gels, indicated that the in vitro model had greater sensitivity than the clinical method in detecting small differences between two products (Franz et al. 2009). It is unclear, however, how this approach can overcome the well-known wide variability in excised human skin permeability. More recently, Lehman and Franz have assessed the bioequivalence of topical retinoid products using pharmacodynamic assays (Lehman and Franz 2012).

23.2 Role of Intended Targets on Topical and Transdermal Delivery

There is a range of skin and systemic sites often targeted by topical and transdermal delivery, summarized in Fig. 23.6. In this section, we illustrate some of the actives and their formulations used to achieve appropriate targeted delivery by topical or transdermal delivery.

23.2.1 Topical Delivery

The skin is the site of application of numerous cosmetics and cosmeceutical preparations. In this section, we will discuss the importance of targets on active delivery choices. Testing for safety of cosmetic preparations will be discussed in the following sections.

A chemist’s role in overcoming an active’s physicochemical properties to make it suitable for topical and transdermal delivery is a complicated task. Formulators have made use of physicochemical characteristics like molecular weight to stop an active from penetrating through the skin. One such example is a high molecular weight (>500 Da) UV A and UVB absorber Tinosorb® M (bisoctrizole, INCI: methylene bis-benzotriazolyl tetramethylbutylphenol (and) aqua (and) decyl glucoside (and) propylene glycol (and) xanthan gum) developed by Ciba Chemicals (now part of BASF, Ludwigshafen, Germany). Similarly L’Oréal’s sunscreen with Uvinul® N539 (2-ethylhexyl 2-cyano-3,3-diphenyl-2-propanoate, INCI: octocrylene), Parsol® 1789 (avobenzone; INCI: butyl methoxydibenzoylmethane) and Mexoryl® SX (MW-563; ecamsule, INCI: terephthalylidene dicamphor sulfon) was formulated to reduce solar-UV-induced skin damage (Seité et al. 2000). Sunscreens containing ecamsule (Mexoryl® SX) are exclusive to L’Oréal and its brands.

Chlorhexidine is another example of a molecule which binds extensively to the skin surface due to its cationic nature (Judd et al. 2013). This ensures that it does not penetrate into the skin and rather provides for a sustained antimicrobial action when used topically (Karpanen et al. 2008). Insect repellents are another good example of actives that...
are not intended to penetrate. Penetration retardants and modifiers have been used for this purpose (Kaushik et al., 2010). On the other hand, terbinafine, a highly lipophilic but equally potent antifungal active, with a log \( P \) of 5.5, needs a different approach. When formulated as a microemulsion to enhance its stratum corneum solubility using a mixture of surfactant, co-surfactant and oil, terbinafine exhibited enhanced permeation parameters. Microbiological studies of the microemulsion showed better antifungal activity against Candida albicans and Aspergillus flavus compared to marketed products (Baboota et al., 2007). Retinol, with a high molecular weight and lipophilicity, is another active that is used for topical treatment of acne. These unfavourable properties were overcome by delivering Retinol using solid lipid nanoparticles (SLN). The suitability of the SLNs was compared to a nanoemulsion of the same size, with respect to the ability to influence active penetration and distribution within the skin. In both formulations, 500 \( \mu \)g retinol was applied to porcine skin. Following the SLN dispersion for 6 h, a high retinol concentration was found in the stratum corneum and upper epidermis (approximately 3400 ng or 0.68% in the top layer). The nanoemulsion, however, only transported 2500 ng (0.5%) into the upper skin strata (Jenning et al., 2000).

For specific disease, conditions where the surface of the skin itself is the target of therapy, formulation and delivery strategy are of greatest importance. Creams, lotions, emulsions and other topical delivery vehicles have been used effectively for this. The challenge here is to modify the formulation according to the active characteristics and skin condition. Being the largest organ, skin is exposed to the damaging effects of UVA and UVB radiation, often leading to erythema. Antioxidants such as Vitamin A, Vitamin E and Vitamin C have shown beneficial effects in reducing oxidative damage to critical cellular components (Trevithick et al., 1993; Stamford, 2012). While the photoprotective effect of topical antioxidants applied before UV exposure is well recognized, the beneficial effect of these compounds when administered after irradiation is less obvious (Wu et al., 2012). Only Vitamin A has been proven to be beneficial in treating long-term photoaged and photo-damaged skin (Cho et al., 2005). The stratum corneum is a major barrier to the penetration of lipophilic Vitamin E (Cassano et al., 2009), and hydrophilic Vitamin C is prone to excessive oxidation. Oxidation of Vitamin C is triggered by its ionization in aqueous solution (Stamford, 2012). Another example where erythema and papular oedema lesions are seen is Herpes labialis. In a recent study, iontophoretic application of 5% acyclovir cream was tested in a placebo-controlled trial for treatment of herpes, among 200 patients with an incipient cold sore outbreak at the erythema or papular oedema lesion stage. A 20 min iontophoretic treatment cycle shortened the median classic lesion healing time by 35 h in the active group when compared to the control (Morrel et al., 2006). Steroids have also been popularly used in the treatment of erythema and other inflammatory skin conditions (Perry and Trafeli, 2009; Paller, 2012). Numerous studies have examined various factors controlling percutaneous absorption rates and dermal clearances of steroids (Siddiqui et al., 1989). The lipophilicity of steroids has been associated with their tissue accumulation and toxic effects. However, Magnusson et al. showed that the tissue accumulation of steroids was not linearly related to their lipophilicity, but the binding of steroids to tissue components led to their accumulation (Magnusson et al., 2006). Long-term corticosteroid usage has been associated with skin deterioration, abnormal skin aging and cutaneous mast cell depletion (Lavker and Schechter, 1985; Gonzalez and Sethi, 2012).

### 23.2.2 Targeting of Keratinocytes

One of the most widely explored aims of cellular targeting has been in the viable epidermis itself. Inflammatory conditions broadly known as dermatitis or eczema are largely reflected in epidermal involvement (Elias et al., 1999), including spongiosis, acantosis and parakeratosis, as well as some dermal involvement (Freeman-Anderson et al., 2008). The classical treatment for such conditions is the use of topical corticosteroids, which
can be classified on a four-point scale of potency (I–IV, mild, moderate, potent, very potent), based on their vasoconstrictor effects in humans. The body site to be treated determines the potency of the steroid to be used. For example, the face should be treated with mild corticosteroids only, such as hydrocortisone and hydrocortisone acetate. Care needs to be taken with the more potent steroids and in the treatment of chronic conditions. Intermittent dosing can be effective in these circumstances.

Salicylic acid has long been used for conditions such as psoriasis and ichthyosis that require keratolytic treatment. At lower concentrations, salicylic acid is known to have keratoplastic effects; however at higher concentrations, it is found to be keratolytic (Gloor and Beier 1984; Schwarb et al. 1999). Coal tar, retinoids and anthralin, as well as corticosteroids are some other commonly used treatments for psoriasis (Mitra and Wu 2010). Psoriatic skin is one such example where the “rigidization” of skin, ascribed to an increase in the levels of cholesterol and fall in the levels of ceramides and the lack of hydration, makes the topical route a big challenge (Wertz et al. 1989). The intricacies of topical delivery into psoriatic skin have lately been addressed by lipoidal carrier systems, such as liposomes. Recent work by Ward et al. describes the delivery of novel immunotherapy using a liposomal platform. Liposomes synthesized to incorporate clodronate were injected intradermally into KC-Tie2 mice. An elimination of the CD11c+, F4/80+ and CD11b+ cells was seen in the skin, and the CD8+ T-cell numbers returned to levels seen in control mice. Further, there was also a marked reduction in the levels of interleukin (IL)-1a, IL-6, IL-23 and tumour necrosis factor (TNFα) (Ward et al. 2011). Peptides and proteins such as elafin and psoriasin are known to be highly upregulated in psoriatic skin (Madsen et al. 1991; Nonomura et al. 1994; Namjoshi et al. 2008). Peptide T analogue, DAPTA, was shown in clinical trials to clear psoriasis lesions and significantly inhibit the monocyte and lymphocyte chemotactic properties of RANTES (a beta chemokine found in increased amounts in psoriatic lesions). A better understanding of the role of these peptides may provide new strategies for the treatment of diseases such as psoriasis (Harder and Schröder 2002).

### 23.2.3 Targeting of Melanocytes

The synthesis and distribution of melanin contribute to skin and hair colour. However, melanin and pigmentation-associated skin cancers are a major problem, while there is also interest in cosmetic skin whitening treatments where increased epidermal melanin synthesis causes unwanted darkening of the skin. Accurate determination of cutaneous melanin in normal skin and in disease conditions requires the collection of skin biopsies. However, our recent work has used multiphoton tomography and fluorescence lifetime imaging to non-invasively measure levels of epidermal melanin, which can be related to the visible skin colour (Dancik et al. 2013a, b). The use of these techniques has implications for lesion diagnosis and assessment of therapeutic progress.

Proteins and peptides play an important role in skin health as structural components of the skin as well as activators, regulators or inhibitors of certain biochemical processes occurring in the skin (Heidl 2009). Peptides as cosmeceuticals are a fast-growing segment of the personal care industry with an increasing trend towards the use of these agents in skin care regimens for protection from radiation and oxidant damage (Brandt et al. 2011). Peptides also play an important role in melanogenesis. Melanocyte-inhibiting factor (also known as Pro-Leu-Gly-NH₂, Melanostatin, MSH release-inhibiting hormone or MIF-1) is an endogenous peptide fragment derived from oxytocin (Taleisnik 1971). Melanostatin is shown to inhibit melanin formation in Streptomyces bikiniensis NRRLB-1049 and B16 melanoma cells. A patent filed in 2010 describes a “one-step” process for production of esters and acetylated forms of peptides, describing melanostatin (MIF-1). This chemical modification approach improved metabolic properties leading to increased efficiency for therapeutic and cosmetic purposes including transdermal and topical administration (Skinner 2007). Melanocyte-stimulating hormone
analogue that function as melanocortin 1 receptor (MC1-R) agonists have been investigated as potential topical agents to prevent skin photocarcinogenesis. In cultured human melanocytes, tetrapeptide \( \alpha \)-MSH analogues, Ac-His-D-Phe-Arg-Trp-NH\(_2\), N-pentadecanoyl and 4-phenylbutyryl-His-d-Phe-Arg-Trp-NH\(_2\) were more potent than \( \alpha \)-MSH in stimulating the activity of melanogenesis, reducing apoptosis and release of hydrogen peroxide and enhancing repair of deoxyribonucleic acid (DNA) photoproducts in melanocytes exposed to UV radiation (Abdel Malek 2006). Melanostatin-5 (Aqua-Dextran-Nonapeptide-1) is a new skin lightening biomimetic peptide. This peptide acts as a \( \alpha \)-MSH antagonist thus preventing and lightening hyperpigmentation (Dhatt 2006). Melanostatin-5 produces noticeable skin lightening by at least 33\% when formulated into a lightening product, and showed continued improvement over time.

### 23.2.4 Targeting of Langerhans Cells

Skin has evolved a highly competent immunological function, in Langerhans cells (LCs – estimated population 500–1000 cells mm\(^{-2}\)) (Berman et al. 1983; Chen et al. 1985), which often serves as the first line of defence against many pathogens (Babiuk et al. 2000). The viable epidermis lacks the blood vessels and sensory nerve endings found in the dermis, which makes it an ideal site for pain-free delivery with minimal damage. There are many approaches for non-invasive targeting of viable epidermal cells and DNA vaccination against major diseases. There have been attempts at displaying foreign peptides and proteins on virus particles and insertion of mammalian cell-active expression cassettes in baculoviruses to express genes efficiently into many different mammalian cell types (Paul et al. 2013). Pearton et al. showed morphological changes in human LCs stimulated with influenza virus-like particulate vaccines delivered via intradermal injection. The LCs were more dispersed throughout the epidermis, often in close proximity to the basement membrane, and appeared vertically elongated, which provides essential evidence of host response (Pearton et al. 2010).

There are many physical approaches, both old and recent for targeting LCs; we will try to mention them in brief. The most well-known technique was: (a) Needle and Syringe: This is an inefficient and indirect way of targeting the dendritic cells with DNA. It has resulted in modest immune responses (Mumper and Ledebur 2001). Other disadvantages include risks due to needle stick injuries (Gill et al. 2009; Sullivan et al. 2010). (b) Liquid Jet Injector: This is a needle-free approach using high-speed liquid jet injectors (Furth et al. 1995) (Mark 2008). This technique has seen a recent resurgence, with liquid delivered around the LCs in gene transfer and DNA vaccination experiments (Furth et al. 1995). Current liquid jet injectors typically disrupt the skin in the epidermal and dermal layers to target LCs. Liquid jet injectors however have been reported to cause pain to the patients. (c) Microneedle Arrays: Unlike current liquid jet injectors, microneedles can accurately target the viable epidermis, are simple to use when built up as patches and are relatively pain free. McAllister et al. (2003) have shown that they can be made from a range of materials, including silicon, metal and other biodegradable polymers. This makes microneedles a cost-effective method for delivering oligonucleotides to epidermal cells for DNA vaccination (Kendall 2010). (d) Biolistic microparticle delivery: In this needle-free technique, pharmaceutical or immunomodulatory agents, formulated as particles, are accelerated in a supersonic gas jet to a sufficient momentum to penetrate the skin (or mucosal) layer and achieve a pharmacological effect. Sanford and Klein (Sanford et al. 1987) pioneered this innovation with systems designed to deliver DNA-coated metal particles (1 \( \mu \)m diameter) into plant cells for genetic modification.

Follicular targeting has been approached as another way of targeting the LCs (Patzelt and Lademann 2013). Vogt et al. (2006) found a high density of LCs around hair follicles that were very susceptible to targeting with the use of nanoparticles. Nanoparticles of 40 nm diameter, but not larger particles in the micron size range, were delivered via the follicles and were able to diffuse through the follicular infundibulum to be taken up by the adjacent Langerhans cells.
23.2.5 Targeting of Fibroblasts

Triggered synovial fibroblasts, as part of a multi-part cellular complex, play an important role in the pathogenesis of rheumatoid arthritis (Pap et al. 2000; Seemayer et al. 2001). Diclofenac is a commonly used non-steroidal anti-inflammatory active for symptom control in osteoarthritis of the knee and soft tissue injuries (Bajaj et al. 2011). Although analgesics applied topically can provide a local enhanced active delivery for the superficial joint tissues by direct diffusion, they are unlikely to be effective for the deeper tissue synovial fluid (Xi et al. 2013). Recently, fibroblasts have been targeted using diclofenac in vesicles containing various penetration enhancers (Manca et al. 2013).

Fibroblasts also play an important role in development of hypertrophic scars, which result from an overgrowth of fibrous tissue at the site of an injury. The development of a hypertrophic scar is a complex interplay between fibroblasts and cytokines (Tredget et al. 1997). Zhao et al. recently delivered small interfering ribonucleic acid (siRNA) – transforming growth factor beta 1 (TGFβ1)-337 from hydrogel pressure-sensitive adhesive patches in mice and demonstrated a down-regulation in collagen and reduction in scar size. The scar fibroblasts were shown to have significantly reduced by undergoing apoptosis (Zhao et al. 2013).

23.2.6 Intradermal and Systemic Targeting

The skin is an ideal route for targeted delivery of steroidal and non-steroidal anti-inflammatory agents and other analgesics for pain and inflammation. This has been achieved with many techniques. Topical NSAIDS and related solutes are often applied to the skin to target tissues directly below the application site. In a 1999 study, Roberts et al. used biopsy and microdialysis techniques to show that most solutes penetrate below dermal capillaries into the subcutaneous and deeper tissues of both rats and human subjects. The selectivity of local penetration is time related, and the concentrations of the NSAIDS in the underlying tissues at longer times are often defined by recirculation from the systemic blood supply (Roberts and Cross 1999). Increased depths of penetration may be achieved by the use of vasoactive agents (Singh and Roberts 1994). On the other hand, steroids have been shown to accumulate in the skin to a greater extent. Magnusson et al. showed a slower penetration and a higher rate of accumulation of progesterone in the viable epidermis of full-thickness skin. These findings were consistent with dermal penetration limitation effects associated with high lipophilicity of the steroids (Magnusson et al. 2006). Previously, Pelchrzim et al. also showed a similar reservoir effect of the stratum corneum on corticosteroid penetration, although they considered this to be formulation dependent (Pelchrzim et al. 2004). Ketorolac has been successfully delivered by iontophoresis to human volunteers using silver electrodes with a current of 2 mA for five treatment sessions of 20 min every day in order to avoid the well-known gastrointestinal side effects of its oral delivery (Saggini et al. 1996).

Transdermal delivery has been successfully used to achieve systemic targeting, by exploiting the rich blood perfusion of the dermis. Fentanyl, a widely used opioid, has potency approximately 100–500-fold greater than morphine. Its physicochemical and pharmacological properties, together with its pharmacokinetics (short half-life; high first pass effect), make it an ideal candidate to be delivered systemically through the skin. Fentanyl has been delivered transdermally using two different techniques. Firstly, it is formulated in transdermal patches to be passively delivered for the treatment of chronic pain. However, this technique cannot provide a rapid bolus active input for the relief of acute pain (Chelly et al. 2004). Iontophoresis has also been investigated for systemic Fentanyl delivery; for example, as a patient controlled system that could be activated for symptomatic relief (Ashburn et al. 1995). The skin also provides a potential route for the non-invasive delivery of peptides and proteins which are in danger of inactivation when delivered orally, due to chemical and enzymatic degradation, although there are significant challenges involved in achieving this (Benson and Namjoshi 2008).
23.3 Role of Safety and Active Toxicity in Transdermal Formulation

Cosmetics and pharmaceuticals must be formulated not only for efficacy, but also for patient or consumer acceptance. This is influenced by a perception of safety or lack of toxicity. Regarding safety and toxicity, it must be understood that for a topically applied active to have either therapeutic or toxic effects, it must first be absorbed through the skin. The toxic effects caused if the chemicals are absorbed can include acute irritation, corrosion and skin sensitization (Grice et al. 2010). Chemicals applied to the skin can also have direct effects on the stratum corneum, resulting in an alteration in its permeability. This concept has been effectively applied in the development of penetration enhancers. However, the harmful effects of excipients on the skin have been evaluated under increasing scrutiny by regulatory authorities. Regulatory considerations will be discussed later in this chapter.

Topically applied actives causing adverse effects can be classified as either allergens or irritants (Zweiman 1990). These allergens and irritants not only affect the skin but can also affect other body sites. Cutaneous active reactions can be classified into three types of contact dermatitis: (a) Allergic contact dermatitis, (b) irritant contact dermatitis and (c) photo contact dermatitis. The role and mechanism of irritation caused by various chemical penetration enhancers has been studied extensively.

23.3.1 Mechanisms of Contact and Photo Allergies and Irritations

Allergic contact dermatitis (ACD) results from an adaptive immune response to the penetration of allergens into the skin. ACD progresses in two phases: (a) in the initial sensitization phase, the host is immunized to the allergen, and (b) in the elicitation phase, a rapid secondary immune response is mounted following re-exposure to the allergen which manifests as ACD (Alenius et al. 2008). Cutaneous photosensitivity can be evoked by both systemic and topical exogenous photo-sensitizers leading to active photosensitivity and photo contact dermatitis, respectively. Photoallergenicity is a well-organized immunological reaction. The current understanding of ordinary contact dermatitis and active hypersensitivity is based on the hapten hypothesis: molecules bind covalently to proteins, and the resulting conjugates can be recognized as immunogenic determinants. Likewise, photosensitive chemicals have a haptenic moiety.

Although the terms structure–activity relationship (SAR) and quantitative structure–activity relationship (QSAR) are widely used, for skin sensitization, it is more meaningful to describe the relationship as being between chemistry and activity. Skin sensitization induction is a multistage process (Jowsey et al. 2006): Stage 1, Translocation of the sensitizer from the skin surface to the epidermal site of action. This depends on the dose given and duration of exposure; Stage 2, Covalent reaction of the sensitizer with skin protein. This is strongly dependent on the chemical nature of the sensitizer, in particular electrophilic reactivity and hydrophobicity. The nature of the skin proteins involved in this process is not established. Possibilities range from any protein encountered in the skin to highly nucleophilic proteins, “purpose-designed” by evolution, associated with epidermal Langerhans cell membranes; Stage 3, Response of epidermal Langerhans cells to the modified protein, resulting in migration to the lymph node and presentation of antigen, leading to antigen recognition by naïve T-lymphocytes resulting in clonal expansion. Regulators classify chemicals as being hazardous after an acute dermal contact if they lead to mortality (owing to absorption), cause acute irritation or corrosion and cause skin sensitization on multiple contacts. In the EU, evaluation of skin irritation/corrosion is mandatory for all products likely to be associated with skin exposure. The section below discusses testing protocols and requirements in the EU.
23.3.2 Testing of Dermatologics, Cosmetics and Their Safety Evaluation

The European Commission Scientific Committee on Consumer Safety (SCCP) adopted its most recent guidelines “The SCCS’s notes of guidance for the testing of cosmetic substances and their safety evaluation, 8th Revision” in December 2012 (SCCP 2012). These set out procedures for the safety evaluation of cosmetic substances (single ingredients or defined mixtures) and finished cosmetic products. In the guidelines, a cosmetic ingredient is defined as “any chemical substance or mixture of synthetic or natural origin, used in the formulation of cosmetic products.” A cosmetic ingredient may be:

1. A chemically well-defined single substance with a molecular and structural formula
2. A complex mixture, requiring a clear definition and often corresponding to a mixture of substances of unknown or variable composition and biological nature
3. A mixture of 1 and 2, used in the formulation of a finished cosmetic product

A finished cosmetic product is defined as “Any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours”.

Table 23.2 illustrates the SCCP requirements for testing of chemical and physical properties of cosmetic substances, and the associated toxicity studies (SCCP 2012).

| Chemical and physical specifications | Relevant toxicity studies                        |
|------------------------------------|-------------------------------------------------|
| Chemical identity                  | Acute toxicity                                  |
| Physical form                      | Corrosivity and irritation                       |
| Molecular weight                   | Skin sensitization                               |
| Characterization and purity of the chemical | Dermal/percutaneous absorption                  |
| Characterization of the impurities or accompanying contaminants | Repeated dose toxicity                           |
| Solubility                          | Reproductive toxicity                           |
| Partition coefficient (log \( P_{ow} \)) | Mutagenicity, genotoxicity                      |
| Additional relevant physical and chemical specifications | Carcinogenicity                                  |
| Homogeneity and stability           | Toxicokinetic studies                           |
| UV–VIS absorption spectrum         | Photo-induced toxicity                          |
| Isomer composition                 | Human data                                       |
| Functions and uses                 |                                                 |

SCCP recognizes the increasing use of synthetic and semisynthetic ingredients, in addition to the ingredients derived from natural products that have been traditionally used in cosmetics. In addition, their requirements for evaluating the safety requirements for cosmetic products are increasingly being brought into line with those for therapeutic products.

23.3.3 In Vitro Skin Irritancy Tests: A Need for Triangulation

While dermal toxicity to exogenous materials is seldom associated with mortality, it may involve significant morbidity. Ethical concerns have brought about a paradigm shift in the way the skin irritancy of a cosmetic ingredient is evaluated. Prior to its phasing out and eventual ban by the European Commission, the scientific community has traditionally used in vivo animal testing methods to evaluate the safety of a topically
applied product. There are many in vitro testing models that have been developed and commercialized to provide an alternative to animal testing. Models such as EpiSkin® (Episkin SNC, Lyon, France), SkinEthic® (SkinEthic laboratories (Nice, France) and EpiDerm® (Mat-Tek Inc., Ashland, USA) have reasonable similarities to human tissue in terms of morphology, lipid composition and biochemical markers (Netzlaff et al. 2005). Numerous reviews and research articles have been written comparing the different skin models to each other and to human skin (Lotte et al. 2002; Netzlaff et al. 2005; Schafer-Korting et al. 2006, 2008; Neis et al. 2010; Gotz et al. 2012; Brinkmann et al. 2013). Lotte et al. (2002) studied the permeation of lauric acid, caffeine and mannitol in EpiSkin®, SkinEthic® and EpiDerm® and observed that although there was huge variability in permeation data among the skin models, the order of permeation was in line with that observed in ex vivo skin. Enzymatic equivalence and gene expression were also compared (Hu et al. 2010; Neis et al. 2010). In microarray analysis, Hu et al. (2010) found that the expression of a large percentage of the genes was consistent between EpiDerm® and human skin indicating the presence of similar metabolic pathways. In a 2005 review, Netzlaff et al. evaluated the morphology of commercially available skin models and compared their suitability for testing phototoxicity, irritancy, corrosivity and active transport (Netzlaff et al. 2005). They tabulated comparative morphological features and test results for these parameters. They commented that currently the models come close to reproducing human skin, but only in certain aspects. These skin models are useful in toxicity testing, including phototoxicity testing, and to an extent for drug transport studies. The variability in transport can be attributed to the incompletely developed barrier.

Before the enforcement of the EU chemicals policy REACH (Registration, Evaluation and Authorization of Chemicals) in 2009, Basketter et al. (2007) published report and recommendations of European Centre for the Validation of Alternative Methods (ECVAM’s) Workshop 59. This workshop was part of a concerted effort in recognizing the role of percutaneous/dermal absorption process in sensitization. They defined epidermal disposition as the specific location and stressed the quantification of chemicals in the epidermal compartment, which are relevant to local toxicity endpoints/skin sensitization. OECD Test Guideline (TG) 428 (OECD 2004), for in vitro skin absorption, provides general rules on how to measure systemic availability and concentrations of chemicals that permeate through all layers of the skin and the overall kinetics of skin absorption but does not allow for separate evaluation of epidermal disposition to be derived for local skin toxicity endpoints, such as skin sensitization. Hence, this report emphasizes the need to relate the in vivo dosimetry of sensitizers that penetrate into the viable epidermis via the stratum corneum of human skin to the concentrations used in in vitro applications. This requires the need to

Table 23.3  Safety evaluation procedure of cosmetic substances as applied by the SCCP: Further requirements for cosmetic ingredients (SCCP 2012)

| Toxicological requirements for inclusion of a substance in one of the Annexes to Regulation (EC) No 1223/2009 (to be evaluated by the SCCP) Dir. 76/768/EEC |
| Basic requirements for cosmetic substances present in finished cosmetic substances (which are to be evaluated by individual safety assessors) |
| General principles for the calculation of the Margin of Safety and lifetime cancer risk for a cosmetic substance |
| The specific assessment of hair dyes and hair dye components |
| The Threshold of Toxicological Concern (TTC) |
| Aspects to consider with respect to the risk assessment for the inhalation route |
| Human biomonitoring |

Table 23.4  SCCP evaluation of finished cosmetic products: Safety and microbiological quality (SCCP 2012)

| Toxicological profile of the substances |
| Stability and physical and chemical characteristics of the finished cosmetic product |
| Evaluation of the safety of the finished product |
| Microbiological quality: quantitative and qualitative limits |
| Challenge testing |
| Good manufacturing practice |
estimate both the applied concentration of chemical onto the skin surface for given finite dose exposure conditions and to relate it to what is applied to cells in vitro as being representative of the target dose of chemical in human skin.

The models proposed by the SCCP for skin safety tests have been validated against in vivo Draize tests (SCCP 2012). However, these standalone methods rely heavily on quantification of optical density and colorimetry, which lead to a one-dimensional analysis. Moreover, since results obtained from previously conducted human tests studies on model compounds are necessary as reference values for in vitro assays, attention should also be paid to the generation of in vivo data and standardization of in vivo test protocols (Ponec 2002).

In the last decade, numerous committees and taskforces have been set up to constantly review the performance of in vitro irritancy and genotoxicity assays (Fentem et al. 1998; Eskes et al. 2012; Pfuhler et al. 2014). In vitro assays have shown a very high rate of positive genotoxicity results that do not correlate with in vivo genotoxicity or carcinogenicity (Kirkland et al. 2007). Due to the inability to follow up the in vitro tests with in vivo assays, many potential new products have been de-selected. The Cosmetics Europe (formerly COLIPA) Genotoxicity Task Force has driven and funded projects to help address the high rate of misleading positives in in vitro genotoxicity tests (Pfuhler et al. 2014). The ongoing “3D skin model” project is now validating the use of human reconstructed skin (RS) models in combination with the micronucleus (MN) and Comet assays. Non-testing methods like [quantitative] structure–activity relationships ([Q]SARs) or in silico methods are also a valuable way of gaining more toxicological information. Formation of chemical categories facilitates the application of read across between similar chemicals based on the assumption that their behaviour will be consistent for their class (Adler et al. 2011).

Even from before the complete ban on animal testing, alternative techniques to test dermal irritancy and immunogenicity caused by pharmaceutical and cosmetic products were being developed. Responses to irritants have been studied in cell culture for a significant period (Deleo et al. 1996). There are other approaches that can be used to complement the validated in vitro methods and satisfy the need for triangulation in safety evaluation. Human ex vivo skin is often used as a realistic model for in vivo human skin in transdermal active delivery. The study of detailed cellular and sub-cellular processes in the skin was previously not possible using conventional light microscopic methods, such as wide-field and confocal microscopy. Although these tools work reliably in cell cultures and thin tissue sections, their application in thick biological samples such as skin is greatly limited due to light-scattering and absorbing properties of biological tissues. However, this is being addressed with the increasing use of multiphoton or two photon microscopy (MPM/TPM). Understanding of the process of epidermal and transdermal transports of xenobiotics is important for rational design of topical active delivery and to avoid exposure to toxic and allergenic compounds. MPM allows 3D visualization of penetration and distribution with minimal sample preparation. Sanchez et al. have used cellular autofluorescence to show that ex vivo skin viability, including metabolic activity, can be preserved up to 7 days at 4 °C (Sanchez et al. 2010). MPM has also been used to demonstrate the importance of stratum corneum, in the sensitization phase of contact allergy (Samuelsson et al. 2009). One of the present drawbacks of MPM is that it tends to be qualitative rather than quantitative; however, the use of fluorescence lifetime imaging (FLIM) holds promise for quantitative analysis in tissue samples (Benati et al. 2011; Koenig et al. 2011). Excised skin obtained from elective abdominoplasties is a convenient resource, which when used within its viability provides an excellent model. However, not all research facilities have access to freshly excised skin. Skin from elective surgery is donated in goodwill to assist scientific research, and there are ethical constraints on its use for commercial testing. The EU prohibits financial gain through the use of human tissue (Netzlaff et al. 2005). Progress is ongoing in the development of advanced skin equivalents with immune and inflammatory equivalency.
Better culture media to maintain the viability of ex vivo skin and skin biopsies are also under development.

Chau et al. (2013) recently described an advanced static 3D skin equivalent which combines human keratinocytes, human dendritic cell and fibroblasts combined into a three-layer CellGrown™ tissue culture insert comprising keratinocyte and fibroblast layers at a thickness of 100 μm and 30 μm, respectively. Dinitrochlorobenzene (DNCB), an established skin sensitizer, was used topically to stimulate this skin model and showed extensive cell response with up-regulation of the cell surface antigens CD86 (cluster of differentiation 86) and HLA-DR (an MHC Class II antigen expressed mainly on antigen-presenting cells) on the dendritic cells. Another approach to emulate the human skin composition of both somatic and immune cell populations is the use of skin biopsies for in vitro cultures. Skin biopsies contain all the immune cell types relevant for the donor at the place of biopsy extraction (Giese and Marx 2014). A biopsy from the skin sample can then be used to test for the presence of inflammation-related human genes using real-time quantitative polymerase chain reaction (RQ-PCR). The de novo construction of full skin equivalents and the use of skin biopsies are two complementary and scientifically grounded approaches to model human skin immunity in vitro (Giese and Marx 2014).

Conclusions

In this chapter, we have discussed some of the major issues facing the formulator: efficacy, safety, toxicity and skin targets. Efficacy, defined as the product of potency of the active and the amount delivered to its site of action, and formulation dictate the dosage of a given active. The formulator must be guided by the physicochemical properties of the particular active and knowledge of skin physiology to create a delivery system to target particular skin regions or cell populations. For all topically applied actives and cosmetics, safety and irritancy testing is required. Suitable predictive methods of in vitro testing must be established and validated to replace in vivo animal testing that can no longer be used. A triangulation approach is suggested to deal with the problem of false positives in the in vitro tests.

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