The Tape-Stripping Technique as a Method for Drug Quantification in Skin

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ABSTRACT - Quantification of drugs within the skin is essential for topical and transdermal delivery research. Over the last two decades, horizontal sectioning, consisting of tape stripping throughout the stratum corneum, has become one of the traditional investigative techniques.

Tape stripping of human stratum corneum is widely used as a method for studying the kinetics and penetration depth of drugs. This paper shows the applications of the tape stripping technique to quantify drug penetration through the skin, underlining its versatile application in the area of topical and transdermal drugs.

INTRODUCTION

Tape stripping with adhesive tape is a widely accepted and used method to examine the localization and distribution of substances within the stratum corneum (SC) [1-7]. This is a minimally invasive technique to sequentially remove SC by the repeated application of appropriate adhesive tapes [8]. This technique can be used to investigate SC cohesion *in vivo* by quantifying the amount of SC removed [9]. Today, weighing with precision balances is the most frequently used method to determine the amount of SC removed on a tape strip. The method is also used to provide information about the kinetics of transdermal drug delivery, offering an apparently easy and quite non-invasive methodology for skin tissue sampling, and is the basis of the FDA's so-called dermatopharmacokinetic (DPK) approach to the assessment of topical bioavailability and bioequivalence [10]. However, validation and optimization of the procedure have not come quickly and the proposed guidance document has been withdrawn for re-evaluation. More recent work has addressed at least some of the important limitations of the DPK approach [11-13] and has proposed modifications in order to incorporate it into an improved protocol.

A number of excellent reviews that have been published contain detailed discussions concerning many aspects of the tape stripping technique [14-16]. The present review shows an updated overview of the use of the tape stripping technique (TS) in the pharmaceutical field, specifically in the area of topical and transdermal drugs. This focus is justified due to the magnitude of the experimental data available with the use of this technique. The use of the tape stripping technique in experimental medicine and pharmaceutical sciences has a long history.

The skin

The skin is the largest organ of the body [17-19], accounting for more than 10% of body mass, and the one that enables the body to interact more intimately with its environment. Essentially, the skin consists of four layers: The SC, that is the outer layer of the skin (non-viable epidermis), and forms the rate-controlling barrier for diffusion for almost all compounds. It is composed of dead flattened, keratin-rich cells, the corneocytes. These dense cells are surrounded by a complex mixture of intercellular lipids, namely, ceramides, free fatty acids, cholesterol, and cholesterol sulphate. Their most important feature is that they are structured as ordered bilayer arrays [20]. The predominant diffusional path for a molecule crossing the SC appears to be intercellular [21-23]. The other layers are: the remaining layers of the epidermis (viable epidermis), the dermis, and the subcutaneous tissues (Figure 1). There are also several associated appendages: hair follicles, sweat ducts, apocrine glands and nails.

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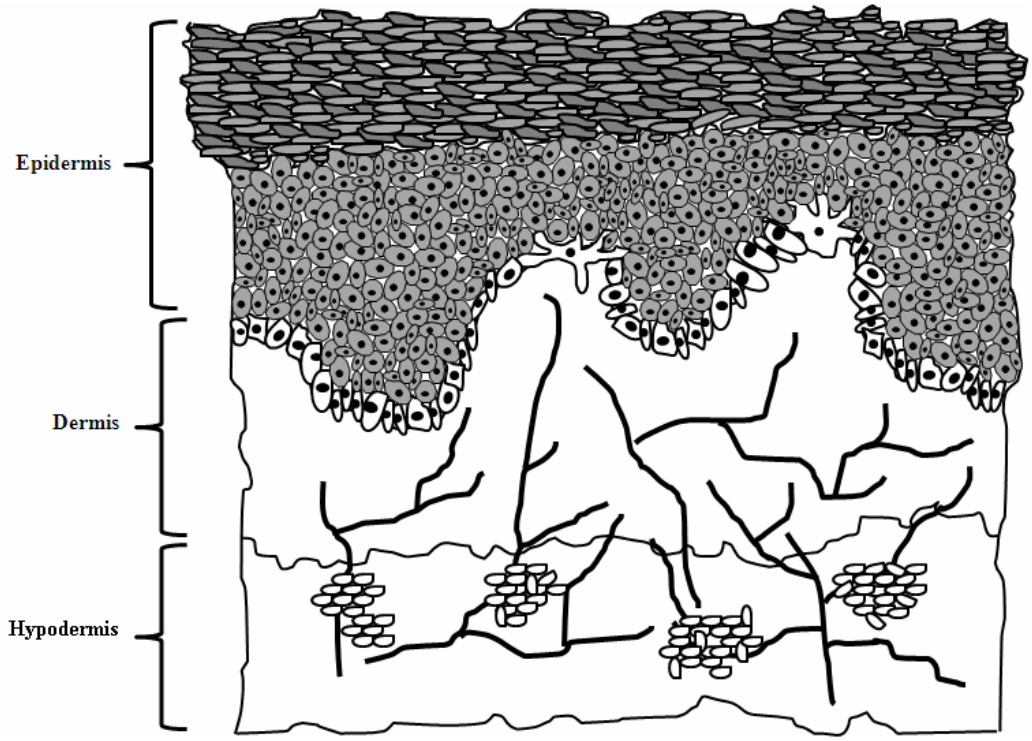

Figure 1. Layers of human skin.

In a general context, the skin's functions may be classified as protective, homeostasis maintaining functions, or sensing [25].

Many agents are applied to the skin either deliberately or accidentally, with either beneficial or deleterious outcomes. The main interest in dermal absorption assessment is related to: a) Local effects in dermatology (e.g., corticosteroids for dermatitis); b) transport through the skin seeking a systemic effect (e.g., nicotine patches, hormonal drug patches, etc.); c) surface effects (e.g., sunscreens, cosmetics, and anti-infectives) [26]; d) targeting of deeper tissues (e.g., nonsteroidal anti-inflammatory agents) [7, 27-35]; and e) unwanted absorption (e.g., solvents in the workplace, pesticides or allergens) [36,37]. Figure 2 summarizes the process of percutaneous absorption.

The skin became popular as a potential site for systemic drug delivery, on the one hand, because of the possibility of avoiding the problems of stomach emptying, pH effects, enzyme deactivation associated with gastrointestinal passage, and hepatic first-pass metabolism; and on the other hand, due to its capability to enable input control.

Tape stripping technique overview

The simplest method for reducing the barrier imposed by the SC is to remove it. Theoretically, an adhesive tape removes a layer of corneocytes. *In vivo,* removal of the SC by TS is performed by the repeated application of adhesive tapes to the skin's surface. In Figure 3 we can observe a detailed procedure of the tape stripping technique. It has been found that on the flexor surface of the forearm, about 30 tape strips are needed to strip off most of the horny layer [8]. Multiple strips remove a substantial skin barrier, as evidenced by 20 to 25-fold increases in transepidermal water loss (TEWL) [38].Usually, the amount of SC removed by TS is not linearly proportional to the number of tapes removed [8]. TS appears to be simple and easy to perform [39-40], however there are different parameters that can influence the quantity of SC removed by a piece of tape, and these include TS mode [38, 41], skin hydration, cohesion between cells (which increases with SC's depth), the body site and inter-individual differences [9, 42]. The impact of these factors has been frequently investigated [8,38-44].

After its description by Pinkus [8], TS has become a standard method in dermatological research [45].

This method can be used to obtain a more susceptible skin, e.g., prior to the application of an irritant [46] or an allergen [47–49]. Similarly, TS is performed to induce a defined disruption of the water barrier, e.g., to evaluate the effect of a subsequently applied skin care product in barrier restoration [50]. It may be also used to obtain cells for mycological culture [51,52] or to investigate SC quality [53]. In dermatopharmacology, the SC barrier function [50,54] and the bioavailability and bioequivalence of topical drugs [39,55-57] can be evaluated with the use of this technique [58,59].

Tape stripping appears to be simple and easy to perform. However, there are parameters which have to be defined, as they may change the outcome. Because various brands of tape differ in shape, surface area, composition and adhesive properties, the influence of the tape brand on the outcome seems apparent [38,41]. Other parameters which influence the procedure can be subsumed in the intrinsic properties of the SC [38]. Although these properties are often investigated, little is known about the anatomical sites (intrinsic factor) as well as the pressure with which the tape is applied on to the skin, the duration of pressure and the removal process (extrinsic factors) influencing SC removal.

In the case of bioequivalence studies, topical bioavailability can be estimated from the drug concentration within the SC, which is expected to be related to the drug concentration at the target site (i.e., usually viable epidermis or dermis) since the SC is the rate limiting barrier for percutaneous absorption. Similarly to the determination of the drug concentration in blood and/or urine as surrogate for the concentration at the target tissue, the determination of the drug concentration in the SC may serve as a surrogate for the concentration in the viable (epi-)dermis [58]. A typical profile obtained from a skin permeation study with sodium naproxen is shown in figure 4 [7]. TS, which enables the removal of the SC layer by layer, is a useful DPK technique for the assessment of drug amounts in SC as a function of time [59].

Applications of the *Tape-stripping* technique

Removal by TS of the outermost skin layer, the SC, has become a common practice in recent years [60,61]. The determination of the kinetics and penetration depth of different kind of permeants by tracing the concentration profiles in SC, has been facilitated by the use of the virtually non-invasive method of SC stripping with adhesive tape [1,2,5,43,44]. For this reason, TS also offers the possibility of evaluating bioequivalence of topical dermatological dosage forms [5].

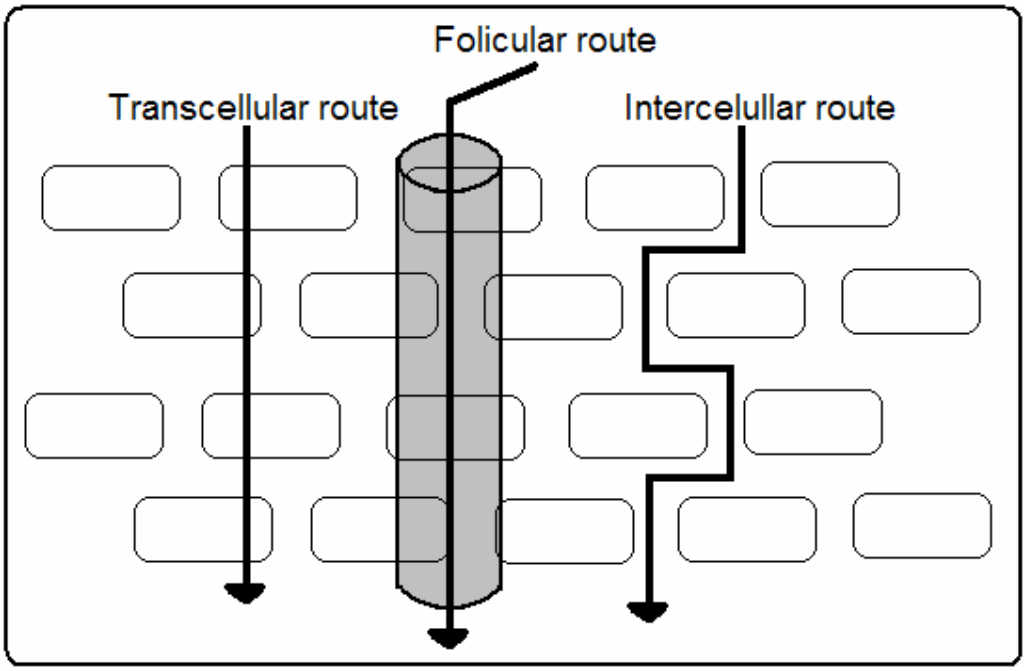

Figure 2. Processes of percutaneous absorption and transdermal delivery.

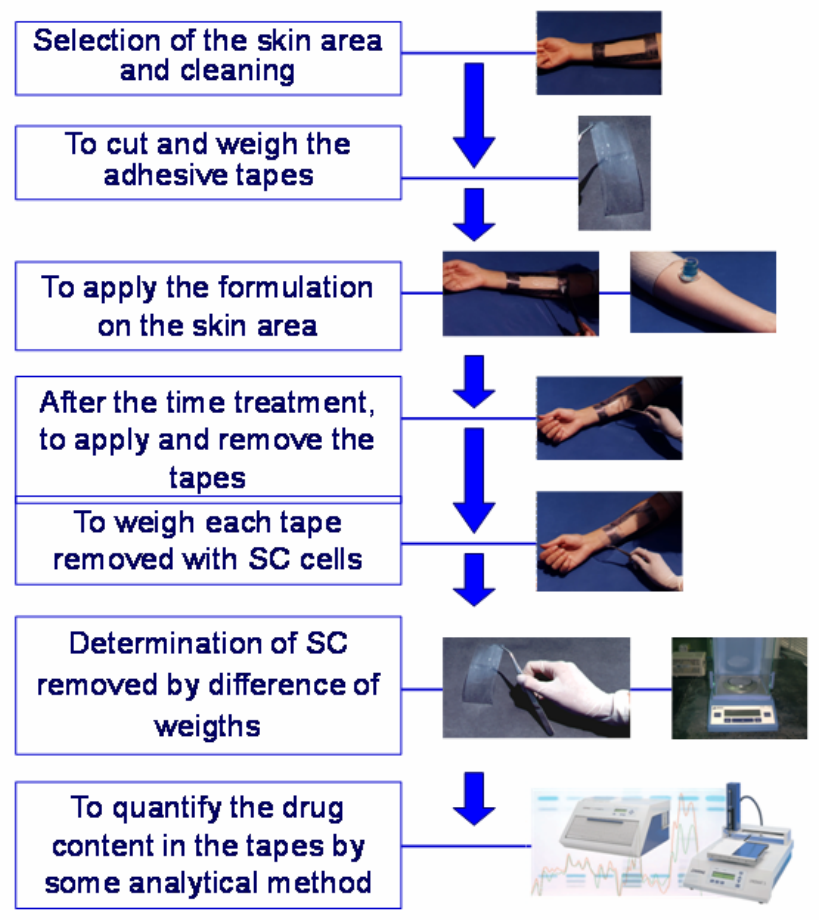

Figure 3. The tape stripping technique procedure

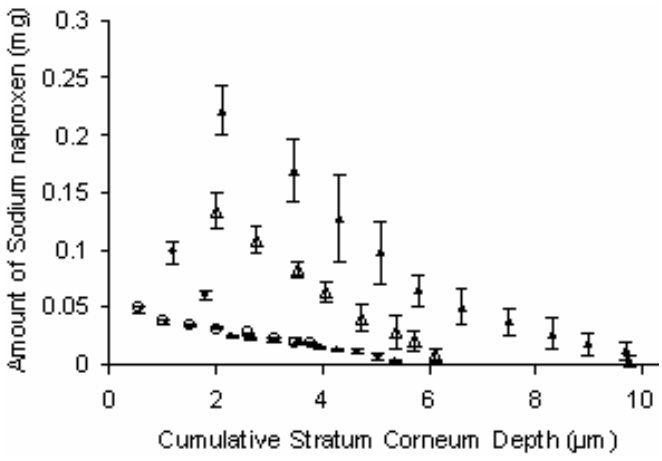

Figure 4. Penetration profiles across human SC of Sodium naproxen formulated in PF-127 gels with different penetration enhancers [Azone®-Transcutol® mixture▲, Transcutol® Δ applying an infinite dose, and a film with Azone®-Transcutol® mixture◆, Transcutol® ○] (Mean± SD; n = 6).

DPK characterization of active drugs in human volunteers has been suggested to be able to replace comparative clinical trials as a means of documenting bioequivalence [59]. Moreover, *in vitro* methods are encouraged by regulatory agencies regarding the provision of percutaneous absorption data for drugs, pesticides and cosmetics [62]. All these points are emphasized in Table 1, which summarizes the research with the TS technique to determine the kinetics and penetration depth of permeants (drugs and toxic chemicals) [7, 26, 35, 37, 44, 63-97], in order to evaluate the factors that influence the physiology of the SC [42, 44, 105,106], to determine the composition of the SC [108], superficial infections in the skin [110, 111], and evaluate skin regeneration [112, 113], etc.

1) Kinetics and penetration depth of drugs

1.1 Analgesic and anti-inflammatory drugs

Arima et al. [63] investigated the effect of hydroxypropyl-P-cyclodextrin (HP-P-CD) on the cutaneous penetration and activation of ethyl 4-biphenylyl acetate (EBA), a prodrug of the non-steroidal anti-inflammatory drug 4-biphenylylacetic acid (BPAA), from hydrophilic ointment, using hairless mouse skin *in vitro*. When the hydrophilic ointment containing a complex of EBA with HP-P-CD was applied to full-thickness skin, HP-P-CD facilitated the penetration of EBA into the skin. When the ointment containing the EBA:HP-P-CD complex was applied to the skin, the BPAA flux through the tape-stripped skin was greater than that through full-thickness skin, while the activation of the prodrug in the skin was slowed by TS. Their results suggest that the enhancing effect of HP-P-CD on the cutaneous penetration of EBA would be largely attributed to an increase in the effective concentration of EBA in the ointment.

Curdy et al. [35] administered piroxicam from a commercially available gel to human volunteers, both passively and under the application of an iontophoretic current. After treatment, the SC at the site of application was progressively tape-stripped and piroxicam transport into the membrane was assessed by UV-analysis of drug extracted from the tape-strips. Current application enhanced drug uptake into the SC, as indicated by both increased piroxicam concentrations in the horny layer and detectable concentrations at greater depths in the membrane. The total amount of drug recovered in the SC post-iontophoresis was significantly higher than that found following passive diffusion for each application time.

Escobar-Chávez et al. [7] determined the penetration of sodium naproxen, formulated in Pluronic F-127 gels containing Azone® and Transcutol as penetration enhancers, through human skin *in vivo* by using the TS technique. It was found that the combination of Azone® and Transcutol in PF-127 gels enhanced sodium naproxen penetration, with up to two-fold enhancement ratios compared with the formulation containing Transcutol only. These results were confirmed by TEWL and ATR-FTIR spectroscopy, suggesting a synergistic action for Azone® and Transcutol®.

Esposito et al. [64] produced and characterized monoleine (MO) dispersions as drug delivery systems for indomethacin. An *in vitro* diffusion study was conducted using Franz cells associated to SC epidermal membrane on cubosome dispersions viscosized by carbomer. *In vivo* studies based on skin reflectance spectrophotometry and TS were performed to better investigate the performance of cubosome as an indomethacin delivery system.

Indomethacin incorporated in viscosized MO dispersions exhibited a lower flux with respect to the analogous formulation containing the free drug in the aqueous phase and to the control formulation based on carbomer gel. Reflectance spectroscopy demonstrated that indomethacin incorporated into MO dispersions can be released in a prolonged fashion. TS experiments corroborated this finding. MO dispersions can be proposed as nanoparticulate systems able to control the percutaneous absorption of indomethacin

Ganem-Quintanar et al. [65] used naproxen-loaded nanoparticles to prepare, in a one-step process, unilaminar films of Eudragit E-100. Nanoparticle films and conventional films were characterized *in vitro* by drug release studies through a cellulose membrane using Franz-type cells, and *in vivo* by penetration experiments with the TS technique. Concerning *in vivo* penetration studies, no statistical differences were found for the amount of naproxen penetrated across the SC and the depth of penetration for the two films.

Herkenne et al. [66] investigated pig ear skin as a surrogate for human skin in the assessment of topical drug bioavailability by sequential TS of the SC.

Ex vivo experiments on isolated pig ears were compared with *in vivo* studies in human volunteers. Four formulations including ibuprofen in different propylene glycol (PG)-water mixtures

(25:75, 50:50, 75:25 and 100:0), were compared. Derived DPK parameters characterizing the diffusion and partitioning of the drug in the SC *ex vivo* were consistent with those *in vivo* following a 30-minute application period. Furthermore, non-steady-state *ex vivo* results could be used to predict the *in vivo* concentration profile of the drug across the SC when a formulation was administered for 3 h (i.e., close to steady-state). Taken together, the results obtained suggest that pig ear skin *ex vivo* is promising as a tool for topical formulation evaluation and optimization.

Hostýnek et al. [67] sheded light on the long-standing controversy on whether wearing copper bangles benefits patients suffering from inflammatory conditions such as arthritis. Sequential TS was performed on healthy volunteers to examine the diffusion of copper through human SC *in vivo,* following application of the metal as powder on the volar forearm for periods of up to 72 h. Exposure sites were stripped 20 times, and the strips were analyzed for metal content by inductively coupled plasma-mass spectroscopy.

The results indicate that, in contact with skin, copper will oxidize and may penetrate the SC after forming an ion pair with skin exudates. The rate of reaction seems to depend on contact time and oxygen availability. A marked inter-individual difference was observed in baseline values and amounts of copper absorbed.

Lodén et al. [68] compared the bioavailability of ketoprofen in a photostabilised gel formulation without photoprotection using a new DPK tape stripping model and an established *ex vivo* penetration method using human skin. Analyses of the SC showed that during the first 45 minutes, about 12 μg/cm^2 of ketoprofen were absorbed into the skin from the formulations. The area under the ketoprofen concentration–time curve ($AUC_{0–6\ h}$) for the photo-stabilised gel/transparent gel ratio was 73%. The rate of penetration of ketoprofen through isolated skin was approximately 0.2 μg/cm^{2}h for both formulations. The ratio's $AUC_{0–36\ h}$ was 84%. Thus, the two methods did not disagree in terms of the relative efficacy of the two gels. The comparison of the amount of ketoprofen in the skin after 45 min with the amount penetrated through the excised skin during 36 h, suggests a change in the thermodynamic activity of ketoprofen during exposure. A supersaturated formulation may have been formed initially due to evaporation of ethanol.

Wagner Steffen et al. [69] studied the penetration kinetics of sesquiterpene lactones (SLs) in *Arnica montana* preparations; a stripping method with adhesive tape and pig skin as a model was used. For the determination of SLs in the stripped layers of the SC, a gas chromatography/mass spectrometry method was developed and validated.

The penetration behavior of one gel preparation and two ointment preparations was investigated. The SLs of all preparations showed a comparable penetration and permeation through the SC, in the uppermost layer of the skin. Interestingly, the gel preparation showed a decreased penetration rate over 4 h, whereas the penetration rate of ointments remained constant over time. Moreover, they could demonstrate that the total amount of SLs penetrated depends only on the kind of formulation and the SLs-content in the formulation, but not on SLs composition or the extraction agent used.

Wagner Heike et al. [70] carried out penetration experiments to investigate several incubation times with three different skin flaps using the Saarbruecken penetration model and the lipophilic model drug flufenamic acid. Drug distribution within SC was obtained by the TS technique, while the drug present in deep skin layers was determined by cryosectioning. In addition, for the lipophilic drug flufenamic acid, a direct linear correlation was found between SC/water partition coefficients and the drug amounts penetrated into the SC for all the time intervals tested. The authors concluded that SC/water-partition coefficients offer the possibility to predict drug amounts within the SC of different donor skin flaps, without a time-consuming determination of the lipid composition of the SC.

1.2 Corticosteroids

The aim of Pellanda et al. [71] was to investigate the effect of i) dose and ii) application frequency on the penetration of triamcinolone acetonide (TACA) into human SC *in vivo*. The experiments were conducted on the forearms of 15 healthy volunteers, with i), single TACA doses (300 μg/cm^2 and 100 μg/cm^2), and ii) single (1 x 300 μg/cm^2) and multiple (3 x 100 μg/cm^2) TACA doses. SC samples were collected by TS after 0.5, 4 and 24 h (i) and after 4, 8 and 24 h (ii). In Experiment 1, TACA amounts within SC after application of 1 x 300 μg/cm^2 compared to 1 x 100 μg/cm^2 were only significantly different immediately after application, and were similar at

4 and 24 h. In ii), multiple applications of 3 x 100 μg/cm^2 yielded higher TACA amounts compared to a single application of 1 x 300 μg/cm^2 at 4 and 8 h. At 24 h, no difference was observed. In conclusion, by using this simple vehicle, considerable TACA amounts were retained within the SC, independently of dose and application frequency.

1.3 Disinfectants

Lboutounne et al. [72] investigated the sustained bactericidal activity of chlorhexidine base loaded poly(Є-caprolactone) nanocapsules against *Staphylococcus epidermidis* inoculated onto porcine ear skin. The antimicrobial activity of these colloidal carriers was evaluated (i) *in vitro* against eight strains of bacteria, and (ii) *ex vivo* against *Staphylococcus epidermidis* inoculated for 12 h onto porcine ear skin surface treated for 3 min either with 0.6% chlorhexidine base loaded or unloaded nanocapsules suspended in hydrogel, or 1% chlorhexidine digluconate aqueous solution. Chlorhexidine absorption into the SC was evaluated by the TS technique. The results showed that chlorhexidine nanocapsules in aqueous suspension with a 200–300 nm size and a positive charge exhibited similar minimum inhibitory concentrations against several bacteria, compared with chlorhexidine digluconate aqueous solution. *Ex vivo*, there was a significant reduction in the number of colony forming units from skin treated with chlorhexidine nanocapsules suspension for 3 min compared to chlorhexidine digluconate solution after an 8-h artificial contamination. Interestingly, nanocapsules were present in porcine hair follicles. Topical application of chlorhexidine base-loaded positively charged nanocapsules in an aqueous gel achieved a sustained release of bactericide against *Staphylococcus epidermidis* for at least 8 h.

1.4 Drugs for keratinization disorders

Fresno-Contreras et al. [73] designed an all-*trans* retinoic acid (RA) topical release system that modifies drug diffusion parameters in the vehicle and the skin in order to reduce systemic absorption and side-effects associated with the topical application of the drug to the skin. RA, either in free form or encapsulated in SC lipid liposomes, was included in hydrogels prepared with Carbopol® UltrezTM 10 and hyaluronic acid. *In vitro* permeability experiments with [^{3}H]-*t*-RA were carried out using a Franz-type diffusion cell in abdominal rat skin samples. Accumulation of the drug in the surface and skin layers was evaluated by both the TS technique and a dissection technique. The results show that RA encapsulation not only prolongs drug release, but also promotes drug retention in viable skin. At the same time, interaction between RA and hyaluronic acid has an obstructive effect on diffusion, which contributes to the formation of a reservoir.

1.5 Anesthetics

Padula et al. [74] studied the behavior of a skin bioadhesive film containing lidocaine, *in vitro* and *in vivo*. Film characterization included *in vitro* and *in vivo* drug transport studies with and without iontophoresis. The release rate was compared with a lidocaine commercial gel. The permeation kinetics across the skin was not linear, but the patch acted as a matrix controlling drug delivery. Additionally, permeation rate increased with drug loading. The *in vivo* experiments with TS indicated that the presence of water during film application is essential to achieve not only the proper adhesion, but also an effective accumulation. The application of an electric current to the patch can further increase the amount of drug accumulated in the SC.

1.6 Keratolytics

Bashir et al. [75] studied the keratolytic efficacy of topical preparations containing salicylic acid (SA) in humans by the TS technique, quantifying SC removal by protein analysis. In combination with TS, squamometry was used to evaluate the influence of SA on skin surface scaliness and desquamation. Furthermore, skin barrier perturbation and skin irritability were recorded and related to the dermatopharmacological effect of the preparations. In contrast to squamometry, TS combined with protein analysis was sensitive in detecting the keratolytic effect of SA within hours of application. Importantly, whereas the pH of the preparations had only a minimal influence on efficacy, local dermatotoxicity was significantly increased at an acidic pH.

This indicates that the intent to increase the amount of free, non-dissociated SA is, in fact, counterproductive, as more acidic preparations resulted in skin irritation and barrier disruption.

1.7 Retinoids and antioxidants

Abdulmajed et al. [76] used a novel synthetic technique to synthesize the co-drug retinyl ascorbate (RA-AsA) ester from all-*trans*-retinyl chloride (RA) and l-ascorbic acid (AsA) suspended in ethanol at low temperature. The flux and permeation coefficient were determined using heat separated human skin membrane, and skin penetration was determined by TS using full-thickness human skin. All experiments were performed in parallel with retinyl palmitate and ascorbyl palmitate. Overall, the data suggest the potential value of RA-AsA co-drug for treating damage to skin resulting from UV-induced production of free radicals.

1.8 Aquaporine-3

Hara et al. [77] showed that glycerol replacement corrects each of the defects in aquaporine-3 (AQP3)-null mice. SC water content, measured by skin conductance and ^3H_2O accumulation, was 3-fold lower in AQP3-null vs. wild-type mice, but was similar after topical or systemic administration of glycerol in amounts that normalized glycerol content in the SC. Orally administered glycerol fully corrected reduced skin elasticity in AQP3-null mice, as measured by the kinetics of skin displacement after suction, and the delayed barrier recovery, as measured by TEWL after TS. The analysis of [^{14}C]glycerol kinetics indicated a reduced blood-to-SC transport of glycerol in AQP3-null mice, resulting in slowed lipid biosynthesis. These data provide functional evidence for a physiological role of glycerol transport by aquaglyceroporin, and indicate that glycerol is a major determinant of SC water retention and of mechanical and biosynthetic functions. Their findings establish a scientific basis for the >200 year old empirical practice of including glycerol in cosmetic and medicinal skin formulations.

1.9 Antiviral drugs

Morgan et al. [78] measured the contribution of SC barrier and microvascular perfusion in determining dermal tissue levels of two hydrophilic drugs (aciclovir and penciclovir) *in vivo*. Removal of the SC by TS resulted in a 1300-fold increase in penciclovir absorption and a 440-fold increase in aciclovir absorption, confirming that SC is the major barrier to hydrophilic drug absorption.

1.10 Anti-Varicella Zoster virus nucleoside

Jarvis et al. [79] determined the *in-vitro* dermal delivery of a new class of lipophilic, highly potent and uniquely selective anti-Varicella Zoster virus nucleoside (VZV) analogue compared with aciclovir. Three test compounds (Cf1698, Cf1743, and Cf1712) and acyclovir were formulated in propylene glycol/aqueous cream, and finite doses were applied to full-thickness pig ear skin for 48 hours in vertical Franz-type diffusion cells. Depth profiles were constructed following TS and membrane separation. All three test compounds reached the target basal epidermis in concentrations suggesting they would be highly efficacious in reducing viral load. Furthermore, the data showed that each of the test compounds would have a far superior performance than aciclovir. The dermatomal site of viral replication during secondary infection —the basal epidermis— was successfully targeted.

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1.11 Vaccines

The skin-associated lymphoid tissue, formed by powerful antigen-presenting cells (APCs), such as Langerhans cells (LCs), dermal dendritic cells (DCs), re-circulating T cells, and regional LNs, ensures the efficient presentation of antigen to immunocompetent cells and the induction of strong immune responses. LCs and dermal DCs commonly exist in the skin and are easy to target [80]. The TS technique has been used to study the effect of oligodeoxynucleotides on the immune response [81] and expression of immune receptors [82].

Inoue et al. [81] examined the effect of CpG-oligodeoxynucleotide (CpG-ODN) on the immune response to an antigen applied to tape-stripped mouse skin, by evaluating the production of cytokines and Ig isotypes. Confocal laser scanning microscopy revealed that the OVA (model antigen) and CpG-ODN easily penetrated the tape-stripped skin. Co-administration of CpG-ODN and OVA to the disrupted skin elicited an antigen-specific, Th1-predominant immune response, and enhanced the production of Th1-type cytokines, IL-12 and IFN-γ. On the other hand, the production of a Th2-type cytokine, IL-4, was drastically suppressed. In terms of antigen-specific antibody production, the IgG2a level, which is regulated by IFN-γ, was increased by CpG-ODN, but IgE production regulated by IL-4 was suppressed. Furthermore, the administration of CpG-ODN through the skin drastically attenuated the production of IgE in mice

experiencing IgE-type immune response. Administration of CpG-ODN through the skin may shift the immune response from a Th2 to a Th1-like response.

Continuing with their studies, Inoue et al. [82] also demonstrated that TS induces the expression of toll-like receptor (TLR)-9 in the skin, and enhances the Th1-type immune response triggered by CpG-ODN administered through the tape-stripped skin. TS induces the expression of TLR-9 and tumor necrosis factor (TNF)-α in the skin, and CpG-ODN treatment through the tape-stripped skin enhances the migration of antigen-presenting cells to the draining lymph nodes. On the other hand, TLR-9 mRNA and TNF-α mRNA were not observed in the skin when CpG-ODN was injected intradermally, or in Th1-type immune response. The transdermal application of CpG-ODN with an antigen through the tape-stripped skin is an effective way to induce a Th1-type immune response, and is also a simple, cost-effective and needle-free vaccination system.

1.12 Other kind of permeants (Polyethylene glycols, 4-cyanophenol)

Ayala-Bravo et al. [83] investigated the effect of sucrose esters (sucrose oleate and sucrose laureate in water or Transcutol®, TC) on the SC barrier properties *in vivo*, and examined the impact of these surfactant-like molecules on the *in vivo* percutaneous penetration of a model penetrant, 4-hydroxybenzonitrile (4-cyanophenol, 4CP). The effect of the enhancers on 4CP penetration was monitored *in vivo* using ATR-FTIR spectroscopy in conjunction with TS of the treated site. A combination of sucrose esters (oleate or laureate) and TC is able to temporarily alter the SC barrier properties, thereby promoting 4CP penetration.

Results from TS experiments can be affected significantly by chemical diffusion into the SC during the time required to apply and remove all of the TSs, t_{TS} (period of time required to completely remove the SC by tape stripping). For this reason, Reddy et al. [84] studied dermal absorption of 4CP in humans using TS experiments to assess the conditions under which diffusion alters TS results. Mathematical models were developed to assess the effects of diffusion on parameter estimation. In an experiment with $t_{TS} > t_{lag}$ (i.e., the lag time for a chemical to cross the SC), the permeability coefficient for 4CP, Psc,v, calculated including t_{TS}, was consistent with the values from the literature. When diffusion during stripping was not included in the model, Psc,v, was 70% smaller. Calculations show that chemical concentrations in TSs can be affected by diffusion during TS, but with $t_{TS} < 0.2$ t_{lag} and an exposure time > 0.3 t_{lag}, TS concentrations are not significantly affected by t_{TS}.

Jakasa et al. [85] developed a sensitive method for the determination of polyethylene glycols (PEGs) with different molecular weights (MW) in the human SC obtained by TS. The analysis is based on derivatization with pentafluoropropionic anhydride and gas chromatography–electron capture detection. The method showed to be suitable for studying permeability in normal and impaired skin with respect to MW in the range of 150–600 Da.

In order to obtain more data to assess the barrier function of uninvolved skin in atopic dermatitis (AD) patients, Jakasa et al. [86] determined the percutaneous penetration of PEGs of various molecular sizes *in vivo* in AD patients and control subjects using TS of the SC. The apparent diffusion coefficient of PEGs through atopic skin was twice as high as through normal skin, and decreased with increasing MW in both groups. The partition coefficient in the skin of AD patients was half of that for normal skin, but as for normal skin, there was no MW dependence. Although atopic skin exhibited an altered barrier with respect to diffusion and partitioning, the permeability coefficients were nearly the same for atopic and normal skin. The results support the assumption of an altered skin barrier in AD patients, even if the skin is visibly unaffected by the disease.

Tsai et al. [87] further investigated the dependence of permeability on MW with different forms of barrier disruption. A series of PEGs with a MW ranging from nearly 300 to over 1000 Da were used to study the effects of TS and sodium dodecyl sulphate (SDS) treatment on MW permeability profiles of mouse skin *in vitro*. The total penetration of PEG oligomers across control skin and tape-stripped skin and SDS-treated to different degrees of barrier disruption progressively decreased with increasing MW. Penetration enhancement relative to control skin was more prominent with larger molecules. The MW cut-off for skin penetration increased with the degree of barrier disruption, irrespectively of the treatment applied, and was 986 Da (TS) and 766 Da (SDS treatment) at TEWL levels in the range of 10–20 g/m^2 per h, compared with 414 Da for control skin. The results strongly suggest that, regardless of the form of barrier disruption applied, not only higher amounts, but also more

varieties of chemicals (larger molecules), may penetrate into the skin in the presence of a compromised barrier compared with normal skin.

1.13 UV absorbers

Alvárez-Román et al. [88] determined whether encapsulation of lipophilic compounds in polymeric nanoparticles is able to improve topical delivery to the skin. The penetration of octyl methoxycinnamate (OMC) encapsulated in poly(Є-caprolactone) nanoparticles, into and across porcine ear skin *in vitro*, was investigated using TS.

Quantification of OMC in the skin using TS demonstrated that nanoparticulate encapsulation produced a 3.4-fold increase in the level of OMC within the SC. Nanoparticulate encapsulation of OMC increased its "availability" within the SC.

Olvera-Martínez et al. [26] prepared polymeric nanocapsules (NCs) containing OMC, and their *in vivo* distribution profile through the SC was determined by the TS technique. The penetration degree of OMC formulated in NCs was compared with that obtained for a nanoemulsion (NE) and a conventional oil-in-water (o/w) emulsion (EM). *In vivo* percutaneous penetration, evaluated by the TS technique, demonstrated that NE increased the extent of OMC penetration relative to the penetration achieved by NCs or EM. Likewise, OMC accumulation in the skin was significantly greater with NE than with EM or NCs.

Sarveiya et al. [89] developed a reverse HPLC assay to quantify four common sunscreen agents, namely, 2-hydroxy-4-methoxybenzophenone, 2-ethylhexyl-*p*-methoxycinnamate, octylsalicylate and salicylic acid 3,3,5-trimethcyclohexyl ester in a range of biological matrices. This assay was further applied to study skin penetration and systemic absorption of sunscreen filters after topical application to human volunteers. The assay allows the analysis of sunscreen agents in biological fluids, including bovine serum albumin solution, plasma and urine, and in human epidermis by using the TS technique. The results from the preliminary clinical study demonstrate a significant penetration of all sunscreen agents into the skin.

Lademann et al. [90] determined the amount of sunscreen present on the skin of people at the beach. The amounts of sunscreen applied to different body sites were quantitatively determined by TS. The actual amounts of sunscreen applied were compared with the COLIPA (European Cosmetic Toiletry and Perfumery Association) standard. Most volunteers had applied 10% or less of the COLIPA standard amount to all body sites assessed.

Mavon et al. [91] assessed the penetration of titanium dioxide (TiO_2) and methylene bis-benzotriazoyl tetramethylbutylphenol (MBBT), included in a broad-spectrum sunscreen formulation, into human skin *in vivo*, using the TS technique, and *in vitro*, using a compartmental approach. More than 90% of both sunscreens were recovered in the first 15 tape strippings. In addition, they have shown that the remaining 10% did not penetrate the viable tissue, but was localized in the furrows. Less than 0.1% of MBBT was detected in the receptor medium, and no TiO_2 was detected in the follicles, the viable epidermis or the dermis. Thus, this *in vivo* and *in vitro* penetration study showed an absence of TiO_2 penetration into viable skin layers through either transcorneal or transfollicular pathways, and a negligible transcutaneous absorption of MBBT. However, differences in distribution within the SC reinforced the need for a complementary approach, using a minimally invasive *in vivo* methodology and an *in vitro* compartmental analysis. This combination represents a well-adapted method for testing the safety of topically applied sunscreen formulations in real-life conditions.

1.14 Fragances

In-vitro human skin permeation and distribution of geranyl nitrile (GN) were determined by Brian et al. [92] using epidermal membranes, following application in 70% ethanol, under non-occlusive conditions, at maximum in-use concentration (1%). Levels of GN in the epidermis (plus any remaining in SC after TS), filter paper membrane support, and receptor fluid were combined to produce a total absorbed dose value of 4.72±0.32%. The systemic exposure resulting from the use of GN as a fragrance ingredient, under unoccluded conditions, would be low based on the currently reported use levels.

1.15 Dyes

Teichmann et al. [93] developed a method to investigate the effectiveness of reservoir closure by different formulations. Patent Blue V in water was used as a model penetrant. Its penetration, with and without barrier cream treatment, was

analyzed by TS in combination with UV/VIS spectroscopic measurements. The investigations showed that the SC represents a reservoir for topically applied Patent Blue V in water. Furthermore, the barrier investigations showed that vaseline and bees wax form a 100% barrier on the skin surface. The third barrier cream, containing waxes and surfactant, only partially showed a protective effect against the penetration of Patent Blue V in water. Strong inter-individual differences were observed for this barrier product. In conclusion, it was assumed that the application of barrier creams cannot replace other protective measures, and should be used to inhibit low-grade irritants or in combination with other protectants, or in body areas where other protective measures are not applicable.

Jacobi et al. [94] studied the penetration of highly hydrophilic (Patent blue V) and lipophilic (curcumin) dyes into the skin using pure oil (o) or water (w), and comparing them with an o/w emulsion. The penetration and localization of both dyes were investigated *in vivo* using TS and microscopy techniques. Differences in the distribution and the localization of both dyes within the SC were observed. These differences depend on the physicochemical properties of both the vehicles and the dyes. The vehicle appears to affect, in particular, the penetration pathways.

As we can observe, there is an ongoing search for the identification of testing methods to optimize topical dosage forms and to assess topical drug bioavailability. While *in vitro* screening continues to play an important role (and is relatively inexpensive and easy to use), regulatory approval of drug delivery systems to the skin, with few exceptions, requires clinical trials to be performed. For many drugs used topically, the problem remains unsolved, since an easily visualized pharmacodynamic response is not elicited.

As a consequence, various alternative techniques have been considered, of which SC tape-stripping is being given the greatest attention [7,26,35,63-94]. While the former is technically more challenging, the potential reward is a drug concentration-time profile in a compartment presumed to be in close communication with the site of action of most dermatological drugs. The latter, in contrast, offers an apparently easy and quite non-invasive methodology for skin tissue sampling, and is the basis of the FDA's so-called DPK. Validation and optimization of the procedure have not come quickly. The goal of the research described here is not only to contribute to further establishing the credibility of the tape stripping technique, but also to demonstrate that useful and relevant measurements can be made on a surrogate, *ex vivo* skin model.

2) Dermal absorption of toxic or irritant chemicals

The rate and extent of dermal absorption are important in the analysis of risk from dermal exposure to toxic chemicals, and for the development of topically applied drugs, barriers, insect repellents, and cosmetics, and the TS technique has been widely used to determine the penetration of these kind of substances [36, 37, 95-97].

Mattorano et al. [36] developed and tested a simple, non-invasive dermal sampling technique on 22 human volunteers to estimate acute dermal exposure to jet fuel (JP-8). Two sites on the ventral surface of each forearm were exposed to 25 µl of JP-8, and the SC was sequentially tape-stripped using an adhesive tape. The analysis of the first tape strips indicated that JP-8 was rapidly removed from the SC over the 20-min study period. On average, after 5 min of exposure, the first two tape strips removed 69.8% of the applied dose. The amount recovered with two tape strips decreased over time, to a recovery of 0.9% 20 min after exposure. By fitting a mixed-effect linear regression model to the TS data, the authors were able to accurately estimate the amount of JP-8 initially applied. This study indicates that naphthalene has a short retention time in the human SC and that the TS technique, if used within 20 min of initial exposure, can be used to reliably measures the amount of naphthalene initially present in the SC due to a single exposure to jet fuel.

Chao et al. [37] presented a TS method for the removal and quantification of keratin from the SC for normalization of extracted concentrations of naphthalene (as a marker for jet fuel exposure) from 12 human volunteers before and after exposure to jet fuel (JP-8). Due to the potential for removal of variable amounts of squamous tissue from each tape strip sample, keratin was extracted and quantified using a modified Bradford method. Naphthalene was quantified in the sequential tape strips collected from the skin between 10 and 25 min after a single dose of JP-8 was initially applied. The penetration of jet fuel into the SC was demonstrated by the fact that the average mass of naphthalene recovered by a tape strip decreased with increasing exposure time and subsequent

tape strips. The actual concentration of naphthalene (as a marker for JP- 8 exposure) per unit of keratin in a tape-strip sample can be determined by using this method, and may prove necessary when measuring occupational exposures under field conditions.

Van der Merwe et al. [95] described a physiologically based pharmacokinetic model developed to simulate the absorption of organophosphate pesticides, such as parathion, fenthion, and methyl parathion through porcine skin with flow-through cells. Three parameters were optimized based on experimental dermal absorption data, including solvent evaporation rate, diffusivity, and a mass transfer factor. Diffusion cell studies were conducted to validate the model under a variety of conditions, including different dose ranges (6.3–106.9 μg/cm^2 for parathion; 0.8–23.6 μg/cm^2 for fenthion; 1.6–39.3 μg/cm^2 for methyl parathion), different solvents (ethanol, 2-propanol and acetone), different solvent volumes (5–120 μL for ethanol; 20–80 μL for 2-propanol and acetone), occlusion versus open to atmosphere dosing, and corneocyte removal by TS. The study demonstrated the utility of PBPK models for studying dermal absorption. The similarity between the overall shapes of the experimental and model-predicted flux/time curves and the successful simulation of altered system conditions for this series of small, lipophilic compounds, indicated that the absorption processes described in the model successfully simulated important aspects of dermal absorption in flow-through cells. These data have a direct relevance in the assessment of topical organophosphate pesticides' risk.

Jongh et al. [96] studied whether sodium lauryl sulphate (SLS) penetration rate into the SC is related to an impairment of skin's water barrier function and inflammation. The penetration of SLS into the SC was assessed using a non-invasive TS procedure in 20 volunteers after a 4-h exposure to 1% SLS. Additionally, the effect of a 24-h exposure to 1% SLS on the skin water barrier function was assessed by measuring TEWL. A multiple regression analysis showed that the baseline TEWL, SC thickness and SLS penetration parameters K (SC /water partition coefficient) and D clearly influenced the increase in TEWL after the 24-h irritation test. They found that variation in barrier impairment and inflammation of human skin depends on SLS' penetration rate, which was mainly determined by SC thickness.

Lundgren et al. [97] modified and tested a vacuuming sampler for removing particles from the skin. The sampler was compared with two other skin and surface exposure sampling techniques. These were based on surrogate skin (a patch sampler-adhesive tape on an optical cover glass) and a TS removal procedure. All three samplers measure the mass of dust on the skin. Dust containing starch was deposited onto the skin in a whole-body exposure chamber. Samples were taken from forearms and shoulders and analysed using optical microscopy. With the different sampling techniques, small differences in results were obtained. There was a good agreement between the vacuuming sampler and the TS technique.

3) Evaluation of factors that influence the physiology of the stratum corneum

Many factors are known to influence the physiology of the SC. In this way, increasing age is related to decreased skin thickness [98] and a variation in skin lipids [99] and flora [100]. The anatomical region also influences lipid distribution [101, 102], microflora [103] and physical parameters such as TEWL [104]. However, there are already a few studies reporting conflicting results for the effect of gender [105] on skin physiology, as well as for the effect of anatomical site, pressure, pressure duration and tape removal rate in skin physiology [42], or the effect of skin transport technology, as iontophoresis, on human skin [106].

Jacobi et al. [105] studied the effect of gender on the physiology of the SC. The physiological parameters: TEWL, pH value, hydration and sebum content were determined on the flexor forearms of 6 female and 6 male volunteers. In addition, SC samples, removed by TS, were studied for amount, spectroscopic properties, protein content, and mass. The skin of women was characterized by a significantly higher pH value (5.6±0.4) than that of men (4.3±0.4). Protein absorption was the only other parameter significantly dependent on gender. Both effects might be caused by differences in human biology, such as hormonal status. Therefore, volunteers' gender should be considered in dermatologic studies.

Löffler et al. [43] investigated the influence of the procedures (anatomical site, pressure, pressure duration, tape removal rate) inherent to each stripping protocol on changes in skin physiology. A significant influence of all parameters on TEWL increase, as a function of

tape strip number was observed. The fastest increase was demonstrated on the forehead, followed by the back and, lastly, the forearm. Rapid removal produced a protracted increase compared with slow removal. Pressure for 10s induced a faster increase in TEWL than 2s pressure. Likewise, pressure at 330 g cm^{-2} induced an earlier increase than pressure at 165 g cm^{-2}. Skin hydration was not influenced by the variables tested.

Van der Molen, et al. [44] investigated the efficacy of TS in removing complete cell layers from the superficial part of the human SC. A histological section of skin that was tape-stripped 20 times, clearly showed non-stripped skin in the furrows, indicating persistent incomplete TS. Replicates of tape-stripped skin surface demonstrated that even after removing 40 tape strips, furrows were still present. They emphasize that the results from studies using the TS method have to be viewed from the perspective that cells on one tape strip of the SC may come from different layers, depending on the position of the tape strip in relation to furrow slope, and such results should be interpreted with considerable caution.

Fatouros et al. [106] investigated the local changes in the ultrastructure of human skin after iontophoresis in human skin *in vitro* and *in vivo*. Human dermatomed skin was subjected to passive diffusion for 6 hours, followed by nine hours of iontophoresis at $0.5mA/cm^2$. In addition, iontophoresis patches were applied to healthy volunteers for 3.5h with 0.5h of passive delivery followed by 3h of iontophoresis at a current density of $0.25mA/cm^2$. Subsequently, a series of TSs were performed, and were visualized by freeze-fracture transmission electron microscopy. *In vitro/in vivo* studies suggest that iontophoresis results in the formation of intercellular water pools, and in a weakening of the desmosomal structure only in the upper part of the SC. However, no changes in lipid organization were observed *in vitro* and *in vivo* at the 0.5 and $0.25mA/cm^2$ current densities, respectively. Therefore, even at relatively high current densities, no drastic changes in the ultrastructure of the SC are observed. As far as structural changes in SC are concerned, iontophoresis is a safe method under the experimental conditions used.

4) Stratum corneum composition

Weerheim et al. [107] established a suitable analytical method for the determination of local SC lipid composition. For this purpose, SC samples were collected by sequential stripping with Leukoplex tape in five healthy volunteers. Lipids were extracted with an ethyl acetate: methanol mixture (20:80) and separated by means of HPTLC. The results of this study revealed that the free fatty acid level is higher, and cholesterol and ceramide levels are lower in the uppermost SC layers. Levels remained unchanged in the underlying SC layers. In these layers, the ceramide level was about 60 wt % and free fatty acid and cholesterol levels were about 20 wt % each. Ceramides could be separated into seven different fractions, and the relative amounts of individual ceramide fractions did not significantly change with the SC depth. The method developed allowed to study the differences in the SC lipid profile in healthy and diseased human skin relative to the SC lipid organization and the skin barrier function *in vivo*.

5) Determination of stratum corneum thickness

Alberti et al. [108] evaluated, using attenuated total reflectance Fourier transform infrared spectroscopy, the SC bioavailability of terbinafine (TBF) following topical treatment with four different formulations, based on a vehicle consisting of 50% ethanol and 50% isopropyl myristate. Three of these formulations included a percutaneous penetration enhancer: either 5% oleic acid, 10% 2-pyrrolidone or 1% urea. The SC concentration profile of TBF was measured by repeated infrared spectroscopic measurements while sequentially stripping off the layers of this barrier membrane with adhesive tape. TEWL measurements were also performed, to permit facile estimation of SC thickness. The SC concentration profiles of TBF were fitted to the appropriate solution of Fick's second law of diffusion. This analysis enabled the efficacies of the different formulations tested to be compared to the non-enhancer control. While it was found that the formulation containing 5% oleic acid significantly enhanced the SC availability of TBF, the other formulations did not improve the apparent drug delivery.

Kalia et al. [109] determined whether a structurally heterogeneous biomembrane, human SC, behaved as a homogeneous barrier to water transport. Impedance spectra (IS) of the skin and measurements of the rate of TEWL were recorded sequentially *in vivo* in human subjects as layers of the SC were progressively removed by the serial application of adhesive tape strips. The low-frequency impedance of skin was much more

significantly affected by TS than the higher frequency values; removal of the outermost SC layer had the largest effect. In contrast, TEWL changed little as the outer SC layers were stripped off, but increased dramatically when 6-8 microns of the tissue had been removed. It follows that the two noninvasive techniques probe SC barrier integrity in somewhat different ways. After SC removal, recovery of barrier function, as assessed by increasing values of the low-frequency impedance, apparently proceeded faster than TEWL decreased to the pre-stripping control. The variation of TEWL as a function of SC removal behaved in a manner entirely consistent with a homogeneous barrier, thereby permitting the apparent SC diffusivity of water to be found. Skin impedance (low frequency) was correlated with the relative concentration of water within the SC, thus providing an *in vivo* probe for skin hydration. Finally, the SC permeability coefficient to water, as a function of SC thickness, was calculated and correlated with the corresponding values of skin admittance derived from IS.

6) Determination of superficial infections and viruses

Topical infections due to *S. aureus* and *S. pyogenes* are clinically relevant and cause a variety of serious symptoms, including toxic shock syndrome and skin lesions [110], that can progress to sepsis and systemic shock if they are left untreated [111]. These bacterial species are also the most common causes of impetigo in humans [111]. The TS technique has offered the possibility of studying superficial infections on the skin [112], as well as viruses in skin tumors [113].

Kugelberg et al. [112] presented a new animal model for the purpose of studying superficial infections. In this model, an infection is established by disruption of the skin barrier by partial removal of the epidermal layer by TS and subsequent application of the pathogens *Staphylococcus aureus* and *Streptococcus pyogenes*. The infection and the infection route were purely topical. Thus, the present model is considered more biologically relevant for the study of superficial skin infections in mice and humans. Established topical antibiotic treatments are shown to be effective. The procedures involved in the model are simple, a feature that increases throughput and reproducibility. This new model should be applicable to the evaluation of novel antimicrobial treatments for superficial infections caused by *S. aureus* and *S. pyogenes*.

Forslund et al. [113] investigated 229 immunocompetent patients tested for human papilloma virus (HPV) DNA in swab samples collected on top of skin tumors and in biopsies of the same tumors, obtained after stripping with tape to remove superficial layers. HPV DNA was detected on top of 69% of the lesions, and in 12% of the stripped biopsies. A difference was seen for the four types of tumors studied. Seborrheic keratosis had 79% HPV positivity on top of lesions versus 19% in biopsies; actinic keratosis had 83% HPV positivity on top of lesions versus 11% in biopsies; basal cell carcinoma had 63% on top of lesions versus 8% in biopsies; and squamous cell carcinoma had 58% on top of lesions versus 19% in biopsies. HPV DNA is common in superficial layers of lesions, but is not necessarily present in tumors.

7) Skin regeneration

Malminen et al. [114] investigated the expression of tight junction components during reepithelialization of suction blisters and regeneration of the corneal layer after TS. Suction blisters were induced in eight healthy volunteers, and skin biopsies were taken 4 or 6 days afterwards. The restoration of epidermal barrier function was evaluated by measuring water evaporation (WE) from the wound area. TS was performed on three volunteers to remove the corneal layer. Prior to the biopsies, WE from the blister wounds was markedly elevated compared with normal skin. In the epidermis surrounding the blister, occludin and ZO-1 (membrane-associated guanylate kinase homologue protein family) were expressed in the granular cell layer only. In the hyperproliferative zone adjacent to the border of the blister, the expression of ZO-1 was redistributed into several spinous cell layers, while occludin expression was restricted to the upper epidermis. In the leading edge of migrating keratinocytes, both proteins were expressed solely in the most superficial layer of keratinocytes. Double labelling for ZO-1 and involucrin showed expression of both proteins in the same layers of hyperproliferative keratinocytes, while the expression patterns were clearly different in migrating keratinocytes. Tight junctions of regenerating epidermis may provide a functional barrier prior to regeneration of the corneal layer.

Sekkat et al. [115] developed an *in vitro* model for the developing skin of the premature neonate. Barriers of different levels of efficiency were produced by differential tape-stripping of the SC from the skin of excised porcine ears, and

were characterized by measurements of TEWL. In this way, it was possible to express the recorded TEWL as a function of percentage SC thickness (F), generating the following relationship: TEWL = 2.7+41.exp [-0.028.F]. These data were then compared to previously published *in vivo* measurements of TEWL obtained from a population of premature neonates at various post-conceptional ages (PCA). The former showed a remarkably parallel relationship to that found *in vitro* with the porcine skin model, namely TEWL = 3.3+41 exp [-0.026.(PCA-160)]. Therefore, it can be suggested that the empirically adjusted PCA (i.e., PCA-160) has a close correlation with the developing thickness of the neonate's SC. Consequently, porcine skin *in vitro*, tape-stripped to a particular level, can provide a barrier corresponding to a specific degree of neonate maturation, and thus, can serve as a useful tool to explore whether transdermal drug delivery in this unique patient population may be beneficial.

Zhai et al. [116] used an *in vivo* human model to define the irritation potential of a topical agent after partial removal of the SC by cellophane TS. The tape was applied to and removed approximately 50 times (mean, 50.0 +/- 16.7) from each test site on the volar aspect of the forearm. TEWL was measured before and daily for 5 days. The TEWL values at baseline after stripping represented the point of maximal stripping barrier disruption. The barrier disruption and irritation potential were assessed with TEWL measurements. The results showed that the model topical agent had no adverse effect on barrier repair, i.e. did not interfere with TEWL normalization. This model provides a method for the prediction, with exaggerated sensitivity, of chemical irritation and proclivity to enhance or retard water barrier repair. They believe that the model may predict the response of low irritation materials and may be more sensitive than patch testing on normal skin, particularly for products to be used on certain areas, e.g. the face, anus, etc., or even mucous membranes.

Conclusions

The quantification of drugs within the skin is essential for topical and transdermal delivery research. Over the last two decades, horizontal sectioning, consisting of TS, has been the traditional investigative technique.

Many *in vivo* methods for measuring dermal absorption of chemicals are invasive (e.g., blood sampling) or slow (e.g., urine samples collected for extended periods). TS of the outermost skin layer, the SC, is a fast and relatively non-invasive technique for measuring the rate and extent of dermal absorption. Tape stripping data have been used to estimate permeability coefficients and partition coefficients, SC mass, barrier function, drug reservoir from *in vivo* dermal exposures, and even to explain the SC physiology. TS has also been proposed as a method for evaluating the bioequivalence of topical dermatological dosage forms. DPK characterization of the penetration of active drugs in human volunteers has been suggested to be able to replace comparative clinical trials as means of documenting bioequivalence. It is suggested that DPK assessment of drug concentrations in the SC is comparable to blood/urine measurements of systemically administered drugs, where the concentration of a drug in the SC is expected to relate to its concentrations in viable tissue. Short-contact DPK experiments can be used to obtain diffusion and partitioning parameters that may subsequently be able to predict drug penetration into the SC following longer application periods.
Although tape stripping is widely used to determine dermal absorption through the SC, several factors can influence the actual technique. Recent reviews on this topic provide updated and additional insights (117, 118). The investigation of variations in skin condition (dry versus moist skin, skin defects, etc.) to determine their potential impact on the sampling method is warranted. For these reasons, the tape stripping technique requires further development.

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Table 1. Research on the tape stripping technique as a method to determine skin permeation of different kind of permeants

1) Kinetics and penetration depth of drugs

Research	Outcome	Author (Ref.)-Year
Effect of Azone® and Transcutol® on skin permeation of sodium naproxen formulated in PF-127 gels.	The combination of Azone® and Transcutol® in PF-127 gels enhanced sodium naproxen penetration, with up to two-fold enhancement ratios compared with the formulation containing Transcutol® only.	Escobar-Chávez et al. (7), 2005.
Administration of piroxicam from a commercially available gel to human volunteers, both passively and under the application of an iontophoretic current.	The total amount of drug recovered in the SC post-iontophoresis by TS was significantly higher than that found following passive diffusion for each application time.	Curdy et al. (35), 2001
Effect of hydroxypropyl-P-cyclodextrin (HP-P-CD) on the cutaneous penetration and activation of ethyl 4-biphenylyl acetate (EBA), a prodrug of non-steroidal anti-inflammatory drug 4-biphenylylacetic acid (BPAA), from hydrophilic ointment, using hairless mouse skin *in vitro*.	The enhancing effect of HP-P-CD on the cutaneous penetration of EBA would be largely attributable to an increase in the effective concentration of EBA in the ointment.	Arima et al. (63), 1998
Production and characterization of monoleine (MO) dispersions as drug delivery systems for indomethacin.	Reflectance spectroscopy demonstrated that indomethacin incorporated into MO dispersions can be released in a prolonged fashion. TS experiments corroborated this finding.	Esposito et al. (64), 2005
Unilaminar films of Eudragit E-100 prepared from naproxen-loaded nanoparticles vs. conventional films.	*In vivo* penetration studies showed no statistical differences for the penetrated amount of naproxen across the SC and the depth of penetration for the two films. The films formulated from nanoparticle dispersions were shown to be effective for the transdermal administration of naproxen.	Ganem-Quintanar et al. (65), 2006
Investigation of pig ear skin as a surrogate for human skin in the assessment of topical drug bioavailability by sequential TS of the SC.	Pig ear skin *ex vivo* is promising as a tool for topical formulation evaluation and optimization.	Herkenne et al. (66), 2006
Examination of the diffusion of copper through human SC *in vivo* following application of the metal as powder on the volar forearm for periods of up to 72 h.	Copper will oxidize and may penetrate the stratum corneum after forming an ion pair with skin exudates. The rate of reaction seems to depend on contact time and oxygen availability. A marked	Hostýnek et al. (67), 2006

	inter-individual difference was observed in baseline values and the amounts of copper absorbed.	
Comparison of the bioavailability of ketoprofen in a photostabilised gel formulation without photoprotection using a new dermatopharmacokinetic TS model and an established *ex vivo* penetration method using human skin.	The comparison of the amount of ketoprofen in the skin after 45 min with the amount penetrated through the excised skin during 36 h, suggests a change in the thermodynamic activity of ketoprofen during exposure.	Lodén et al. (68), 2004
Penetration kinetics of SLs (sesquiterpene lactones) in *Arnica montana* preparations, by using a stripping method with adhesive tape and pig skin as a model.	Gel preparation showed a decrease in penetration rate, whereas the penetration rate of ointments remained constant over time. The total amount of SLs penetrated depends only on the kind of formulation and the SLs-content, but not on SLs composition or on the extraction agent used.	Wagner Steffen et al. (69), 2006
Penetration experiments investigating several incubation times with three different skin flaps, using the Saarbruecken penetration model and the lipophilic model drug flufenamic acid.	A direct linear correlation was found between the SC/water partition coefficients and the drug amounts penetrated into the SC for all time intervals tested.	Wagner Heike et al. (70), 2002
Effect of dose and application frequency on the penetration of triamcinolone acetonide (TACA) into human SC *in vivo*.	Considerable TACA amounts were retained within the SC, independently of dose and application frequency.	Pallenda et al. (71), 2006
Sustained bactericidal activity of chlorhexidine base loaded poly(Є-caprolactone) nanocapsules against *Staphylococcus epidermidis* inoculated onto porcine ear skin.	Topical application of chlorhexidine base-loaded positively charged nanocapsules in an aqueous gel achieved a sustained release of bactericide against *Staphylococcus epidermidis* for at least 8 h.	Lboutounne et al. (72), 2002
Design of an all-*trans* retinoic acid (RA) topical release system that modifies drug diffusion parameters in the vehicle and the skin, in order to reduce the systemic absorption and side-effects associated with the topical application of the drug to the skin.	RA encapsulation not only prolongs drug release, but also promotes drug retention in viable skin.	Fresno-Contreras et al. (73), 2005
Behaviour of a skin bioadhesive film containing lidocaine *in vitro* and *in vivo*.	*In vivo* experiments with TS indicated that the presence of water during film application is essential to achieve not only the proper adhesion, but also an effective accumulation.	Padula et al. (74), 2003

Keratolytic efficacy of topical preparations containing salicylic acid in humans by TS, and quantification of SC removal by protein analysis.	TS combined with protein analysis was sensitive in detecting the keratolytic effect of salicylic acid within hours of application.	Bashir et al. (75), 2005
Novel synthetic technique to synthesize the co-drug retinyl ascorbate (RA-AsA) ester from all-*trans*-retinyl chloride (RA) and l-ascorbic acid (AsA) suspended in ethanol at low temperature.	The data suggest the potential value of RA-AsA co-drug for treating damage to skin resulting from UV-induced production of free radicals.	Abdulmajed et al. (76), 2004
Glycerol replacement corrects each of the defects in aquaporine-3 (AQP3)-null mice.	The findings establish a scientific basis for the >200-yr-old empirical practice of including glycerol in cosmetic and medicinal skin formulations due to its influence on water retention and the mechanical and biosynthetic functions of the SC.	Hara et al. (77), 2003
Contribution of SC barrier and microvascular perfusion in determining dermal tissue levels of hydrophilic drugs (aciclovir and penciclovir) *in vivo*.	There was no relationship between fibre depth and the amount of drug dialysed, which suggests free movement of antiviral drug on reaching the aqueous environment of the dermis.	Morgan et al. (78), 2003
Determination of the *in-vitro* dermal delivery of a new class of lipophilic, highly potent and uniquely selective anti-VZV nucleoside analogue compared with aciclovir.	Topical delivery of these compounds is highly promising as a new first line treatment for VZV infections.	Jarvis et al. (79), 2004
Effect of CpG oligodeoxynucleotide (CpG-ODN) on the immune response to an antigen applied to tape-stripped mouse skin by evaluating the production of cytokines and Ig isotypes.	Administration of CpG ODN through skin is a simple strategy for patients with diseases like atopic dermatitits, which is characterized by Th2-dominated inflammation.	Inoue et al. (81,82), 2005, 2006
Effect of sucrose esters (sucrose oleate and sucrose laureate in water or Transcutol®, TC) on the SC barrier properties *in vivo.* Impact of these molecules on the *in vivo* percutaneous penetration of 4-hydroxybenzonitrile (4-HB).	A combination of sucrose esters (oleate or laureate) and TC is able to temporarily alter the SC barrier properties, thereby promoting 4-HB penetration.	Ayala-Bravo et al. (83), 2003
Absorption of 4-cyanophenol (4CP) in humans using TS experiments to assess the conditions under which diffusion alters tape stripping results.	Chemical concentrations in TSs can be affected by diffusion during tape stripping, but with $t_{TS} < 0.2\ t_{lag}$ and an exposure time $> 0.3\ t_{lag}$, TS concentrations are not significantly	Reddy et al. (84), 2002

	affected by t_{TS}.	
Development of a sensitive method for the determination of polyethylene glycols with different molecular weights (MW) in the human SC obtained by TS.	The method showed to be suitable for studying permeability in normal and impaired skin with respect to MW in the range of 150–600 Da.	Jakasa et al. (85, 86), 2004, 2007
Dependence of permeability on molecular weight with different forms of barrier disruption.	Irrespectively of the form of barrier disruption, not only higher amounts, but also more varieties of chemicals (larger molecules) may penetrate into the skin in the presence of a compromised barrier, compared with normal skin.	Tsai et al. (87), 2003
Penetration of octyl methoxycinnamate (OMC) encapsulated in poly(Є-caprolactone) nanoparticles, into and across porcine ear skin *in vitro.*	Nanoparticulate encapsulation of OMC increased its "availability" within the SC.	Alvarez-Román et al. (88), 2004
In vivo distribution profile of OMC contained in nanocapsules (NCs) through the SC. Comparison with a nanoemulsion (NE) and a conventional o/w emulsion (EM).	NE increased the extent of OMC penetration relative to the penetration achieved by NCs or EM.	Olvera-Martínez et al (26), 2005
Quantification of four common sunscreen agents, namely 2-hydroxy-4 methoxybenzophenone, 2-ethylhexyl-*p*-methoxycinnamate, 2-ethylhexylsalicylate (octylsalicylate) and salicylic acid 3,3,5-trimethcyclohexyl ester in a range of biological matrices.	A preliminary clinical study demonstrates a significant penetration of all sunscreen agents into the skin, as well as of oxybenzone and its metabolites across the skin.	Sarveiya et al. (89), 2004
Amount of sunscreen present on the skin of people at the beach.	The best protected areas were the upper arm and décolleté, but even in these areas, most volunteers had applied only 10% of the COLIPA standard amount.	Lademann et al (90), 2004
Penetration of titanium dioxide (TiO_2) and methylene bis-benzotriazoyltetramethylbutyl-phenol (MBBT), included in a broad-spectrum sunscreen formulation, into human skin in vivo, using the TS method, and *in vitro*, using a compartmental approach.	*In vivo* and *in vitro* penetration studies showed an absence of TiO_2 penetration into viable skin layers through either transcorneal or transfollicular pathways, and a negligible transcutaneous absorption of MBBT.	Mavon et al. (91), 2007
In vitro human skin permeation and distribution of geranyl nitrile (GN)	Systemic exposure resulting from the use of GN as a fragrance ingredient, under unoccluded conditions, would be	Brian et al. (92), 2007

	low based on the currently reported use levels.	
Development of a method to investigate the effectiveness of reservoir closure by different formulations. Model penetrant: Patent Blue V.	Application of barrier creams cannot replace other protective measures and should be maximally used to inhibit low-grade irritants or in combination with other protectants, or in body areas where other protective measures are not applicable.	Teichmann et al. (93), 2006
Penetration of highly hydrophilic and lipophilic dyes into the skin using pure oil or water, comparing them with an o/w emulsion.	Differences in the distribution and the localization of both dyes within the SC were observed. These differences depend on the physicochemical properties of both the vehicles and the dyes.	Jacobi et al. (94), 2006

2) Dermal absorption of toxic or irritant chemicals

Research	Outcome	Author (Ref.)-Year
Development and testing of a simple, non-invasive dermal sampling technique on human volunteers under laboratory conditions to estimate acute dermal exposure to jet fuel (JP-8).	Naphthalene has a short retention time in the human SC and the tape stripping method, if used within 20 min of the initial exposure, can be employed to measure the amount of naphthalene in the SC due to a single exposure to jet fuel.	Mattorano et al. (36), 2004
Normalization of extracted concentrations of naphthalene (as a marker for jet fuel exposure) from human volunteers, before and after exposure to jet fuel (JP-8). Removal and quantification of keratin by stratum corneum TS	The amount of keratin removed with tape strips was not affected by an exposure of up to 25 min to JP-8, and there was a substantial decrease in the amount of keratin removed with consecutive tape strippings from the same site; thus, adjusting the amount of naphthalene to the amount of keratin measured in a tape-strip sample should improve the interpretation of the amount of this analyte by using this sampling approach.	Chao et al. (37), 2004
Description of a physiologically based pharmacokinetic (PBPK) model developed to simulate the absorption of organophosphate pesticides, such as parathion, fenthion, and methyl parathion, through porcine skin with flow-through cells.	The study demonstrated the utility of PBPK models for studying dermal absorption, which can be useful as explanatory and predictive tools.	Van der Merwe et al. (95), 2006
Study of whether the sodium lauryl sulphate (SLS) penetration rate into the SC is related to an	Variation in barrier impairment and inflammation of human skin depends on SLS penetration rate, which was	Jongh et al (96), 2006

impairment of skin's water barrier function and inflammation.	mainly determined by SC thickness.	
Modification and testing of a vacuuming sampler for removing particles from the skin.	Agreement between the vacuuming sampler and the TS technique.	Lundgren et al. (97), 2006

3) Evaluation of factors that influence the physiology of the stratum corneum.

Research	Outcome	Author (Ref.)
Effect of gender on the physiology of the SC.	The skin of women was characterized by a significantly higher pH value (5.6±0.4) than that of men (4.3±0.4). Protein absorption was the only other parameter significantly dependent on gender.	Jacobi et al. (105), 2005
Influence of procedures inherent to each stripping protocol on changes in skin physiology.	Skin hydration was not influenced by the variables tested.	Löffler et al. (43), 2004
Efficacy of TS in removing complete cell layers from the superficial part of human SC.	Furrows in the skin can present difficulties when performing depth penetration studies. Although the largest part of the skin surface will be stripped properly, it has to be realized that small areas, represented by furrows, may still contain high concentrations of the substance applied.	Van der Molen et al. (44), 1997
Local changes in the ultrastructure of human skin after iontophoresis in human skin *in vitro* and *in vivo*.	No drastic changes in the ultrastructure of the SC were observed.	Fatouros et al. (106), 2006

4) Stratum corneum composition

Research	Outcome	Author (Ref.)-Year
Establishment of a suitable analytical method for the determination of the local SC lipid composition.	Study of the differences in the SC lipid profile in healthy and diseased human skin relative to the SC lipid organization and to the skin barrier function *in vivo*.	Weerheim et al. (107), 2001

5) Stratum corneum thickness

Research	Outcome	Author (Ref.)-Year
Evaluated, using attenuated total reflectance Fourier transform infrared spectroscopy, the SC bioavailability of terbinafine (TBF) following topical treatment with four different formulations, based	It was found that the formulation containing 5% oleic acid significantly enhanced the SC availability of TBF.	Alberti et al. (108),2001

on a vehicle consisting of 50% ethanol and 50% isopropyl myristate.		
Determined whether a structurally heterogeneous biomembrane, human SC, behaved as a homogeneous barrier to water transport.	The variation of TEWL as a function of SC removal behaved in a manner entirely consistent with a homogeneous barrier, thereby permitting the apparent SC diffusivity of water to be found.	Kalia et al. (109), 1996

6) Determination of superficial infections and viruses

Research	Outcome	Author (Ref.)- Year
New animal model for the purpose of studying superficial infections.	Evaluation of novel antimicrobial treatments for superficial infections caused by *S. aureus* and *S. pyogenes*.	Kugelberg et al. (110), 2005
Immunocompetent patients were tested for human papilloma virus (HPV) DNA in swab samples collected on top of skin tumors and in biopsies of the same tumors, obtained after stripping with tape to remove superficial layers.	HPV DNA is common in superficial layers of lesions, but is not necessarily present in tumors.	Forslund et al. (111), 2004

7) Skin regeneration

Research	Outcome	Author (Ref.)-Year
Expression of tight junction components during the reepithelialization of suction blisters and the regeneration of the corneal layer after TS.	Tight junctions of regenerating epidermis may provide a functional barrier prior to regeneration of the corneal layer.	Malminen et al. (112), 2003
In vitro model for the developing skin of the premature neonate.	Porcine skin, *in vitro*, tape-stripped to a particular level, can provide a barrier corresponding to a specific degree of neonate maturation, and thus, can serve as a useful tool to explore whether transdermal drug delivery in this unique patient population may be beneficial.	Sekkat et al. (113), 2002
An *in vivo* human model was utilized to define the irritation potential of a topical agent after partial removal of the stratum corneum by cellophane tape stripping	This model provides a method for the prediction, with exaggerated sensitivity, of chemical irritation and proclivity to enhance or retard water barrier repair.	Zhai et al. (114), 1998

REFERENCES

[1]. Bommannan D, Potts RO, Guy RH. Examination of stratum corneum barrier function in vivo by infrared spectroscopy. *J Invest Dermatol* 95: 403–408, (1990).

[2]. Higo N, Naik A, Bommannan DB, Potts RO, Guy R. H. Validation of reflectance infrared spectroscopy as a quantitative method to measure percutaneous absorption in vivo. *Pharm Res.*10:1500–1505, (1993).

[3]. Lotte C, Wester RC, Rougier A, Maibach HI. Racial differences in the *in vivo* percutaneous absorption of some organic compounds: a comparison between black, Caucasian and Asian subjects. *Arch Dermatol Res.* 284:456–459, (1993).

[4]. \Pershing LK, Silver BS, Krueger GG, Shah VP, Skelley JP. Feasibility of measuring the bioavailability of topical betamethasone dipropionate in commercial formulations using drug content in skin and a skin blanching bioassay. *Pharm Res.* 9:45–51, (1992).

[5]. Rougier A, Dupuis D, Lotte C, Roguet R, Wester RC, Maibach HI. Regional variation in percutaneous absorption in man: measurement by the stripping method. *Arch Dermatol Res.* 278:465–469, (1986).

[6]. Tojo K, Lee AC. A method for prediction of steady-state of skin penetration in vivo. *J Invest. Dermatol.,* 92:105–108, (1989).

[7]. Escobar-Chávez JJ, Quintanar-Guerrero D, and Ganem-Quintanar A. *In vivo* skin permeation of sodium naproxen formulated in PF-127 gels: Effect of Azone® and Transcutol®, *Drug Develop Ind Pharm.* 31:447-454, (2005).

[8]. Pinkus H. Examination of the epidermis by the strip method of removing horny layers. I. Observation on thickness of the horny layer, and on mitotic activity after stripping. *J. Invest. Dermatol.,* 16:383–386, (1951).

[9]. King CS, Barton SP, Nicholls S, Marks R. The change in properties of the stratum corneum as a function of depth. *Br J Dermatol.,* 100:165–172, (1979). Repetido 43

[10]. Shah VP. Topical Dermatological Drug Product NDAs and ANDAs-*In Vivo* Bioavailability, Bioequivalence, *In Vitro* Release and Associated Studies, US Department of Health and Human Services, Rockville, (1998).

[11]. Conner DP: Differences in DPK Methods. http://www.fda.gov/ohrms/dockets/ac/01/slides/3804s2_05_conner/index.htm, Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, Maryland, November 29, (2001).

[12]. Franz TJ. Study #1, Avita Gel 0.025% vs Retin-A Gel 0.025%, Advisory committee for pharmaceutical sciences meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, Maryland, November 29, (2001).

[13]. Pershing LK. Bioequivalence assessment of three 0.025% tretinoin gel products: dermatopharmacokinetic vs clinical trial methods, advisory committee for pharmaceutical sciences meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA), Rockville, Maryland, November 29, (2001).

[14]. Touitou E, Meidan VM, Horwitz E. Methods for quantitative determination of drug localized in the skin. *J Control Rel.* 56:7–21, (1998).

[15]. Moser K, Kriwet K, Naik A, Kalia YN, Guy R. H. Passive skin penetration enhancement and its quantification in vitro. *Eur J Pharm Biopharm.* 52:103-112, (2001).

[16]. Pinkus H. Tape stripping in dermatological research. A review with emphasis on epidermal biology. *G Ital Dermatol Minerva Dermatol.* 107(5):1115-26, (1966).

[17]. Forslind BA. Domain mosaic model of skin barrier. *Acta Derm Venereol.* 74:1-6, (1994).

[18]. Potts RO, Francoeur ML. The influence of stratum corneum morphology on water permeability. *J Invest Dermatol*. 96, 495-499, (1991).

[19]. Potts RO, Guy RH. Predicting skin permeability. *Phar. Res,* 9(5): 663-669, (1992).

[20]. Ellias PM. Epidermal barrier function: intercellular lamellar lipid structures, origin, composition and metabolism, *J Control Rel.* 15, 199-208, (1991).

[21]. Barry BW. Dermatological Formulations: Percutaneous Absorption. In: Swarbick J, ed. Drugs and the Pharmaceutical Sciences. New York and Basel: Marcel Dekker, Inc. p.202, (1983).

[22]. Hadgraft J. Skin, the final frontier. *Int J Pharm.* 224 (1-2):1-18, (2001).

[23]. Guy RH and Hadgraft J. Transdermal drug delivery. New York: Marcel Dekker, Inc., p. 1-23, (2003).

[24]. Walters KA and Roberts MS. Dermatological and transdermal formulations. New York: Marcel Dekker, Inc., p. 1-39, (2002).

[25]. Nevill AM. The need to scale for differences in body size and mass: and explanation of Klieber´s 0.75 mass exponent. *Am Physiol Soc.* 2870-2873, (1994).

[26]. Olvera-Martínez BI, Cazares-Delgadillo J, Calderilla-Fajardo SB, Villalobos-García R, Ganem-Quintanar A, Quintanar-Guerrero D. Preparation of polymeric nanocapsules containing octyl methoxycinnamate by the emulsification–diffusion technique: Penetration across the stratum corneum. *J Pharm Sci.* 94:1552–1559, (2005).

[27]. Escobar-Chávez JJ, López-Cervantes M, Naïk A, Kalia YN, Quintanar-Guerrero D, Ganem-

Quintanar A. Applications of the thermoreversible Pluronic F-127 gels in pharmaceutical formulations, *J Pharm Pharmaceut Sci.* 9(3):339-358, (2006).

[28]. Miyazaki S, Yokouchi Ch, Nakamura T, Hashiguchi N, Hou W-M, and Takada M. Pluronic F-127 gels as a novel vehicle for rectal administration of indomethacin, *Chem Pharm Bull.*34, 1801-1808, (1986).

[29]. Chi SCh, Do K, Tan HK, and Chun HW. Anti-inflammatory and analgesic transdermal gel, *United States Patents.*, Patent number 5,527,832, (1996).

[30]. Fang JY, Leu YL, Wang YY, and Tsai YH. *In vitro* topical application and *in vivob* phramacodynamic evaluation of nonivamide hydrogels using Wistar rat as an animal model, *Eur. J Pharm Sci.* 15(5):417-423, (2002).

[31]. Shin SC, Cho CW, and Oh IJ. Effects of non ionic surfactants as permeation enhancers towards piroxicam from the poloxamer gel through rat skins, *Int J Pharm.* 222(2), 199-203, (2001).

[32]. Liaw J, and Lin Y-Ch. Evaluation of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) gels as a release vehicle for percutaneous fentanyl, *J Control. Rel.* 68:273-282, (2000).

[33]. Wang YY, Hong CT, Chiu WT, and Fang JY. *In vitro* and *in vivo* evaluations of topically applied capsaicin and nonivamide from hydrogels, *Int J Pharm.* 224,1-2, (2001).

[34]. Kattan El, A. F., Asbill, C. S., Kim, N., and Michniak, B. B. Effect of formulation variables on the percutaneous permeation of ketoprofen from gel formulations, *Drug Deliv.* 7,3, (2000).

[35]. Curdy C, Kalia YN, Naïk A, Guy RH. Piroxicam delivery into human stratum corneum in vivo: iontophoresis versus passive diffusion. *J Control Rel.* 76, 73-79, (2001).

[36]. Mattorano DA, Kupper LL, Nylander-French LA. Estimating dermal exposure to jet fuel (naphthalene) using adhesive tape strip samples. *Ann Occup Hyg*. 48(2): 139–146, (2004).

[37]. Chao Y-Ch, Nylander-French LA. Determination of Keratin Protein in a Tape-stripped Skin Sample from Jet Fuel Exposed Skin. *Ann Occup Hyg*. 48(1):65–73, (2004).

[38]. Tsai J-C, Weiner ND, Flynn GL, Ferry J. Properties of adhesive tapes used for stratum corneum stripping, *Int J Pharm.* 72: 227-231, (1991).

[39]. Sheth NV, McKeough MB, Spruance SL. Measurement of the stratum corneum drug reservoir to predict the therapeutic efficacy of topical iododeoxyuridine for herpes simplex virus infection. *J Invest Dermatol.* 89: 598–602, (1987).

[40]. Ohman H, Vahlquist A. In vivo studies concerning a pH gradient in human stratum corneum and upper epidermis. *Acta Derm Venereol (Stockh).* 74: 375–379, (1994).

[41]. Bashir, S. J., Chew, A. L., Anigbogu, A. Physical and physiological effects of stratum corneum tape stripping. *Skin Res Technol.* 7:40–48, (2001).

[42]. Marttin E, Neelissen-Subnel MTA, De Haan FHN, Boddé HE. A critical comparison of methods to quantify stratum corneum removed by tape-stripping. *Skin Pharmacol.* 9:69–77, (1996).

[43]. Löffler H, Dreher F, Maibach HI. Stratum corneum adhesive tape stripping: influence of anatomical site, application pressure, duration and removal. *Br J Dermatol.* 151: 746–752, (2004).

[44]. Van der Molen RG, Spies F, Van ‘t Noordende JM, Boelsma E, Mommaas AM, Koerten HK. Tape stripping of human stratum corneum yields cell layers that originate from various depths because of furrows in the skin. *Arch Dermatol Res.* 289:514–518, (1997).

[45]. Surber C, Schwarb FP, Fmith EW. Tape stripping technique. In: Percutaneous Absorption – Drug – Cosmetics – Mechanisms – Methodology (Bronough H, Maibach HI, eds), 3rd ed. New York: Marcel Dekker, 395–409, (1999).

[46]. Nangia A, Camel E, Berner B. Influence of skin irritants on percutaneous absorption. *Pharm Res.*10:1756–1759, (1993).

[47]. Van Voorst Vader PC, Lier JG, Woest TE. Patch tests with house dust mite antigens in atopic dermatitis patients: methodological problems. *Acta Derm. Venereol (Stockh).*,71:301–305, (1991).

[48]. Surakka J, Johnsson S, Rosen G. A method for measuring dermal exposure to multifunctional acrylates. *J Environ Monit.*1:533–540, (1999).

[49]. Kondo H, Ichikawa Y, Imokawa G. Percutaneous sensitization with allergens through barrier-disrupted skin elicits a Th2-dominant cytokine response. *Eur J Immunol.* 28: 769–779, (1998).

[50]. Fluhr JW, Gloor M, Lehmann L. Glycerol accelerates recovery of barrier function in vivo. *Acta Derm Venereol (Stockh).* 79:418–21, (1999).

[51]. Pechere M, Krischer J, Remondat C. Malassezia spp. Carriage in patients with seborrheic dermatitis. *J Dermatol.* 26:558–61, (1999).

[52]. Pechere M, Remondat C, Bertrand C. A simple quantitative culture of Malassezia spp. in HIV-positive persons. *Dermatology.*191:348–349, (1995).

[53]. Ghadially R, Brown BE, Sequeira-Martin SM. The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. *J Clin Invest.*95:2281–90, (1995).

[54]. Van der Valk PG, Maibach HI. A functional study of the skin barrier to evaporative water loss by means of repeated cellophane-tape stripping. *Clin Exp Dermatol.*15:180–2, (1990).

[55]. Hostynek JJ, Dreher F, Pelosi A. Human stratum corneum penetration by nickel. In vivo study of depth distribution after occlusive application of the metal as powder. *Acta Derm Venereol (Stockh).*212 (Suppl.):5–10, (2001).

[56]. Lademann J, Otberg N, Richter H. Investigation of follicular penetration of topically applied substances. *Skin Pharmacol Appl Skin Physiol.* 14 (Suppl.1):17–22, (2001).

[57]. Wilhelm KP, Surber C, Maibach HI. Effect of sodium lauryl sulfate- induced skin irritation on *in vivo* percutaneous penetration of four drugs. *J Invest Dermatol.* 97:927–32, (1991).

[58]. Schafer U. Topical Absorption of Dermatological Products. Robert L. Bronaugh and Howard I. Maibach (Editors), Marcel Dekker, New York, Basel, 544 p, (2001).

[59]. Shah VP, Flynn GL, Yacobi A, Maibach HI, Bon C, Fleischer NM, Franz TJ, Kaplan SAJK, Lesko LJ, Marty JP, Pershing LK, Schaefer H, Sequeira JA, Shrivastava SP, Wilkin J, Williams RL. Bioequivalence of topical dermatological dosage forms-methods of evaluation of bioequivalence. *Pharm Res.* 15, 167–171, (1998).

[60]. Surakka J, Lindh T, Rosen G. Workers' dermal exposure to UV-curable acrylates in the furniture and parquet industry. *Ann Occup Hyg*. 44:635–44, (2000).

[61]. Nylander-French LA. A tape-stripping method for measuring dermal exposure to multifunctional acrylates. *Ann Occup Hyg.* 44:645–51, (2000).

[62]. Howes D, Guy R, Hadgraft J, Heylings J, Hoeck U, Kemper F, Maibach H, Marty JP, Merk H, Parra J, Rekkas D, Rondelli I, Schaefer H, Täuber U, Verbiese N. Methods for assessing percutaneous absorption. The report and recommendations of ECVAM workshop 13. ATLA 24, 81–106, (1996).

[63]. Arima H, Miyajib T, Irie T, Hirayama F, Uekamaa K. 1998. Enhancing effect of hydroxypropyl-P-cyclodextrin on cutaneous penetration and activation of ethyl 4-biphenylyl acetate in hairless mouse skin. *Eur J Pharm Sci.* 6:53-59, (1998).

[64]. Esposito E, Cortesi R, Drechsler M, Paccamiccio L, Mariani P, Contado C, Stellin E, Menegatti E, Bonina F, Puglia C. Cubosome dispersions as delivery systems for percutaneous administration of indomethacin. *Pharm Res.,* 22(12):2163-2173, (2005).

[65]. Ganem-Quintanar A, Silva-Alvarez M, Alvarez-Roman R, Casas-Alancaster N, Cazares-Delgadillo J, Quintanar-Guerrero D. Design and evaluation of a self –adhesive naproxen-loaded film prepared from a nanoparticle dispersión. *J Nanosci Nanotechnol*. 6(9-10):3235-3241, (2006).

[66]. Herkenne C, Naik A, Kalia YN, Hadgraft J, Guy RH. Pig ear skin ex vivo as a model for in vivo dermatopharmacokinetic studies in man. *Pharm Res*. 23(8), 1850-1856, (2006).

[67]. Hostýnek JJ, Dreher F, Maibach HI. 2006. Human stratum corneum penetration by copper: In vivo study after occlusive and semi-occlusive application of the metal as powder. *Food Chem Toxicol.* 44:1539–1543, (2006).

[68]. Lodén M, Akerstrom U, Lindahl K, Berne B. 2004. Bioequivalence determination of topical ketoprofen using a dermatopharmacokinetic approach and excised skin penetration. *Int J Pharm.* 284:23–30, (2004).

[69]. Wagner S and Merfort I. Skin penetration behaviour of sesquiterpene lactones from different *Arnica* preparations using a validated GC-MSD method. *J Pharm Biomed Anal. In press,* (2006).

[70]. Wagner H, Kostka K-H, Lehr C-M, Schaefer UF. Correlation between stratum corneum/water-partition coefficient and amounts of flufenamic acid penetrated into the stratum corneum. *J Pharm Sci.* 91(8):1915-1921, (2002).

[71]. Pellanda C, Ottiker E, Strub C, Figueiredo V, Rufli T, Imanidis G, Surber C. Topical bioavailability of triamcinolone acetonide: Effect of dose and application frequency. *Arch Dermatol Res.* 298:221–230, (2006).

[72]. Lboutounne H, Chaulet JF, Ploton C, Falson F, Pirot F. Sustained ex vivo skin antiseptic activity of chlorhexidine in poly(e-caprolactone) nanocapsule encapsulated form and as a digluconate. *J Control Rel.*82:319–334, (2002).

[73]. Fresno-Contreras MJ, Jiménez-Soriano MM, Ramírez-Diéguez A. In vitro percutaneous absorption of all-*trans* retinoic acid applied in free form or encapsulated in stratum corneum lipid liposomes. *Int J Pharm.* 297:134–145, (2005).

[74]. Padula C, Colombo G, Nicoli S, Catellani PL, Massimo G, Santi P. Bioadhesive film for the transdermal delivery of lidocaine: in vitro and in vivo behaviour. *J Control Rel.* 88: 277–285, (2003).

[75]. Bashir SJ, Dreher F, Chew AL, Zhai H, Levina C, Stern R, Maibach HI. 2005. Cutaneous bioassay of salicylic acid as a keratolytic. *Int J Pharm.* 292:187–194, (2005).

[76]. Abdulmajed K and Heard ChM. Topical delivery of retinyl ascorbate co-drug 1. Synthesis, penetration into and permeation across human skin. *Int J Pharm*. 280:113–124, (2004).

[77]. Hara M, Verkman AS. Glycerol replacement corrects defective skin hydration, elasticity, and barrier function in aquaporin-3-deficient mice. *PNAS.* 100(12):7360–7365, (2003).

[78]. Morgan CJ, Renwick AG, Friedmann PS. The role of stratum corneum and dermal microvascular perfusion in penetration and tissue levels of water-soluble drugs investigated by microdialysis. *Br J Dermatol.* 148:434–443, (2003).

[79]. Jarvis CA, McGuigan C, Heard CM. In vitro delivery of novel, highly potent anti-Varicella Zoster virus nucleoside analogues to their target site in the skin. *Pharm Res.* 21(6):914-919, (2004).

[80]. Strid J, Hourihane J, Kimber I, Callard R, Strobel S. Disruption of the stratum corneum allows potent epicutaneous immunization with protein antigens resulting in a dominant systemic Th2 response. *Eur J Immunol.*34:2100–9, (2004).

[81]. Inoue J, Yotsumoto S, Sakamoto T, Tsuchiya S, Aramaki Y. Changes in immune responses to antigen applied to tape-stripped skin with CpG oligodeoxynucleotide in mice. *J Control Rel.* 108:294– 305, (2005).

[82]. Inoue, J. and Aramaki, Y. 2006. Toll-like receptor-9 expression induced by tape-stripping triggers on effective immune response with CpG-oligodeoxynucleotides. *Vaccine*. In press, (2006).

[83]. Ayala-bravo HA, Quintanar-Guerrero D, Naik A, Kalia YN, Cornejo-Bravo JM, Ganem-Quintanar A. 2003. Effects of sucrose oleate and sucrose laureate on *in vivo* human stratum corneum permeability. *Pharm Res.*20 (8):1267-1273, (2003).

[84]. Reddy MB, Stinchcomb AL, Guy RH, Bunge AL. Determining dermal absorption parameters *in vivo* from tape strip data. *Pharm. Res.,* 19(3):292-298, (2002).

[85]. Jakasa I, Calkoen F, Kezic S. Determination of polyethylene glycols of different molecular weight in the stratum corneum. *J Chromatogr B*. 811:177–182, (2004).

[86]. Jakasa I, Verbek MM, Esposito M, Bos JD, Kezic S. Altered penetration of polyethylene glycols into uninvolved skin of atopic dermatitis patients. *J Investigative Dermatol.* 127:129–134, (2007).

[87]. Tsai J-C, Shen L-C, Sheu HM, Lu C-C. Tape stripping and sodium dodecyl sulfate treatment increase the molecular weight cut-off of polyethylene glycol penetration across murine skin. *Arch Dermatol Res.* 295:169–174, (2003).

[88]. Alvarez-Román R, Naik A, Kalia YN, Guy RH, Fessi H. Enhancement of topical delivery from biodegradable nanoparticles. *Pharm Res*. 21(10):1818-1825, (2004).

[89]. Sarveiya V, Risk S, Benson HAE. Liquid chromatographic assay for common sunscreen agents: application to in vivo assessment of skin penetration and systemic absorption in human volunteers. *J Chromatogr B.* 803:225–231, (2004).

[90]. Lademann J, Schanzer S, Richter H, Pelchrzim RV, Zastroe L, Golz K, Sterry W. Sunscreen application at the beach. *J Cosmet Dermatol.*3(2):62-68, (2004).

[91]. Mavon A, Miguel C, Lejeune O, Payre B, Moretto P. In vitro percutaneous absorption and in vivo stratum corneum distribution of an organic and mineral sunscreen. *Skin Pharmacol Physiol.* 20(1):10-20, (2007).

[92]. Brian KR, Green DM, Lalko J, Api AM. In-vitro human skin penetration of the fragrance material geranyl nitrile. *Toxicology in Vitro* 21:133–138, (2007).

[93]. Teichmann A, Jacobi U, Waibler E, Sterry W, Lademann J. An in vivo model to evaluate the efficacy of barrier creams on the level of skin penetration of chemicals. *Contact Dermatitis.* 54:5–13, (2006).

[94]. Jacobi U, Tassopoulos T, Surber C, Lademann J. Cutaneous distribution and localization of dyes affected by vehicles all with different lipophilicity. *Arch Dermatol Res.* 297:303–310, (2006).

[95]. Van der Merwe D, Brooks JD, Gehring R, Baynes RE, Monteiro-Riviere NA, Riviere JE. A physiologically based pharmacokinetic model of organophosphate dermal absorption. *Toxicol Sci*. 89(1):188–204, (2006).

[96]. De Jongh CM, Jakasa I, Verberk MM, Kezic S. Variation in barrier impairment and inflammation of human skin as determined by sodium lauryl sulphate penetration rate. *Br J Dermatol.* 154:651–657, (2006).

[97]. Lundgren L, Skare L, Lidén C. Measuring dust on skin with a small vacuuming sampler—A comparison with other sampling techniques. *Ann Occup Hyg.*50(1):95–103, (2006).

[98]. Leveque JL, Corcuff P, de Rigal J, Agache, P. In vivo studies of the evolution of physical properties of the human skin with age. *Int J Dermatol.* 23:322–329, (1984).

[99]. Denda M, Koyama J, Hori J, Horii I, Takahashi M, Hara M, Tagami H. Age- and sex-dependent change in stratum corneum sphingolipids. *Arch Dermatol Res.* 285:415–417, (1993).

[100]. Somerville DA. The normal flora of the skin in different age groups. *Br J Dermatol.* 81:248–258, (1980).

[101]. Schurer NY, Plewig G, Elias PM. Stratum corneum lipid function. *Dermatologica.* 183:77–94, (1991).

[102]. Cua AB, Wilhelm KP, Maibach HI. Skin surface lipid and skin friction: Relation to age, sex and anatomical region. *Skin Pharmacol*. 8:246–251, (1995).

[103]. Marples RR. Sex, constancy, and skin bacteria. *Arch Dermatol Res.* 272: 317–320, (1982).

[104]. Fluhr JW, Dickel H, Kuss O, Weyher I, Diepgen TL, Berardesca E. Impact of anatomical location on barrier recovery, surface pH and stratum corneum hydration after acute

barrier disruption. *Br J Dermatol.* 146: 770–776, (2002).

[105]. Jacobi U, Gautier J, Sterry W, Lademann J. Gender-Related Differences in the Physiology of the Stratum Corneum. *Dermatology.* 211:312–317, (2005).

[106]. Fatouros DG, Groeninka HWM, De Graaff AM, Van Aelst AC, Koertenc HK, Bouwstra JA. Visualization studies of human skin in vitro/in vivo under the influence of an electrical field. *Eur J Pharm Sci.*29:160–170, (2006).

[107]. Weerheim A. Ponec M. Determination of stratum corneum lipid profile by tape stripping in combination with high-performance thin-layer chromatography. *Arch Dermatol Res.*293:191–199, (2001).

[108]. Alberti, I., Kalia, Y. N., Naik, A., Bonny, J. D., & Guy, R. H. In vivo assessment of enhanced topical delivery of terbinafine to human stratum corneum. J Control Rel. 71(3), 319–327, (2001).

[109]. Kalia, Y. N., Pirot, F., & Guy, R. H. Homogeneous transport in a heterogeneous membrane: water diffusion across human stratum corneum in vivo. *Biophys J.* 71(5), 2692–2700, (1996).

[110]. Alouf JE, Muller-Alouf H. Staphylococcal and streptococcal superantigens: molecular, biological and clinical aspects. *Int J Med Microbiol.*292:429–440, (2003).

[111]. George A, Rubin G. A systematic review and meta-analysis of treatments for impetigo. *Br J Gen Pract.* 53:480-487, (2003).

[112]. Kugelberg E, Norström T, Petersen TK, Duvold T, Andersson DI, Hughes D. Establishment of a superficial skin infection model in mice by using *Staphylococcus aureus* and *Streptococcus pyogenes. Antimicrob Agents Chem.*49(8):3435–3441, (2005).

[113]. Forslund O, Lindelöf B, Hradil E, Nordin-Stenquist B, Kirnbauer R, Slupetzky K, Dillner J. High prevalence of cutaneous human papillomavirus DNA on the top of skin tumors but not in ‘‘Stripped’’ biopsies from the same tumors. *J Invest Dermatol.*123:388-394, (2004).

[114]. Malmminen M, Koivukangas V, Peltonen J, Karvonen S-L, Oikarinen A, Peltonen S. Immunohistological distribution of the tight junction components ZO-1 and occludin in regenerating human epidermis. *Br J Dermatol.* 149:255–260, (2003).

[115]. Sekkat N, Kalia YN, Guy RH. Biophysical study of porcine ear skin in vitro and its comparison to human skin in vivo. *J Pharm Sci.* 91(11):2376-2381, (2002).

[116]. Zhai H, Poblete N, and Maibach HI. Stripped skin model to predict irritation potential of topical agents in vivo in humans. Int J Dermatol, 37(5), 386-389, (1998).

[117]. Choi MJ, Zhai H, Kim J-H, Maibach HI. Tape stripping method versus stratum corneum. In: Zhai H, Wilhelm KP, & Maibach HI (eds.): Dermatotoxicology, 7th edition.CRC Press, Boca Raton, 327-337, (2008).

[118]. Löffler H, Weimer C, Dreher F, Maibach HI. Parameters influencing stratum corneum removal by tape stripping. In: Zhai H, Wilhelm KP, & Maibach HI (eds.): Dermatotoxicology, 7th edition. CRC Press, Boca Raton, 339-342, (2008).