15 The Correlation Between Transepidermal Water Loss and Percutaneous Absorption: An Overview

Jackie Levin
AZCOM, Glendale, Arizona, U.S.A.

Howard I. Maibach
Department of Dermatology, University of California School of Medicine, San Francisco, California, U.S.A.

INTRODUCTION

Transepidermal water loss (TEWL) is the outward diffusion of water through skin (1). TEWL measurements are used to gauge the skin's water barrier function. An increase in TEWL reflects impairment of the water barrier (2). TEWL measurements allow parametric evaluation of the effect of barrier creams against irritants and characterization of skin functionality in clinical dermatitis and in irritant and allergic patch test reactions (3). An evaporimeter determines TEWL by measuring the pressure gradient of the boundary layer, resulting from the water gradient between the skin surface and ambient air. TEWL measurements can be affected by the anatomical site, sweating, skin surface temperature, inter-and intraindividual variation, air convection, ambient air temperature and humidity, and instrument-related variables, to name a few. Although TEWL is influenced by many variables, experiments show that evaporimeter measurements are reproducible in vitro and in vivo (3,4).

Percutaneous Absorption

Percutaneous absorption refers to the rate of absorption of a topically applied chemical through the skin. A compound's absorption rate is important for determining the effectiveness and/or potential toxicity of topically applied compounds. Since many topical formulations are used on diseased skin, where the integrity of the permeability barrier is in doubt, the dose absorbed into the body could vary greatly (5). The rate of absorption in vivo through the stratum corneum (SC) cannot be described by a zero-or first-order mathematical rate equation, because the SC is a complex system variable in its penetration properties. Many factors contribute to the percutaneous absorption of a given chemical. One rate limiting the step of a compound's absorption through the skin is the rate of diffusion through the SC. This review discusses the three main categories that give rise to percutaneous absorption rate variation, namely, methodology (including the effects of application time, method of measurement, and physicochemical properties of the topical compound), interindividual variation (including the effects of skin condition, age of individual, and blood flow), and intraindividual variation (including the differences between anatomic sites) (6,7).

Why Do We Want to Correlate TEWL and Percutaneous Absorption?

The extensive procedure required to measure percutaneous absorption versus TEWL enhances the desire to find a correlation between the two measurements to more easily assess skin barrier function. Experimentation of the correlation between TEWL and percutaneous absorption has resulted in studies concluding significant quantitative correlation and a few concluding no quantitative correlation.

The majority of studies investigating TEWL and percutaneous absorption correlation observe a quantitative correlation. It is our hypothesis that the papers that did not observe a quantitative correlation (8,9) or observed a weak correlation (1,10) do so because of assumptions made in the experiment's design.

Many of the experiments investigating TEWL and percutaneous absorption make large assumptions, which could affect the results of experimentation, and hence be the source of the controversy. For example, Tsai et al. (11) and Chilcott et al. (9) assume that an in vitro

Table 1 Summary of the Permeability and Lipophilicity of all the Compounds Tested on the Barrier-Disrupted Hairless Mouse

Compound	Partition coefficient ($K_{o/w}$)	Correlation coefficient (r)
Sucrose	−3.7	0.82
Caffeine	−0.02	0.86
Hydrocortisone	1.5	0.82
Estradiol	2.7	0.72
Progesterone	3.9	0.01

Source: From Ref. 11.

measurement of TEWL and percutaneous absorption is equivalent to in vivo measurements, whereas Lamaud et al. (12) assume that animal skin may serve as a permeability model for human skin. Great sources of error and variation can also be induced depending on the measurement method and type of absorption compound used in obtaining percutaneous absorption rates. As we do not completely understand the qualitative relationship between TEWL and percutaneous absorption, it is hard to determine, which assumptions made during the experiment could be affecting the correlation results. This section investigates the probable causes that could influence the results of the correlation experiments. Provided in Table 1 is a summary of the major assumptions made by the studies discussed.

In this section, we review some major studies defining the correlation between TEWL and percutaneous absorption and discuss major assumptions made in these experiments, which could significantly affect those studies that did not conclude a quantitative correlation. Provided in Table 2 is a summary of the major assumptions made by the studies discussed.

Main Review Correlation Studies

Oestmann et al. (1) investigated the correlations between TEWL and hexyl nicotinate (HN) penetration parameters in man. HN penetration was indirectly measured by laser Doppler flowmeter (LDF), which quantifies the increase in cutaneous blood flow (CBF) caused by the penetration of HN, a vasoactive substance. Lipophilic HN was chosen over hydrophilic methyl nicotinate because HN is a slower penetrant, hence, making it easier to distinguish an intact barrier from an impaired barrier.

Table 2 A Summary of the Major Assumptions Made by the Studies Discussed in This Review

Ref.	In vivo vs. in vitro (precabs)[a]	Skin type	Percutaneous absorption measurement method	Type of absorption compound[b]	Healthy skin versus damaged skin	Correlation results
(1)	Vivo	Human	LDF	Lipophilic	Healthy	Yes
(13)	Vivo	Human	Urinary	Lipophilic	Healthy	Yes
(14)	Vivo	Human	Urinary	Hydrophilic and lipophilic	Healthy	Yes
(15)	Vivo	Human	Plasma cortisol level	Lipophilic	Damaged	Yes
(11)[c]	Vitro	Animal	Diffusion cell	Hydrophilic and lipophilic	Damaged	Yes
(11)[c]	Vitro	Animal	Diffusion cell	Highly lipophilic	Damaged	No
(8)	Vivo	Human	LDF	Lipophilic	Damaged	Yes
(9)	Vivo	Animal	Urinary	Lipophilic	Both	Yes
(9)	Vitro	Both	Diffusion cell	Hydrophilic and lipophilic	Both	No

[a]As TEWL in vivo and in vitro measurements are considered equivalent, the authors are only concerned with how percutaneous absorption measurements were taken.
[b]Type of absorption compound was determined by their octanol-water partition coefficient, $K_{o/w}$ (Table 1). Values less than one are hydrophilic and more than three is very lipophilic.
[c]Reference Tsai et al. (11) was divided into two experiments in this table, as the study found a correlation between TEWL and percutaneous absorption with some compounds and no correlation with others.
Abbreviation: LDF, laser Doppler flowmeter.

LDF parameters t_0 and t_{max} were compared with corresponding TEWL values, and a weak quantitative negative correlation was made ($r = -0.31$ and -0.32). This correlation suggests that when an individual's response time, t_0, was fast, the skin barrier was impaired. The weak negative correlation found may be because of the percutaneous absorption method used. The LDF method has some negative attributes and is not as reproducible as other methods. Further research should investigate this weak correlation between TEWL and penetration of HN.

Lamaud et al. (12) investigated whether permeability changes of hydrophilic compounds (TEWL) are correlated to those of lipophilic compounds (hydrocortisone). In the first part of the experiment, penetration of 1% hydrocortisone and TEWL rates were recorded for the hairless rats in vivo before and after UV irradiation (660 J/cm^2). Both the results, before and after UV irradiation, correlated well with the TEWL values for application periods up to one hour.

In the second part, drug penetration was evaluated by urinary excretion five days after a single 24-hour application on normal, stripped, or UV-irradiated skin of hairless rats. The quantity of drug eliminated correlated with the level of TEWL for up to two days.

These results suggest that TEWL can predict the changes of skin permeability to lipophilic drugs in normal and some damaged skin.

Lavrijsen et al. (8) characterized the SC barrier function in patients with various keratinization disorders using two noninvasive methods, namely, measuring outward transport of water through skin by evaporimetry (TEWL) and the vascular response to HN penetration into the skin determined by LDF. Three of the five types of keratinization disorders studied, autosomal dominant ichthyosis vulgaris (ADI), X-linked recessive ichthyosis (XRI), and autosomal recessive congenital ichthyosis (CI), have impaired barrier function and are a type of ichthyosis, whereas the other two keratinization disorders studied, dyskeratosis follicularis (DD) and erythrokeratoderma variabilis (EKV), have no prior information available on barrier impairment. In this experiment, the two methods of barrier function assessment, TEWL and LDF, were correlated.

TEWL measurements and the LDF parameter, t_0, showed a high negative correlation in the patient group ($r = -0.64$) and a weaker negative correlation among the control group ($r = -0.39$). As TEWL reflects the SS-flux of a compound across SC, and parameter t_0 is a function of the duration of the lag phase (non-SS), this study suggests that these two methods should not be considered as exchangeable alternatives but rather as complementary tests. Each method reflects a different aspect of the barrier function.

This paper concludes that TEWL and HN penetration injunction are suitable methods to monitor skin barrier function in keratinization disorders and are helpful in discriminating between some of these disorders.

Rougier et al. (13) attempted to establish the relationship between the barrier properties of the horny layer (percutaneous absorption and TEWL) and the surface area of the corneocytes according to anatomic site, age, and sex in man. The penetration of benzoic acid (BA) was measured in vivo at seven anatomic sites and compared with its TEWL measurement taken on the contralateral site. The amount of BA penetrated was measured through urinary extraction up to 24 hours after application. It was discovered that irrespective of anatomic site and gender, a linear relationship ($r = 0.92$, $p < 0.001$) exists between total penetration of BA and TEWL.

Comparing corneocyte surface area to permeability, the study found a general correlation of increasing permeability for both H_2O and BA with decreasing corneocyte size. The smaller the volume of the corneocyte, the greater is the intercellular space available to act as a reservoir for topically applied molecules (10). This thinking is because of other studies that have shown that the smaller the capacity of the reservoir, the less the molecule is absorbed (10,14–16). However, for certain anatomic sites where corneocyte size was similar (980–1000 mm^2), there were large differences in permeability. Therefore, showing that, when percutaneous absorption and TEWL are quantitatively correlated, corneocyte size only partially explains the difference in permeability between the different anatomic sites and age of the skin.

Lotte et al. (17) examined the relationship between the percutaneous penetration of four chemicals (acetyl-salicylic acid, BA, caffeine, and sodium salt of BA) and TEWL in man as a function of anatomic site. The amount of chemical penetrated was measured by urinary excretion for up to 24 hours after application. For a given anatomic site, the permeability varies widely with the nature of the molecule administered because of the physicochemical

interactions that occur between the molecule, vehicle, and SC. For all anatomic sites investigated, irrespective of physicochemical properties of the molecules administered, there was a linear relationship between TEWL and percutaneous absorption.

Aalto-Korte and Turpeinen (18) attempted to find the precise relationship between TEWL and percutaneous absorption of hydrocortisone in patients with active dermatitis. Percutaneous absorption of hydrocortisone and TEWL were studied in three children and six adults with dermatitis. All the subjects had widespread dermatitis covering at least 60% of the total skin area. Plasma cortisol concentrations were measured before and two and four hours after hydrocortisone application by radioimmunoassay. TEWL was measured in six standard skin areas immediately before application of the hydrocortisone cream. Each individual TEWL value was calculated as a mean of these six measurements.

The concordance between the postapplication increment in plasma cortisol and mean TEWL was highly significant, resulting in a correlation coefficient of $r = 0.991$ ($p < 0.001$). In conclusion, this study found a highly significant correlation between TEWL and percutaneous absorption of hydrocortisone.

Tsai et al. (11) investigated the relationship between permeability barrier disruption and the percutaneous absorption of various compounds with different lipophilicity values. Acetone treatment was used in vivo on hairless mice to disrupt the normal permeability barrier, and in vivo TEWL measurements were used to gauge barrier disruption. The hairless mouse skin was then excised and placed in diffusion cells for the in vitro percutaneous absorption measurements of five model compounds. The permeability and the lipophilicity of all the compounds tested on the barrier-disrupted hairless mouse are summarized in Table 1.

The permeability barrier disruption by acetone treatment and TEWL measurements significantly correlated with the percutaneous absorption of the hydrophilic and lipophilic drugs, sucrose, caffeine, and hydrocortisone. However, acetone treatment did not alter the percutaneous penetration of the highly lipophilic compounds, estradiol and progesterone, hence suggesting that there is no correlation between TEWL and the percutaneous absorption of highly lipophilic compounds. The results imply the need to use both TEWL and drug lipophilicity to predict alterations in skin permeability.

Chilcott et al. (9) investigated the relationship between TEWL and skin permeability to tritiated water (^3H_2O) and the lipophilic sulfur mustard (^{35}SM) in vitro. No correlation was found between basal TEWL rates and the permeability of human epidermal membrane to ^3H_2O ($p = 0.72$) or ^{35}SM ($p = 0.74$). Similarly, there was no correlation between TEWL rates and the ^3H_2O permeability of full-thickness pigskin ($p = 0.68$). There was no correlation between TEWL rates and ^3H_2O permeability following up to 15 tape strips ($p = 0.64$) or four needlestick punctures ($p = 0.13$). These data indicate that under these experimental circumstances TEWL cannot be used as a measure of skin's permeability to topically applied compounds.

More on Assumptions

There is no doubt that the best experimental conditions are those that are closest to reality; in our case, those are TEWL and percutaneous absorption measured in vivo, on human skin, and using the most reliable percutaneous absorption method of measurement available. It is not a coincidence that all the studies, which used these ideal experimental conditions, came up with the same result that TEWL and percutaneous absorption are quantitatively correlated. It is only the studies, which veered from these most ideal conditions by measuring in vivo or using animal skin to model human skin or using alternate and less reliable methods or percutaneous measurement that found no significant quantitative correlation between the two skin barrier indicators. In the sections below, we will discuss the possible repercussions of varying experimental conditions that form the ideal.

Using In Vitro Methods to Model In Vivo Experiments

Skin permeation can be measured in human or in vitro by using excised skin in diffusion cells. In theory, studies using excised skin are feasible models for in vivo experiments, because passage through the skin is a passive diffusion process and the SC is composed of nonliving tissue. Many studies comparing in vivo and in vitro TEWL and percutaneous absorption measurements have been conducted, and the results from those experiments support the contention that reliable measurements can be obtained from in vitro methodology (6,19–25).

Although the consensus is that in vitro experiments are reasonable models for in vivo human experiments, some experiments note significant differences between these methods for measuring skin permeation. The most significant study by Bronaugh and Stewart (23) found that the effects of UV irradiation could not be duplicated using an in vitro experimentation model, hence suggesting that in vitro experiments examining the TEWL and percutaneous absorption after barrier damage may not be an acceptable model for in vivo experimentation. In vitro damage to the SC barrier may not be an accurate model to in vivo SC damage, because in vivo exposure to skin irritants results in a cascade of reactions that do not occur in human cadaver skin (19).

Chilcott et al. (9) investigated TEWL and percutaneous absorption correlation in vitro after inducing different types of barrier damage. This was also one of the only studies reviewed, which did not observe a correlation between TEWL and percutaneous absorption. Perhaps, using in vitro methodology in the experimental design may be responsible for the lack of correlation to skin damage reported in this study.

Using Animal Skin to Model Human Skin

Comparing the skin morphology and chemical absorption of human versus animal skin, it is clear that human skin is unique in both aspects and should be used for the most meaningful results (26). Yet an experiment by Bronaugh et al. (27) found that depending on the compound of interest and the vehicle used, permeability values obtained using animal skin can be well within an order of magnitude of the permeability values for human skin.

Independently, in vitro methods and animal skin models prove to be reliable models for human in vivo absorption. Therefore, it seems logical to assume that in vitro and animal methods may be used in unison to accurately model in vivo human absorption. However, Rougier et al. (28) documented a distinct difference between animal studies done in vivo versus in vitro when compared with human absorption. This experiment compares the skin permeability of humans to the hairless rat (29) and the hairless mouse (22) using molecules of widely different physicochemical properties. The results show that, in vivo, for whatever the molecule tested the permeability ratios remained relatively constant, whereas in vitro they do not. Therefore, when application conditions are strictly identical in humans and animals, it may be possible to model human in vivo absorption by measuring in vivo animal absorption but not using in vitro animal absorption. The inaccurate results obtained when conducting experiments in vitro using animal skin may have affected the results studied by Tsai et al. (11) and Chilcott et al., (9), which were the only two papers to conclude no correlation between TEWL and percutaneous absorption, and these were the only two papers using in vitro animal methodology.

Percutaneous Absorption Measurement Methods

A major factor affecting percutaneous absorption measurements is methodology (30,31). All methods for percutaneous absorption measurements are not equal and hence can give different results. The fourth column of Table 2 summarizes the percutaneous absorption methods used in these correlation studies.

The most common method for determining percutaneous absorption in vivo is measuring the radioactivity of excreta, following topical application of a labeled compound. Determination of percutaneous absorption from urinary radioactivity does not account for metabolism by skin, but has been proven to be a reliable method for absorption measurement and is widely accepted as the ''gold standard'' when available.

The most commonly used in vitro technique involves placing a piece of excised skin in a diffusion chamber, applying radioactive compound to one side of the skin, and then assaying for radioactivity in the collection vessel on the other side (32). The advantages of using this in vitro technique are that the method is easy to use and the results are obtained quickly. The disadvantage is that the fluid in the collection bath, which bathes the skin, is saline, and though it may be appropriate for studying hydrophilic compounds, it is not so for hydrophobic compounds. If the parent compound is not adequately soluble in water, then determining in vitro permeability into a water receptor fluid will be self-limiting.

When conducting in vitro experiments, animal skin is often substituted for human skin. Because animal skin has different permeability characteristics from human skin, one should be careful, which type of animal skin is used (refer to section Using Animal Skin to Model Human

Skin). In addition, proper care should be taken in skin preparation of excised skin to make sure not to damage skin barrier integrity. Anatomical site is as important as using of many different skin samples.

The only two experiments, which did not find a correlation between TEWL and percutaneous absorption, by Tsai et al. (11) and Chilcott et al. (9), were those, which measured percutaneous absorption in vitro. Perhaps, using a diffusion cell to measure percutaneous absorption is the reason for not finding a correlation.

Oestmann et al. (1) and Lavrijsen et al. (8) used LDF to measure HN penetration. LDF measures the increase in CBF caused by the penetration of HN, a vasoactive substance. One problem with this method is that LDF measurements are on the amount of HN absorbed but also on the individual's vasoreactivity, gender, and age. This may be the reason that Oestmann et al. (1) and Lavrijsen et al. (8) obtained only a weak correlation between TEWL and percutaneous absorption of HN. Another disadvantage of this method is that LDF measurements have many sources of variation, which make it difficult to compare interlaboratory results. If an attempt should be made, note that LDF parameters t_0 and t_{max} are the function of HN concentration, the vehicle used, and the application time; the LDF parameters LDF_{base} and LDF_{max} are relative values depending on the type of LDF used.

Type of Compound Used to Measure Percutaneous Absorption

The percutaneous absorption rate and/or total absorption of a compound varies greatly depending on the compound and its lipophilicity. Yet, many of the papers reviewed did not consider how lipophilicity of the test compound would affect percutaneous absorption and hence affect correlation results. Feldmann and Maibach (20) measured both the total absorption and maximum absorption rate for 20 different compounds of different lipophilicities. The range for total absorption for the 20 compounds tested was >250 times, whereas the difference in maximum absorption rate was >1000-fold (20). Because of the extreme range of absorption for topically applied compounds, it seems reasonable to assume that the correlation between TEWL and percutaneous absorption may not be independent of the physicochemical properties of the compound applied. Namely, can TEWL measurements predict the skin barrier's permeability changes to both hydrophilic and very lipophilic compounds?

Correlation results from many studies, Oestmann et al. (1), Lamaud et al. (12), Lavrijsen et al. (8), Lotte et al. (17), Aalto-Korte and Turpeinen (18), and Tsai et al. (11), suggest that TEWL can predict the changes in skin permeability to hydrophilic and slightly lipophilic topical drugs. Tsai et al. (11) also discovered that the percutaneous absorption of highly lipophilic compounds does not correlate with TEWL.

The highly lipophilic compounds are the compounds that did not show evidence of a correlation between percutaneous absorption and TEWL, whereas the moderately lipophilic compounds, such as hydrocortisone and BA, did. This should be further investigated. In the future, it may be necessary to use both TEWL and drug lipophilicity to predict alterations in skin permeability.

EXPLORING THE QUALITATIVE REASONING FOR THE CORRELATION BETWEEN PERCUTANEOUS ABSORPTION AND TEWL

Yet, despite the significant quantitative correlation demonstrated in some experiments, the precise qualitative relationship between percutaneous absorption and TEWL remains unsettled. Is the quantitative correlation just a coincidence or have we not discovered the link between the two indicators?

Experiments investigating the correlation between TEWL and percutaneous absorption have found a quantitative correlation between the two skin barrier indicators, yet have failed to find their precise qualitative relationship. Most experiments looking for an explanation of skin permeability examine and compare trends in physical aspects of the skin such as SC membrane thickness, corneocyte size, area of the horny layer, transcorneal routes, sebum lipid film, intercellular volume, to name a few. Yet we remain clueless about the structure-function relationship of the SC, because there is no morphological aspect that explains the permeability of the SC. Skin has particular features, which combine together in varying degrees to produce

different experimental values of TEWL and percutaneous absorption (17). Further investigation needs to be done regarding the relationship between TEWL and percutaneous absorption in skin structure and morphology.

CONCLUSION

Although it is not certain why studies by Tsai et al. (11) and Chilcott et al. (9) showed no quantitative correlation, we can postulate some estimations.

The study by Tsai et al. (11) is the only paper demonstrating a clear distinction between highly lipophilic compounds and slightly lipophilic compounds, when correlating percutaneous absorption and TEWL. Acetone treatment could affect a certain aspect of the skin barrier that mostly affects and interacts with hydrophilic compounds, hence having no affect on the highly lipophilic compounds such as estradiol and progesterone. It would be interesting to ascertain if the same results were obtained when selecting a different form of barrier damage such as physical tape stripping. Or it could be the fact that the lipophilic compounds chosen were even more hydrophobic than those used in other experiments, and indeed, TEWL and percutaneous absorption of highly lipophilic compounds are not correlated.

It is difficult to understand why Chilcott et al. (9) found no correlation between TEWL and percutaneous absorption. The results could have been affected, because the experiment was done in vitro, partly on animal skin, using an extremely lipophilic compound, ^{35}SM. It would be interesting to ascertain if TEWL and percutaneous absorption of ^{35}SM correlated with the results up to one hour after application.

Taken together, the weight of evidence confirms a relationship between TEWL (water transport) and percutaneous penetration, yet much remains before this can fully be generalized and the mechanism understood. Future experiments should take into consideration the effects of modeling realistic situations using alternative methods to the ideal.

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16 Role of Calcium in the Regulation of Skin Barrier Homeostasis

Hanafi Tanojo, Gena Y.Y. Chang, Jiun-Wen Guo, and Xinfan Huang
Genepharm, Inc., Sunnyvale, California, U.S.A.

Howard I. Maibach
Department of Dermatology, University of California School of Medicine, San Francisco, California, U.S.A.

INTRODUCTION

Some natural products have been shown to benefit the skin, especially for the restoration of skin barrier. Dead Sea mud and water, balneotherapeutic water preparations, deep sea sponges, milk, and pearl, for examples, have been used in ancient to modern formulations for topical application to provide healthy ageless skin. Results were not always well documented, but the effects have been observed and triggered many investigations. Among many components within these materials, calcium is one notable ingredient in common.

Calcium is important for human body and involved in many life processes. For instance, this element plays a crucial role in the growth, death, differentiation, and function of immune cells. Calcium is also important in the regulation of skin barrier homeostasis, as calcium is involved in the regeneration process of skin barrier components (1). The role of calcium in skin is more complex than previously assumed. The elucidation of calcium regulation mechanism in skin could be useful to understand and solve skin problems.

MECHANISM OF CALCIUM CELL SIGNALING IN SKIN

Calcium is the most abundant metal ion and fifth (after H, O, C, and N) most abundant element in human body on both an atom and weight basis. Over 98% of body calcium resides in bones and tooth enamel. The rest, in form of ion Ca^{2+}, is found throughout body fluids and takes part in various processes, including muscle contraction, blood clotting, nerve excitability, intercellular communication, membrane transport of molecules, hormonal responses, exocytosis, and cell fusion, adhesion, and growth (2).

Calcium ion is used as a universal messenger for living things, even in simple organisms and plants. The unique combination of its ionic radius and double charge allows Ca^{2+} to be specifically recognized and to yield tighter binding to receptors to the exclusion of other ions, leading to strong, specific binding (3). The specificity enables cells to form special receptors to assess signals from calcium. For many parts of the body, Ca^{2+} often acts as a second messenger in a manner similar to cyclic adenosine monophosphate (cAMP). Transient increases in cytosolic Ca^{2+} concentration trigger numerous cellular responses, including muscle contraction, release of neurotransmitters, and glycogen breakdown (glycogenolysis), also as an important activator of oxidative metabolism (4). Ca^{2+} does not need to be synthesized and degraded with each message transmission, so it is an energy-efficient signal for the cell (5).

In skin, calcium can provide signals for the cells, either extracellular or intracellular (in the cytosol). The extra- and intracellular signals are connected to each other, but may also act separately. In keratinocytes, extracellular Ca^{2+} levels influence growth and differentiation (6,7). At low extracellular Ca^{2+} levels (<0.1 mM), keratinocytes proliferate as a monolayer, rapidly becoming confluent (6,7). In this condition, keratinocytes never stratify, but show an undifferentiated, basal cell-like phenotype (8). The cells synthesize keratin proteins and are connected by occasional gap junctions but not by desmosomes. The cells also synthesize mainly ceramide type 2 (Cer-NS) and a small amount of ceramide type 3 (Cer-NP) (9). Keratinocytes grown in low-calcium medium (0.02 mM) maintained intracellular Ca^{2+} levels adequate for arachidonic acid metabolism and actually showed increased prostaglandin (PGE_2 and PGF_2) production up to 4.5 times compared with cells grown at normal Ca^{2+} level

(1.2 mM) (10). If this is true for the in vivo condition, a low level of extracellular Ca^{2+}, for instance, due to a defective skin barrier may cause an increase in prostaglandin synthesis, leading to hyperproliferative epidermal disorders, such as psoriasis, which are often associated with abnormalities in prostaglandin production (11).

Extracellular Ca^{2+} levels at equal or more than 0.1 mM trigger the differentiation of keratinocytes and synthesis of a complex pattern of free and covalently bound ceramides (12). The mRNA levels of keratin 10 (K10) and profilaggrin as well as those of ceramide glucosyltransferase and glucosylceramide-β-glucosidase increased (9). The early differentiation markers, K1 and K10, are observed within 8 to 24 hours, then the late markers, loricrin and filaggrin, are shown after 24 to 48 hours (8). Keratinocytes rapidly flatten, form desmosomes, and differentiate with stratification, while cornified envelopes form in cells of the uppermost layers (6,7).

The response to signaling is also shown in a progressive way. Keratinocytes grown in a low-calcium media proliferate. Increased extracellular Ca^{2+} inhibits proliferation, while it induces differentiation (13). With the increase, K14 expression is downregulated (8). On the other hand, differentiation of keratinocytes caused a decrease in responsiveness to extracellular Ca^{2+}, which may facilitate the maintenance of the high level of intracellular Ca^{2+} required for differentiation (14).

Raised extracellular Ca^{2+} increases intracellular Ca^{2+} (15–17). This implies that increased intracellular Ca^{2+} is the actual signal to trigger keratinocyte differentiation. Intracellular Ca^{2+} signals are assessed through calcium-binding proteins to induce responses. The major calcium-binding protein in skin is calmodulin. Calmodulin regulates target protein by modulating protein–protein interactions in a calcium-dependent way. Calmodulin regulates many enzymes, for example, adenyl and guanyl cyclase, phosphodiesterase, ornithine decarboxylase, calcium-calmodulin–dependent protein kinase, transglutaminase, and phospholipase, which are also found in skin (5).

Both intracellular release and transmembrane flux contribute to the rise in intracellular Ca^{2+} (16,17). The rise in keratinocyte intracellular Ca^{2+} in response to raised extracellular Ca^{2+} has two phases: (*i*) an initial peak, not dependent on extracellular Ca^{2+}, and (*ii*) a later phase that requires extracellular Ca^{2+} (16). An early response of human keratinocytes to increases in extracellular Ca^{2+} is an acute increase in intracellular Ca^{2+}. Stepwise addition of extracellular Ca^{2+} to neonatal human keratinocytes is followed by a progressive increase in intracellular Ca^{2+}, where the initial spike of increased intracellular Ca^{2+} is followed by a prolonged plateau of higher intracellular Ca^{2+} (18). The response of intracellular Ca^{2+} to increased extracellular Ca^{2+} in keratinocytes is saturated at 2-mM extracellular Ca^{2+} (18,19). The response of intracellular Ca^{2+} to increased extracellular Ca^{2+} in keratinocytes resembles the response in parathyroid cells, in that a rapid and transient increase in intracellular Ca^{2+} is followed by a sustained increase in intracellular Ca^{2+} above basal level. This multiphasic response is attributed to an initial release of Ca^{2+} from intracellular stores followed by an increased influx of Ca^{2+} through voltage-independent cation channels. The keratinocyte and parathyroid cell contains a similar cell membrane calcium receptor thought to mediate this response to extracellular Ca^{2+}. This receptor can activate the phospholipase-C pathway, leading to an increase in the levels of inositol 1,4,5-triphosphate (IP_3) and *sn*-l,2-diacylglycerol (DAG)—both of which are important messengers—as well as stimulating Ca^{2+} influx and chloride currents (20,21). IP_3 causes release of Ca^{2+} from internal stores, such as endoplasmic reticulum, further increasing intracellular level to precede a number of calcium-stimulated cellular events (22). DAG forms a quarternary complex with phosphatidylserine, calcium, and protein kinase C to activate the kinase, which will accelerate terminal differentiation (13). The signal transduction mediated through calmodulin induces other proteins, for example, desmocalmin, which is associated with the formation of desmosomes (23).

REGULATION OF CALCIUM GRADIENT

The regulation of calcium in skin shows an ingenious adaptation of living organisms to the presence of this ion. As Ca^{2+} cannot be metabolized like other second-messenger molecules, cells tightly regulate intracellular levels through numerous binding and specialized extrusion proteins (24). The concentration of calcium in extracellular spaces (generally $\sim$1.5 mM) is four orders of magnitude higher than in the cytosol ($\sim$0.1 μM). In excitable cells, for example,

muscle cells, the extracellular concentration of calcium must be closely regulated to keep it at its normal level of ~1.5 mM, so that it cannot accidentally trigger the muscle contraction, the transmission of nerve impulses, and blood clotting (4). In other cells, including keratinocytes, the extracellular level is similarly maintained in a specific equilibrium with the intracellular concentration.

Ca^{2+} also regulates melanin production in melanocyte; one way is through its ability to act as a cofactor for phenylalanine hydroxylase, which catalases the conversion of L-phenylalanine to L-tyrosine, the precursor of melanin (25). As with keratinocytes, low extracellular Ca^{2+} concentrations increase the proliferation of melanocytes, whereas high concentration does not show effect (26). Elevations in intracellular Ca^{2+} concentration have an inhibitory effect on the melanin production (27), but if coupled with the increase of cAMP, elevated intracellular Ca^{2+} level stimulates melanogenesis (28).

It is important for the cells to keep the intracellular calcium level low. A low-calcium concentration makes the use of the ion as an intracellular messenger energetically inexpensive. The movement of calcium ions across membranes requires energy, usually supplied by adenosine triphosphate (ATP). If the resting level of calcium in the cell were high, a large number of ions would need to be transported into the cytoplasm to raise the concentration by the factor of 10, which is ordinarily needed to activate an enzyme; afterward the excess calcium would have to be expelled from the cell. The normally low-calcium level means that relatively few ions need to be moved, with a relatively small expenditure of energy, to regulate an enzyme. In contrast, energetic cost of regulation by the other important intracellular messenger, cAMP, is high; it must be synthesized and broken down each time it carries a message, and both steps requires a significant investment of energy (3). Furthermore, low intracellular calcium is a necessary condition for the phosphate-driven metabolism characteristic of higher organisms. The energy-rich fuel for most cellular processes is ATP. Its breakdown releases inorganic phosphate. If the intracellular concentration of Ca^{2+} were high, the phosphate and the calcium would combine to form a precipitate of hydroxyapatite crystals, the same stony substance found in bone, and the calcification would ultimately doom the cell (3).

The large concentration gradient between extracellular spaces and cytosol is maintained by the active transport of Ca^{2+} across the plasma membrane, the endoplasmic reticulum (or the sarcoplasmic reticulum in muscle), and the mitochondrial inner membrane. Generally, plasma membrane and endoplasmic reticulum each contain a Ca^{2+}-ATPase that actively pumps Ca^{2+} out of the cytosol at the expense of ATP hydrolysis (4). Mitochondria act as a "buffer" for cytosolic Ca^{2+}. If cytosolic concentration of calcium rises, the rate of mitochondrial Ca^{2+} influx increases while that of Ca^{2+} efflux remains constant, causing the mitochondrial concentration of Ca^{2+} to increase, while the cytosolic concentration of Ca^{2+} decreases to its original level (its set point). Conversely, a decrease in cytosolic concentration of Ca^{2+} reduces the influx rate, causing net efflux of concentration of Ca^{2+} and an increase of cytosolic concentration of Ca^{2+} back to the set point (4). In melanocytes, Ca^{2+} homeostasis is regulated by melanin (29). Addition of high Ca^{2+} concentration to melanocytes kept in Ca^{2+}-free medium shows different type of increase between poorly and well-melanized melanocytes. This may be the result of the different content of melanin, which provides clearance of cytoplasmic Ca^{2+} into melanosomes (29). The strong Ca^{2+}-binding capacity of melanin (particularly inside melanosomes) is evident in its protective characteristic against DNA damage induced by reactive oxygen species (ROS) in both melanocytes and keratinocytes (30). It was reported that H_2O_2 and other reactive oxygen compounds induce increases in intracellular Ca^{2+} concentration and disrupt intracellular Ca^{2+} homeostasis, causing DNA strand breaks (31). On the other hand, the presence of melanin reduces intracellular Ca^{2+} level and stabilizes intracellular Ca^{2+} homeostasis (29).

Besides the already mentioned Ca^{2+}-ATPase, the transport of Ca^{2+} is regulated by a series of calcium pumps, transport systems, and ion channels. The availability of certain regulatory systems is dependent on the activity of the cells. In excitable cells such as cardiac muscle, the influx of Ca^{2+} to cytosol is regulated by voltage- (or potential-) dependent channels, while the efflux (out of cytosol) is regulated by cation exchanger, such as Na^{+}-Ca^{2+} exchanger (5). Undifferentiated keratinocytes in the basal layer have different sets of Ca^{2+} transport system than differentiated cells in the upper layers. In basal layer, the system consists of 14-pS nonspecific cation channels (NSCC) (32) and does not possess functional voltage-sensitive Ca^{2+} channels (17). Differentiated keratinocytes are likely to possess at least two and possibly three pathways of Ca^{2+} influx: (*i*) nicotinic channel (nicotinic acetylcholine receptor or

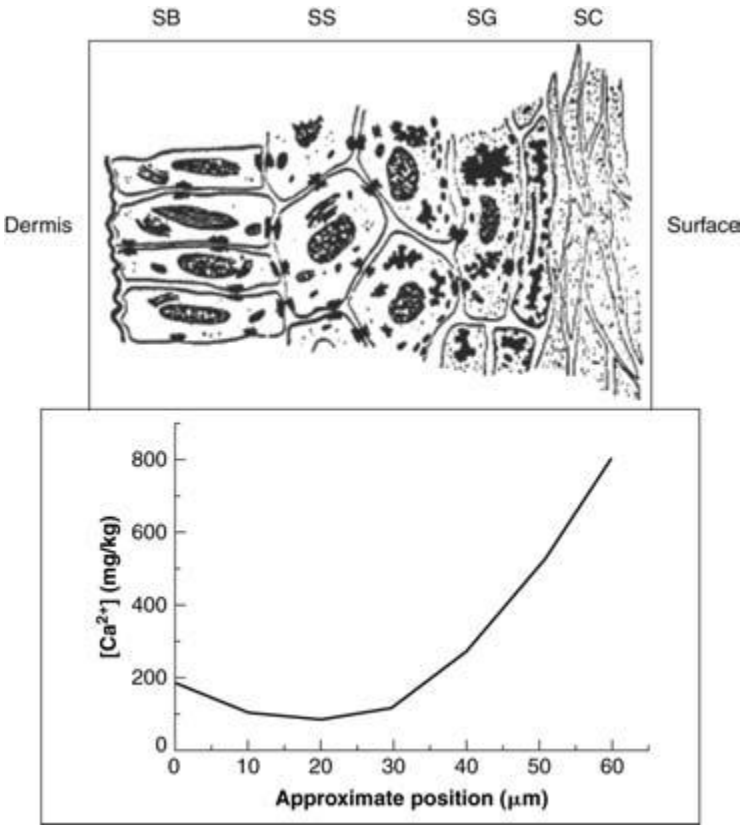

Figure 1 Illustration of calcium gradient in epidermis based on literature data. *Abbreviations*: SB, stratum basale/basal layer; SS, stratum spinosum; SG, stratum granulosum; SC, stratum corneum. *Source*: From Ref. 36.

nAChR); (*ii*) voltage-sensitive Ca^{2+} channels (VSCC) which can be blocked by nifedipine or verapamil; and (*iii*) NSCC, which is not activated by nicotine (33).

Other than the high-calcium gradient between extra- and intracellular domains of keratinocytes, a calcium gradient is present within the epidermis, with higher quantities of Ca^{2+} in the upper than in the lower epidermis, as the cell moves from the basal layer to the stratum granulosum (SG) (34). Ca^{2+} concentration increases steadily from the dermal-epidermal junction to the region just below the stratum corneum (SC), while this is not the case with other ions (35). Figure 1 illustrates the calcium gradient in human skin in comparison with an actual literature data (36). Such a gradient is not observed in skin abnormalities related to the formation of abnormal barrier function, such as psoriasis (37). Studies in mice and rats showed that this gradient exists at the same time as the formation of a maturing skin barrier at the end of gestation. The gradient is then maintained from the newborn throughout the adult life (38), although it tends to change with aging. It is not yet clear whether the calcium gradient leads to the formation of a mature barrier or the barrier caused the gradient. It may even be both if the regulation uses a feedback mechanism, as the differentiation will eventually form a barrier leading to the accumulation of Ca^{2+} in the upper epidermis. This high level of Ca^{2+} will, in turn, guarantee the ongoing process of differentiation toward the formation of corneocytes, fully differentiated keratinocytes in SC. The mechanism is thus almost completely autonomous and perpetual and, if it runs smoothly, requires little correction from the body.

SKIN BARRIER HOMEOSTASIS AND REPAIR

The skin barrier function is connected to the chemical and physical condition of SC, the uppermost layer of the epidermis, where the final phase of keratinocyte differentiation into corneocytes takes place. Skin barrier gives protection against desiccation and environmental

challenge by regulating water flux and retention (39). The optimal level of hydration maintained in skin barrier layer is largely dependent on three components, which are constantly regenerated particularly in SC, namely, (*i*) intercellular lamellar lipids, as an effective barrier to the passage of water; (*ii*) corneocytes, which provide the tortuous diffusion path created by the SC layers and corneocyte envelopes that retard water loss, and (*iii*) natural moisturizing factor (NMF), a complex mixture of low–molecular weight, water-soluble compounds first formed within the corneocytes by degradation of the histidine-rich protein known as filaggrin. Disturbance to the regeneration processes of these components, in which calcium plays a significant role as mentioned above, results in dry, flaky skin conditions (40). At normal calcium gradient condition, Ca^{2+} induces synthesis of intercellular lipid (41), full terminal differentiation into corneocytes (42), and the formation of the cornified envelope (43). Abnormal calcium distribution in aging people has been linked to fragile skin barrier in elderly (44).

Disruption of the barrier with acetone treatment or tape stripping depletes Ca^{2+} from the upper epidermis, resulting in the loss of the Ca^{2+} gradient (45–47). This is due to accelerated water transit that leads to the increased passive loss of Ca^{2+} into and through SC (45,47), because the permeability of SC to Ca^{2+} dramatically increased after SC was pretreated with acetone or sodium lauryl sulfate solution (48). The permeability of skin to Ca^{2+} ions has been known from some dermatoses, such as calcinosis cutis (49–51) and perforating verruciform collagenoma (52). In a shorter term, calcinosis cutis developed after a 24-hour (at least) topical application of an electrode paste containing saturated calcium chloride solution, bentonite, and glycerin, which are used for examination by electroencephalography or electromyography (53,54). The permeability of human skin to Ca^{2+} ions in vitro shows a marked dependence on anatomic site. In agreement with the data observed for nonelectrolytes, permeation decreased in the following order: foreskin > mammary > scalp > thigh. Mouse and guinea pig skin show comparable permeability to that of human scalp. Ca^{2+} transport from dermis across epidermis is higher than that from epidermis to dermis (55,56). Using a technique to continuously monitor the low level of Ca^{2+} flux across human SC in vitro, the flux through untreated human SC was shown to be sigmoidal. After SC was pretreated with acetone or sodium lauryl sulfate, the shape of the curve was similar, but the Ca^{2+} flux was significantly higher (48).

The decrease in Ca^{2+} levels in the outer epidermis is associated with enhanced lamellar body secretion and lipid synthesis (important components in repair responses) (45,57). Experiment in mice shows that after the calcium gradient disappears following acute permeability barrier disruption, the gradient returns after six hours in parallel with barrier recovery. This indicates that skin barrier formation (through restriction of transcutaneous water movement) could regulate the formation of the epidermal calcium gradient (58). Note that the barrier repair in response to the skin barrier disruption is not the same as the normal barrier regeneration process. The response is an emergency step to quickly reduce the transepidermal water loss to its set point and thereby returning the calcium gradient to its natural condition (45). Once the calcium gradient is normalized, the normal skin barrier regeneration takes place. The process of barrier repair in connection with transepidermal water loss and calcium gradient is illustrated in Figure 2.

Addition of high calcium concentration during the barrier disruption process will induce higher influx of calcium into epidermal keratinocytes, which delays the emergency skin barrier repair process (59). Also, if Ca^{2+} gradient can be preserved after skin barrier disruption by the addition of Ca^{2+} into the media, or occlusion of barrier-disrupted skin with water vapor-impermeable membrane, lamellar body secretion, lipid synthesis, and emergency barrier recovery are inhibited (57,60). The inhibition raised by high extracellular concentration of Ca^{2+} is potentiated by high extracellular potassium (K^+) (61). However, during this delay, and if the applied calcium concentration is within the right physiological range, the normal skin regeneration process can take place and the normal barrier function is restored without the formation of intermediate emergency barrier. This is indicated in a study on the cultured keratinocytes that extracellular calcium in physiological range of concentration is not a sufficient signal for growth arrest when other growth conditions are optimized (62).

Another study confirmed that barrier recovery is accelerated by the decreased level of Ca^{2+} and also K^+ during an increased water loss, since water loss may induce a decrease in the Ca^{2+} concentration in the upper epidermis, which, in turn, may stimulate lamellar body secretion and barrier repair (63). Furthermore, the inhibition raised by high extracellular Ca^{2+} concentration is reversed by nifedipine or verapamil, which are specific calcium channel

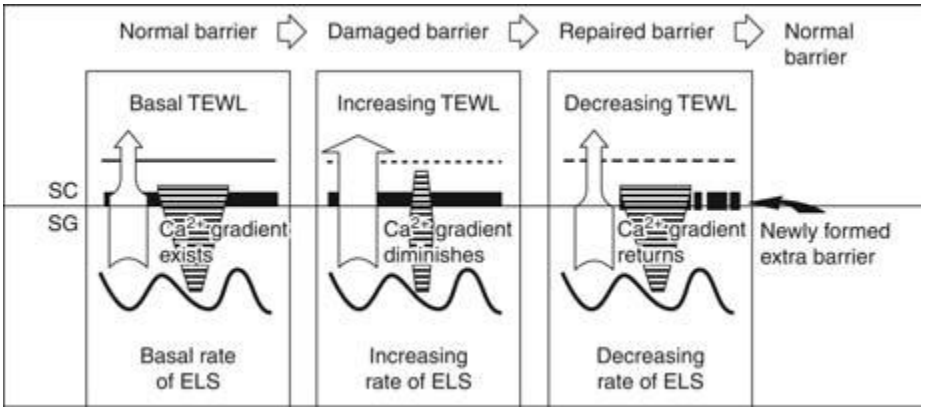

Figure 2 Illustration of skin barrier repair in epidermis. *Abbreviations*: SC, stratum corneum; SG, stratum granulosum; TEWL, transepidermal water loss; ELS, epidermal lipid synthesis.

blockers (61). In another study, administration of Ca^{2+}-free solutions by sonophoresis resulted in a marked decrease in Ca^{2+} content in the upper epidermis, and subsequently the loss of the Ca^{2+} gradient was accompanied by accelerated lamellar body secretion (a sign of emergency skin barrier repair) (64).

Dry, itchy, and scaly skin symptoms are frequently linked to an impaired skin barrier function, as observed in psoriasis, ichtyosis, atopic skin, and contact eczemas (65). Psoriatic lesions have been directly related to the loss of the normal calcium gradient in epidermis (37). The abnormal calcium gradient is shown in the people with atopic skin (66). In chronic hemodialysis patients, the commonly incident of uremic pruritus is found linked to the disrupted calcium gradient, especially with higher Ca^{2+} deposition in the extracellular fluid and cytoplasm of basal cells, and in the extracellular fluid, nuclei and cytoplasm of spinous cells compared with the non-pruritus group (67). On the other hand, studies on reconstructive epidermis have clearly demonstrated that once Ca^{2+} distribution profile is restored to normal, the terminal differentiation and SC barrier formation is improved (68). These facts indicate that restoration of Ca^{2+} gradient may lead to alleviation of dry, itchy, scaly, and other adverse skin symptoms related to skin barrier function.

TOPICAL APPLICATION OF CALCIUM

With the understanding that decreased Ca^{2+} level at the suprabasal cell layers results in abnormal differentiation, it is logical to attempt calcium supplementation by topical application. However, there are two difficulties in this approach. Topical application of high level of calcium alone is not recommended, because it may lead to calcitosis cutis, as seen in long-term occupational exposure to high levels of dissolved calcium, for example, in miners (49), agricultural laborers (50), and oil field workers (51). Secondly, if Ca^{2+} level in the basal cell layer increases after such application, then it causes disturbance of keratinocyte proliferation, reducing epidermal growth rate, and also may cause symptoms such as detected in uremic pruritus patients (67). The normalization of distribution of calcium ion requires high concentration below SG and SC interface (68), thus requires delivery of calcium below the skin barrier region in SC. As learned from the therapy using natural resources, topical application of calcium apparently should be accompanied in certain balance with other ions, such as sodium, potassium, magnesium, chlorides, and bromides, and also the delivery of calcium should be targeted only to the suprabasal cell layers (69).

As mentioned earlier, Dead Sea mud and water, balneotherapeutic water preparations, deep sea sponges, milk, and pearl are among natural products that contain high-calcium level in balance with other ions and demonstrate beneficial effects for skin barrier–related disorders. The restoration of normal barrier function during the application of high concentration of calcium is evident from the effect of bathing in the calcium-rich Dead Sea water to improve skin diseases related to skin barrier impairment (70) as well as to enhance skin hydration and

reduce inflammation in atopic dry skin (71). Other products such as milk and pearl have been used for specialty cosmetics for centuries in many countries. Although many components in milk may also contribute to the effects on skin, such as its biopeptide (72), milk is generally known as natural resource for calcium. In China, pearl powder has been investigated for various treatments (73).

Skin therapy with natural mineral waters has been intensively studied. The analysis of various water sources with clear benefits revealed unanimously high content of Ca^{2+}, compared with other natural water springs (74). One study of spa therapy has been reported on the basis of well-documented records on spa treatment in the 18th and 19th century in Bath, England. One of the factors that contributed to the success of this spa therapy is attributed to the large quantities of water rich in calcium found in the area (75).

It is possible that the effect of other ions also contributes to the positive outcome of the therapy. Magnesium, another divalent cation abundantly found in the body and in beneficial mineral waters, provides vasodilation, thereby lowering blood pressure effect, supposedly through its competition with cellular calcium (76). Bromides found in the thick haze overhanging the Dead Sea are also cited to have particularly improved psoriatic conditions (74). Sodium and potassium can also contribute to the ionic balance in the epidermis, as shown in the beneficial study of seawater to skin disorders (77). Some other elements such as selenium, zinc, rubidium, and sulfur may also provide additional effects, although their concentrations in mineral waters are generally low (74).

Specific topical formulations containing calcium in a mixture with other ions, sodium, potassium, magnesium, chloride, and bromide, have been used as adjunctive treatment for skin barrier restoration, which is also applicable for post treatment of cosmetical procedure, such as microdermabrasion or photothermolysis. The formulation is shown to accelerate the restoration of a quality skin barrier and alleviate scaly skin symptoms related to skin barrier disruption in relatively short time because of its ability to restore epidermal calcium gradient (78). This type of therapy might be considered safer than the application of calcium channel modulators or growth factors because of the additional adverse effects.

CONCLUSION

Calcium ions play an important role in the homeostasis of skin barrier. A change in the barrier will change the calcium ion gradient in skin and lead to disturbance in the skin barrier regeneration process. A severe change might lead into a high degree of calcium signaling, which may induce the activation of various processes, from increased synthesis of skin components or messengers to the inflammatory reactions. All these are important factors leading to impaired skin conditions. The regulation of calcium in skin is therefore necessary to maintain a good skin barrier function and to avoid abnormal skin symptoms. Application of topical preparations containing relatively high level of calcium in balance with other ions and targeted delivery to suprabasal cell layers has been shown to help the skin barrier recovery and homeostasis. Ranging from natural products to laboratory compositions, the preparations are getting more acknowledgments from dermatological experts, not only because of the safe but effective results for therapy but also for more understanding on the effects of calcium on skin health in general.

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17 Percutaneous Penetration Enhancers: An Overview[a]

Haw-Yueh Thong, Hongbo Zhai, and Howard I. Maibach
Department of Dermatology, University of California School of Medicine, San Francisco, California, U.S.A.

INTRODUCTION

Skin is an optimal interface for systemic drug administration. Transdermal drug delivery (TDD) is the controlled release of drugs through intact and/or altered skin to obtain therapeutic levels systematically and to affect specified targets for the purpose of, for example, blood pressure control, pain management, and others. Dermal drug delivery (DDD) is similar to TDD except that the specified target is the skin itself (1). TDD has the advantages of bypassing gastrointestinal incompatibility and hepatic "first pass" effect; reduction of side effects due to the optimization of the blood concentration time profile; predictable and extended duration of activity; patient-activated/patient-modulated delivery; elimination of multiple dosing schedules, thus enhancing patient compliance; minimization of inter- and intrapatient variability; reversibility of drug delivery allowing the removal of drug source; and relatively large area of application compared with the mucosal surfaces (1).

After nearly four decades of extensive study, the success of this technology remains limited, with many problems waiting to be solved, one of which is the challenge of low skin permeability hindering the development of TDD for macromolecules. To overcome the skin barrier safely and reversibly while enabling the penetration of macromolecules is a fundamental problem in the field of TDD and DDD.

Several technological advances have been made in the recent decades to overcome skin barrier properties (2). Examples include physical means such as iontophoresis, sonophoresis, and microneedles; chemical means such as penetration enhancers (PEs); and biochemical means such as liposomal vesicles and enzyme inhibition.

We overview physical and biochemical means of penetration enhancement, and focus on the common chemical PEs. We discuss the classification and mechanisms of chemical PEs, its applications in TDD, and trends and development in penetration enhancement.

PHYSICAL PENETRATION ENHANCEMENT

Physical means of penetration enhancement mainly incorporate mechanisms to transiently circumvent the normal barrier function of SC and to allow the passage of macromolecules. Although the mechanisms are different, these methods share the common goal to disrupt SC structure to create "holes" big enough for molecules to permeate. Table 1 summarizes the commonly investigated technologies of physical penetration enhancement. Two of the better-known technologies are iontophoresis and sonophoresis, and the holes created by these methods are generally believed to be of nanometer dimensions, permissive of transport of small drugs (3). A new and exciting technology for macromolecule delivery is microneedle-enhanced delivery. Theses systems use arrays of tiny needlelike structures to create transport pathways of microns' dimensions, and should be able to permit transport of macromolecules, possibly supramolecular complexes and microparticles. These systems have greatly enhanced (up to 100,000 fold) the penetration of macromolecules through skin (4), while also offering painless drug delivery (5,6).

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Table 1 Physical Methods of Penetration Enhancement

Method	Definition	Mechanism(s)	Examples of drugs	Reference
Ionotophoresis	The electrical driving of charged molecules into tissue by passing a small direct current through a drug-containing electrode in contact with skin	1. Electrical repulsion from the driving electrode drives charged molecules 2. The flow of electric current enhances skin permeability 3. Electroosmosis affect uncharged and large polar molecules	Calcitonin, trans-nail delivery of salicylic acid, transdermal delivery of peptides, proteins, and oligonucleotides	(4,7–10)
Electroporation	A method of reversibly permeabilizing lipid bilayers by the application of an electric pulse	Application of short (micro- to millisecond) electrical pulses of ~100–1000 V/cm creates transient aqueous pores in the lipid bilayers	Methotrexate, timolol, fentanyl, tetracaine, nalbuphine, cyclosporin-A	(11–17)
Sonoporation	Ultrasound-mediated delivery of therapeutic agents into biological cells	1. (Low-energy frequency): disturbs the lipid packing in SC by cavitation 2. (Shock waves): increase free volume space in bimolecular leaflets thus enhancing permeation	Insulin, cutaneous vaccination, transdermal heparin delivery, transdermal glucose monitoring, delivery of acetyl cholinesterase inhibitors for the treatment of Alzheimer's disease, treatment of bone diseases and Peyronie's disease and dermal exposure assessment	(4,18,19)
Microneedle-enhanced delivery systems	A method using arrays of microscopic needles to open pores in SC thus facilitating drug permeation	Bypasses the SC and delivers drugs directly to the skin capillaries. Also has the advantage of being too short to stimulate the pain fibers.	Oligonucleotide, insulin, protein vaccine, DNA vaccine, methyl nicotinate	(3,6)

Abbreviation: SC, stratum corneum.

BIOCHEMICAL PENETRATION ENHANCEMENT

Biochemical means of penetration enhancement include using prodrug molecules (20), chemical modification (21), enzyme inhibition (22), and the usage of vesicular systems or colloidal particles (23). Among these strategies, special formulation approaches, based mainly on the usage of colloidal carriers, are most promising. Liposomes (phospholipids-based artificial vesicles) and niosomes (nonionic surfactant vesicles) are widely used to enhance drug delivery across the skin. In addition, proliposomes and proniosomes, which are converted to liposomes and niosomes upon simple hydration are also used in TDD (24). Generally, these colloidal carriers are not expected to penetrate into viable skin. Most reports cite a localizing effect whereby the carriers accumulate in SC or other upper skin layers (4).

More recently, a new type of liposomes called transferosomes has been introduced (25,26). Transferosomes consist of phospholipids, cholesterol and additional "edge activators"—surfactant molecules such as sodium cholate. The inventors claim that 200- to 300-nm sized transferosomes are ultradeformable and squeeze through pores less than one-tenth of their diameter, and are thus able to penetrate intact skin. Penetration of these colloidal particles works best under in vivo conditions and requires a hydration gradient from the skin surface toward the viable tissues to encourage skin penetration under non-occluded conditions.

In addition, ethosomes, which are liposomes high in ethanol content (up to 45%), penetrate skin and enhance compound delivery to deep skin strata or systematically. The mechanism suggested is that ethanol fluidizes both ethosomal lipids and lipid bilayers in the SC, allowing the soft, malleable vesicles to penetrate through the disorganized lipid bilayers (27).

In general, six potential mechanisms of actions of these colloidal carriers were proposed (4):

1. Penetration of SC by a free drug process—drug releases from vesicle and then penetrates skin independently
2. Penetration of SC by intact liposomes
3. Enhancement due to release of lipids from carriers and interaction with SC lipids
4. Improved drug uptake by skin
5. Different enhancement efficiencies control drug input
6. The role of protein requires elaboration

CHEMICAL PENETRATION ENHANCERS

Substances that help promote drug diffusion through the stratum corneum (SC) and epidermis are referred to as PEs, accelerants, adjuvants, or sorption promoters (28). PEs have been extensively studied given its advantages such as design flexibility with formulation chemistry and patch application over large area. PEs improve drug transport by reducing the resistance of SC to drug permeation. To date, none of the existing chemical penetration enhancers (CPEs) have proven to be ideal. In particular, the efficacy of PEs toward the delivery of high–molecular weight drugs remains limited. Attempts to improve enhancement by increasing the potency of enhancers inevitably lead to a compromise on safety issues. Achieving sufficient potency without irritancy has proved challenging.

CLASSIFICATION OF CPEs

The diverse physicochemical properties and variation in mechanisms of action of compounds investigated for their penetration enhancement effects made a simple classification scheme for PEs difficult to set up. Hori et al. (29) proposed a conceptual diagrammatic approach based on Fujita's data (30) for the classification of PEs. In this approach, they determined organic and inorganic values for PEs, and the resultant plot of organic versus inorganic characteristics grouped PEs into distinct areas on the diagram—area I encloses enhancers, which are solvents; area II designates PEs for hydrophilic drugs; and area III contains PEs for lipophilic compounds. On the other hand, Lambert et al. (31) grouped most PEs into three classes: solvents and hydrogen bond acceptors (e.g., dimethylsulfoxide, dimethylacetamide, and dimethylformamide), simple fatty acids and alcohols, and weak surfactants containing a moderately sized polar group (e.g., Azone®, 1-dodecylazacycloheptan-2-one); whereas Pfister et al. (28) classified PEs as either polar or nonpolar. To date, there is no consensus as to which classification to adopt. Table 2 classifies commonly

(*text continues on page 191*)

Table 2 Chemical Penetration Enhancers

Category and examples	Cosolvent/vehicle	Mechanism	Examples of drugs (33)	Comment	Reference
Sulfoxides					
DMSO		1. Increase lipid fluidity 2. Promote drug partitioning	DMSO: theophylline, salicylic acid, hydrocortisone, testosterone, scopolamine, antimycotics, fluocinolone acetonide, flufenamic acid		(34,35)
DCMS		Protein-DCMS interactions, resulting in a change in protein conformation, creating aqueous channels	DCMS: methotrexate, naloxone, pyridostigmine bromide, hydrocortisone, progesterone	DCMS enhance polar drug more effectively	
Alkanones					
N-heptane, n-octane, n-nonane, n-decane, n-undecane, n-dodecane, n-tridecane, n-tetradecane, n-hexadecane		Extensive barrier alteration of SC	Propanolol, diazepam		(36)
Alcohols					
Alkanol: E, propanol, butanol, 2-butanol, pentanol, 2-pentanol, hexanol, octanol, nonanol, decanol, BA		1. Low–molecular weight alkanols ($C \leq 6$) may act as solubilizing agents 2. More hydrophobic alkanols may extract lipids from SC[a], leading to increased diffusion	E: tacrine, metrifonate, dichlorvos, ketolorac, nitroglycerin, tazifylline, betahistine, cyclosporin A		(37–40)
Fatty alcohol: caprylic, decyl, LA, 2-lauryl, myristyl, cetyl, stearyl, oleyl, linoleyl, linoleyl alcohol			LA: buprenorphine		(41)
Polyols					
PG, PEG, ethylene glycol, diethylene glycol, triethylene glycol, dipropylene glycol, G, propanediol, butanediol, pentanediol, hexanetriol	43× enhancement of diazepam and 86× enhancement of midazolam maleate seen in PG and 5% Azone in a PG:ethanol: water (2:2:1) vehicle.	PG may solvate α-keratin and occupy hydrogen bonding sites, reducing drug-tissue binding	PG: 5-fluorouracil, tacrine, ketorolac, isosorbide dinitrate, clonazapem, albuterol, verapamil, betahistine, estradiol, dihydroergotamine, methotrexate, steroids, midazolam maleate, diazepam PEG: terbutaline G: diazepam, terbutaline, 5-fluorouracil	Inclusion of 2% Azone or 5% oleic acid to PG produced a more bioactive formulation	(42,43)

Amides				
Urea, DMA, diethyltoluamide, DMF, dimethyloctamide, dimethyldecamide	Urea: hydration of SC, keratolytic, creating hydrophilic diffusion channels DMA/DMF: (low conc.): partition to keratin, (high conc.): increase lipid fluidity, disrupt lipid packaging	Urea: ketoprofen, 5-fluorouracil DMA/DMF: griseofluvin, betamethasone 17-benzoate, caffeine	Urea analogues in PG enhanced permeability of 5-fluorouracil 6×	(41,44)
Biodegradable cyclic urea: 1-alkyl-4-imidazolin-2-one		Indomethacin	Comparable to or better than Azone	(45)
Pyrrolidone derivatives: 1M2P, 2-pyrrolidone, 1-lauryl-2-pyrrolidone, 1-methyl-4-carboxy-2-pyrrolidone, 1-hexyl-4-carboxy-2-pyrrolidone, 1-lauryl-4-carboxy-2-pyrrolidone, 1-methyl-4-methoxycarbonyl-2-pyrrolidone, 1-hexyl-4-methoxycarbonyl-2-pyrrolidone, 1-lauryl-4-methoxycarbonyl-2-pyrrolidone, NMP, *N*-cyclohexylpyrrolidone, *N*-dimethylaminopropylpyrrolidone, *N*-cocoalkypyrrolidone, *N*-tallowalkylpyrrolidone	Interact with both keratin in the SC and with lipids in the skin structure	1M2P: griseofulvin, theophylline, tetracycline, ibuprofen, betamethasone 17-benzoate NMP: prazosin		(41,46)
Biodegradable pyrrolidone derivatives: Fatty acid esters of *N*-(2-hydroxyethyl)-2-pyrrolidone				(31)

(*Continued*)

Table 2 Chemical Penetration Enhancers (*Continued*)

Category and examples	Cosolvent/vehicle	Mechanism	Examples of drugs (33)	Comment	Reference
Cyclic amides: 1-dodecylazacycloheptane-2-one(Azone), 1-geranylazacycloheptan-2-one, 1-farnesylazacycloheptan-2-one, 1-geranylgeranylazacycloheptan-2-one, 1-(3,7-dimethyloctyl) azacycloheptan-2-one, 1-(3,7,11-trimethyldodecyl) azacycloheptan-2-one, 1-geranylazacyclohexane-2-one, 1-geranylazacyclopentan-2,5-dione, 1-farnesylazacyclopentan-2-one	Azone: enhancer effect can be increased by use of a cosolvent such as PG.	Azone: 1. Affects lipid structure of SC 2. Increases partitioning 3. Increases membrane fluidity	Azone: 5-fluorouracil, antibiotics, glucocorticoids, peptites, clonazepam, albuterol, estradiol, levonorgestrel, HIV protease inhibitor (LB-71148), betahistine, dihydroergotamine	Azone: significant accelerant effects at low conc. (1–5%), can be applied undiluted to skin without significant discomfort, effective for both hydrophilic and hydrophobic drugs	(47–49)
Hexamethylenelauramide and its derivatives					(50)
Diethanolamine, triethanolamine					(42)
Fatty acids Linear: LIA, valeric, heptanoic, pelagonic, caproic, CA, LAA, myristic, stearic, OA, caprylic Branched: isovaleric, neopentanoic, neoheptanoic, neononanoic, trimethyl hexanoic, neodecanoic, isostearic		Selective perturbation of the intercellular lipid bilayers OA: decreases the phase transition temperatures of the lipid, increasing motional freedom or fluidity of lipids	Naloxone, mannitol, betamethasone 17-benzoate, hydrocortisone, acyclovir, nitroglycerin OA: galanthamine, estradiol, levonorgestrel CA: buprenorphine, albiterol LAA: buprenorphine, betahistine	Among stearic, oleic, and linoleic acids, maximum enhancement was observed with linoleic acid	(41,51,52)

Fatty acid esters Aliphatic: isopropyl n-butyrate, isopropyl n-hexanoate, isopropyl n-decanoate, IPM, isopropyl palmitate, octyldodecyl myristate Alkyl: EA, butyl acetate, methyl acetate, methylvalerate, methylpropionate, diethyl sebacate, ethyl oleate		IPM: direct action on SC, permeating into liposome bilayers, increasing fluidity Aliphatic: increase diffusivity in the SC and/or the partition coefficient Alkyl: increase lipid fluidity (similar to DMSO)	IPM: galanthamine, ketorolac, chlorpheniramine, dexbrompheniramine, diphenhydramine, theophylline, pilocarpine, verapamil EA: levonorgestrel, 17β-estradiol, hydrocortisone, 5-fluorouracil, nefedipine		(53,54)
Surfactants Anionic: sodium laurate, sodium lauryl sulfate, sodium octyl sulfate		Alter the barrier function of SC, allowing removal of water-soluble agents that normally act as plasticizers		Greater damage and permeation enhancement with anionic surfactants than with nonionic surfactants	(55,56)
Cationic: Cetyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, octyltrimethylammonium bromide, benzalkonium chloride, octadecyltrimethylammonium chloride, cetylpyridinium chloride, dodecyltrimethylammonium chloride, hexadecyltrimethylammonium chloride	Significant increases in the flux of lidocaine from saturated systems in PG-water mixtures	Adsorb at interfaces and interact with biological membranes, causing damage to skin		Cationic surfactants are more destructive to skin than anionic surfactants.	(56–58)
zwitterionic surfactants hexadecyl trimethyl ammoniopropane sulfonate, oleyl betaine, cocamidopropyl hydroxysultaine, cocamidopropyl betaine					(59)
Nonionics: Polyxamer (231, 182, 184), Polysorbate (20, 60), Brij (30, 93, 96, 99), Span (20, 40, 60, 80, 85), Tween (20, 40, 60, 80), Myrj (45, 51, 52), Miglyol 840	Polysorbate 20 and 60 increased lidocaine flux in the presence of PG	Emulsify sebum, enhancing the thermodynamic activity of coefficients of drugs	Tween 80: ketoprofen Polysorbate 20, 60: lidocaine		(41,60,61)

(*Continued*)

Table 2 Chemical Penetration Enhancers (*Continued*)

Category and examples	Cosolvent/vehicle	Mechanism	Examples of drugs (33)	Comment	Reference
Bile salts: sodium cholate, sodium salts of TC, glycolic, desoxycholic acids			TC: elcatonin and vit. D_3, estradiol and vit. D_3,		(62)
Lecithin					(63)
Terpenes					
Hydrocarbons: D-Limonene, α-pinene, β-carene Alcohols: α-Terpineol, terpinen-4-ol, carvol Ketones: Carvone, pulegone, piperitone, menthone Oxides: Cyclohexene oxide, limonene oxide, α-pinene oxide, cyclopentene oxide, 1,8-cineole Oils: Ylang ylang, anise, chenopodium, eucalyptus		1. Increases diffusivity of drugs within SC due to disruption of intercellular lipid barrier 2. Opens new polar pathways within and across the SC	5-Fluorouracil, aspirin, haloperidol	Hydrocarbon terpenoids were least effective, oxides moderately effective, and the alcohols, ketones, and cyclic ethers most effective accelerants of 5-fluorouracil permeation	(36,64,65)
Organic acids					
Salicylic acid and salicylates (including their methyl, ethyl, and propyl glycol derivatives), citric and succinic acid					(66)
Cyclodextrins					
HPβCD DIMEB	Higher penetration of liarzole in DIMEB with PG/oleic acid compared with HPβCD	Form inclusion complexes with lipophilic drugs and increase their solubility of in aqueous solutions	Liarzole		(67,68)
Proprietary chemical enhancers					(69)
Alkyl-2-(N,N-disubstituted amino)-alkanoate ester (NexAct®)					
2-(n-nonyl)-1,3-dioxolane (SEPA®)			Ibuprofen, ketoprofen, alprostadil, testoterone		

[a]SC: stratum corneum.

Abbreviations: DMSO, dimethylsulfoxide; DCMS, decylmethylsulfoxide; BA, benzyl alcohol; LA, lauryl; PG, propylene glicol; E, ethanol; PEG, polyethylene glicol; G, glicerol; DMA, dimethylacetamide; DMF, dimethyformamide; 1M2P, 1-methyl-2-pyrrolidone; NMP, *N*-methyl-pyrrolidone; LIA, linoleic acid; CA, capric acid; LAA, lauric acid; OA, oleic acid; IPM, isopropyl myristate; EA, ethyl acetate; TC, taurocholic; HPβCD, 2-hydroxypropyl-β-cyclodextrin; DIMEB, 2,6-dimethyl-β-cyclodextrin; conc., concentration; vit., vitamin.

Source: From Ref. 9.

investigated PEs based on the chemical classes to which the compounds belong (32). Only representative compounds are listed to avoid an exhaustive list. Note that a perfect classification is yet to be developed, and the key lies in a comprehensive understanding of the mechanisms and the physicochemical parameters of CPEs.

MECHANISM OF CPEs

The mechanisms of action proposed for commonly seen CPEs are listed in Table 2. Basically, transdermal penetration of most drugs is a passive diffusion process (70). There are three major potential routes for penetration—appendageal (through sweat ducts and/or hair follicles with associated sebaceous glands), transcellular permeation through the SC, or intercellular permeation through the SC (4). The appendageal route usually contributes negligibly to steady-state drug flux given its small available fractional area of 0.1%. This route may be important for short diffusional times and for ions and large polar molecules, which have low penetration across SC. The intact SC thus comprises the predominant route through which most molecules penetrate.

Kanikkannan et al. (71) suggested three pathways for drug penetration through the skin: polar, nonpolar, and both. The mechanism of penetration through the polar pathway is to cause protein conformational change or solvent swelling; whereas the key to penetrate via the nonpolar pathway is to alter the rigidity of the lipid structure and fluidize the crystalline pathway. Some enhancers may act on both polar and nonpolar pathways by dissolving the skin lipids or denaturing skin proteins. On the other hand, Ogiso and Tanino (72) proposed the following mechanisms for the enhancement effect: (*i*) an increase in the fluidity of the SC lipids and reduction in the diffusional resistance to permeants, (*ii*) the removal of intercellular lipids and dilation between adherent cornified cells, (*iii*) an increase in the thermodynamic activity of drugs in vehicles, (*iv*) the exfoliation of SC cell membranes, the dissociation of adherent cornified cells, and elimination of the barrier function.

Ogiso et al. (73) also proposed examples of PEs with different relative enhancement capabilities due to differences in the chemical structure and other parameters. In their study, the relative ability to enhance transdermal penetration of indomethacin into hairless rat skin was studied. The results were summarized in Table 3 (69).

Furthermore, Kanikkannan et al. (71) proposed that on the basis of the chemical structure of PEs (such as chain length, polarity, level of unsaturation, and presence of specific chemical groups such as ketones), the interaction between the SC and PEs may vary, contributing to the different mechanisms in penetration enhancement. A comprehensive understanding of the mechanisms of action and a judicious selection of CPE would be helpful in the successful development of TDD and DDD products.

FDA-APPROVED TDD

There has been an increased focus on the potential of TDD as evident from the increase in the number of patents as well as scientific publications on TDD systems. Many drugs have been evaluated for TDD in prototype patches, either in vitro permeation studies using mouse, rat, or

Table 3 Examples of Penetration Enhancers with Different Relative Enhancement Capabilities due to Differences in the Chemical Structure and other Parameters

Mechanisms	Comparison
Extraction of intercellular lipids and dilations between cornified cells, permitting percutaneous passage of polar substances	1-dodecylazacycloheptane-2-one (Azone) > n-octanol > d-limonen > oleic acid > cíñelo
Increase in partitioning into skin	1-dodecylazacycloheptane-2-one > n-octanol > cineol > d-limonen > oleic acid > isopropyl myristate > monooleate
Increase in the fluidity of SC lipids and reduction in diffusional resistance	1-dodecylazacycloheptane-2-one > isopropyl myristate > monoolein > oleic acid > cineol, sodium oleate
Increase in thermodynamic activity in vehicles	n-octanol > sodium oleate > d-limonen > monoolein > cineol > oleyl oleate > isopropyl myristate

human skin or have reached varying stages of clinical testing. Examples are listed in Table 2. Despite a wide array of TDD systems undergoing research and development, only a small percentage of the drugs reach the market successfully because of three limitations: difficulty of penetration through human skin, skin irritation and allergenicity, and clinical need. In addition, it is generally accepted that the best drug candidates for passive adhesive transdermal patches must be nonionic; must have low molecular weight (<500 Da), adequate solubility in oil and water (log P in the range 1–3), and a low melting point (<200°C); and must be potent (dose <50 mg/day, and ideally <10 mg/day) (74–76). Given these operating parameters, the number of drug candidates, which fits the criteria, may seem low. Nevertheless, with the development of novel technologies, such constraint may be overcome.

Since the introduction of a TDD for scopolamine in 1981, several new products have been introduced. The U.S. TDD market approached $1.2 billion in 2001 and was based on 11 drug molecules: fentanyl, lidocaine, prilocaine, nitroglycerin, estradiol, ethinyl estradiol, norethindrone acetate, testosterone, clonidine, nicotine, and scopolamine (77). Barry (4) reported that 40% of drug delivery candidate products that were under clinical evaluation and 30% of those in preclinical development in the United States were TDD or DDD systems.

Examples of Food and Drug Administration (FDA)-approved transdermal patches and their applications are given in Table 4. Despite a plethora of candidate CPEs to choose from, all currently available TDD products adopt skin occlusion as the primary mechanism for penetration enhancement, perhaps due to its simplicity and convenience, and the following effects on SC (78,79): an increase in SC hydration and a reservoir effect in penetration rates of the drug due to hydration, an increase in skin temperature from 32°C to 37°C, and the prevention of accidental wiping or evaporation (volatile compound) of the applied compound.

Table 4 Examples of FDA-Approved Transdermal Patches, Their Applications, and the Mechanisms/Compounds Used for Penetration Enhancement

Drug	Application(s)	Example of commercially available product(s)	Penetration enhancement effect and PEs
Scopolamine	Motion sickness	Transderm Scop	Occlusive effect
Fentanyl	Moderate-to-severe chronic pain	Duragesic	Occlusive effect
Lidocaine	Anesthesia	Lidoderm	Occlusive effect, urea, propylene glycol
Prilocaine	Anesthesia	EML anesthetic disc	Occlusive effect, polyoxyethylene fatty acid esters
Testosterone	Hormone replacement therapy	Androderm	Occlusive effect, glycerol monooleate
Estradiol/norethindrone acetate	Hormone replacement therapy	Combipatch	Occlusive effect, silicone, oleic acid, dipropylene glycol
Estradiol	Symptomatic relief of postmenopausal symptoms and prevention of osteoporosis	Alora, Climera, Esclim, Vivelle, Vivelle-dot	Occlusive effect; Climera: fatty acid esters; Vivelle: 1,3-butylene glycerol, oleic acid, lecithin, propylene glycol, dipropylene glycol; Vivelle-dot: oleyl alcohol, dipropylene glycol
Norelgestromin/ethinyl estradiol	Contraception	Ortho Evra	Occlusive effect, lauryl lactate
Nitroglycerin	Angina pectoris	Nitro-Dur, Nitrodisc, Transderm-Nitro	Occlusive effect, fatty acid esters
Clonidine	Hypertension	Catapres-TTS	Occlusive effect
Nicotine	Smoking cessation	Nicoderm CQ	Occlusive effect
Methyphenidate	Attention deficit hyperactive disorder	Daytrana	Occlusive effect
Selegiline	Depression	Emsam	Occlusive effect
Oxybutynin	Urge/urinary incontinence	Oxytrol	Occlusive effect

Abbreviations: PEs, penetration enhancers; EMLA, eutectic mixture of local anesthetic.

FUTURE TRENDS

The protective function of human SC imposes physicochemical limitations to the type of molecules that can traverse the barrier. As a result, commercially available products based on TDD or DDD have been limited. Various strategies have emerged over the last decade to optimize delivery. Approaches such as the optimization of formulation or of drug-carrying vehicle to increase skin permeability do not greatly improve the permeation of macromolecules.

On the contrary, physical or mechanical methods of enhancing delivery have been more promising. Improved delivery has been shown for drugs of differing lipophilicity and molecular weight, including proteins, peptides, and oligonucleotides, using electrical methods (iontophoresis and electroporation), mechanical (abrasion, ablation, and perforation), and other energy-related techniques such as ultrasound and needleless injection (80).

Another strategy for penetration enhancement is to exploit the synergistic effects offered by combined techniques. Karande et al. (81) reported the discovery of synergistic combinations of penetration enhancers (SCOPE), which allow permeation of 10-kDa macromolecules with minimal skin irritation using high-throughput screening method. Kogan and Garti (51) also showed that the combination of several enhancement techniques led to synergetic drug penetration and decrease in skin toxicity. In essence, the possibilities seem endless in the field of TDD and DDD.

CONCLUSION

TDD would avoid problems associated with the oral route as well as the inconvenience and pain associated with needle delivery and has thus competed with oral and injection therapy for the accolade of the innovative research area for drug delivery. Yet there remains a paucity of candidates for TDD or DDD to be marketed. The reasons are twofold: (*i*) most candidate drug molecules have low permeation rates through the skin to ever reach clinically satisfactory plasma level; (*ii*) risk of skin irritation and allergic contact dermatitis may be increased by skin occlusion (79,82) and/or the application of potent PEs (81). The ideal characteristics of PEs include the following (28):

- Be both pharmacologically and chemically inert
- Be chemically stable
- A high degree of potency with specific activity, rapid onset, predictable duration of activity, and reversible effects on skin properties
- Show chemical and physical compatibility with formulation and system components
- Be nonirritant, nonallergenic, nonphototoxic, and noncomedogenic
- Be odorless, tasteless, colorless, cosmetically acceptable, and inexpensive
- Be readily formulated into dermatological preparations, transdermal patches, and skin adhesives
- Have a solubility parameter approximating that of skin (83)

Future studies on the mechanisms of penetration enhancement, the metabolic processes of chemicals within the skin, skin toxicity, as well as the development of novel technologies will improve our knowledge on penetration enhancement. While the current TDD and DDD technologies still offer significant potential for growth, next-generation technologies will enable a much broader application of TDD to the biopharmaceutical industry.

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18 Tests for Skin Protection: Barrier Effect

Heidi P. Chan, Hongbo Zhai, and Howard I. Maibach
Department of Dermatology, University of California School of Medicine, San Francisco, California, U.S.A.

INTRODUCTION

One important skin function is protecting us from environmental toxicity. This is evident in certain occupations where there is constant exposure to hazardous substances. Precautionary measures such as glove use minimizes the risk of incurring contact dermatitis (CD), though at times the gloves themselves may cause this skin disease. Barrier creams (BCs) may play an important role in the prevention of CD (1–6), and various in vitro and in vivo methods have been developed to evaluate their efficacy. In practice, their utilization remains the subject of a lively debate; some suggest that the inappropriate BC application may exacerbate rather than prevent irritation (1–3,6–9). The accuracy of measurements depends on the use of appropriate methodology.

This chapter provides the investigative details of pertinent scientific literature and summarizes methodology and efficacy of BC.

IN VITRO METHODS

In 1946, Sadler and Marriott (10) introduced facile tests to evaluate the efficiency of BC. One method used the fluorescence of a dyestuff and eosin as an indicator to measure penetration and the rates of penetration of water through BC; this is rapid and simple, but provides only a qualitative estimate.

Suskind (11) performed a simple method to measure the relative efficacy or repellency of several formulations with film immersion test in a specific exposure. Formulation containing 52.5% silicone in bentonite and 30% silicone in petrolatum were effective against a range of aqueous irritants and sensitizers.

Langford (12) conducted in vitro studies to determine that the efficacy of the formulated fluorochemical (FC)-resin complex included solvent penetration through treated filter paper, solvent repellency on treated pigskin, and penetration of radio-tagged sodium lauryl sulfate (SLS) through treated hairless mouse skin. He also conducted an in vivo study on 75 persons who had previously experienced irritation on their hands because of continued contact with solvents. Eighty-three percent of the panelists stated the cream was effective in protecting their hands.

Reiner et al. (13) examined the protective effect of ointments on guinea pig skin in vitro and in vivo. The permeation values of a toxic agent through unprotected and protected skin within 10 hours as a function of time was determined radiologically and enzymatically. Permeation of the toxic agent was markedly reduced by polyethylene glycol ointment base and ointments containing active substance. In in vivo experiments on guinea pigs, mortality was greater after applying the toxic agent to unprotected skin. All formulations with nucleophilic substances markedly reduced the mortality rate.

Loden (14) evaluated the effect of BC on the absorption of (^{3}H) water (^{14}C)-benzene and (^{14}C)-formaldehyde into excised human skin. The control and the BC-treated skins were exposed to the test substance for 30 minutes, whereupon absorption was determined. The experimental "water barrier" cream reduced the absorption of water and benzene, but not formaldehyde. One cream slightly reduced benzene and formaldehyde absorption. Two other creams did not affect the absorption of the substances studied.

Treffel et al. (15) measured in vitro on human skin the effectiveness of BC against three dyes (eosin, methylviolet, and oil red O) with varying n-octanol/water partition coefficients (0.19, 29.8, and 165, respectively). BC efficacy was assayed by measurements of the dyes in the epidermis of protected skin samples after 30 minutes' application. The efficacy of BC against the three dyes showed in several cases data contrary to manufacturer's information. There was

no correlation between the galenic parameters of the assayed products and the protection level, indicating that neither the water content nor the consistency of the formulations influenced the protection effectiveness.

Fullerton and Menne (16) tested the protective effect of various ethylenediaminetetraacetate (EDTA) barrier gels against nickel contact allergy using in vitro and in vivo methods. In an in vitro study, about 30 mg of barrier gel was applied on the epidermal side of the skin, and a nickel disc was placed above the gel. After 24-hour application, the nickel disc was removed and the epidermis separated from the dermis. Nickel content in epidermis and dermis was quantified by adsorption differential pulse voltametry (ADPV). The amount of nickel in the epidermal skin layer on barrier gel–treated skin samples was significantly reduced compared with the untreated control. In vivo patch testing of nickel-sensitive patients was performed using nickel discs with and without barrier gels. Test preparations and nickel discs were removed one day post application, and the test sites were evaluated. Reduction in positive test reactions was highly significant on barrier gel–treated sites.

Zhai et al. (17) used an in vitro diffusion system to measure the protective effect of quaternium-18 bentonite (Q18B) gels to prevent 1% concentration of [^{35}S] SLS penetration by human cadaver skin. The accumulated amount of [^{35}S]-SLS in receptor cell fluid was measured to evaluate the efficacy of the Q-18B gels over a 24-hour period. These test gels significantly decreased SLS absorption when compared with the unprotected skin control samples. The percentages of protection effect of three test gels against SLS percutaneous absorption were 88%, 81% and 65%, respectively.

IN VIVO METHODS

Schwartz et al. (18) introduced an in vivo method to evaluate the efficacy of a vanishing cream against poison ivy extract using visual erythema on human skin. The test cream was an effective prophylaxis against poison ivy dermatitis as compared to unprotected skin.

Lupulescu and Birmingham (19) observed the ultrastructural and relief changes of human epidermis following exposure to a protective gel and acetone and kerosene on humans. Unprotected skin showed cell damage and a disorganized pattern in the upper layers of epidermis. Protective agent prior to solvent exposure substantially reduced the ultrastructural and relief changes of epidermis cells.

Lachapelle et al. (3,20–23) used a guinea pig model to evaluate the protective value of BC and/or gels by laser Doppler flowmetry and histological assessment. The histopathological damage after 10 minutes of contact with toluene was mostly confined to the epidermis, while the dermis was almost normal. The dermal blood flow changes were relatively high on the control site compared with the gel-pretreated sites.

Frosch et al. (1,8,9,24,25) developed the repetitive irritation test (RIT) in the guinea pig and in humans to evaluate the efficacy of BC using bioengineering techniques. The cream-pretreated and untreated test skin (guinea pig or humans) were exposed daily to the irritants for two weeks. The resulting irritation was scored on a clinical scale and assessed by biophysical techniques' parameters. Some test creams suppressed irritation with all test parameters; some failed to show such an effect or even exacerbated (9).

Zhai (2) used an in vivo human model to measure the effectiveness of BC against dye indicator solutions: methylene blue in water and oil red O in ethanol, representative of model hydrophilic and lipophilic compounds. Solutions of 5% methylene blue and 5% oil red O were applied to untreated and BC-pretreated skin with the aid of aluminum occlusive chambers for zero and four hours. At the end of the application time, the materials were removed, and consecutive skin surface biopsies (SSBs) obtained. The amount of dye penetrating into each strip was determined by colorimetry. Two creams exhibited effectiveness, but one cream enhanced cumulative amount of dye.

Zhai et al. (5) introduced a facile approach to screening protectants in vivo in human subjects. Two acute irritants and one allergen were selected: SLS representative of irritant household and occupational CD, the combination of ammonium hydroxide (NH_4OH) and urea to simulate diaper dermatitis, and Rhus to evaluate the effect of model-protective materials. Test materials were spread over onto the test area, massaged, allowed to dry for

30 minutes, and reapplied with another 30-minute drying period. The model irritants and allergen were applied with an occlusive patch for 24 hours. Inflammation was scored with an expanded 10-point scale at 72 hours post application. Most test materials statistically suppressed the SLS irritation and Rhus allergic reaction and not NH_4OH- and urea-induced irritation.

Wigger-Alberti et al. (26) determined which areas of the hands were likely to be skipped on self-application of BC by fluorescence technique at workplace. Results showed that the application of BC was incomplete, especially on the dorsal aspects of the hands.

Draelos (27) conducted a randomized, double-blind, split-body study in 80 men, women, and children (neonate–80 years) with the following dermatological conditions: household dermatitis (21), occupational hand dermatitis (18), latex glove irritant CD (9), diaper dermatitis (5), cutaneous wounds (17), and allergic CD (10). The subjects were given two identical jars (1 jar containing petrolatum-based cream, and the other contained hydrogel-based barrier/repair cream) and were instructed to apply one cream to half of their bodies, while the other cream to the other half for four weeks. Results showed that 62% of the subjects preferred hydrogel-based barrier/repair cream over the petrolatum-based cream ($p \leq 0.005$) as well as the investigator's assessment ($p \leq 0.00001$) in terms of the overall skin appearance.

McCormick et al. (28) performed a double-blind, randomized trial comparing a novel BC versus an oil-containing lotion in 54 health care workers for two months. Results showed that both creams substantially protected the health care workers against drying and chemical irritation, preventing skin breakdown and promoting more frequent hand washing.

The skin protection efficacy of dexapanthenol was investigated by Biro et al. (29) in a double-blind, randomized, placebo-controlled study design in 25 healthy volunteers (18–45 years). They compared a cream containing 5% dexapanthenol with its vehicle-moisturizing base and applied to the flexor forearms twice daily for 26 days—one arm treated by the test product, while the other treated with placebo. In days 15 to 25, 2% SLS was applied on both forearms. Measures of skin physiology included sebumetry, corneometry, pH values, and clinical appearance (photographs). Results showed, though not significantly, a decreasing trend of the pH values and sebum content during SLS treatment but normalized when SLS was discontinued. Hydration of the stratum corneum remained stable throughout the study in the dexapanthenol group, while corneometry for the placebo group showed a significant ($p < 0.05$) decrease at the end of the SLS treatment on day 23. This study demonstrates the capability of dexapanthenol to protect skin from experimentally induced skin irritation.

Perrenoud et al. (30) conducted a double-blind crossover study comparing a new registered BC containing 5% aluminum chlorhydrate as active ingredient with its vehicle in 21 apprentice hairdressers who are frequently exposed to repeated shampooing and hair care products for a period of two months. The subjects were randomly assigned two groups; then, each subject was given identical 50-g tubes at the onset of the study, after two weeks, and at the start of the second phase. The contents of the tubes were unknown to the investigators and subjects. The participants recorded their daily comments. Evaluation of the creams' efficacy included: (*i*) clinical scores (dryness, redness, and breaks rated as 0 = none−3 = maximum) assessed by the researchers; (*ii*) biometric measurements using evaporimetry, corneometry, and chromametry; and (*iii*) recording of subjective opinions. Result for clinical evaluation showed low scores—nearly everyone had a "0" or "1" score. Only corneometric values showed a significant difference, i.e., the scores for the control group were significantly ($p < 0.01$) higher than the test product.

De Paepe et al. (31) investigated the beneficial effects of a skin tolerance−tested moisturizing cream on the barrier function in experimentally elicited irritant and allergic CD in 24 white female volunteers. Skin compatibility tests with the raw cosmetic materials and the final test product were initially performed in a large population to verify that the test product was well tolerated. Irritant CD was elicited using 1.25% SLS patch tested for 24 hours on the volar forearms of 12 white female volunteers in two sites (1 site for treatment with the test cream, while the other site left untreated). A third site was patch tested with filter paper soaked in pure water. Following patch removal, the forearms were washed, and application of 0.03-mL test cream was initiated the next day, twice daily for 14 consecutive days. There was a significant ($p < 0.05$) decrease in transepidermal water loss (TEWL) values of the treated site

on days 3, 8, and 15 as compared with the untreated site. Allergic CD was elicited using nickel-mediated contact allergy patch (CAP) test in another 12 white female volunteers with well-established histories of nickel (Ni)-contact allergies. Two patches contained 0.3 mL of 5% nickel sulfate in petrolatum and a third patch contained 0.3 mL of physiological serum (0.9% NaCl) to serve as control. Patches were removed after 48 hours, and test sites were cleaned with dry tissue, then 0.3 mL of the test cream was applied on the test sites twice a day for four consecutive days. Results revealed a significant ($p < 0.05$) decrease in TEWL values of the treated site when compared with the untreated site on days 3, 8, and 15.

Diepgen et al. (32) investigated six skin care products (Locobase® Pro cream, Debba® Wet, Taktosan®, Pluctect® Dual, Locobase® fatty cream, and Kerodex® 71) for their compatibility with normal and diseased skin, as well as their efficacy as protective skin barriers in 40 healthy volunteers in a double-blind study. The chamber scarification test (33) was used to compare the test products with known positive (aqueous SLS 0.5%) and negative (paraffin oil) controls and to rank the irritancy potential of products in 20 healthy volunteers. Approximately 0.1 mL of each product was applied to the scarified normal skin of the flexor forearms of the participants using Finn Chambers®. Patches were removed after 23 hours (day 1) and read an hour later and immediately before reapplication of the samples for days 2 and 3. Reactions were scored visually using a 5-point scale ("0" = no reaction to "4" = confluent, severe redness with edema or bullae). Results revealed that out of the eight samples applied, Debba Wet had the highest sum of scores ("12") in five subjects, while positive control only reached a maximum score of "10" in three subjects. Both Debba Wet and SLS 0.5% were considerably more irritating ($p < 0.0001$, $x^2 = 87$, df = 7) than the other test products. The ranking of the test products were: Debba Wet (score average = 11) $\geq$ aqueous SLS 0.5% (score average = 7.4) $\geq$ Taktosan cream (score average = 3.7) $\geq$ Locobase fatty cream (score average = 3.3) $\geq$ Kerodex 71, Pluctect Dual, Locobase Pro cream, and paraffin oil (score average = 2.2−3.0). On the other hand, the short-time repeated exposure occlusive irritation test (ROIT) was used to assess the efficacy of the six products and yellow Vaseline® as protective skin barriers in another 20 healthy volunteers. ROIT involved multiple short application times using low concentration of irritants. Aqueous SLS 0.5% was used as the irritant and was patch tested using Large Finn Chambers on the volar forearms of the subjects. For each site, the following were applied: irritant alone and water alone; one site was left blank, while the rest of the sites were first pretreated with the seven test creams 10 minutes before irritant application. The placing of the test products was changed from person to person according to a rotation system. The whole procedure was done every 3 to 3.5 hours for three consecutive days. Parameters used were TEWL (measured by Tewameter® TM 210), erythema (measured by ChromaMeter® CR 300), and clinical visual scoring (numerical scale: "0" = no reaction to "3" = pronounced erythema and edema, extensive scaling, possibly vesicles, bullae, pustules, and/or pronounced crusting). The comparison of the differential TEWL values between the test areas and the untreated sites showed significantly ($p < 0.05$) increased values for Vaseline, Taktosan, and Debba Wet. There was no significant difference among the TEWL values for Locobase Pro Cream, Plutect Dual, Locobase fatty cream, and Kerodex 71 when compared to normal skin. The increase in TEWL values was not significant ($p > 0.05$) between SLS-exposed sites and pretreated sites. Clinically, treatment with the SLS increased the visual scores. Likewise, Vaseline, Taktosan, and Debba Wet did not offer protection from skin irritation.

Modak et al. (33) demonstrated that the use of topical formulation with zinc gel delayed or prevented latex sensitivity in 22 volunteers known to have mild-to-moderate latex intolerance. Three centiliters of both zinc gel formulation and placebo creams were applied to the subjects divided into three groups: group A (zinc gel formulation applied on the right hand and placebo cream on the left hand in 10 subjects who used powdered latex gloves); group B (no cream on the right hand and zinc gel formulation on the left hand in another 10 subjects who used powdered latex gloves); and group C (no cream on the right hand and zinc gel formulation on the left hand in 2 volunteers who used powder-free latex gloves). Latex gloves were then worn by the subjects until they perceived discomfort or until three to four hours had passed without symptoms. Investigators rated the subjects using numerical scale: "0" = no visible reaction to "3" = severe itching, redness, and papules all over the hand within 30 minutes. Results showed that zinc gel formulation protected 21 out of 22 volunteers

from latex sensitivity. Only one subject had a score of "1" belonging to group A. Additionally, the investigators extracted latex proteins from the gloves and treated with zinc gel formulation diluted in distilled water. Results revealed that zinc gel formulation–treated latex proteins decreased (mean = 0.28) as compared with the untreated ones (mean = 1.14) by ~74%. Lastly, zinc gel formulation was compared with three other creams and a control (no cream applied) to evaluate its barrier efficacy. Zinc gel formulation proved superior among the three creams.

IN VITRO AND IN VIVO METHODS

Teichmann et al. (34) investigated the reservoir and barrier functions of the skin in two study designs because the former function is dependent on the latter function. Study design A was carried out in six healthy volunteers according to the method described by Teichman (35) and in pigskin to quantify stratum corneum penetration. Patent Blue V ($C_{54}H_{26}CaN_4O_{14}S_4$) in water (the penetrant) was applied to the human skin in increasing amounts—10 and 40 μg/cm^2 of the 0.5% concentration and 40 μg/cm^2 of the 2% concentration. After one hour, substances were wiped to avoid occlusion, and then tape stripping was performed on the fifth hour. Results for the 10 μg/cm^2 of the 0.5% concentration revealed that the amount of stratum corneum extracted was 5.10 ± 1.25 μg/cm^2—no penetrant was recovered, i.e., no excess amount developed. However, after the applications of 40 μg/cm^2 of the 0.5% concentration and 40 μg/cm^2 of the 2% concentration (21.5 ± 1.0 μg/cm^2 and 27.7 ± 1.5 μg/cm^2 of extracted stratum corneum, respectively), excess amounts of penetrants were recovered (6.7 ± 2.8 μg/cm^2 and 27.7 ± 1.5 μg/cm^2, respectively). The same procedure was performed on the porcine skin to obtain a histological diagnosis and showed that a large amount of Patent Blue V was located on the skin surface and the upper parts of the stratum corneum, and greater amounts were also found in the furrows.

Study B was performed in another six healthy volunteers and the three BCs—commercial BC, beeswax, Vaseline—were investigated using the penetration behavior of Patent Blue V in water in the different BC-pretreated skin, and one untreated site by tape stripping. Results revealed that the commercial BC did not demonstrate barrier function—similar to the untreated site ($p > 0.05$), while beeswax and Vaseline were significant ($p < 0.05$) in their efficacy of barrier function.

Chilcott et al. (36) conducted an in vivo and in vitro study evaluating the efficacy of a BC (70% w/w Fomblin^{TM} HC/R and 30% w/w lubricant grade polytetrafluoroethylene) versus chemical warfare agent in domestic white pigs. The in vivo study involved 18 pigs, prepared as previously described (37), and divided into three groups: control group (no agent, no BC), positive control group (with agent, no BC), and pretreated group (application of agent 15 minutes post application of the BC). An amount of 40 μL of the BC and ^{14}C-VX (~6-hour 2LD$_{50}$) was applied over the inner ear of the animals. Indicators of mortality included a decrease in serum acetylcholinesterase (AchE) and a large pupil diameter. Animals in the control and BC-treated groups survived the three-hour exposure period, while five of the six animals in the positive control group died after a mean time of 65 ± 13 minutes. Correspondingly, there was a significant ($p < 0.05$) decline in serum AchE, while there was no significant ($p > 0.05$) change in pupil diameter. On the other hand, the in vitro study involved the contralateral (unexposed) ears of the postmortem pigs from the in vivo study. Twelve pigskins were placed in Franz-type glass diffusion cells filled with phosphate-buffered saline (PBS) receptor chamber fluid. An amount of 25.4 μL of the BC was applied onto the skin using a 25-μL positive displacement pipette and spread using a piston from a 1-mL syringe, as previously described (37), to give a nominal thickness of 0.1 mm. Each diffusion cell was subjected to the same decontamination procedure similar to the in vivo study. Pretreatment with the BC significantly ($p < 0.05$) decreased skin surface spreading of ^{14}C-VX and lowered the total amount penetrated, similar to the in vivo study results. On the other hand, the three-dose parameters (i.e., unabsorbed, skin, and receptor/systemic) were significantly ($p < 0.05$) different except for one parameter (i.e., total amount absorbed) between the two systems.

Recent BC experiments are summarized in Table 1.

Table 1 Brief Data of Recent Experiments of Barrier Creams

Models						
In vitro	In vivo					
Animals or humans		Irritants or allergens or penetrants	Barrier creams	Evaluations by	Efficacy	Reference
Human skin		Dyes (eosin, methyl violet, oil red O)	16 BCs	Amount of dyes in the epidermis	Various % protection effects	Treffel et al. (15)
Human skin	Nickel-sensitive patients	Nickel disc	Ethylenediaminetetraacetate (EDTA) gels	Nickel content	Significantly reduced the amount of nickel in the epidermis in vitro, and significantly reduced positive reactions in vivo	Fullerton and Menne (16)
Human skin		[^{35}S]-SLS	3 quaternium-18 bentonite (Q-18B) gels	Amount of [^{35}S]-SLS	% protection effect was 88%, 81%, and 65%, respectively	Zhai et al. (17)
	Guinea pigs	n-Hexane, trichlorethylene, toluene	3 water-miscible creams	Morphological assessment	Limited protective effects	Lachapelle et al. (23)
	Guinea pigs and humans	SLS, sodium hydroxide, toluene, lactic acid	Several BCs	Various bioengineering techniques	Some of them suppressed irritation, some failed	Frosch et al. (1,8,24,25)
	Humans	Dyes (methylene blue and oil red O)	3 BCs	Amount of dye penetrating into strips	Two of them exhibited effectiveness, one enhanced cumulative amount of dye	Zhai and Maibach (2)
	Humans	SLS, ammonium hydroxide (NH_4OH) and urea, Rhus	Several protectants	Clinical scores	Most of them suppressed the SLS irritation and Rhus-allergic reaction, failed to suppress NH_4OH and urea irritation	Zhai et al. (5)
	Humans	Self-application of BC	An oil-in-water emulsion	Fluorescence technique	Self-application of BC was incomplete	Wigger-Alberti et al. (26)
	Humans	Skin with dermatitis	Hydrogel Barrier/repair creams	Questionnaire	62% of the subjects' and 75% of the investigators' assessments favored the BC	Draelos (27)

	Humans	Antiseptics, gloves	Novel barrier cream and cream with oil-containing lotion	Clinical scores	Both creams offered protection	McCormick et al. (28)
	Humans	SLS on days 15–25	5% dexpanthenol	Various bioengineering techniques and photography	Capable to protect skin in experimentally elicited irritation	Biro et al. (29)
	Humans	Shampoos and other hair care products	5% aluminum chlorhydrate	Clinical scores, bioengineering techniques, subjects' personal assessment	Very little difference between BC and its vehicle	Perrenoud et al. (30)
	Humans	SLS and nickel	Skin tolerance–tested moisturizing cream	TEWL	Significant decrease in TEWL values of treated sites	Paepe et al. (31)
Ears of the domestic white pigs	Domestic white pigs	VX chemical warfare	AG-7 (70% w/w Fomblin™ HC/R plus 30% w/w lubricant grade polytetrafluoroethylene)	Acetylcholinesterase, pupil diameter;	Treated groups survived the 3-hr exposure; Pretreatment of BC lowered the amount of VX penetration	Chilcott et al. (36)
	Humans	SLS	6 skin care products	Chamber scarification test and ROIT	Debba Wet and SLS were more irritating; Vaseline, Takstosan, and Debba Wett did not offer protection from skin irritation.	Diepgen et al. (32)
Dissolved latex proteins	Humans	Latex gloves	Topical formulation with zinc	Clinical Scores	Protected 95% of subjects; Decreased dissolved latex proteins by ~74 %	Modak et al. (33)
Pigskin	Humans	Patent Blue V	Quantify stratum corneum penetration; and comparison of 3 BCs for efficacy	Penetration behavior of Patent Blue V	Higher concentrations of the penetrant yielded excess amount recovered; and Vaseline and beeswax are effective BCs	Teichman (34)

Abbreviations: SLS, sodium lauryl sulfate; BC, barrier cream; TEWL, transepidermal water loss; ROIT, repeated occlusive irritation test.

CONCLUSIONS

Some BCs reduce CD under experimental conditions. But, inappropriate BC application may enhance irritation rather than benefit. To achieve the optimal protective effects, BC should be used with careful consideration based on a specific exposure conditions; also, the proper use of BC should be instructed.

In vitro methods are simple, rapid, and safe and are recommended in screening procedures for BC candidates. With radiolabeled methods, we may determine the accurate protective and penetration results even in the lower levels of chemicals because of the sensitivity of radiolabeled counting when BCs are to be evaluated. Animal experiments may be used to generate kinetic data because of a similarity between humans and some animals (pigs, monkeys, etc.) in percutaneous absorption and penetration for some compounds. But no one animal, with its complex anatomy and biology, will simulate the penetration in humans for all compounds. Therefore, the best estimate of human percutaneous absorption is determined by in vivo studies in humans. The histological assessments may define what layers of skin are damaged or protected and may provide the insight mechanism of BC. Noninvasive bioengineering techniques may provide accurate, highly reproducible, and objective observations in quantifying the inflammation response to various irritants and allergens when BCs are to be evaluated that could assess subtle differences to supplement traditional clinical studies.

To validate these models, well-controlled field trials are required to define the relationship of the model to the occupational setting. Finally, the clinical efficacy of BC should be assessed in the workplace rather than in experimental circumstance. A recent review of evaluating the efficacy of BC provides additional insights (38).

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19 Electron Paramagnetic Resonance Studies of Skin Lipid Structure

Kouichi Nakagawa
RI Research Center, Fukushima Medical University, Hikarigaoka, Fukushima, Japan

INTRODUCTION

Stratum corneum (SC) is the outermost layer of skin and the skin barrier against chemicals, surfactants, UV irradiation, and environmental stresses.The SC has a heterogeneous structure composed of corneocytes embedded in the intercellar lipid lamellae, as illustrated in Figure 1. The morphology of the SC lipids is closely associated with the main epidermal barrier. Knowledge of the lipid structure is important in understanding the mechanism of irritant dermatitis and other SC diseases. The structural properties of the SC lipid are obtained by the analyses of aliphatic spin probes incorporated into intercellular lamella lipids using electron paramagnetic resonance (EPR) (1–7). The EPR spin probe method measures nondistractively the ordering of the lipid bilayer of SC.

EPR (or electron spin resonance, ESR) utilizes spectroscopy, which measures the freedom of an unpaired electron in an atom or molecule. The principles behind magnetic resonance are common to both EPR and nuclear magnetic resonance (NMR), but there are differences in the magnitudes and signs of the magnetic interactions involved. EPR probes an unpaired electron spin, while NMR probes a nuclear spin. EPR can measure 10^{-9} molar concentration of the probe and is one of the most sensitive spectroscopic tools. Therefore, EPR is able to elucidate skin lipid structures as well as dynamics.

EPR in conjunction with the spin probe (or label) method has considerable advantages in the study of lipid structures as well as behaviors. The macroscopic and local viscosity of the environment profoundly influences the rate of lipid molecular reorientation. The physicochemical properties of intercellar lipids of SC as a function of various surfactants (1,2), water contents (3), various kinds of spin probes (4), and ordering (or fluidity) change of the SC lipid (5) were investigated. These studies provided the fluidity-related behaviors of SC at the different conditions by measuring EPR signal intensities and hyperfine coupling values. Furthermore, quantitative analysis of the experimental spectra can be achieved by a modern slow-tumbling simulation, which showed that the spectral simulation provided insight into the quantitative ordering of human lipid structure (6,7). In this chapter, the quantitative evaluations of SC lipid structure as a function of skin depth are described.

EPR APPARATUS

EPR apparatus consists of a klystron to generate microwaves, electromagnet, resonant cavity, microwave detector, amplifier, A/D converter, and PC (Fig. 2). The microwaves from the klystron have a constant frequency, and those microwaves reflected from the resonant cavity are detected, changed to an electronic signal, amplified, and then recorded. In contrast to NMR, substances that contain unpaired spin can be observed by EPR. Paramagnetic substances including transition metal complexes, free radicals, and photochemical intermediates are observed. Approximately 10^{-13} mole of a substance gives an observable signal, thus EPR has great sensitivity.

EPR OF SPIN PROBES (or SPIN LABELS)

Nitroxide Probes for EPR

Momentum of electron spin in a magnetic field orients only two quantum states: $m_s = ½$ and $m_s = -½$. Application of an oscillating field perpendicular to a steady magnetic field (H) induces transitions between the two states, provided the frequency (ν) of the oscillating field

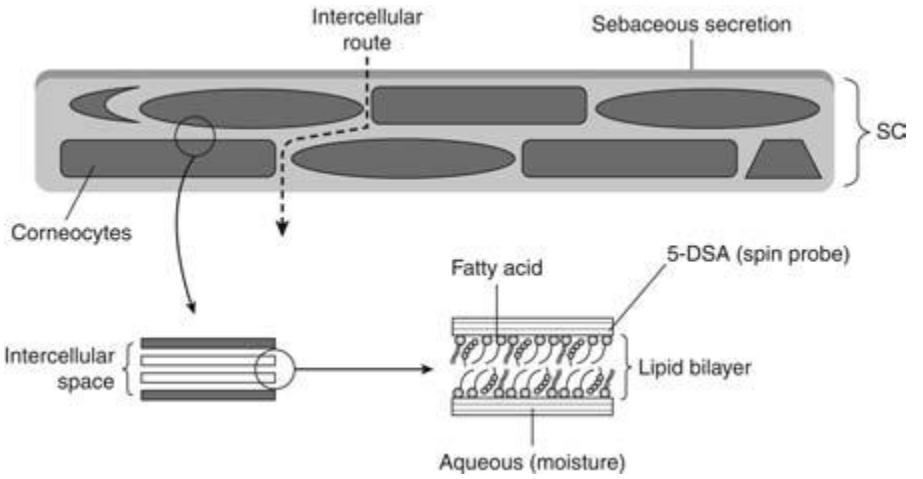


Figure 1 Schematic representation of the "Brick and Mortar" model of the stratum corneum is shown. Also, the most likely probe location in the lipid bilayer and pathways of drug permeation through intact stratum corneum is shown.

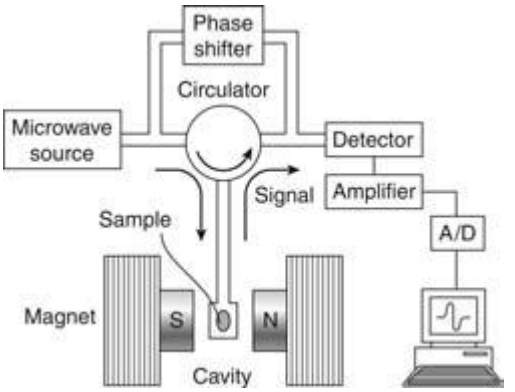


Figure 2 Block diagram of EPR spectrometer.

satisfies the resonance condition:

$$\Delta E = h\nu = g\beta H \tag{1}$$

where ΔE is the energy-level separation, h is Planck's constant, g is a dimensionless constant called the g-value, β is the electron Bohr magneton, and H is the applied magnetic field.

The interaction of an electron spin in resonance with a neighboring nuclear spin in a molecule is called hyperfine coupling. In the case of nitroxide spin probe, ^{14}N of the probe has three quantum states: $+1$, 0, and -1. Each quantum state interacts with an electron spin and further splits into two sets of energy states (Fig. 3). The selection rules for transitions in hyperfine coupling are $\Delta m_s = 1$ and $\Delta m_I = 0$. Thus, one can observe three transition (resonance) lines for fast-tumbling nitroxide spin probe in a spectrum. The interval of the resonance lines is called the hyperfine coupling constant. The EPR spectra are usually recorded as the first derivative of the absorption spectrum as shown in lower part of Figure 3.

Single-Chain Aliphatic Spin Probes

The ordering (or fluidity) of the lipid bilayer is obtained with doxyl stearic acid (DSA), which is most commonly used. The chemical structures of DSAs are depicted in Figure 4. Changes of lipid chain ordering are able to monitor using the probes. The orientation of spin probe reflects the local molecular environment and should serve as indicator of conformational changes in lipid bilayers.

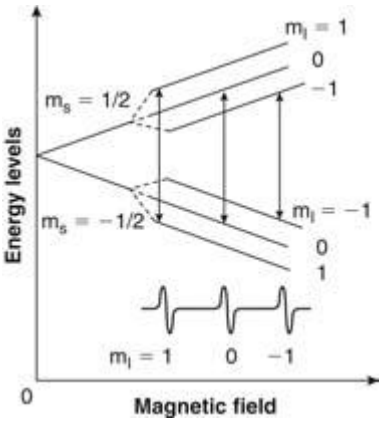


Figure 3 Hyperfine levels and transitions for a nitroxide nitrogen nucleus (^{14}N) of $I = 1$ with positive coupling constant.

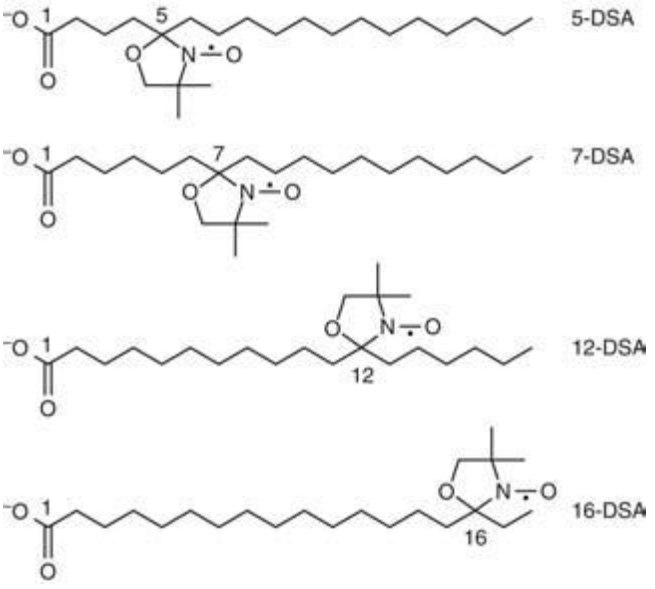
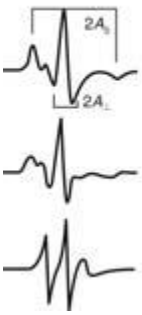


Figure 4 Chemical structures of various doxyl stearic acid (DSA) spin probes.

The ordering at different position of the lipid bilayer is obtained with 5-, 7-, 12-, and 16-DSA. The 5-DSA is usually used for extraction of information near surface region in a lipid membrane. The 16-DSA is for near the end of the lipid chain. It is notable that other spin probes are also commercially available.

EPR Line Shapes Due to Spin Probe Motion

The line widths can vary under certain spin probe environments. When line broadening arises from incomplete averaging of the *g*-value and the hyperfine coupling interactions within the limit of rapid tumbling in a medium, EPR line shape starts changing from the triplet pattern. EPR spectra of nitroxide radicals for different tumbling times as well as different order parameters are presented in Figure 5. If a spin probe is oriented (immobilized) in a membrane, EPR spectrum is an anisotropic pattern, which clearly shows parallel and perpendicular hyperfine coupling structures (the top spectrum in Fig. 5). The order parameter is approximately 0.7 or higher. If a spin probe tumbles relatively fast (weakly immobilized) in a membrane, EPR spectrum is a triplet pattern with unequal intensities. The order parameter is usually very small (~0.1).



Description of spectra	Approx. tumbling time (ns)	Approx. order parameter
Immobilized	0.5	0.7
Moderately immobilized	2.5	0.3
Weakly immobilized	5.0	0.1

Figure 5 Nitroxide EPR line shape as a function of tumbling time and order parameter. Parallel and perpendicular hyperfine couplings, $2A_{||}$ and $2A_{\perp}$, are also indicated for an anisotropic (immobilized) EPR spectrum.

CALCULATION OF ORDER PARAMETER

Conventional Order Parameter (S)

The order parameter indicates the lipid fluidity and microenvironment of the medium in which the spin probe is incorporated. The conventional order parameter (S) is determined by the hyperfine coupling (A) of the EPR spectrum according to the following relations (8):

$$S = \frac{A_{II} - A_{\perp}}{A_{ZZ} - (1/2)(A_{XX} + A_{YY})} \frac{a}{a'}, \tag{2}$$

$$a' = \frac{A_{II} + 2A_{\perp}}{3}, \tag{3}$$

where a is the isotropic hyperfine coupling value, $(A_{XX} + A_{YY} + A_{ZZ})/3$; A_{XX}, A_{YY}, and A_{ZZ} are the principal values of the spin probe. The experimental hyperfine couplings of $2A_{||}$ and $2A_{\perp}$ are obtained from the EPR spectrum. In a calculation of the order parameter, the principal components of A_{XX}, A_{YY}, $A_{ZZ} = (0.66, 0.55, 3.45)$ mT and g_{XX}, g_{YY}, $g_{ZZ} = (2.0086, 2.0063, 2.0025)$ were used for 5-DSA (9).

Note that the conventional analysis measuring $2A_{||}$ and $2A_{\perp}$ gives limited information concerning the probe moiety in the lipid. Changes in the probe behavior are reflected in the EPR line width as well as the line shape, besides hyperfine values. In some cases, S-values do not represent the subtle difference in overall EPR spectral changes related to the lipid chain ordering (6). Thus, the conventional calculation is qualitative analysis.

Order Parameter (S_0) by Slow-Motional EPR Simulation

In general, the large ordering value indicates the anisotropy of the probe site in the lipid (Fig. 5). For example, the spin probe is incorporated in the highly oriented intercellular lipid bilayer in normal skin; the probe cannot move freely because of the rigid lipid structure. Once the normal lipid structure is completely destroyed by chemical and/or physical stress, the clear triplet spectrum yields the small ordering value.

The slow-tumbling motions of the spin probes can be exactly calculated using a nonlinear least square–fitting program called NLLS, which analyzes the experimental EPR spectra on the basis of stochastic Liouville's equation (10–12). The simulation of the EPR spectra for spin probes incorporated into multilamella lipids is carried out using a microscopically ordered but macroscopically disordered (MOMD) model introduced by Meirovitch et al. (13). This model is based on the characteristics of the dynamic structure of lipid dispersions.

The order parameter, S_0, is defined as (14,15):

$$S_0 = \langle D_{00}^2 \rangle = \left\langle \frac{1}{2}(3\cos^2\gamma - 1) \right\rangle = \frac{\int d\Omega \exp(-U/kT) D_{00}^2}{\int d\Omega \exp(-U/kT)}, \tag{4}$$

which measures the angular extent of the rotational diffusion of the nitroxide moiety. Gamma (γ) is the angle between the rotational diffusion symmetry axis and the z-axis of the nitroxide axis

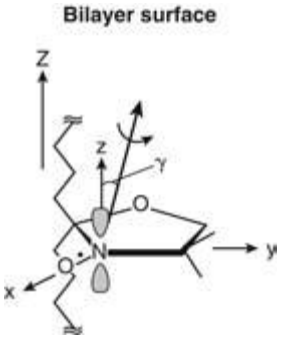


Figure 6 A schematic representation of a conformation of DSA spin probe in the SC membrane, where z-axis of the acyl chain is parallel to z-axis of the nitrogen $2P_z$ orbital.

system; z is the axis of the nitrogen $2p_z$ orbital, and x-axis is along the N–O bond (Fig. 6). The local or microscopic ordering of the nitroxide spin probe in the membrane is characterized by the S_0 value. A larger S_0 value indicates very restricted motion in the membrane. It is notable that the angle in relation with the S_0 value is discussed later in the next section.

Conventional (S) and Simulated Order Parameter (S_0)

The "Brick and Mortar" model of the SC is illustrated in Figure 1. SC intercellular lipids arrange themselves into bilayer and pack into lamellae. The single-chain 5-DSA normally dissolves into lipids and fat phases. The most likely location of the single-chain probe in the SC is shown in Figure 1. The aliphatic probe will be located in the lipid phase and fat-like sebaceous secretion of the SC.

Stripped SC was examined to characterize the lipid chain ordering using two methods: conventional order parameter and simulated order parameter (5,6). One piece of stripped SC (~7×37 mm^2) was incubated in ~50 μm 5-DSA aqueous solution for about 1 hour at 37°C. After rinsing with deionized water to remove excess spin probe, the SC sample was mounted on an EPR cell. A commercially available X-band (9 GHz), EPR spectrometer, was used to measure the ordering of the SC sample. The typical spectrometer settings were the following: microwave power, 10 mW; time constant, 1 second; sweep time, 480 seconds; modulation, 0.2 mT; and sweep width, 15 mT. The detailed sample preparations are described elsewhere (7).

Figure 7 shows the experimental and simulated EPR spectra of 5-DSA in the SC. The reasonable agreement of the experimental and simulated spectra suggests that simulation analysis can provide detailed information regarding the SC lipids. The S_0 value changes from 0.61 to 0.96, while the S value is in the range of 0.56 to 0.59. The conventional S value was obtained by Eq. (2) measuring the hyperfine values from the observed spectrum.

There are significant differences between the conventional and simulated order parameters. Because the slow-tumbling simulation calculates the total line shape of the

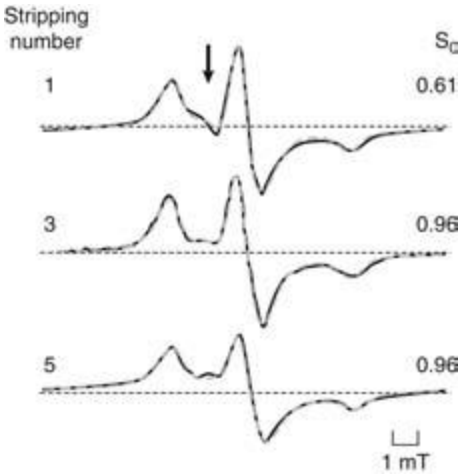


Figure 7 Experimental (*solid line*) and simulated (*dashed line*) EPR spectra of 5-DSA probe. Stripping numbers show consecutively stripped SC from the surface downward. The arrow of stripping number 1 indicates the characteristic peak.

spectrum, it is able to extract more detailed information about the SC structure than the conventional analysis, which is normally ambiguous in distinguishing the two hyperfine components (parallel and perpendicular) from the experimental spectrum because of the presence of weak and broad signals (6). Thus, the S_0 values (0.2–0.5) obtained by the simulation suggest that the outermost SC layers are less rigid (or more mobile), while the deeper lipid layers (S_0~0.9) have more rigid and oriented structures.

The arrow in the spectrum indicates the characteristic peak, which is prominent only for the first stripping (Fig. 7). This peak diminishes in intensity with increasing depth in the SC. The marked peak appears near the center of the spectrum because the probe embedded in the first stripping sample has greater freedom of motion. The other two lines of the nitroxide probe overlaid the central region of the spectrum. The results imply that signals can originate from sebaceous secretion.

Further investigation of the characteristic peak was performed. Figure 8A shows the EPR spectrum of the first stripping from SC. The strong and broad peak observed for the SC sheet from the human forehead is shown in Figure 8B. The peak intensity decreases after washing the SC with soap (Fig. 8C). Thus, the signal can be attributed to sebaceous secretion (7). The strength of the signal is considered to reflect the abundant sebaceous secretion at the forehead compared with that of the forearm.

Furthermore, one can calculate the angle (γ in Fig. 6) between the rotational diffusion symmetry axis (the lipid in SC) and the z-axis of the nitroxide axis system. Figure 9 represents the schematic illustration of the bilayer distance in relation to the angle. The simulated S_0 value of 0.61 can be the angle of 30. The value of 0.96 is the angle of 9.4. The angle suggests that the SC lipids align nearly perpendicularly to the bilayer surface. The larger S_0 value yields larger distance between the lipid bilayer. The analysis implies that the long distance of the lipid bilayer can be related to the well-oriented SC structure.

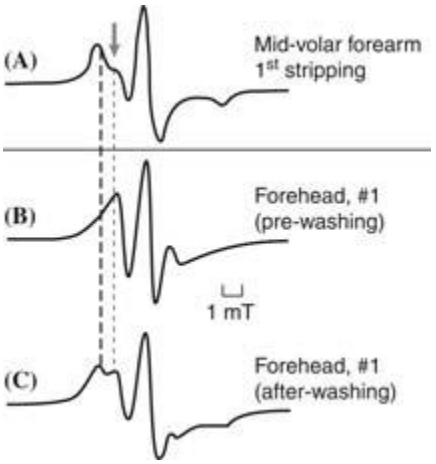


Figure 8 Experimental EPR spectra of 5-DSA in (**A**) the first stripped SC from human mid-volar forearm, (**B**) the first stripping SC from human forehead prewashing, and (**C**) the first stripping SC from human forehead after-washing. The short dashed line corresponds to the characteristic signal. The long dashed line corresponds to the probe incorporated into the SC lipids.

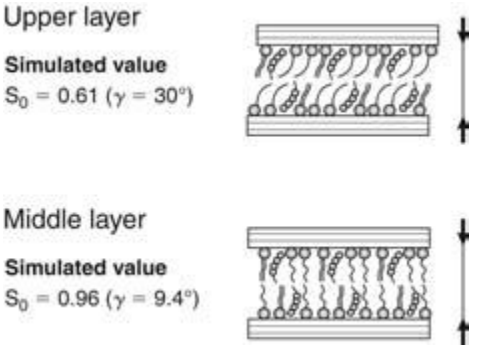


Figure 9 The bilayer distances and the values of simulated order parameter related to the angles between the bilayer surface and the single-chain probe.

OTHER APPLICATIONS OF THE EPR METHOD

Effects of Surfactants

Different types as well as mixtures of surfactants change the fluid structure of lipid bilayer differently. Kawasaki et al. examined the influence of anionic surfactants, sodium lauryl sulfate (SLS) and sodium lauroyl glutamate (SLG), on human SC by the EPR spin label method (1). The order parameter obtained by 1.0% wt SLS-treated cadaver SC (C-SC) was 0.52. On the other hand, the high S value of 0.73 for 1.0% wt SLG was obtained. The results suggest clear surfactant effects on the structure of lipid bilayer. In addition, a reasonable correlation between order parameters and human clinical data (visual scores and transepidermal water loss values) was shown.

Effects of Skin Penetration Enhancers

Interaction of skin penetration enhancer correlates with the fluidity of the intercellular lipid bilayers. Quan and Maibach investigated the effects on a C-SC at three concentrations of laurocapram (1-dodecylazacyclo-heptan-2-one) utilizing the EPR spin probe method (16). The EPR spectra of laurocapram-treated human SC were totally different from those of untreated C-SC. The results suggest that laurocapram causes an increase in the flexibility and polarity of local bilayers surrounding 5-DSA.

CONCLUSION

EPR along with a modern computational analysis provides quantitative insight into the SC structure as a function of the depth. The EPR spectral pattern contains important information regarding the probe moiety as well as the SC structure. Satisfactory agreement between the experimental and calculated spectrum can provide a quantitative S_0, which gives the microscopic lipid ordering in the SC lipid. The spectral simulation offers a reliable value of the lipid ordering where conventional order parameter cannot reveal the detailed ordering (6). In addition, the EPR method recognizes sebaceous exudates (7). Therefore, EPR together with a computational analysis is a powerful method to investigate various SC.

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20 Human Skin Buffering Capacity: An Overview

Jackie Levin
AZCOM, Glendale, Arizona, U.S.A.

Howard I. Maibach
Department of Dermatology, University of California School of Medicine, San Francisco, California, U.S.A.

INTRODUCTION

When dilute aqueous acid or alkaline solutions come into contact with the skin, the change in pH is generally temporary, and the original skin pH (a measure of the hydronium ion concentration) is rapidly restored indicating that the skin has significant buffering capacity.

A buffer is a chemical system that can limit changes in pH when an acid or a base is added. Buffer solutions consist of a weak acid and its conjugated base. The system has its optimum buffering capacity when about 50% of the buffer is dissociated or, in other words, at a pH about equal to its pKa (1). The pKa is the negative of the common logarithm of the acid dissociation constant (Ka) and is a measure for the strength of the acid. The buffer capacity is further dependent on the concentration of the system.

SKIN'S ACID CHARACTER AND BUFFER CAPACITY

The acidic character of the skin was first mentioned by Heuss (2) and later by Schade and Marchionini (3) who introduced the term "acid mantle" for the skin's acidic outer surface pH. The importance of the skin's acidic character has more recently been recognized as playing a crucial role in barrier homeostasis and immune function (4–6). The skin was further shown to partially resist acidic/alkaline aggression to some extent (7).

This article provides a review of studies investigating the skin's buffering capacity, specifically the epidermis, via alkali/acidic aggression tests. This review tries to discern which components of the epidermis are most likely responsible for the skin's buffering capacity.

Alkali/Acidic Aggression Tests

An acid/alkali aggression test is a way to measure the acid/alkali resistance (i.e., buffering capacity) of the skin. Alkali/acidic resistance tests were commonly used in the 1960s to detect workers who may likely develop occupational diseases in certain chemical work environments (7). A mild variation of the alkali/acidic resistance tests, also called acid/alkali neutralization test, assesses how quickly the skin is able to buffer applied acids/bases without the occurrence of skin corrosion. Repetitive applications of acid or base demonstrate that the skin's buffering capacity is limited and may be overcome; as illustrated by the long time required for neutralization (8–11).

The next section focuses on the aggression tests aiming to study which components of the epidermis are responsible for the skin's buffering capacity.

Free Fatty Acids/Sebum

Early experimentation hypothesized that the sebum contributes to the buffering capacity of the skin in two ways: first, it protects the epidermis against the influence of alkali by slowing down the exposure and penetration of acids or alkalis applied to the skin (12–14), and second, the fatty acids in sebum may act as a buffer system (15,16).

Later experiments by Lincke et al. (17) refuted the second hypothesis by demonstrating that the sebum had no relevant acid and a negligible alkali-buffering capacity of around pH 9. Further challenging the hypothesis, a quicker neutralization was observed on the delipidized skin than the untreated skin (12,14).

Vermeer concluded similarly when comparing the neutralization on soles and forearm with and without sebum removal (16). However, when comparing these skin regions,

differences in the sebum content and stratum corneum (SC) thickness may have also contributed to the observed effect.

Vermeer (14) and Neuhaus (18) believe that the increased rate of neutralization is due to a higher carbon dioxide (CO_2) diffusion. This theory, discussed later in detail, is generally not accepted but is also not clearly substantiated either way. After lipid removal, the skin starts to increase acid production, which may account for the faster neutralization. The same investigators also found that the increase in neutralization after lipid removal is temporary and limited to the first few minutes, which is probably related to the activity of sebaceous glands to produce relevant amounts of sebum.

Because of the negligible buffering capacity of sebum and to standardize the experiment (limit inter- and intra-individual variability), today most neutralization experiments are performed after cleansing the skin with solvents, which remove most of the sebum, including fatty acids.

Epidermal Water-Soluble Constituents

Vermeer et al. (16) first demonstrated the importance of water-soluble constituents to the skin's buffering ability. Water-soaked skin, where the water-soluble constituents were extracted, demonstrated a significantly reduced neutralization capacity, indicating that water-soluble substance constituent(s) of the skin is a major contributor to the buffering capacity (10,19,20). Water soaking may have induced also other changes to the skin, altering buffering capacity.

The significance of water-soluble constituents of the epidermis to the buffering capacity of the skin further supports the theory of minimal contribution from the sebum of the skin due to its lipid-soluble nature (16).

Sweat

Eccrine sweat initially accelerates the neutralization of alkalis (8–11,16,19,21,22). Spier and Pascher (23) suggest that the main buffering agents of sweat are lactic acid and amino acids (AAs). The lactic acid-lactate system in sweat has a highly efficient buffering capacity between pH 4 and 5 (13). However, it has not been completely demonstrated that lactic acid is the main buffering agent in sweat or at the surface of the skin. Conversely, the contribution of AAs to the buffering capacity of sweat and of the horny layer surface has been investigated thoroughly (16,19,22).

By comparing sweating and non-sweating persons, Vermeer et al. (16) found that AAs play a significant role in neutralization during the first five minutes while lactic acid does not. This confirms that AAs are key elements contributing to the buffering capacity of the skin.

Keratin

The contribution of keratin to the buffering capacity of the skin remains questionable. Keratin is an amphoteric protein with the ability to neutralize acids and alkalis in vitro (8–11,17,24–26) and hence may participate in the skin's buffering capacity. Scales scraped from the normal skin bind small amounts of alkali in vitro (27,28). However, Vermeer and coworkers showed that water-soluble constituents of the epidermis participate more in the skin's buffering capacity than the insoluble constituents of the skin such as keratin.

While insoluble keratin filaments on the skin may have only little buffering capacity (16,29), keratin hydrolysates and free AAs might contribute to the water-soluble portion of the epidermis. However, AA's composition of keratin (30,31) does not correspond with AA found in the water-soluble portion of the SC (23), which implies that keratin is not a major contributor to the pool of free AA.

Despite little evidence of keratin's role in the buffering capacity, a modifying action of keratin is assumed (17). Without an intact keratin layer, neither a physiological surface pH nor normal neutralization capacity can be maintained (32). Further research remains to be conducted to determine keratin's role in the buffering capacity of the epidermis.

Stratum Corneum Thickness

Differences in thickness of the SC may explain the interindividual differences in buffering capacity (33). The thicker the SC, the better the buffering (10,19), which is likely related to a better barrier for acids/alkalis within a thicker SC (33). In addition, as the skin ages, its

thickness diminishes and its buffering ability diminishes (34). Current technology, allowing more accurate SC measurement, may help in clarifying this point (35).

CO_2

Little is known about the role of CO_2/HCO_3^- participating in the skin's buffering capacity. Burckhardt's studies were the first to suggest that the CO_2 diffusing from the epidermal layer may be responsible for neutralizing alkali in contact with the skin (8–11). He demonstrated (8,9) that when a five-minute alkali neutralization experiment was repeated subsequently several times on the same skin area, the neutralization times were becoming longer, but finally reached an approximately constant time. He suggested that the shorter neutralization times at the beginning were due to acids present on the skin surface rapidly neutralizing the alkali. He further suggested that, after successive alkali exposure, the endogenous acids were not anymore present on the skin surface, which resulted in longer neutralization times, and that through skin diffusing, CO_2 took over the role in neutralizing the alkali. At this time, Burckhardt's hypothesis of the role of CO_2 as a buffering agent was accepted by others despite the rather weak experimental evidence (17,25,36,37).

For instance, the increased neutralization time after lipid removal of the skin surface with the help of soaps or neutral detergents was believed to be the consequence of a greater diffusion of CO_2, although this has never been quantified (13,36,37). It was also postulated that the hydrated SC retains CO_2 and limits its diffusion, whereas a moderate hydration level was regarded best for an effective alkali neutralization, although this has also never been analyzed in further details (37).

Clearly, the above studies fail to provide quantitative support for their conclusions concerning CO_2 as a relevant buffering agent. More likely, the constant neutralization time after successive alkali exposure may be related to the destruction of the "skin barrier" and unlimited penetration of the applied alkali as suggested by others (18,19).

Knowing that several authors considered CO_2 a relevant contributor in alkali neutralization without having quantitative data to sustain their hypothesis, Vermeer et al. (19) demonstrated that CO_2 is unlikely of great importance for alkali neutralization on the skin.

His experiment was focused on the first minutes of the neutralization process in contrast to the previous experiments mentioned (18,25,36,37), which paid attention to the later neutralization process. For example, Piper (25) analyzed the neutralization process for up to one hour and concluded that for the first half an hour alkalis are neutralized on the skin by the skin's own amphoteric substances (such as AAs) but in the second half hour diffusing CO_2 takes over. Piper's conclusions are not necessarily contradictory to the results obtained by Vermeer above and may actually be in agreement. According to Piper, "the longer the contact between the skin and alkali, the greater the importance of CO_2." Supported by the recent discoveries of relatively low level of CO_2 production in the epidermis and the limited activity of the Kreb's cycle suggesting that a minimal amount of CO_2 would be available for neutralization (30), it seems likely that CO_2 does not significantly contribute in the alkali neutralization process. Further studies should help to clarify the relevance of CO_2 in the skin's buffering capacity.

Free AAs

The free AAs in the water-soluble portion of the epidermis seem to play a significant role in the neutralization of alkalis within the first five minutes of experimentation (16,25,26).

Piper (25) found a good buffering capacity of the skin between pH 4 and pH 8, with an optimum at 6.5 well corresponding to the pKa of AA. This observation further indicates that lactic may be less relevant in alkali neutralization of the skin.

Despite the general agreement about the role of AAs in the neutralization of alkalis, which AAs are the key buffering agents remains an open question. The AAs' composition of the upper SC was reported by Spier and Pascher (23).

Spier and Pascher reported that the free AAs of the SC account for 40% of the water-soluble substances extracted from the SC removed by tape stripping (23,29). From the AAs present, 20% to 32% was serine and 9% to 16% was citrulline. Aspartic acid, glycine, threonine, and alanine were 6% to 10%. The smallest concentration of AAs accounted for glutamic acid at 0.5% to 2%.

The water-soluble, free AAs on the skin surface may originate from three possible sources.

1. Eccrine sweat
   Sweat contains 0.05% AAs, which remain on the surface of the skin after evaporation. The specific AA found in sweat was not investigated.
2. Degradation of skin proteins
   Degradation of skin proteins, including proteins constituting the desmosomes, may be a source for AAs such as serine, glycine, and alanine.
3. Hair follicle
   Citrulline is recognized as a constituent of protein synthesized in the inner root sheath and medulla cells of the hair follicle. Specific proteases release citrulline. Citrulline was also found in proteins in the membrane of the corneocytes as well as free floating (30).
   Further research needs to be completed to identify which AAs contribute to the buffering capacity of the skin and what is their main source.

DISCUSSION

The buffering capacity depends on many factors such as the following.

Alkali/Acid Agression

The nature and content of epidermal constituents available for buffering is likely a function of the acid/alkali aggression. For example, the more corrosive the compounds, the more the destruction of the skin, which results in an increased level of skin substances potentially available for buffering. In addition, a corrosive compound damages the skin's barrier, leading to increased penetration of the acid/alkali, which may further influence the skin's buffering capacity.

Skin Condition

Skin conditions were shown to greatly influence buffering capacity. Besides the skin's barrier properties, which were shown to vary between subjects and depend on the skin conditions and health, the presence of free AAs participating in the neutralization process may also play a role in the buffering capacity of the skin. Subjects with low buffering capacity are especially susceptible to the irritating effect of acids and/or bases, and predisposed to contact occupational eczema and dermatitis (29).

CONCLUSION

Whereas the skin's exquisite buffering capacity has been widely studied in vitro and in vivo, further research needs to be completed to better understand the exact mechanisms.

Experimentation reviewed here suggests that AAs are primarily responsible for the neutralization capacity of the skin. The exact sources of the AAs as well as the types of AAs that are primarily responsible for the neutralization capacity remain still rather speculative. In addition, it seems that a sweat component increases the neutralization capacity of the epidermis. Whether the buffering component of sweat is additional AA or lactic acid remains unknown.

While additional components of the epidermis such as sebum, keratin, and CO_2 seem not to significantly participate as buffering agents of the epidermis, they still do seemto play a role in the protection of the skin from the harm of acids and bases. Sebum may slow down the initial penetration of applied substances. Keratin is important for the hydration of the skin and may contribute some of the free AAs responsible for buffering of applied acids/alkalis. Finally, CO_2 may play a role in the buffering capacity of certain compounds under certain circumstances such as after prolonged or repetitive exposure to an alkali.

When the buffering capacity is exhausted, the pH of the skin becomes significantly altered; repair mechanism similar to wound healing may step in to restore the normal skin's pH (38). After thorough review of studies investigating the buffering capacity of the skin and

other studies investigating the endogenous mechanisms for restoring and maintaining the skin pH, it is interesting to note that the two topics have been investigated separately without looking for a commonality. It would not be surprising if the mechanisms responsible for maintaining the skin pH influence the processes responsible for maintaining the skin's buffering capacity. The above rationale may shed light on skin diseases in persons with diminished buffering capacity, an increased sensitivity to acids and/or bases, and an increased skin surface pH.

Taken together, we interpret this rich experimental literature, even if often quite old, as leading the way to the use of contemporary methods to further refine our insight into the skin's buffering capacity. This capacity, when fully understood, may lead not only to the potential for decreasing threat to the skin of exogenous acids and bases, but for establishing an experimental base for optimal pH in many pharmacologic, metabolic, and toxicologic situations.

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21 Skin pH and Skin Flora

Shamim A. Ansari
Colgate-Palmolive Company, Piscataway, New Jersey, U.S.A.

INTRODUCTION

The skin being the largest organ covers the entire exterior of the body and thus forms a protective barrier in between the human body and its environment. This tough and dry exterior signifies the physical character of the skin. The uppermost layer of the skin is a multilayered structure called the stratum corneum. The top three to five layers of stratum corneum undergo progressive desquamation. The morphology and thickness of stratum corneum is different at various body sites (1–3). The skin maintains characteristic physicochemical features such as structure, hydration, temperature, pH, and oxygen and carbon dioxide gradients. Changes in any of these factors impact the overall physiology of the skin. The acidic nature of the skin was discovered by Heuss in 1892 (4) and was later validated by Schade and Marchonini in 1928, (5) who underlined acidity as its protective feature and called it the "acid mantle." Current literature indicates that the skin surface pH is largely acidic between 5.4 and 5.9 (6).

The skin surface pH plays an important role in skin physiology and directly or indirectly influences various other factors such as composition of stratum corneum lipids, stratum corneum hydration, barrier function of the skin, and the skin's microbiota (7–15). The acidic pH of skin provides optimal pH for enzymes, e.g., glucocerebrosidase (16) and phospholipases (17), to work on extracellular lipids and a vitamin A–esterifying enzyme (18). Conversely, acidic pH of the skin has also been shown to accelerate the repair process of barrier function when damaged with acetone or extensive tapestripping (19). Also, the acidic skin pH has been shown to correlate with enhanced resistance against sodium lauryl sulfate (SLS)-induced irritant dermatitis (15,20).

An intraday variation (circadian rhythm) of skin pH was reported at some body sites, e.g., shin, forearms, and axilla (21,22). The skin pH was higher (pH 5.3) in the afternoon and lower (pH 4.9) at night (21–23). Investigations on seasonal differences in skin surface pH are limited (24). During summer, the pH of the skin surface is usually 0.5 units below pH values during the rest of the year (25).

Acidic pH of the skin is the result of the physiology of human body, which in turn regulates endogenous skin flora (15,26,27). The skin further provides a habitat for resident microbiota, which under normal conditions protects the skin from pathogenic organisms. Soon after birth, bacteria start to colonize the skin and other body sites. Despite wide variations in environmental conditions, the skin is capable of maintaining a stable microbial ecosystem (28). The skin temperature tends to be cooler than normal body temperature, slightly acidic, and mostly dry, whereas most bacteria prefer neutral pH, 37°C temperature, and moisture for optimal growth. Therefore, skin's microenvironment greatly dictates the microbial spectrum and population density. Some of the resident bacteria play an active role in maintaining acidic pH of the skin and preventing colonization by pathogenic bacteria.

THE ORIGIN OF THE SKIN pH

It is now well accepted that the acid mantle of the stratum corneum is very important for normal skin physiology and its bacterial flora. What makes the skin surface "acidic" is still not fully understood (14). Many endogenous and exogenous factors have been proposed, which influence the skin pH, e.g., eccrine and sebaceous secretions, anatomic sites, moisture, proton pumps, genetic predispositions, and age (10,11,14). Active and passive energy, bioenergetic processes, have also been suggested as sources for the acidic pH of the skin (11,29,30). For

example, lactic acid produced by passive process acidifies the superficial layers of skin (31). Other important components of passive metabolic processes include free fatty acids, cholesterol sulfate, urocanic acid, pyrrolidone carboxylic acid, which also contribute to the skin's acidic pH (32). Active proton pumps (e.g., the sodium/hydrogen anion exchanger proteins or NHE1) present in the membranes of the lamellar bodies are responsible for acidification of the intracellular space in the lower layers of stratum corneum (33). Free fatty acids generated by lipases of bacterial and/or pilosebaceous origin are partly implicated in the genesis of acid mantle (34).

The pores of the skin are made up of a combination of sebaceous and sudoriferous glands. When in balance, the combined excretion of oil and sweat from these pores has a pH of about 5.5. However, occlusive dressing has been shown to significantly increase the skin surface pH, moisture content, and bacterial density (35), indicating the role of endogenous factors in these changes. Exogenous factors such as skin cleansers, cosmetics, occlusive treatments, and topical antibiotics/antiseptics have been shown to alter the skin surface pH (36–39). Altered skin pH has been associated with dermatological conditions such as irritant contact dermatitis, atopic dermatitis, ichthyosis, acne vulgaris, and *Candida albicans* infections (31,40–42).

There could be many factors affecting the overall pH of the skin depending on the subject, body sites, and other biochemical factors. The skin of newborns and small infants differ from adults in some characteristics (43,44). The pH of infant skin is higher (e.g., 6.6 ± 0.25) than the adults (45–48). The pH of the skin differs at various anatomical sites, the superficial pH on the nose was the lowest among the regions tested (49). Regions with higher *Staphylococcus epidermidis* concentrations are slightly more acidic. In general, the skin surface pH is relatively similar at different body sites; however, slightly higher pH was reported in areas with higher moisture such as intertriginous areas (axillae, inguinal and submammary folds, and finger webs) (6,14,50).

A slight person-to-person variation in skin pH occurs because not everyone's skin is exposed to the same conditions such as weather and harsh detergents. Recently, in a large multicenter study, the skin surface pH of the volar forearm was assessed before and after refraining from shower and cosmetic product application for 24 hours (15). The average pH dropped from 5.12 ± 0.56 to 4.93 ± 0.45. The authors concluded that the "natural" skin pH is on average 4.7, which is below the generally reported pH range between 5.0 and 6.0. Interestingly, the study also suggested that showering with plain tap water in Europe, which has a pH around 8.0, could increase the skin pH for >four hours. The skin surface pH not only varied at different locations (Table 1), but also the lipid composition in the stratum corneum differs as a function of skin region and could influence the pH profile across the stratum corneum (51,52). Other reports (11,29,31,53,54) suggest that the pH of the skin follows a sharp gradient across the stratum corneum, which is possibly involved in controlling enzymatic actives and skin renewal (55).

Table 1 The pH Values on Human Skin at Various Locations as Reported in Selected Literature

Skin surface pH	Location	Reference
4.0–5.5	Forehead	50,59
4.0–5.5	Forehead and cheek	57
4.1–4.2	Forearm	62
4.4	Volar forearm	63
4.4–5.1	Volar forearm	64
4.5–5.6	Forehead	65
4.2–4.5	Forearm	65
5.5–5.8	Forehead	66
5.56–5.96	Back of the wrist	66
4.8–5.0	Volar forearm	67
4.93–5.12	Volar forearm	15
5.0–5.4	Volar forearm	68
5.0–5.5	Ventral forearm	30
5.4–5.9	Lower arm	6
5.5–5.8	Forearm	61

AGE, RACE, AND GENDER DIFFERENCES

Reports on the differences and/or similarities in the skin surface pH among various age, race, and gender are scanty. The newborn baby's skin pH recorded to be neutral soon becomes acidic within a month (56). The higher skin pH in infants is well documented (45–48) and may be associated with different chemical composition of the skin lipids (44); however, within a month, the baby's skin attains an acidic pH similar to adult skin. The available literature on skin surface pH indicates that the pH remains constant between 18 and 60 years of age (21,57,58). Men and women older than 80 years showed increased pH values (57,59). In the older age group of over 70 years, the mean pH value of the forehead was measured to be 5.6 as opposed to 5.3 in the younger age group (59). Anatomical differences in pH have also been reported (Table 1), which also influence the microbial composition and density (see later in the text and Table 3). In one of the studies (57), among 89% of the subjects, the skin surface pH on the cheek was higher than that on the forehead. In subjects younger than 80 years, the average pH is ranged between 4.0 and 5.5 on the forehead and 4.2 to 5.9 on the cheek (57). In another study, facial pH at different sites did not differ significantly between subjects with and without acne (60). Unlike in women, in men, the area close to the wrist had significantly lower pH values compared with the proximal sites (61).

Skin pH has been reported to vary with race, gender, and genetic background. Black people have a lower skin surface pH when compared with Caucasians (58,68), which has been attributed to pigmentation effects (29). Gupta et al. 1987 (65) measured the skin surface pH of 55 brown-skinned Indians comprising of 30 males and 25 females in the age range 12 to 58 years. Indian skin was slightly more alkaline, though the data are not definitive because the groups tested were small (65). The average pH values on the forehead of male and female were 5.51 and 5.73, respectively.

The differences between male and female skin surface pH have not been fully established. Studies published to date show contradictory results (Table 2). In most studies, significantly more acidic skin pH was found in men when compared with women (60,64,68–71), while other studies (61) showed the reverse situation, i.e., more acidic pH for women rather than men, while others showed no gender differences (21,57,58,61,72). A study conducted in India found that the male skin was slightly but significantly more acidic than the female. The same study (65) reported that the pH values at the axilla, umbilicus, palm, foot, sole, and cheek were consistently higher than those at scalp, forehead, retroauricular and popliteal fossae, anterior arm, anterior forearm, posterior neck, back, dorsum of hand, anterior leg, and anterior thigh. The highest pH was recorded in axilla (5.98 for male and 6.00 for female). The study notes that high density of both sweat glands and bacterial flora leads to a high skin pH, whereas lower pH was observed in area with high concentration of sebaceous glands and bacterial flora.

In the underarm region, the skin surface pH is significantly different between men and women, more acidic pH values were found in women than in men (71). The baseline pH value before washing was 6.58 ± 0.63 (right armpit) and 6.67 ± 0.65 (left armpit) in men versus 5.8 ± 0.53 (right armpit) and 5.94 ± 0.62 (left armpit) in women. Interestingly, washing of armpits with pure tap water further increased the difference between male and female pH values (71). The pH difference between right and left armpit was not statistically significant or (71) similar to some earlier reports of no difference in skin pH between the dominant and nondominant forearms or hands (61,62).

Table 2 Gender Differences in Skin pH

	pH		
Anatomical sites	Female	Male	Reference
Forehead	5.4–5.8	5.1–5.5	60
Forehead	5.73	5.51	66
Axilla	5.80–5.94	6.58–6.67	72
Volar forearm	4.8–5.8	4.3–4.7	53
Volar forearm	5.60–5.88	4.76–4.93	68
Volar forearm	4.97–6.09	5.44–6.16	61
Back of wrist	5.84	5.56	66
Back skin surface	5.43–5.73	4.96–5.12	22

One of the prevalent hypotheses about the role of skin pH is its putative importance in antimicrobial defense (63,73). Possible explanations are that (*i*) the top layer of the skin is very dry and densely packed, which makes this first line of defense inhospitable to many bacteria; (*ii*) salty secretions from sweat glands create an environment that is hyperosmotic and thus unfavorable for bacteria; and (*iii*) normal flora grow best at a more acidic pH, whereas pathogenic bacteria, such as *Staphylococcus aureus*, grow best at neutral pH (74). A more acidic pH helps to protect skin against colonization by nonresident and pathogenic bacteria because many of them survive well in a narrow pH range near neutral.

The acidic condition of the skin is caused by secretions from sweat glands, skin oil, and the breakdown of fatty acids by *S. epidermidis*. Thus, a resident microflora is partly responsible for the acidic pH of skin. A multicenter study also found that the acidic pH of the skin surface (4.0–4.5) keeps the resident bacteria attached to the skin, whereas an alkaline pH (8,9) increased the dispersal of bacteria from the skin (11,15,27). The importance of pH for antimicrobial function is further supported by neonatal eczematous and atopic skin, which displays a neutral pH (41,75,76).

SKIN FLORA

The skin provides the largest organ (about 2 m^2 skin surface in average human adult) and an intricate habitat for a complex microbial ecosystem comprising resident and transient microflora, mainly bacteria (77,78), to a lesser extent fungal and possibly viral agents. Bacteria-skin relationship can be commensal, symbiotic, or parasitic relative to the host's overall physical and immune status. Persistant colonization is the result of alterations in the host's immune status, resulting in a significant impact on the balance of the bacteria-skin relationship.

The acid mantle, level of mineral and moisture, and use of skin cleansers and cosmetics influence the growth and maintenance of resident flora; and the state of resident flora influence the acquisition of transient bacteria (77). This acid mantle, a fine film with a slightly acidic pH on the surface of the skin, provides a protective barrier to the skin. The microbial population dynamics on various parts of the skin is determined by the anatomical location, the amount of sebum and sweat production, local pH, humidity, temperature, light exposure, etc. (71,79). Host factors such as age, immune status, hormonal status, and other habits also influence the composition and density of the skin flora (80,81). The development of bacterial flora on skin from birth to adulthood has not been systematically studied. During the prenatal stage, the skin remains sterile but soon becomes colonized by bacteria after birth. Not all bacteria are welcomed onto the skin. The skin allows the colonization and growth of those bacteria, which protect the host from pathogenic bacteria both directly and indirectly. These bacteria can act by producing antibiotics (e.g., bacteriocin), toxic metabolites, inducing a low reduction-oxidation potential, depleting essential nutrients, preventing attachment of competing bacteria, inhibiting translocation, by degrading toxins, etc (81,82).

Microbial status on skin can be temporary or transient, short-term resident and long-term resident biota. Establishment of a resident status depends on the ability of the bacteria to adhere to the skin epithelium, grow in a relatively dry and acidic environment, and establish a relationship that is more mutualistic than commensalistic (11,15,82). Bacterial adhesion or detachment from the skin could be mediated by (*i*) specific interactions via lectin or sugar binding; (*ii*) hydrophobic interactions; and (*iii*) electrostatic interactions (83,84). Hand washing with a skin cleanser containing microbial anti-attachment ingredients has also been shown to prevent bacterial adherence to skin, which may be working via electrostatic interaction (85, unpublished data). Recently, using 1% lactic acid (pH 3.0) and 1% sodium carbonate decahydrate (pH 11.0) under acidic conditions, the dispersal rate of the resident bacteria from volar forearm was much lower than under alkaline conditions, suggesting the role of electrostatic interaction between bacteria and positive charges of the skin under acidic pH. The differences in dispersal rate under acidic and alkaline pH have not been fully understood. Various explanations (15) are put forward for high dispersal rate under alkaline condition: (*i*) Under alkaline conditions both keratines and the bacterial surfaces are negatively charged resulting in repulsion. (*ii*) Net negative charge of the keratins created by alkaline treatment would lead to the swelling of the skin, which may open up the sponge-like corneocytes, allowing the bacteria to diffuse to the surface. A laboratory-based study has shown that washing hands with plain soap spreads bacteria on the entire hands (personal observation). It has also been reported that repeated washing could not diminish numbers of bacteria (86); therefore, the practice of rigorous preoperative washing of the hands in hospitals has been questioned (87,88). Because of the inefficacy

of washing regimens, especially in health care settings, selection of an effective skin cleanser for routine hand hygiene is very important (88,89).

Bacterial species commonly isolated from normal skin include *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Brevibacteria*, *Propionibacteria*, and *Acinetobacter* (79,81,90,91). *S. aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* are transient colonizers (91,92). The gram-negative bacteria are the minor constituents of the normal skin flora, and *Acinetobacter* is one of the few gram-negative bacteria commonly found on skin. The presence of *E. coli* on the skin surface is indicative of fecal contamination. Yeasts are uncommon on the skin surface, but the lipophilic yeast *Pityrosporum ovalis* is occasionally found on the scalp. Racial and gender differences in skin microflora are not fully examined (93). A more recent study using molecular techniques has provided better understanding on microbial ecology of the skin (94). Gao et al. (94), using molecular techniques, have identified 182 species of bacteria on human forearm skin, of which 8% were unknown species that had never been described before (94). This study also shed some light on the gender differences of skin microbiota, the microbial mix, and the possible role of pH (61). Roughly, half of the bacteria identified in the samples represented the genera of *Propionibacteria*, *Corynebacteria*, *Staphylococcus*, and *Streptococcus*, which are generally considered as the resident flora of human skin. Among the six individuals sampled, only four species of bacteria were in common: *Propionibacterium acnes*, *Corynebacterium tuberculostearicum*, *Streptococcus mitis*, and *Finegoldia AB109769*. Interestingly, three bacterial species were found only in the male subjects: *Propionibacterium granulosum*, *Corynebacterium singulare*, and *Corynebacterium appendixes* (95).

The skin surface pH influences various factors for the growth of resident and pathological microorganisms (7,11,71,95). The acidic pH of the skin is regarded as one of the major factors in making the skin a less favorable habitat for bacteria (96). A high density of bacteria was found in skin area with less acidic pH such as genitocrural area, anal regions, toe webs, submammary fold, and axillae (55,71). Those areas of the skin, which are relatively dry and exposed, have lower pH and lower microbial population density as well. For example, volar forearm skin has bacterial population about 10^2 to 10^3 cfu/cm^2 (colonies forming units/cm^2) (63), compared with 10^5 cfu/cm^2 in relatively moist underarms area (78). Artificial occlusion of the forearm skin leads to significant changes in skin pH and in the composition and density of bacterial species (35,63). For example, before occlusion, the skin pH value was 4.38, and after five days of occlusion, the pH increased to 7.05 (63). Similarly, the average bacterial count before occlusion was 1.8×10^2 cfu/cm^2, which increased to 4.5×10^6 cfu/cm^2 on the fifth day (63). It is evident that moist skin environment promotes bacterial growth and colonization. The distribution and composition of bacterial species on the skin vary at different body sites (Table 3). In intratrigenous area, the skin surface pH is somewhat higher, which in turn favors higher bacterial density (90,91).

Table 3 Normal Skin Microflora in Areas with High Density

Bacteria	Area
S. epidermidis	Upper trunk
S. hominis	Glaborous skin
S. capitis	Head
S. saccharolyticus	Forehead/antecubital
S. saprophyticus	Perineum
M. crococcus luteus	Forearm
Corynebacterium xerosis	Axilla, conjuctiva
C. minutissimum	Intertriginous (e.g., axilla)
C. jeikeium	Intertriginous (e.g., axilla)
P. acnes	Sebaceous gland, forehead
P. granulosum	Sebaceous gland, forehead, axilla
P. avidum	Axilla
Brevibacterium spp.	Axilla, toe webs
Dermabacter spp.	Forearm
Acinetobacter spp.	Dry area
Pityrosporum spp.	Uppermost part of sebaceous gland follicle

Abbreviations: *S. epidermidis*, Staphylococcus epidermidis; *S. hominis*, Staphylococcus hominis; *S. capitis*, Staphylococcus capitis; *S. saccharolyticus*, Staphylococcus saccharolyticus; *S. Saprophyticus*, Staphylococcus saprophyticus; *C. minutissimum*, Corynebacterium minutissimum; *C. jeikeium*, Corynebacterium jeikeium; *P. acnes*, Propionibacterium acnes; *P. granulosum*, Propionibacterium granulosum; *P. avidum*, Propionibacterium avidum.

Table 4 Effects of Skin pH on Skin Microflora

Effects	Reference
Acidic pH (4–4.5) keeps the resident flora attached to the skin.	15
Alkaline pH (8,9) promotes dispersal of bacteria from the skin.	
Less acidic pH promotes bacterial growth, especially gram-negative bacteria and propionibacteria.	63,74
Skin candidal infection was more inflammatory when the SC was buffered to pH 6.0 versus 4.5, indicating that pH may mediate immune reaction to infections.	113
High pH in the axilla promotes high bacterial growth and malodor.	114
Acidic pH boosts the activity of antibacterial lipids and peptides.	26,104,107,108
Acidic pH facilitates production of natural antimicrobial peptides, wound healing, and regulating keratinization and desquamation processes.	9,53,105,106,107

Abbreviation: SC, stratum corneum

The normal flora also acts as a barrier to prevent invasion and growth of pathogenic bacteria (34,97). The relevance of normal skin flora as a defensive barrier can be articulated with the finding that intensive use of antimicrobial skin cleansers could lead to an increased susceptibility to skin infections by gram-negative bacteria (98–100). A healthy growth and maintenance of the resident bacteria effectively deny the colonization by transient bacteria (e.g., *E. coli*, *Pseudomonas*, coagulase positive *S. aureus*, *C. albicans*). The skin's antimicrobial defenses include the mechanical rigidity of the stratum corneum, its low moisture content, stratum corneum lipids, lysozyme, acidity (pH 5), and defensins (29,101–103). Recent studies suggest that increased enzyme activity of phospholipase A2 is related to the formation of the acid mantle in the stratum corneum (29,31).

PROTECTIVE ROLE OF ACIDIC pH OF THE SKIN

Besides other physicochemical roles of the skin pH, it is now generally accepted that the normal skin surface pH has a beneficial role in relation to skin microflora (Table 4). Acidic pH of the skin (pH 4.0–4.5) helps the resident bacterial flora to remain attached to the skin and prevents cutaneous invasion by pathogenic microorganisms (7,8,15), whereas alkaline pH (8.0–9.0) is reported to promote dispersal of the bacteria (15). Acids produced by bacteria also contribute to the local protective mechanisms. For example, *S. epidermidis*, *P. acnes*, *Pityrosporum ovale*, and *Corynebacteria* produce lipases and esterases that break triglycerides to free fatty acids, leading to a lower skin surface pH and thereby creating an unfavorable environment for skin pathogens. Acidic pH of the skin also facilitates the production of natural antimicrobial peptides, attributes to the wound healing, and regulates the keratinization and desquamation processes (9,53,104–108). The skin flora also produces proteinaceous or lipidic antibacterial compounds termed "bacteriocins." These bacteriocins are involved in controlling/regulating bacterial competition for survival in this microenvironment. For example, a bacteriocin-Pep 5 produced by *S. epidermidis* is particularly active against other staphylococci, specifically *S. aureus* (109). Interestingly, the acidic pH of the skin boosts the activity of these antibacterial lipids and peptides possibly by enhancing the interaction with the bacterial membrane (26,105,108,109).

EFFECTS OF THE SKIN pH ON SKIN FLORA AND PATHOLOGY

Cutaneous pH plays an important role in maintaining the normal bacterial flora of the skin and preventing cutaneous invasion by pathogens (26,110). The acidic pH of the skin surface has long been regarded as the result of exocrine secretions of the skin glands, which in turn is involved in regulating the skin flora. Furthermore, a number of recent investigations published on the pH gradients in deeper layers of skin indicate a close relationship among the barrier function, a normal maturation of stratum corneum, and desquamation. Initially, work done in test tubes clearly demonstrated the effect of pH on bacterial growth (111,112). The study found that *S. aureus* grew equally well at pH 5, 6, and 7; normal micrococci showed somewhat, but

not significantly, better growth at pH 6 and 7 than at pH 5. On the other hand, aerobic diphtheroids grew significantly better at pH 7 than at lower pH levels (113). The acidic pH of skin provides a balanced environment for the resident bacteria. Changes in the skin pH and other organic factors play a role in certain skin pathogenesis and in their prevention and treatment (Table 4).

P. acnes is a classical example of how a slight increase in the skin pH can facilitate the resident bacteria to become pathogenic. Under normal pH of 5.5, growth of *P. acnes* is at its minimum; however, a slight shift toward alkaline pH would make it a more favorable environment, resulting in increased growth of this organism (112,115). As mentioned earlier, prolonged occlusion of skin significantly affects the growth of the normal skin flora, skin pH, and the rate of transepidermal water loss (TEWL) and carbon dioxide emission (35,63).

Recent studies have shown the relationship between a change in skin pH and its consequences in atopic dermatitis, particularly disturbances in skin barrier function and increased colonization with *S. aureus* (116). However, other studies (105,117) have suggested that in atopic dermatitis, increased colonization by *S. aureus* and other bacteria could be associated with a decrease in sphingosine and ceramide production. In atopic eczema, not only the skin surface pH was significantly higher than in normal healthy skin (41,118) but also the growth of *S. aureus* and exotoxin production were increased, which have been shown to induce eczema on intact skin (119).

Changes in the skin pH from acidic to alkaline could also be a risk factor for the development of candidal infections (113). A laboratory-based study, where right and left forearms were respectively buffered at pH 6.0 and 4.5, inoculated with a suspension of *C. albicans* and occluded for 24 hours showed more pronounced skin lesions with the higher pH suggesting that the higher pH may increase yeast virulence and/or modulate the host's defence capability (66). Yosipovitch et al. (67) found that the pH values in the intertrigenous skin among 50 noninsulin-dependent diabetic patients were significantly higher than in normal healthy volunteers and attributed the higher pH as a risk factor for candidal infection (66). Patients on dialysis also showed significant increase in their skin surface pH.

In a moist intertrigenous area, such as axilla, the pH is physiologically higher than in other skin regions (78,90,114), which promotes the growth of local flora. It has been established that underarm odor is created by the action of indigenous bacteria on axillary apocrine gland secretions (78, personal observations). Application of a deodorant product showed significant reduction in axillary pH, which in turn inhibited the growth of underarm bacteria (114).

EFFECTS OF SKIN CLEANSERS AND COSMETICS ON SKIN pH AND FLORA

As mentioned earlier, there are many external factors that influence the skin surface pH. Some of the external factors include the use of soap, detergents, and cosmetic products. Long-term use of these agents has been shown to alter the skin surface pH and to some extent affect the skin microflora at least for a short duration (37,114,120). Alterations in skin pH could cause irritation or interfere with the keratinization process as well (11,121).

Frequent hand washing with soap may damage the skin and facilitate more bacterial colonization. In fact, water and soap washing of damaged skin were not effective in reducing the bacterial contamination (122). Use of an alkaline soap with pH 10.5 to 11.0 resulted in higher skin surface pH and marked increase in the number of *Propionibacteria*, but the counts of coagulase negative *Staphylococci* were not much changed (48,96). In acne-prone young adults, washing of facial skin with an alkaline cleansing agent was reported to cause more inflammatory reaction than the acidic syndet bar (42). On the other hand, washing with an acidic skin cleanser (pH of 5.5) similar to the normal skin pH in adults increased the skin surface pH but significantly less than the alkaline soap (48,74,98,123). At the forehead, there was a clear correlation between bacterial counts and the skin pH, both with *Propionibacteria* and *Staphylococci*, but on the forearm only *Propionibacteria* count was higher with higher pH. The skin surface pH was significantly higher when neutral preparations were used. The number of *Propionibacteria* was significantly linked to the skin pH (74). The use of synthetic detergents with pH similar to the skin surface pH led to a rise in the skin surface pH for a shorter duration (36,42), and such temporary changes in skin pH were limited to the top layers of the stratum corneum (55).

Korting et al. (96) were among the first to examine the effect of different skin cleansing treatments on the bacterial flora and the skin surface pH in healthy volunteers (37,96) using a crossover clinical design. Essentially, volunteers in one group washed their foreheads and forearm with an alkaline soap twice daily for one minute and those in the other group used an acidic soap (syndet). After four weeks, both groups switch their soaps, respectively, in a crossover fashion. The skin pH and bacterial density were determined at the beginning of the study and at the end of every week (96). The pH was increased when alkaline soap was used first and the pH dropped with the changeover to syndet. When syndet was used first, the pH dropped slightly, and then increased when alkaline soap was used. Long-term use of syndet lowered the skin pH by about 0.3 units. In general, washing with alkaline soap resulted in an increase in skin pH and a marked increase in *Propionibacteria* without any significant change in counts of coagulase negative *Staphylococci*.

A more recent study found the natural skin surface pH below 5, which is on the lower end of many studies reported to date in the literature (15). They assessed the pH on volar forearm before and after refraining from shower and using any cosmetic products on skin for 24 hours. The baseline pH before taking shower was 5.12 ± 0.56. After 24 hours without any product application or contact with water, the pH value dropped to 4.93 ± 0.45. On average, the authors estimated that the natural pH value of the volar forearm skin to be 4.7 (15), which is in contrast to general assumption that the average skin pH ranges between 5.0 and 6.0. Interestingly, the study also found that plain tap water with pH around 8.0 as generally found in Europe could increase the skin pH up to six hours after application.

CONCLUSION

Since the first report in 1892 by Heuss (4) on the acidic nature of the skin, significant progress has been made in the field of skin biochemistry/microbiology, yet a number of areas remain to be fully explored. The exact origin of the skin acidity is still being investigated, but recent studies appear to indicate that several endogenous factors, including the presence of lactic acid, free fatty acids, urocanic acid, pyrrolidone carboxylic acid in sweat and sebaceous secretions are involved. The skin is the primary organ protecting the human body from external physical and chemical assaults. Overall, the skin surface is acidic with subtle differences between race and gender. It is not yet clear whether the skin has an inherently acidic pH that provides a hospitable environment for certain organisms or whether the organisms are attracted by other factors to colonize the skin. A recent study on human forearm superficial bacterial flora using molecular techniques highlighted the subtle differences in skin bacteria between men and women and possible relation with the skin pH (94), and suggested the need for further studies including host-and site specificities of the bacterial species on skin and their role (if any) in the pathogenesis of skin diseases. The acidic pH of the skin provides an optimal environment for resident bacteria and their enzymatic activities. Together, the acidic pH of the skin and the resident flora of the skin play an important role in maintaining skin health. The acidic pH of the skin is a key factor in the barrier function (14,19) and plays a key role in the mutualistic relationship with resident microflora (80,124–126). It is well recognized that an increased skin surface pH may be associated with the pathogenesis or the severity of many skin disorders, including acute eczema, irritant contact dermatitis, atopic dermatitis, ichthyosis, acne vulgaris, and *C. albicans* infections (11,20,26). It is becoming more evident that the repeated use of alkaline skin cleansing products, detergents, and even hard water (pH 8.0) can adversely affect the natural skin pH and disturb the normal flora. To maintain the normal physiology and microflora of the skin, use of cosmetics and skin cleansing products, which do not alter the skin pH or adversely affect the skin flora should be considered. Additionally, more research on pre- and probiotics in regulating healthy skin flora and maintaining optimal skin biochemistry is needed.

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