Part III

Skin Measurements

32 Tribological Studies on Skin: Measurement of the Coefficient of Friction

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CONTENTS

32.1 INTRODUCTION

Studying the friction of skin supplements other mechanical tests. Friction studies can be conducted with noninvasive methods and give a measure of the skin's health — skin hydration, for example: Naylor[1] showed that moistened skin has an elevated friction response and El-Shimi[2] demonstrated that drier skin has a lowered friction response. Friction provides a quantitative measurement to assess skin.

The friction parameter generally measured is the coefficient of friction. To measure the friction coefficient, a surface is brought into contact with another and moved relative to it. When the two surfaces are brought into contact, the perpendicular force is defined as the normal force (N). The friction force (F) is that force, which opposes relative movement between the two surfaces. From Amonton's law, the coefficient of friction (μ) is defined as the ratio of the friction force to the normal force:

$$\mu = F/N.$$

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The friction coefficient can be measured in two ways: the static friction coefficient (μ_s) and the dynamic or kinetic friction coefficient (μ_k). The static friction coefficient is defined as the ratio of the force required to *initiate* relative movement and the normal force between the surfaces; the dynamic or kinetic friction coefficient is defined as the ratio of the friction force to the normal force when the two surfaces are moving relative to each other. For simplicity, much of the research has focused on the dynamic friction coefficients wherein the two surfaces move at a relative *constant velocity*. Most of the friction studies on skin have dealt with the dynamic friction coefficient and the subscript k is usually dropped. This overview references the dynamic coefficient of friction unless otherwise noted.

According to Amonton's Law, the dynamic friction coefficient remains unchanged regardless of the probe velocity or applied normal load in making the measurement. Amonton's laws hold true in the case of solids with limited elastic properties. Although Naylor[1] concluded Amonton's Law to be true, later studies by El-Shimi[2] and Comaish and Bottoms,[3] and Koudine et al.[4] have found that skin deviates from Amonton's Law since the friction coefficient increased when the normal load was decreased. El-Shimi[2] and Comaish and Bottoms[3] reasoned that the rise in friction coefficient resulted from the viscoelastic nature of the skin allowing for a nonlinear deformation of the skin with increasing load.

32.1.1 Experimental Designs

Various experimental designs have been devised to measure the friction on skin. They focus on measuring friction by pressing a probe onto the skin with a known normal force, and then detecting the skin's frictional resistance to movement of the probe. The designs fall into two categories:

1. A probe moved across the skin in a linear fashion.
2. A rotating probe in contact with the skin surface.

In the linear designs, the probe movement is accomplished in several ways. Comaish and Bottoms[3] utilized one of the simplest linear designs: they moved the probe across the skin by attaching it to a pan of weights by means of a pulley. Weights are placed in the pan such that the probe slides over the skin at a constant velocity. This allows for the calculation of the dynamic friction coefficient by dividing the total weight in the pan by the normal load on the probe.

More sophisticated linear designs, followed the simple design used by Comaish and Bottoms,[3] but provide motorized unidirectional movement of the probe or the use of a reciprocating motor to move the probe back and forth. In both designs the motorization affords greater control in maintaining the velocity of the probe. Strain gauges measure the friction force as the probe moves along the skin surface. A Biomedical Tribometer, a friction measurement device where the normal load and the probe speed are computer controlled, can also be used.

The second design category measures friction with a rotating wheel pressed onto the surface of the skin with a known normal force. Highley et al.[5] measured the frictional resistance by determining the angular recoil of the instrument as the wheel contacted the skin. They measured this angular recoil by recording the proportion of light that hit a dual element photocell. An electrical signal was then generated in proportion to the frictional resistance. Comaish et al.[6] developed a portable, hand-held device (Newcastle Friction Meter) that relied on a torsion spring to measure the skin's frictional resistance.

An important part of designing a friction measurement apparatus is choosing the probe size, shape, and material. Because friction is an interaction between two surfaces, the probe geometry and material will affect the values calculated for the friction coefficient of the other surface. Also, results will be more accurate when the probe's normal force is maintained at a constant value or continuously monitored; previous methods used to maintain the normal force include spring mechanisms or static weights to weigh down the probe. These parameters are revisited critically later in this article.

Much effort has been spent in understanding how skin friction changes with differing biological conditions and upon the application of various products to the skin surface. These studies have been of interest to various industries that manufacture products meant as skin topical agents because friction measurements can provide clues regarding the effectiveness of their products.

32.1.2 Hydration

Hydration is a complex phenomena influenced by intrinsic (i.e., age, anatomical site) and extrinsic (i.e., ambient humidity, chemical exposure) factors. These factors can affect the mechanical properties of the skin and research has been performed to correlate hydration levels with the skin's friction coefficient.[24] Hydration studies have investigated how increases and decreases in skin hydration correlated with the friction coefficient. In past studies researchers generally induced increases in skin hydration through water exposure. However, decreases in skin hydration were not experimentally induced and dehydration studies were performed between subjects with "normal" skin and subjects that had clinically "dry" skin.[2,12]

32.1.3 Lubricants/Emollients/Moisturizers

Much of the reviewed research has been devoted to ascertaining how the application of certain ingredients influences the skin surface, of interest to the cosmetic/moisturizer and lubricant industries. The studies focused on the effects of talcum powder,[2,3] oils,[2,3,5,14] and skin creams/moisturizers.[7,14,17,24] Hills et al.[15] analyzed how changes in the friction coefficient, following emollient application, differed with temperature.

32.1.4 Probes

Probe geometry and material influence the measured value of the friction coefficient because friction is a probe-skin interaction phenomenon. Few studies have examined probe effects: El-Shimi[2] studied probe roughness and Comaish and Bottoms[3] probe roughness and material.

32.1.5 Normal Load

Friction measurements can offer quantitative insight into changes on the skin surface and the UMT offers technical advances over existing friction measurements. The control of the probe speed and the real-time monitoring of the normal load allow for real-time calculation of the friction coefficient. As seen in Figure 32.1, the control of the load is important because the friction coefficient does not adhere to Amonton's Law. Wolfram[18] theoretically deduced that the friction coefficient would relate to the normal load as follows:

$$\mu \propto N^{-1/3}$$

(where N is the applied normal load to the skin). Sivamani et al.[17] found that the friction coefficient related to the normal load as follows:

$$\mu \propto N^{-0.32}$$

and Koudine et al.[4] found the dependence on the applied normal load to be:

$$\mu \propto N^{-0.28}$$

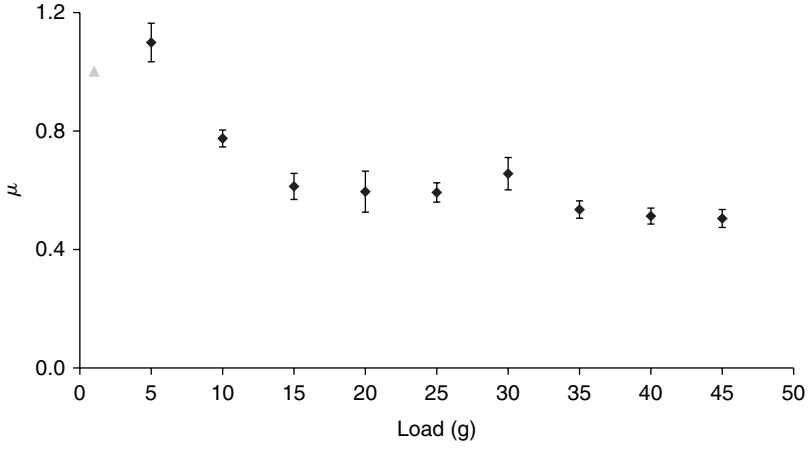

FIGURE 32.1 Friction coefficient versus normal load (50 g Sensor). The friction coefficient increased as the normal load was decreased suggesting that the skin does not follow Amonton's Law. The probe was moved at 5 mm min^{-1} ($n = 4$). Reproduced from Sivamani et al.[24]

32.2 SKIN FRICTION COEFFICIENT VALUES

Friction is an important characteristic of skin because it allows us to execute many of our daily activities. In addition, friction studies offer insight into how skin and the skin surface change across age, gender, race, anatomical site, and chemical applications. This can provide better information about expected skin variations in the population and why certain topical applications are effective. Comparative studies are particularly useful in following how the skin mechanically changes under different conditions.

Previous studies have reported various values for the skin's friction coefficient. Most dynamic friction coefficient measurements most fall in a range of 0.2 to 0.5 (Figure 32.2). Besides natural variations in skin, the wide range in results may be due to differences in probe movement, geometry, controlled monitoring of the normal force, and material chosen to make the friction measurement. In designing the friction measurement apparatuses, the two types of probe movement utilized were rotational and linear. As a result, the linear probe constantly moves over "untested" skin and the rotational probe spins over "tested" skin. This can lead to discrepancies in reported values for the skin friction coefficient. Another important source of variation may be the ability to control the normal force while the probe is testing the skin surface. The skin friction instruments are designed to measure the fictional resistance of the skin and it is assumed that the normal force is constant. During a test the normal force may not stay constant as a result of an uneven skin surface, inaccurate spring, and a nonuniform distribution of static weights placed above the probe head. Therefore, the assumption of a constant normal force may be incorrect and can lead to variation in the calculated friction coefficient. A third source for variation is the choice of the probe material. Because friction is a surface phenomenon between two materials, the choice of the probe influences the numerical value obtained for the friction coefficient.

32.2.1 Hydration

Hydration studies reveal that drier skin had lowered friction while hydrated skin had an increased amount of friction (Table 32.1). However, the skin response is more complex, because very wet skin also has a lowered friction coefficient much like the characteristics of dry skin.[16] Most studies focus on an intermediate zone of hydration where the skin has been moistened without an appreciable "slippery" layer of water on the skin. Results in Table 32.1 show that the increases in friction were

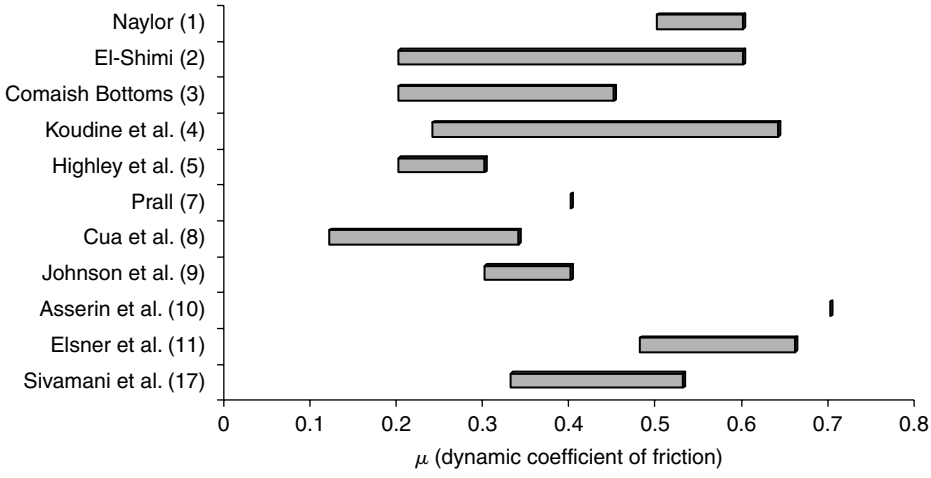

FIGURE 32.2 Outline of the ranges in the dynamic coefficient of friction. These ranges reflect measurement of untreated "normal" skin friction *in vivo*. Reproduced from Sivamani et al.[25]

TABLE 32.1
Comparative Studies of the Changes in Dynamic Friction Coefficient (μ) with Increasing Hydration (Hydration) and Decreasing Hydration (Dryness)

Author	Probe material	% Increase due to hydration $\{(\mu_{moist} - \mu_{normal})/\mu_{normal}\} \times 100$	% Decrease due to dryness $\{(\mu_{normal} - \mu_{dry})/\mu_{normal}\} \times 100$
Naylor[1]	Polyethylene	80	—
El-Shimi[2]	Stainless steel (Rough); stainless steel (smooth)	100–200 (Stainless steel rough) —	28 (Stainless steel rough); 41 (Stainless steel smooth)
Comaish and Bottoms[3]	Wool; teflon	40[a] (Wool); 400[a] (teflon)	—
Highley et al.[5]	Nylon	500	—
Prall[7]	Glass	200	—
Johnson et al.[9]	Glass	100–233	
Lodén et al.[12]	Stainless steel	—	33 (Hand); 41 (Back); 14 (Arm)
Nacht et al.[14]	Teflon	45	—
Sivamani et al.[17]	Stainless steel	55 (*In vitro*)	10 (*In vivo*)

[a]Comaish and Bottoms studied the change in the *static* friction coefficient in their hydration study.

varied and this possibly results from the various probes used. Although the addition of water increases the friction coefficient, this effect lasts for a period of minutes before the skin returns to its "normal" state.[2,5,14,17] The water has an effect of softening the skin and this in turn allows for greater contact area between the probe and the skin. Also, water results in adhesive forces between the water and the probe. Thus, there is more frictional resistance between the skin and the probe and results in a higher friction coefficient.[18] Since the water evaporates in minutes, the skin returns to its "normal" state in the same time frame. For dry skin, the skin becomes less supple and the probe does not achieve as much contact area and this allows the probe to glide more easily over the skin surface. This results in a lowered friction coefficient as seen in the isopropyl study[17] and in prior studies involving subjects

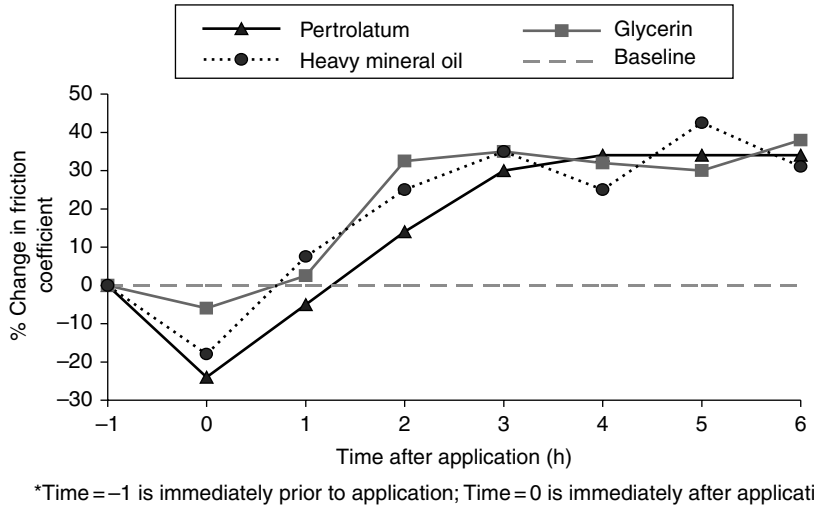

FIGURE 32.3 Effect of lubricant cosmetic ingredient on skin friction coefficient. Amount applied of each material: ~2 mg/cm^2. Reproduced from Nacht et al.[14] (mean of five subjects but *P* value was not published). Time = −1 is immediately prior to application; Time = −0 is immediately after application.

with clinically dry skin.[2,12] The agreement between the experimentally induced dry skin and clinical dry skin is expected.[18]

32.2.2 Lubricants/Emollients/Moisturizers

The studies on lubricants, emollients, and moisturizers are important for cosmetics and products developed to make the skin look and feel healthier. The literature reports that the important qualitative characteristics in skin topical agents are skin smoothness, greasiness, and moisturization.[17,19] Previous research has tried to describe these subjective, qualitative descriptions in a quantitative fashion by correlating them against the friction coefficient. Prall[7] tried to find a quantitative correlation for skin smoothness but was unable to make a direct correlation to the friction coefficient until he added the skin topography and hardness into the analysis. Nacht et al.[14] found a linear correlation between perceived greasiness and the friction coefficient (Figure 32.4).

32.2.2.1 Lubricant Oils

A lowering in the friction coefficient is the initial effect after the application of oils and oil-based lubricants.[2,5,14] Nacht et al.[14] and Highley et al.[5] also showed that after the initial decrease in friction, the oils eventually raised the skin's friction coefficient. The results of the lubricant cosmetic studies by Nacht et al.[14] are shown in Figure 32.3.

32.2.2.2 Emollients and Moisturizers

Prall[6] and Nacht et al.[14] found that the friction coefficient rises with the addition of emollients and creams in a similar fashion to water. However, the effect of the creams lasted for hours while the water effect lasted for about 5 to 20 min.[7,14,17] Sivamani et al.[17] quantified the friction, greasiness, and "stickiness" of the skin following application of creams and treatments (Figure 32.5). Hills et al.[15] also studied emollients, but they examined how different emollients compared against one another and how changes in temperature changed the friction coefficient. At a higher temperature (45°C), most emollients lowered the friction coefficient to a greater degree than at a lower temperature (18°C).

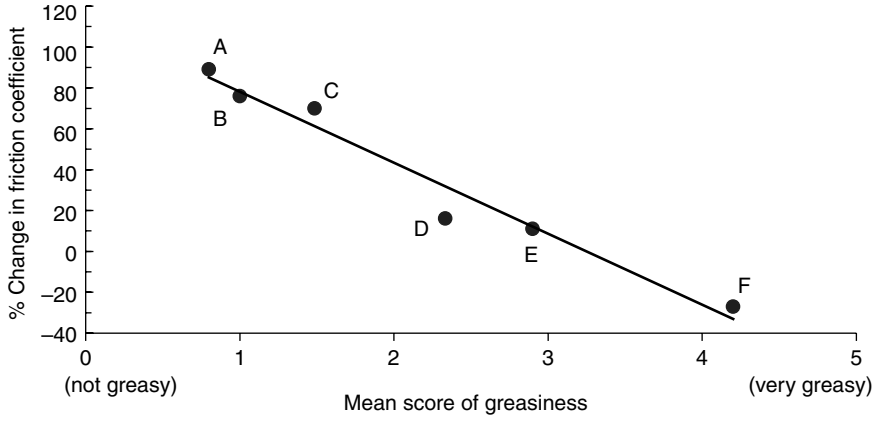

FIGURE 32.4 Correlation between changes in the friction coefficient and the sensory perception of greasiness. A, B, C, D, E, and F represent different creams that were applied to the skin. The reported percent change in the friction coefficient is immediately after application and the greasiness scores were subjective evaluations. Reproduced (from Nacht et al.[14]).

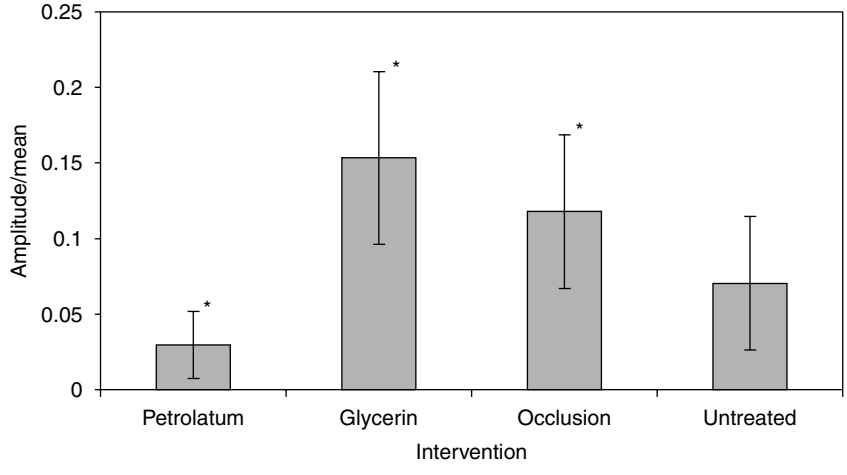

FIGURE 32.5 Amplitude/mean measurements for interventions. The application of glycerin and the PVDC occlusion increased the amplitude/mean of the volar forearm. Also, the addition of glycerin raised the amplitude/mean significantly more than the PVDC occlusion. Petrolatum significantly decreased the amplitude/mean and this is a quantitative evidence of petrolatum's greasiness ($P < .001$). Reproduced from Sivamani et al.[24]

When lubricants/moisturizers are applied to the skin, the skin friction is affected in three general ways.[14,18]

- *A large, immediate increase in the friction coefficient, similar to water application, that follows with a slow decrease in the friction coefficient.* These agents can be interpreted to act by immediate hydration of the skin through some aqueous means to give the immediate increase in friction. In Figure 32.4, creams A, B, and C represent this type of lubricant/moisturizer.
- *An initial decrease in the friction coefficient that is followed by an overall increase in the friction coefficient over time.* These agents are fairly greasy products (Figure 32.3) and this greasiness causes the immediate decrease in the friction coefficient. The eventual rise in

the friction coefficient is probably due to the occlusive effects of these agents.[21] In other words, these products and ingredients act to prevent water loss from the skin, thereby increasing the hydration of the skin. Representations of a few ingredients that elicit this response are in Figure 32.3 and represented as cream F in Figure 32.4.

- *A small, immediate increase in the friction coefficient that then increases slowly with time.* These agents are interpreted to act as a combination of effects seen in the previous two cases. These lubricants/moisturizers have ingredients and agents that serve to both hydrate the skin through some aqueous method, and prevent water loss through some occlusive mechanism. Because of the presence of the these occlusive agents, which tend to be more slippery, the immediate rise in the friction coefficient is lower than in products that fall into the first category listed above. In Figure 32.4, this is seen in creams D and E.

32.2.3 Probes

El-Shimi[2] and Comaish and Bottoms[3] compared probes (Table 32.1) and found that smoother probes gave higher friction coefficient measurements. El-Shimi[2] noted that higher friction coefficient measurements were made with a smoother stainless steel probe as opposed to a roughened stainless steel probe. Comaish and Bottoms[3] found a similar result with two types of nylon probes: a sheet probe and a knitted probe. The sheet probe (the smoother of the two) gave a higher friction coefficient measurement. El-Shimi[2] postulates that the smoother probe forms more contact points with the skin and has a greater skin contact area than the rougher probe, resulting in more resistance from the skin and a larger measurement for the friction coefficient.

32.2.4 Anatomic Region, Age, Gender, and Race

Few studies address the effects of anatomic region, age, gender, or race as they pertain to the friction coefficient. To date, no significant differences have been found with regard to gender[8,22,24] or race.[23,24]

The friction coefficient varies with anatomical site: Cua et al.[8,22] found that friction coefficients varied from 0.12 on the abdomen to 0.34 on the forehead. Elsner et al.[11] measured the vulvar friction coefficient at 0.66, whereas the forearm friction coefficient was 0.48. Sivamani et al.[24] found that the proximal volar forearm had a higher friction coefficient than the distal volar forearm. Manuskiatti et al.[23] studied skin roughness and found significant differences in skin roughness at various anatomical sites. Differences in environmental influences (i.e., sun exposure) and hydration may account for this. Elsner et al.[11] showed that the more-hydrated vulvar skin had a 35% higher friction coefficient than the forearm, and this is in agreement with hydration studies that contend that skin has an increased friction coefficient under increased hydration.

Age-related studies have been contradictory where some authors found no difference[8,22,24] and others found differences.[10,11] Cua et al.[22] showed no differences in friction with respect to age except for friction measurements on the ankle. Elsner et al.[11] also performed age-related tests and found no differences in the vulvar friction coefficient, but observed a higher forearm friction coefficient in younger subjects. They postulate that the skin on parts of the body that become exposed to sunlight can undergo photoaging and thus, forearm skin shows evidence of age-related differences while the light-protected vulvar skin does not.[11] Asserin et al.[10] concluded that younger subjects had a higher forearm friction coefficient than older subjects.

There are few gender-related and racial friction studies; Cua et al.[8,22] and Sivamani et al.[24] found no significant friction differences between the genders. Manuskiatti et al.[23] found no significant racial (black and white skin) differences in skin roughness and scaliness. Sivamani et al.[24] found no differences in volar forearm friction among different ethnicities before and after chemical treatments (Figure 32.7 and Figure 32.8).

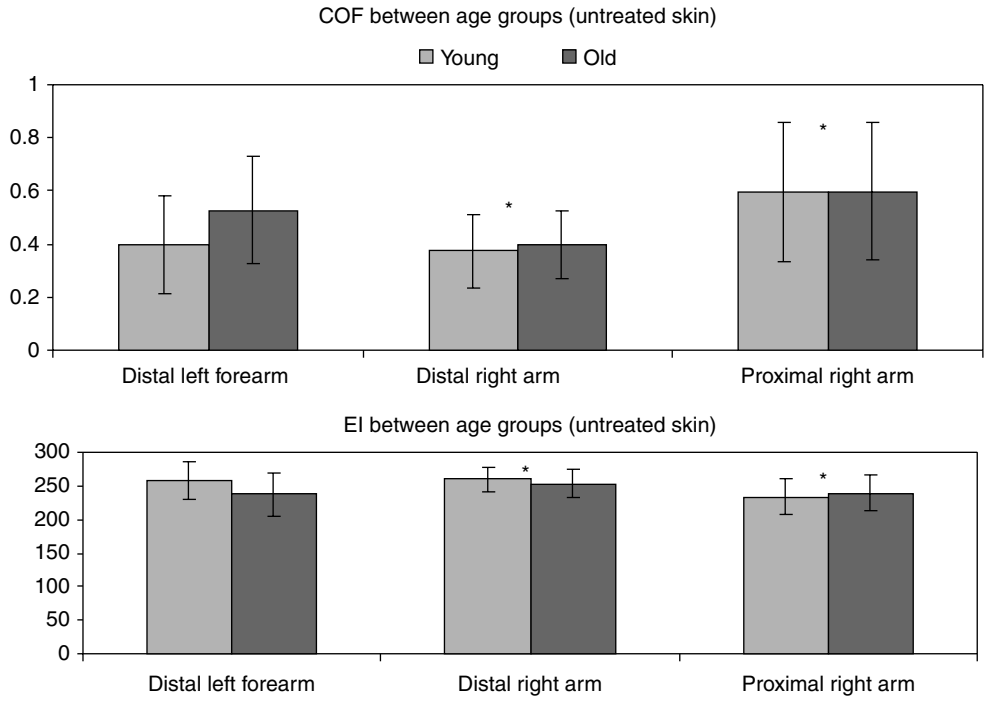

FIGURE 32.6 Age related comparisons of friction and electrical impedance. No significant differences were apparent between old and young skin on the volar forearm. Within each category, the proximal right arm friction and electrical impedance measurements were different from the distal right arm ($P < .001$). Reproduced from Sivamani et al.[24]

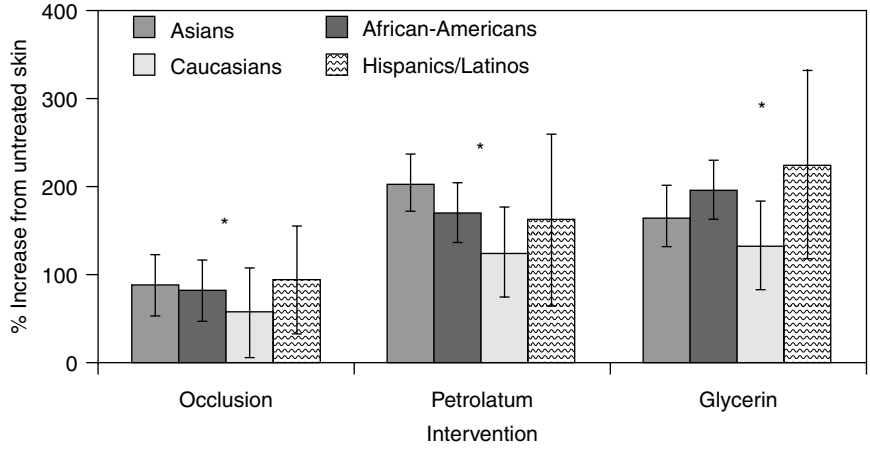

FIGURE 32.7 Coefficient of friction across ethnicity. Data represents increase in friction when compared to untreated skin of the volar forearm. No significant differences were found between the different ethnic groups. Petrolatum and glycerin increased the friction coefficient significantly more than PVDC occlusion ($P < .01$). The increase in the friction coefficient due to petrolatum was not significantly different from the effect of glycerin. Reproduced from Sivamani et al.[24]

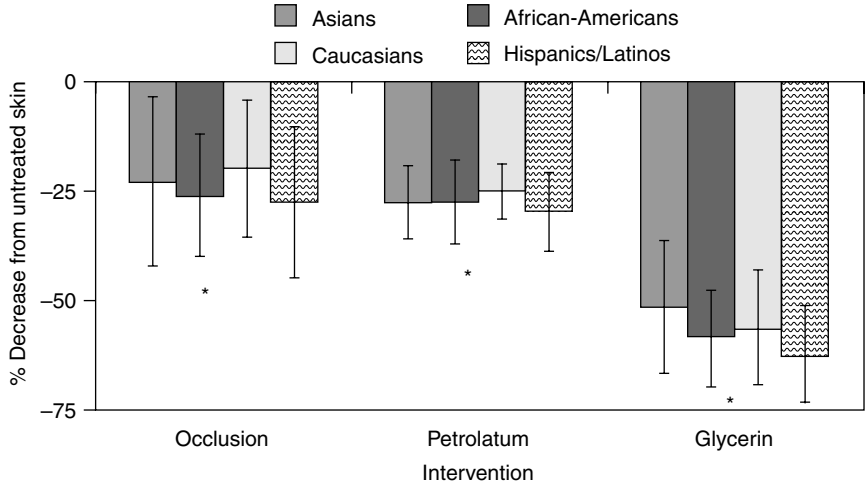

FIGURE 32.8 Change in electrical impedance across ethnicity. Data represents decrease in electrical impedance when compared to untreated skin of the volar forearm. No significant differences were found between the different ethnic groups. Glycerin lowered the electrical impedance significantly more than PVDC occlusion or petrolatum ($P < .01$). The decrease in the electrical impedance due to PVDC occlusion was not significantly different from the effect of petrolatum. Reproduced from Sivamani et al.[24]

32.3 CONCLUSION

Although there have been limited studies dealing with the measurement of the skin friction coefficient, studies demonstrate that differences in skin, because of various factors — such as age and hydration — can be correlated with the friction coefficient. Friction coefficient studies can serve as a quantitative method to investigate how skin differs on various parts of the body and how it differs between different people. It is also a useful method for tracking the changes resulting from environmental treatments — such as sunlight — and when various chemicals are applied to the skin — such as soaps, lubricants, and skin creams. The reviewed studies also indicate that the design of the test apparatus is an extremely important factor, because test design parameters can also have an influence on friction measurements. A better appreciation of the importance of the friction coefficient will become clearer as measurement methods improve and allow for greater accuracy.

REFERENCES

1. Naylor, P.F.D., The skin surface and friction, *Br. J. Dermatol.*, 1955; 67: 239–248.
2. El-Shimi, A.F., *In vivo* skin friction measurements, *J. Soc. Cosmet. Chem.*, 1977; 28: 37–51.
3. Comaish, S. and Bottoms, E., The skin and friction: deviations from Amonton's laws, and the effects if hydration and lubrication, *Br. J. Dermatol.*, 1971; 84: 37–43.
4. Koudine, A.A., Barquins, M., Anthoine, Ph., Auberst, L., and Leveque, J.-L., Frictional properties of skin: proposal of a new approach, *Int. J. Cosmet. Sci.*, 2000; 22: 11–20.
5. Highley, D.R., Coomey, M., DenBeste, M., and Wolfram, L.J., Frictional properties of skin, *J. Invest. Dermatol.*, 1977; 69: 303–305.
6. Comaish, J.S., Harborow, P.R.H., and Hofman, D.A., A hand-held friction meter, *Br. J. Dermatol.*, 1973; 89: 33–35.
7. Prall, J.K., Instrumental evaluation of the effects of cosmetic products on skin surfaces with particular reference to smoothness, *J. Soc. Cosmet. Chem.*, 1973; 24: 693–707.
8. Cua, A., Wilheim, K.P., and Maibach, H.I., Frictional properties of human skin: relation to age, sex and anatomical region, stratum corneum hydration and transepidermal water loss, *Br. J. Dermatol.*, 1990; 123: 473–479.

9. Johnson, S.A., Gorman, D.M., Adams, M.J., and Briscoe, B.J., The friction and lubrication of human *stratum corneum*, thin films in tribology, Dowson, D. et al. (eds.), *Proceedings of the 19th Leeds-Lyon Symposium on Tribology*, Elsevier Science Publishers, B.V., 1993, pp. 663–672.
10. Asserin, J., Zahouani, H., Humbert, Ph., Couturaud, V., and Mougin, D., Measurement of the friction coefficient of the human skin *in vivo*. Quantification of the cutaneous smoothness, *Colloids Surf. B Biointerfaces*, 2000; 19: 1–12.
11. Elsner, P., Wilhelm, D., and Maibach, H.I., Frictional properties of human forearm and vulvar skin: influence of age and correlation with transepidermal water loss and capacitance, *Dermatologica*, 1990; 181: 88–91.
12. Lodén, M., Olsson, H., Axéll, T., and Linde, Y.W., Friction, capacitance and transepidermal water loss (TEWL) in dry atopic and normal skin, *Br. J. Dermatol.*, 1992; 126: 137–141.
13. Sulzberger, M.B., Cortese Jr., T.A., Fishman, L., and Wiley, H., Studies on blisters produced by friction, *J. Invest. Dermatol.*, 1966, 47: 456–465.
14. Nacht, S., Close, J., Yeung, D., and Gans, E.H., Skin friction coefficient: changes induced by skin hydration and emollient application and correlation with perceived skin feel, *J. Soc. Cosmet. Chem.*, 1981; 32: 55–65.
15. Hills, R.J., Unsworth, A., and Ive, F.A., A comparative study of the frictional properties of emollient bath additives using porcine skin, *Br. J. Dermatol.*, 1994; 130: 37–41.
16. Dawson, D. in: Wilhelm, K.-P., Elsner, P., Berardesca, E., Maibach, H., (eds.), *Bioengineering of the Skin: Skin Surface Imaging and Analysis*. Boca Raton, CRC Press, 1997, pp. 159–179.
17. Sivamani, R.K., Goodman, J., Gitis, N.V., and Maibach, H.I., Friction coefficient of skin in real-time, *Skin Res. Technol.*, 2003; 9: 235–239.
18. Wolfram, L.J., Friction of skin, *J. Soc. Cosmet. Chem.*, 1983; 34: 465–476.
19. Denda, M., in: Lodén, M., Maibach, H., (eds.), *Dry Skin and Moisturizers: Chemistry and Function*. Boca Raton, CRC Press, 2000, pp. 147–153.
20. Wolfram, L.J., in: Leveque, J.-L., (ed.), *Cutaneous Investigation in Health and Disease: Noninvasive Methods and Instrumentation*, New York, Marcel Dekker, 1989, Ch 3.
21. Zhai, H. and Maibach, H.I. Effects of skin occlusion of percutaneous absorption: an overview, *Skin Pharmacol. Appl. Skin Physiol.*, 2001; 14: 1–10.
22. Cua, A.B., Wilhelm, K.-P., and Maibach, H.I., Skin surface lipid and skin friction: relation to age, sex, and anatomical region, *Skin Pharmacol.*, 1995; 8: 246–251.
23. Manuskiatti, W., Schwindt, D.A., and Maibach, H.I., Influence of age, anatomic site and race on skin roughness and scaliness, *Dermatology*, 1998; 196: 401–407.
24. Sivamani, R.K., Wu, G.C., Gitis, N.V., and Maibach, H.I., Tribological testing of skin products: gender, age, and ethnicity on the volar forearm, *Skin Res. Technol.*, 2003; 9: 299–305.
25. Sivamani, R.K., Goodman, J., Gitis, N.V., and Maibach, H.I., Coefficient of friction tribological studies in man — an overview, *Skin Res. Technol.*, 2003; 9: 227–234.

33 Smoothness of the Skin, Complexity, and Instrumental Approach

Jean Luc Lévêque

CONTENTS

33.1 INTRODUCTION

In our everyday life, we are used to handling various objects, heavy or light, solid or fragile, rigid or deformable. In general, we neither break them nor deform them. Moreover, we are able to recognize them just by assessing their shape, weight, texture, and temperature. Yet this sense of touch has not been reproduced to date, even with the most advanced robot technology. While modern technology is able to create or reconstitute a virtual environment, to synthesize sounds and fragrances, designing an artificial hand able to permanently adapt the pressure of its fingers to the nature of the object it grasps is hardly possible in most cases. With regard to the simulation of tactile sensations, some haptic systems that generate forces and vibrations corresponding to the hardness and texture of the object touched have begun to appear, but are far too simple to represent the complexity of contact between a finger and a visco-elastic material. These "force feedback" systems have only recently begun to appear in surgery to allow the surgeon performing surgery to sense tactile sensations in the course of telesurgery experiments.

Touching the skin, however, is essential in clinical practice in order to assess certain skin properties that the other senses cannot detect. In cosmetics, this corresponds to a very common gesture performed every day to evaluate the condition of our skin or to check the effect of a cream or shampoo or when applying perfume. In social life too, it is through touching the skin that we express our feelings to convey sympathy, love, and tenderness.

33.2 MECHANISMS INVOLVED

A light touch of the skin by the finger pad, for example, to assess skin smoothness, brings into play a multitude of physical, chemical, neuro-physiological, and cognitive processes.

At the physical and chemical level, the mechanisms of interaction between two skin surfaces, more or less flat, smooth, and adhesive, govern the sliding movement. According to Wolfram,

adhesive forces probably account for the static friction coefficient (μ_s), particularly when a low applied pressure generates a weak indentation of one surface into the other.[1,2] The nonuniform nature of the skin surface in terms of both relief and chemical properties probably also accounts for the kinetic friction coefficient (μ_k) and the amplitude of frictional fluctuations.[3] As we shall see later, objective measurement of these two parameters only represents one aspect of the skin smoothness concept.

The tactile physiological function is mainly a result of the existence of mechano-receptors or transducers in the skin, which transform mechanical energy into the emission of electrical impulses.[4] Each type of receptor is sensitive to a given frequency band. Paccini receptors can detect very small deformations (a few micrometers) up to a frequency of 300 Hz while Meissner receptors work at a lower frequency (50 Hz). These receptors are perfectly adapted to detect the passage of a fingertip over the skin surface. If we consider a scanning velocity of about 10 cm/s and a density of the microrelief lines of 20 cm, this simple gesture would generate an electric signal of about 200 Hz easily detectable by the Paccini receptors.

In actual fact, tactile sensation over the skin surface is highly dependent on finger pressure. The slow adapting receptors (Ruffini and Merkell) probably determine this pressure, which then generates information relative to skin firmness.

The cognitive process includes the many signals emitted by the skin receptors, which then arrive in the brain. They are processed so as to give rise to a particular sensation and to command verbalization and behavior. Research is under progress in this field but the mechanisms of transformation of electric signals into a sensation are still unknown. Some psycho-physical studies tend to demonstrate that the brain probably selects some of the received information according to the subject's intentions. For example, for assessing the smoothness of a surface, sensitivity is greater the lower the shearing forces.[5]

In fact, the external expression of our sensations and feelings is likely to be affected by the sum of our experiences and the cultural background acquired during our life. Skin smoothness, for example, is experienced from birth but the expression of this sensation is largely governed by our personal history.

As a result of this complexity, skin smoothness is therefore difficult to assess globally in an objective manner. At present, attempts to quantify this cosmetically important quality are only partial, either through a purely physical approach (friction measurement), or by objective cognitive measurements (brain activity) or psycho-physical tests.

This chapter concerns, more particularly, a description of the physical approach to measuring skin smoothness.

33.3 FRICTION MEASUREMENTS

Although there are some recent publications describing sophisticated new experimental approaches,[6] research on friction coefficients actually started some 50 years ago. The basic idea is to measure the force needed to drag a pad along the skin surface. Two types of pad movement over the skin were proposed: either rotation or translation. The probe consists of a plastic, steel, or glass material in the form of a disc or sphere. In spite of the great diversity of experimental procedures and conditions, overall these past studies agree on the following conclusions:[7]

- Amonton's law, stipulating a constant value for the friction coefficient whatever the pressure applied to skin, is not verified for skin.
- Hydration increases the friction coefficient although oily materials generally tend to decrease it (lubrication).
- There are no variations in the friction coefficient according to age, gender, and ethnicity. The effect of age is still under debate.

The main issue is to attempt to provide a better interpretation of the results in terms of skin parameters. The friction coefficient depends on several parameters: microrelief, vertical pressure, skin elastic properties, hydration of the surface, presence (or not) of a greasy film at the interface between skin and the measuring pad, nature of the pad. Several publications describe the influence of all these parameters on the measurement of friction coefficients but results are only qualitative because of the complexity of the phenomenon.

A relatively old publication attempted to link skin texture to the measurement of μ_k. Authors found fairly good correlation between the smoothness of chamois leather, as perceived by experts, and the inverse of μ_k.[8] This *in vitro* result was, however, not confirmed *in vivo* by Prall who found other types of correlations.[9] On the other hand, it has been shown that $1/\mu_k$ correlates very well with the slippery effect of oils. Such results reveal the complexity of the quality of "smoothness": smoothness of skin and smoothness perceived when applying a cosmetic product to the skin correspond to two different sensations.

Figure 33.1 is a plot of the variations in the friction coefficient versus time for two applied loads on the pad during a standard experiment. Such curves allow two coefficients to be defined, one corresponding to the maximum of the curve (static friction coefficient μ_s) and the other corresponding to the asymptotic value (dynamic friction coefficient μ_k). This type of variation is driven by the adhesion forces between the pad and the skin surface. The value of μ_k is lower than μ_s because during displacement of the pad, adhesion bonds have no time to reform after rupturing. Fluctuations during continuous displacement correspond to weak increases in μ (adhesion or "sticking") followed by a decrease in μ (bond rupture or "slipping"). This occurs when the stress induced by the adhesive contact and the shearing stress created by the tangential force are equivalent. These oscillations in the μ_k coefficient are sometime called the "stick-slip" process.

It has been hypothesized that the amplitude of this process is linked to the sensation of skin smoothness.[3] The first argument for such a hypothesis comes from a comparison between the

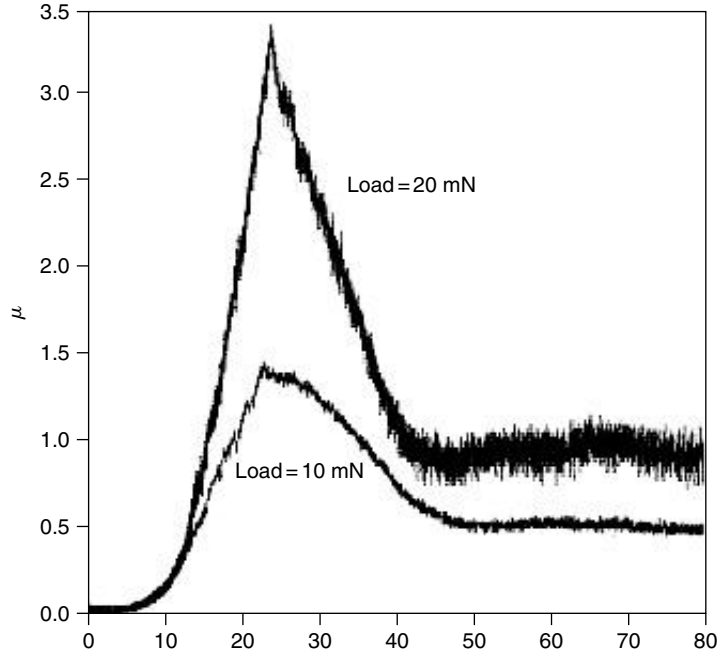

FIGURE 33.1 Variation of the friction coefficient versus time during the recording of an experiment on the forearm. The two curves correspond to two different loads applied on the sliding pad. The maximum of the curves corresponds to the static friction coefficient and the asymptotic value to the kinetic coefficient.[3]

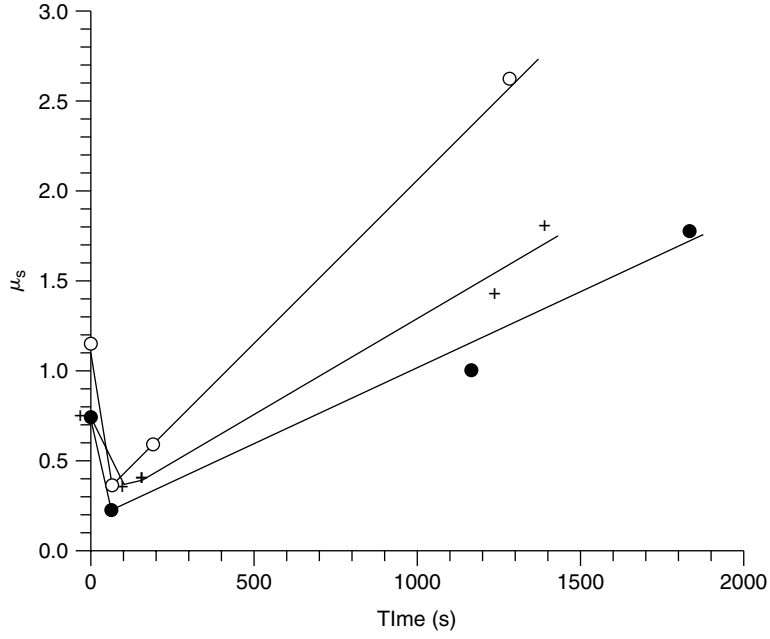

FIGURE 33.2 Variations of the friction coefficient versus time after application of different cosmetic preparations. There is an initial decrease due to lipids followed by a progressive increase due to hydration of the SC.[3]

amplitudes of the stick-slide process of outer and inner forearm skin: this is always higher on the outer part. On the other hand, application of an emulsion, assessed as giving some smoothness to the skin by a panel of experts, decreases this amplitude. This new parameter, called "fluctuations in frictional force amplitude" or (FFFA), could therefore be a good descriptor of skin smoothness.

In fact, such a coefficient also measures nonuniformity of the surface, which is due to the skin microrelief, the presence of squames and hair, and different types and amounts of lipids. These fluctuations are much higher when friction experiments are carried out with a low load on the pad. Measuring FFFA under such conditions could open up new possibilities.

As mentioned earlier, hydration of the skin surface has for a consequence an increase in the dynamic friction coefficient. This increase can be explained by several factors, which are expressed in the formula giving the friction coefficient: $\mu = S(K/E)^{2/3}P^{-1/3}$. Increasing hydration clearly decreases the Young modulus E but also modifies, in an unknown manner, S and K, which are respectively the shear strength of the adhesive contact and the colligative coefficient representing the number of adhesive contacts per unit area. According to Wolfram, water, by dissolving surface protein material, may increase adhesion. When moisturizers are applied to the skin, the increase in μ can be recorded quite rapidly, but follows an initial decrease due to the immediate effect of lipids present in the formulation (Figure 33.2).

33.4 PIEZO-ELECTRIC DETECTION OF THE CONTACT: THE "HAPTIC FINGER"

Dynamic contact between two bodies (e.g., one sliding over the other) generates noise that is characteristic of the hardness of these bodies and of the physical and chemical properties of their interface. Moreover, the velocity of the sliding movement modifies the frequency band of the emitted acoustic waves. The sliding displacement of a finger at the surface of the skin for assessing its smoothness

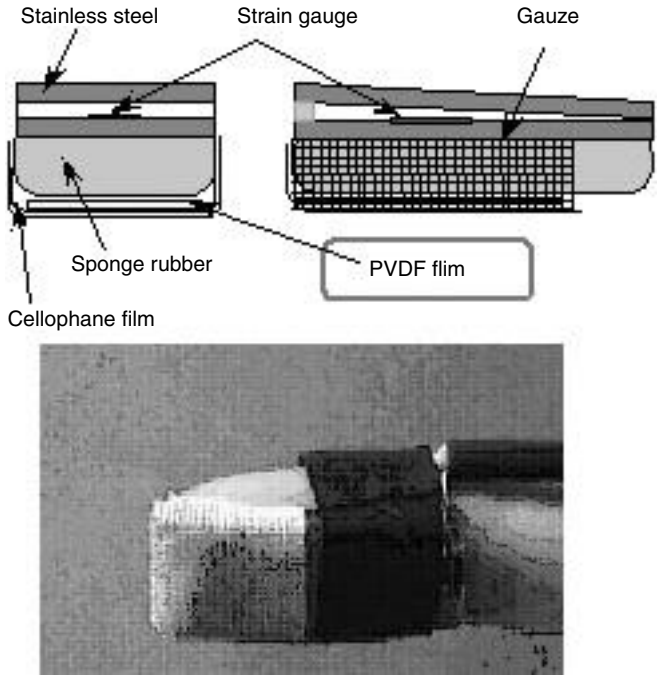

FIGURE 33.3 Structure of the "Haptic Finger."[11]

generates such waves, which can be recorded and analysed. One such promising acoustic method is now under development.[10]

Another way to analyze the waves generated by the sliding movement of the finger over the skin surface is to use a piezo-electric foil, a transducer membrane that generates electrical signals corresponding to the pressure variations of the finger sliding over the skin.

We used a thin foil of poly-vinylidene-fluoride (PVDF) polymer for building a new component, the "Haptic Finger," which, being adjusted at the tip of a finger can be used for touching the skin for assessing its hardness and smoothness, objectively.[11] A strain gauge is mounted in the layered structure of the sensor to control the pressure applied. *In vitro* experiments, carried out on "skin models" made with silicone rubbers of various hardness and roughness, allowed two mathematical parameters to be defined, extrapolated from the electric signal generated in the experiments. The first one is the variance of the signal processed by means of wavelet analysis (WL5-Var), while the second concerns dispersion of the power spectrum density of the signal developed in the frequency domain (FcR). According to our *in vitro* studies, FcR is proportional to the Young modulus (hardness) and WL5-Var is inversely linked to the roughness of the surface under investigation.

An *in vivo* study, carried out on patients with certain skin diseases, allowed us to obtain fairly good correspondence between the measured parameters and the skin assessment given by a clinician. Figure 33.3 represents an example of results obtained *in vivo* from the skin of ten volunteers before and after application of a cosmetic product. With such treatment, only WL5-Var (roughness) was significantly decreased (Figure 33.4).

33.5 COGNITIVE APPROACH

Different types of procedures exist for recording what a person feels during or after application of a cosmetic product, or during or after a given imposed task to evaluate the smoothness of one's own (or another person's) skin. The simplest consists in asking the person to fill in a questionnaire

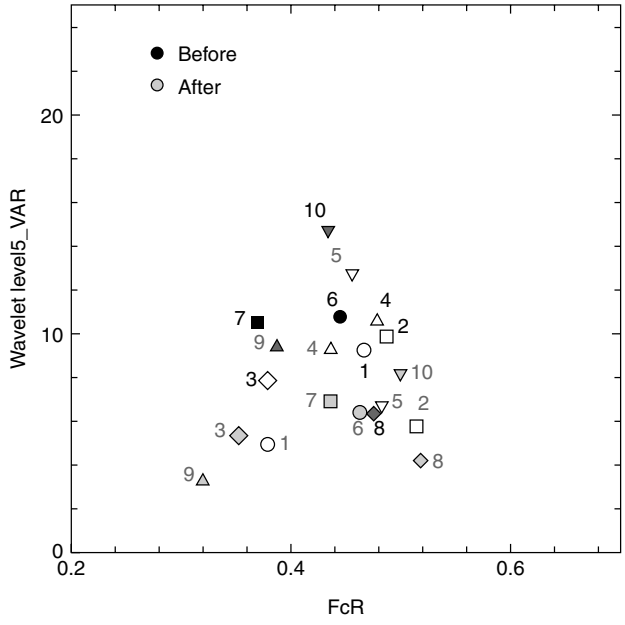

FIGURE 33.4 Measurement of the two parameters generated by the "Haptic Finger," before and 2 h after application of a cosmetic product on ten persons. There is a statistical decrease in "Wavelet Level5_VAR" (Roughness). The FcR parameter (Elastic modulus) is not affected.

where the different attributes of the skin, or of the cosmetic product, are listed with a grading system. There are, for example, standard methods of mood assessment in individuals, such as the "mood adjective check list" (MACL). In certain circumstances, the "mood scale" must be adapted to the population and to the task it has to assess. This approach is more scientifically relevant because the vocabulary chosen corresponds to the most selective and relevant wording, separating out objective from subjective reactions.

For some other tests, psycho-physical methods can be used. For the investigation of skin tactile properties, for example, the stimulus on the skin comes from the application of a series of plastic stimulators presenting two legs separated by different lengths. They are successively applied to the skin of the person who, without seeing the applicator, must decide how many contacts were felt.[12]

As mentioned earlier, any type of stimulation has physiological consequences triggering both personal behavior and verbal expression. To what extent is there a relationship between stimulation and brain activity, on the one hand, and between brain activity and verbalization on the other hand? The first study, which addressed this intriguing yet fundamental question in relation to skin and cosmetic smoothness was conducted in 1999 by Querleux et al.[13] They demonstrated that tactile perception originates in the contra-lateral somatosensory cortex although the subjective part of this perception is marked in the ipsi-lateral sensory region (Figure 33.5). They showed that rough and smooth textures had slightly different localizations in terms of neuronal activity. They also showed that skin-care products amplified sensory perception of the stimulus. This important study is a milestone in this type of research related to sensations generated by the use of a cosmetic product. Indeed, it defines the part played by subjectivity and emotion, an integral part of the cosmetic concept. It also paves the way for a new type of experimental approach in this complex field.

33.6 CONCLUSION

Smoothness of the skin is an important characteristic that cannot be defined and measured simply. Recording the profile of the skin microrelief hardly allows us to predict the perceived feelings

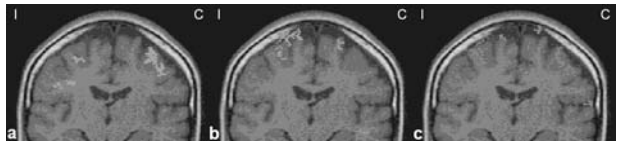

FIGURE 33.5 Activation map superimposed on coronal anatomical MR images in a right-handed subject, in response to passive stimulation. (a) Skin to skin. (b) Perception of a tactile stimulation. (c) Skin to skin with a skin-care product. I: Ipsilateral, C: Contralateral.[13]

we would have by touching it. Besides the microrelief, adhesivity, temperature, and firmness of the surface are all certainly involved in the process. Nowadays, noninvasive methods are able to separately measure each of these parameters, but the human brain works more globally and integrates these physical characteristics of the skin within a more general and cultural background that only some "cognitive" methods can approach. However, measuring the key smoothness-related properties of skin (friction, microrelief, firmness, temperature), in conditions similar to those of everyday life can be of use in designing effective cosmetic or dermatological treatments.

REFERENCES

1. Wolfram, L.J., Friction of the skin, *J. Soc. Cosmet. Chem.*, 1983, 34, 465–476.
2. Wolfram, L.J., Frictionnal properties of skin. *Cutaneous investigation in health and disease* (ed. By J.L. Lévêque), pp. 49–57. Marcel Dekker, New York (1986).
3. Koudine, A., Barquins, M., Anthoine, P.h., Aubert, L., and Lévêque, J.L., Frictional properties of skin: proposal of a new approach, *Int. J. Cosmet. Sci.*, 2000, 22, 11–20.
4. Johanson, R.S. and Vallbo, A.B., Tactile sensory coding in the glabrous skin of the human hand, *Trends Neurosci.*, 1983, 6, 27–31.
5. Smith, A.M., Some shear facts and pure friction related to roughness discrimination and the cutaneous control of grasping, *Can. J. Physiol. Pharmacol.*, 1994, 72, 693–707.
6. Sivamani, R.K., Wu, G.C., Gitis, N.V., and Maibach, H.I., Tribological testing of skin products: gender, age and ethnicity on the volar forearm, *Skin Res. Technol.*, 2003, 9, 299–305.
7. Sivamani, R.K., Goodman, J., Gitis, N.V., and Maibach, H.I., Coefficient of friction: tribological studies in man — an overview, *Skin Res. Technol.*, 2003, 9, 227–234.
8. Cussler, E.L., Zlotnick, S.J., and Shaw, M.C., Texture perceived with fingers, *Percept. Psychophysics*, 1977, 21, 504–510.
9. Prall, J.K., Instrumental evaluation of the effects of cosmetics products on the skin surfaces with particular reference to smoothness, *J. Soc. Cosmet. Chem.*, 1973, 24, 693–707.
10. Zahouani, H., Innovative device to assess *in vivo* the frictional and acoustic properties of the stratum corneum, In Proceedings of "Stratum Corneum IV" Paris, June 17–19, 2004.
11. Tanaka, M., Lévêque, J.L., Tagami, H., Kikuchi, K., and Chonan, S., The Haptis Finger, a new device for monitoring skin condition, *Skin Res. Technol.*, 2003, 9, 131–136.
12. Lévêque, J.L., Dresler, J., Ribot-Ciscar, E., Roll, J.P., and Poelman, C.h., Changes in tactile spatial discrimination and cutaneous coding properties by skin hydration in the elderly, *J. Invest. Dermatol.*, 2000, 115, 454–458.
13. Querleux, B., Gazano, G., Mohen-Domenech, O., Jacquin, J. et al., Brain activation in response to tactile stimulation: functional magnetic resonance imaging versus cognitive analysis, *Int. J. Cosmet. Sci.*, 1999, 21, 107–118.

34 Assessment of Skin Moisturization with Electrical Methods

Enzo Berardesca

CONTENTS

34.1 INTRODUCTION

The importance of water to the proper functioning of the Stratum Corneum (SC) is well-recognized. The reliable quantification of water in the corneum and its interaction with topically applied products is, in fact, essential for understanding skin physiology and developing efficient skin care formulation.

So, it is not surprising that a wide variety of techniques have been developed for measuring SC water content, including electrical measurements (Nova DPM 9003)[1,2]; Skicon 200[2–5]; Corneometer,[2,3] mechanical measurements (Dia-Stron DTM Torque Meter),[6] spectroscopic measurements (Fourier-Transform Infrared Spectroscopy or FTIR)[7]; Nuclear Magnetic Resonance Spectroscopy or NMR,[8] Transient Thermal Transfer or TTT,[8] and direct imaging measurements (Magnetic Resonance Imaging or MRI).[9–11]

Among these different methods, the electrical assessments have become very popular due to their relatively low cost and ease of use.

The use of these techniques has been documented in both normal and diseased skin.[12–18] However, it is also clear that many factors may influence the measurements and can alter the final interpretation of results.[19–21] Furthermore, reproducibility and variability between different instruments and methods can make it difficult to adequately compare data obtained in different laboratories and experiments.

To obtain reliable values, it is necessary to have a standardization of procedures (EEMCO).[21] In this chapter, therefore, attention is focused on the standardization of measurements by determining the variables associated with the method of measuring skin hydration, assessing the extent to which they influence the measured value, and elaborating the techniques by which they may be resolved.

34.2 SOURCES OF ERROR AND ASSOCIATED VARIABLES

The relationship between electrical conductance and water content is not linear, but depends upon the binding state of water molecules to the keratin chains, which is itself described by the water sorption isotherm.[22] It has been customary in the skincare literature to define three types of water according to their strength of binding to the keratin: "tightly-bound" water for water contents from 0 to around 7%, "bound water" between about 7 and 35%, and "free water" beyond. This division can be considered simplistic on the basis of more detailed theory,[23] but is generally helpful. The consequence of variation in water binding strength is that there is no direct proportionality between water content and electrical conductance. Three categories of factors and sources of variation exist in measurements, including instrumental, environmental, and individual-related variables. A detailed account of these influencing variables is given in this section.

34.2.1 Instrument-Related Variables

34.2.1.1 Start-Up and Use

It is very important, independently of the instrument used, that the measurement surface of the probe is absolutely free of dirt particles. Therefore, be aware to clean the measuring head before starting the measurement. In case of cleaning with alcohol it is necessary to dry the measuring surface with a soft tissue, as alcohol and water residues can lead to errors in measurement. The probe should be placed normally to a hair-free skin surface with slight pressure just sufficient to start the measurement process. An inclining position of the probe into the skin or too many hair causes the measuring surface to have an incorrect pressure on the skin.[24] Each of the devices described earlier have a spring in the probe to assure a reproducible pressure. When necessary, shaving or clipping the hair is recommended three days before starting the study.

The instrument should be turned on at least 20 min before measurements are performed. Repeated measurements on the same site can cause occlusion, which results in an increase in the values displayed. Therefore it is recommended to wait for at least 5 sec before repeating a measurement on the same site. It is advisable to measure at least 3 times, once at each of three different but nearby sites, and calculate the median, in order to have more reproducible data.

34.2.1.2 Use of the Probe Protection Covers

The condition of the glass cover of the probe surface has an influence on the measurement results. If this cover has been damaged, it can enable a part of the current flow to reach the skin. This, however, is not dangerous as the current is minor ($>10\ \mu$A) and it will alter the recharge time of the capacitor, and therefore the measured value will increase.

34.2.1.3 Instrumental Variability

The DPM and Skicon readings are usually higher than those of the Corneometer.[2,3,13] Furthermore, it should be remembered that in general different examples of the same instrument type might give variable readings.[5] In general, it appears that the DPM and Skicon are more useful in assessing relatively high water content levels and desorption kinetics, whereas the Corneometer is more

sensitive when assessing relatively dry skin.[4] Furthermore the Skicon is probably measuring SC hydration of more superficial parts of the skin than to the Corneometer. The user should be aware that the electrical-based instruments measure the integer of a field covering a three-dimensional space within the upper parts of the skin. Instead, the DPM readings seem to be less variable than the Corneometer readings.[25] As the Corneometer readings have already been shown to be less variable than the Skicon ones,[13,26] the DPM thus appears as the least variable of the three.[2] Other advantages of a DPM is that it is small, light, portable, and it is not necessary to put it into an electric outlet, thus is very flexible in its use.

34.2.2 Environment-Related Variables

34.2.2.1 Ambient Air Temperature and Humidity

Temperature and relative humidity are known to influence SC water content.[27,28] Under some conditions a linear relationship has been found between "capacitance" and relative humidity.[29] In fact high environmental humidities easily influence the hydration state of the skin surface because the SC is highly hygroscopic. Furthermore chemical changes in environmental humidities have an influence on barrier related factors for example, water holding capacity and thus on SC hydration. Therefore, room humidity should be kept constant. In cases of variation of relative humidity, this variation should be connected in the statistical analysis of variance and, if justified, some suitable normalization procedure such as proposed by Barel and Clarys[29] should be performed. Below 60% relative humidity the influence of the environment is much smaller, however, it is preferable to keep the relative humidity low. Room temperature should be kept constant and at a level to minimize sweat gland activity, hence the range 18 to 20°C is recommended. A steady increase in "capacitance" as room temperature rises above 22°C has been reported,[19] and this may arise from increased perspiration. Seasonal variations due to alterations in temperature and relative humidity occur[28,30–33] particularly in summer the room should be air conditioned. Day-to-day and even diurnal variations are also reported.[34,35]

34.2.3 Individual-Related Variables

34.2.3.1 Anatomical Sites

Regional differences in SC water content have been reported,[13,15,18,25–27,36,37] but when interpreting such results it must be remembered that all these electrical devices give only relative, not absolute, indications of water content expressed in arbitrary units (except by Skicon: μm). The actual relationship between their outputs and true water content is highly complex and also variable. Site-to-site differences in the same anatomical region (e.g., forearm) are reported by some[19] but denied by others.[25] High hydration values appear to exist on the forehead and palms; lower values are observed at the abdomen, thigh, and lower leg.[29] The hydration of contralateral pairs of sites is generally indistinguishable[19] except on the dorsum of the hands. Hence comparative left–right studies of the same selected skin sites are recommended to avoid skin site differences. The influence of body hair should be noted,[24] especially if comparison is made between sites, which are not contralateral.

34.2.3.2 Age and Sex

No significant differences have been reported between males and females when matched by age and tested on the same skin sites.[19,37,38] However, during certain periods of life significant differences may occur. For example, during the initial days of postnatal life, SC hydration is higher than in adults, suggesting that the SC barrier is still in the process of adapting to extrauterine life.[39,40] Based on almost identical values for the parameters of transepidermal water loss (TEWL), SC hydration and pH value, the skin physiology of the child differs very little in SC hydration and barrier function from

that of adults.[41] However, skin aging appears to induce a slow decrease in hydration from around the age of 25 onwards.[19,37,38] In fact, with increasing age, significant decreased levels of all major barrier lipids, changes in cutaneous proteoglycan's size have been described.[42,43] The observation of these dramatic age-related differences suggests that these changes may be involved in the age-related changes in the physical properties of skin.

34.2.3.3 Skin Surface Temperature

It is possible that a skin, which is moist and cool gives exactly the same electrical response to measurements made at a single frequency as a skin, which is dry and warm. To separate and specify potentially confounding influences such as water content, temperature change, and sweat gland activity, it is necessary to use some form of electrical spectroscopic technique, that is, stimulation at three or more different frequencies, or a time-domain approach followed by Fourier transformation.[44–46]

34.2.3.4 Sweating

Thermal sweat gland activity is unlikely if the ambient air temperature is below 20°C, humidity is between 30 and 60%, and the skin temperature is below 30°C, provided that the skin is not exposed to forced convection and no excessive body heat is produced (as a result of physical exercise).[47–49] Subjects in fact, should be relaxed and adapted to the measuring environment for at least 20 min before testing takes place. Therefore, skin should be exposed to the ambient air of the test environment for at least 10 min before taking measurements.[21]

34.3 CONCLUSION

Water plays an important role on the physical properties of the SC, and reliable quantification of water in the corneum and its interaction with topically applied products is essential for understanding skin physiology and developing efficient skin care formulations. Electrical conductance offers a sensitive approach to assessing SC water, even though the exact relationship between instrument readings and actual SC water content is complex and variable, preventing accurate calibration and opening the possibility of misinterpretation.

New methods, more experimental and expensive, are emerging, which can improve the performance of electrical approaches, but for the forseeable future electrical techniques will remain the more basic and widespread methodology, and will remain useful provided their limitations are clearly understood and experimental conditions are designed to overcome the associated difficulties as much as possible.

Finally, it should be remembered that no single type of instrumental test of skin is sufficient; a battery of methods (visual, tactile, instrumental) should always be used to guard against errors of interpretation.

REFERENCES

1. Li, F., Conroy, E., Visscher, M., and Wickett, R.R. The ability of electrical measurements to predict skin moisturization. I. Effects of NaCl and glycerin on short-term measurements. *J. Cosmet. Sci.* 2001; 52: 13–22.
2. Fluhr, J., Gloor, M., Lazzerini, S. et al. Comparative study of five instruments measuring sttratum corneum hydration (Corneometer CM 820 and CM 825, Skicon 200, Nova DPM 9003, DermaLab). Part I. *In vitro*. *Skin Res. Technol.* 1999; 5: 161–170.
3. Barel, A.O. and Clarys, P. *In vitro* calibration of the capacitance method (Corneometer CM 825) and conductance method (Skicon-200) for the evaluation of the hydration state of the skin. *Skin Res. Tecnol.* 1997; 3: 107–113.

4. Van Neste, D. Comparative study of normal and rough human skin hydration *in vivo*: evaluation with four different instruments. *J. Dermatol. Sci.* 1991; 2: 119.
5. Hashimoto-Kumasaka, K., Takahashi, K., and Tagami, H. Electrical measurements of the water content of the stratum corneum *in vivo* and *in vitro* under various conditions: comparison between the skin surface hygrometer and Corneometer in evaluation of the skin surface hydration state. *Acta Derm. Venereol. (Stockh).* 1993; 73: 335.
6. Boyce, S.T., Supp, A.P., Wickett, R.R., Hoath, S.B., and Warden, G.D. Assessment with the dermal torque meter of skin pliability after treatment of burns with cultured skin substitutes. *J. Burn. Care Rehabil.* 2000; 21: 55–58.
7. Brancaleon, L., Bamberg, M.P., Sakamaki, T., and Kollias, N. Attenuated total reflection-Fourier transform infrared spectroscopy as a possible method to investigate biophysical parameters of stratum corneum *in vivo*. *J. Invest Dermatol.* 2001; 116: 380–386.
8. Girard, P., Beraud, A., and Sirvent, A. Study of three complementary techniques for measuring cutaneous hydration *in vivo* in human subjects: NMR spectroscopy, transient thermal transfer and corneometry — application to xerotic skin and cosmetics. *Skin Res. Technol.* 2000; 6: 205–213.
9. Szayna, M. and Kuhn, W. *In vivo* and *in vitro* investigations of hydration effects of beauty care products by high-field MRI and NMR microscopy. *J. Eur. Acad. Dermatol. Venereol.* 1998; 11: 122–128.
10. Ozawa, T. and Takahashi, M. Skin hydration: recent advances. *Acta. Derm. Venereol. Suppl (Stockh).* 1994; 185: 26–28.
11. Querleux, B., Richard, S., Bittoun, J., et al. *In vivo* hydration profile in skin layers by high-resolution magnetic resonance imaging. *Skin Pharmacol.* 1994; 7: 210–216.
12. Leveque, J.L. and de Rigal, J. Impedance methods for studying skin hydration. *J. Soc. Cosm. Chem.* 1983; 34: 419–428.
13. Blichmann, C. and Serup, J. Assessment of skin moisture. *Acta Derm. Venereol. (Stockh).* 1988; 68: 284–290.
14. Serup, J. and Blichmann, C. Epidermal hydration of psoriasis plaques and the relation to scaling. *Acta Derm. Venereol. (Stockh).* 1987; 67: 357–359.
15. Salter, D.C. Instrumental methods for assessing skin moisturization. *Cosmet. Toiletries* 1987; 102: 103–109.
16. Leonardi, G.R., Gaspar, L.R., and Maia Campos, P.M. Application of a non-invasive method to study the moisturizing effect of formulations containing vitamins A or E or ceramide on human skin. *J. Cosmet. Sci.* 2002; 53: 263–268.
17. Martinsen, O.G. and Grimnes, S. Facts and myths about electrical measurement of stratum corneum hydration state. *Dermatology* 2001; 202: 87–89.
18. Black, D., Del Pozo, A., Lagarde, J.M., and Gall, Y. Seasonal variability in the biophysical properties of stratum corneum from different anatomical sites. *Skin Res. Technol.* 2000; 6: 70–76.
19. Rogiers, V., Derde, M.P., Verleye, G., and Roseeuw, D. Standardized conditions needed for skin surface hydration measurements. *Cosmet. Toiletries* 1990; 105: 73–82.
20. Hashimoto-umasaka, K., Takahashi, K., and Tagami, H. Electrical measurement of the water content of the stratum corneum *in vivo* and *in vitro* under various conditions: comparison between skin surface hygrometer and corneometer in evaluation of the skin surface hydration state. *Acta Derm. Venereol.* 1993; 73: 335–339.
21. Berardesca, E. EEMCO guidance for the assessment of stratum corneum hydration: electrical methods. *Skin Res. Technol.* 1997; 3: 126–132.
22. Liron, Z., Clewell, H.J., and Mc Dougal, J.N. Kinetics of water vapor sorption in porcine stratum corneum. *J. Pharm. Sci.* 1994; 83: 692–698.
23. Salter, D.C. Studies in the measurement, form and interpretation of some electrical properties of normal and pathological human skin *in vivo*. Doctor of Philosophy (D.Phil.) thesis, University of Oxford, March 1981 (in 3 volumes containing 694 pages).
24. Loden, M., Hagforsen, E., and Lindberg, M. The presence of body hair influences the measurement of skin hydration with the Corneometer. *Acta Derm. Venereol.* 1995; 75: 449–450.
25. Yamamoto, T. and Yamamoto, Y. Analysis for the change of skin impedance. *Med. Biol. Eng. Comput.* 1977; 15: 219.
26. Barel, A.O., Clarys, P., Wessels, B., and de Romsee, A. Non-invasive electrical measurements for evaluating the water content of the horny layer: comparison between capacitance and conductance

measurements, in: *Prediction of Percutaneous Penetration: Methods, Measurements, Modeling*. Scott, R.C., Guy, R.H., Hadgraft, J., and Boode, H.E., (eds.), IBC Technical Services Ltd., London, Vol. 2, 1991, p. 46.
27. Loden, M., Olsson, H., Axell, T., and Linde, Y.W. Friction, capacitance and transepidermal water loss (TEWL) in dry atopic and normal skin. *Br. J. Dermatol.* 1992; 126: 137.
28. Tagami, H. Impedance measurements for evaluation of the hydration state of the skin surface. in: *Cutaneous Investigation in Health and Disease*, Leveque, J.L., (ed.), Marcel Dekker, New York, 1989, Chapter 5.
29. Barel, A.O. and Clarys, P. Measurements of epidermal capacitance, in: *Handbook of Non-Invasive Methods and the Skin*, Serup, J. and Jemec, G.B.E., (eds.), CRC Press, Boca Raton, 1995, pp. 165–170.
30. Prall, J.K., Theiler, R.F., Bowser, P.A., and Walsh, M. The effectiveness of cosmetic products in alleviating a range of skin dryness conditions as determined by clinical and instrumental techniques. *Int. J. Cosm. Sci.* 1986; 8: 159.
31. Leveque, J.L., Grove, G., de Rigal, J., Corcuff, P., Kligman, A.M., and Saint-Leger, D. Biophysical characterisation of dry facial skin. *J. Soc. Cosm. Chem.* 1987; 82: 171.
32. Wilhelm, K.P. Possible pitfalls in hydration measurements. *Curr. Probl. Dermatol.* 1998; 26: 223–234.
33. Tupker, R.A., Coenraads, P.J., Fidler, V. et al. Irritant susceptibility and weal and flare reactions to bioactive agents in atopic dermatitis. II. Influence of season. *Br. J. Dermatol.* 1995; 133: 365–370.
34. Yosipovitch, G., Xiong, G.L., Haus, E. et al. Time-dependent variations of the skin barrier function in humans: transepidermal water loss, stratum corneum hydration, skin surface pH, and skin temperature. *J. Invest. Dermatol.* 1998; 110: 20–23.
35. Le Fur, I., Reinberg, A., Lopez, S. et al. Analysis of circadian and ultradian rhythms of skin surface properties of face and forearm of healthy women. *J. Invest. Dermatol.* 2001; 117: 718–724.
36. Fluhr, J.W., Dickel, H., Kuss, O. et al. Impact of anatomical location on barrier recovery, surface pH and stratum corneum hydration after acute barrier disruption. *Br. J. Dermatol.* 2002; 146: 770–776.
37. Ya-Xian, Z., Suetake, T., and Tagami, H. Number of cell layers of the stratum corneum in normal skin — relationship to the anatomical location on the body, age, sex and physical parameters. *Arch. Dermatol. Res.* 1999; 291: 555–559.
38. Leveque, J.L. Methodes experimentales d'etude du vieillisement cutane chez l'homme *in vivo*, *Acta. Derm. Venereol. (Stockh).* 1987; 144: 1297.
39. Yosipovitch, G., Maayan-Metzger, A., Merlob, P., and Sirota, L. Skin barrier properties in different body areas in neonates. *Pediatr. Dermatol.* 2000; 106: 105–108.
40. Hoeger, P.H. and Enzmann, C.C. Skin physiology of the neonate and young infant: a prospective study of functional skin parameters during early infancy. *Pediatr. Dermatol.* 2002; 19: 256–262.
41. Fluhr, J.W., Pfisterer, S., and Gloor, M. Direct comparison of skin physiology in children and adults with bioengineering methods. *Pediatr. Dermatol.* 2000; 17: 436–439.
42. De Paepe, K., Vandamme, P., Roseeuw, D., and Rogiers, V. Ceramides/cholesterol/FFA-containing cosmetics: the effect on barrier function. *SOFW J.* 1996; 122: 199–204.
43. Carrino, D.A., Sorrell, J.M., and Caplan, A.I. Age-related changes in the proteoglycans of human skin. *Arch. Biochem. Biophys.* 2000; 373: 91–101.
44. Salter, D.C. Monitoring human skin hydration in vivo using electrical impedance — a model of skin as a solid ionic conductor. *Proceedings IX International Conference on Electrical Bio-Impedance and European Community Concerted Action on Impedance Tomography*, Heidelberg, September 26–30, 1995, pp. 17–20.
45. Salter, D.C. Further hardware and measurement approaches for studying water in the stratum corneum. in: *Bioengineering of the Skin: Water and the Stratum Corneum*, Elsner, P., Berardesca, E., and Maibach, H., (eds.), CRC Press, Boca Raton, FL 1994; pp. 205–215.
46. Egawa, M., Oguri, M., Kuwahara, T., and Takahashi, M. Effect of exposure of human skin to a dry environment. *Skin Res. Technol.* 2002; 8: 212–218.
47. Shahidullah, M., Raffle, E.J., Rimmer, A.R., and Frain-Bell, W. Transepidermal water loss in patients with dermatitis. *Br. J. Dermatol.* 1969; 81: 722.
48. Baker, H. and Kligman, A.M., Measurement of transepidermal water loss by electrical hygrometry. Instrumentation and responses of physical and chemical insults. *Arch. Dermatol.* 1967; 96: 441.
49. Pinnagoda, J., Tupker, R.A., Coenraads, P.J., and Nater, J.P., Transepidermal water loss: with and without sweat gland inactivation. *Contact Dermatitis* 1989; 21: 16.

35 Stratum Corneum Tape Stripping: Relationship with Dry Skin and Moisturizers

Frank Dreher and Howard I. Maibach

CONTENTS

35.1 INTRODUCTION

The outermost skin layer, the *stratum corneum* (SC), consists of corneocytes embedded in lipid layers and represents the main barrier for skin penetration of xenobiotics. Its thickness in healthy adults may vary from 5 to 20 μm, except in the palm and sole where it is much thicker. SC can be removed sequentially by repeated application of appropriate adhesive tapes.[1] This technique, commonly known as "SC tape stripping," is a relatively noninvasive method to investigate the structure, properties, and functions of SC *in vivo*[2] and is the most frequently used for such purposes. Other techniques to remove SC are skin surface biopsy using cyanoacrylate strips and skin scraping.

Since SC was found to be a reservoir for topically applied chemical ingredients,[3,4] its removal by tape stripping has provided useful data on the absorption of products in the skin[5] and may therefore be a particularly powerful method for the evaluation of bioavailability and bioequivalence of topical drug products. The assessment of topical drug bioavailability, which includes the determination of a concentration *versus* time profile of an applied drug in human SC *in vivo* using the tape stripping technique, was described as a dermatopharmacokinetic approach by the U.S. Food and Drug

Administration.[6] The possibility that dermatopharmacokinetic characterization might be an alternative approach to clinical trials in order to assess the bioequivalence of topical drugs, like the use of concentration–time curves for systemically administered drugs, was explored during an AAPS/FDA workshop on "Bioequivalence of Topical Dermatological Dosage Forms — Methods for Evaluating Bioequivalence," in September 1996.[6] However, the draft guidance established during this workshop has still not been finalized nor has been generally accepted; the issue is still under discussion between scientists from industry, academia, and regulatory authorities.

35.2 TAPE STRIPPING TECHNIQUE

35.2.1 Experimental Procedure

The SC tape stripping is carried out by applying an adhesive tape onto the skin surface and further removing it by tearing off. The tape is pressed to a previously delineated skin surface area according to a standardized procedure by applying a constant pressure (e.g., 100 g cm^{-2}), using a weight or spring system over an appropriate time period (e.g., 5 sec). Then the tape is removed with a single continuous motion. The application and removal procedure may be repeated 10 to more than 100 times at the same site. Commonly used tapes for skin tape stripping are stationary tapes (e.g., Scotch® Book Tape no. 810 or 845 from 3M Co., USA), medical tapes (e.g., Transpore® from 3 M Co.) or, for such a purpose specially designed, commercially available tapes (e.g., D-Squame® from CuDerm Inc., USA). Tapes differ in shape, size, composition, and adhesive properties.

Following tape stripping, when performing dermatopharmacokinetic studies, the solute contained in the SC removed by tape strips is extracted and measured using appropriate analytical methods such as HPLC.

35.2.2 Technical Recommendations

Despite the simplicity of tape stripping procedure, numerous artifacts may result in inaccurate conclusions following dermatopharmacokinetic studies. The origin of such artifacts as well as possible ways to avoid them are discussed in the following.

35.2.2.1 Removal of Residual Product

After product application and prior to tape stripping procedure, the product excess has to be removed by wiping with a tissue or cotton swab or by an appropriate skin wash.[6,7] The effectiveness of washing procedure may be validated by simulating skin wash on an inert surface such as the bottom of a glass beaker. Hereby, washing procedure should be modified when the applied product is not completely removed. However, care should be taken to ensure that the washing process (e.g., with an aqueous solution containing a mild detergent) does not bring about subsequent solute extraction or redistribution in the SC. Using such a validated washing procedure, the removal of residual or unabsorbed product is effective after one or at most two tape strips. These tape strips contain superficial product residue, which is not likely to be bioavailable and therefore should not be taken into account when performing dermatopharmacokinetic studies. Improper removal of residual product may consequently have a direct impact on the results obtained in such studies. For instance, inadequate removal of residual topical preparation leads to an overestimation of chemical absorption into the SC.

35.2.2.2 Amount of SC Removed

It is currently recognized, that the amount of SC removed by a single tape strip may be influenced by numerous parameters. Differences in adhesive properties between tape brands[8] as well as between

samples of the same brand may result in different amounts of SC removed per surface unit. Pressure,[9] time course between application and removal,[10] as well as velocity of removal process[9] of the tape further influence SC amount removed. Additionally, SC removal may also depend on intrinsic skin properties related to race, sex, age, skin site, as well as skin condition (e.g., moistened versus dry, sun protected versus photo-aged, healthy versus diseased) or other site-specific inter- and intra-subject differences (e.g., SC thickness). Moreover, it has been recognized that SC amount removed by tape stripping may vary according to the depth. In general, the initial strips remove the largest amount of SC, because they remove the loosely packed squamous cells. Since the cohesiveness between corneocytes increases with depth,[11] decreasing amounts of SC are removed with increasing strips. In addition, product application prior to tape stripping must also be considered as a factor influencing the amount of SC removed by sequential tape stripping.[12] For instance, the vehicle components may alter both adhesive properties of the tape as well as cohesiveness of the corneocytes. Finally, differences in environmental conditions may also affect the amount of SC removed by tape stripping[13] suggesting that tape stripping should be performed under controlled conditions as when measuring transepidermal water loss (TEWL).

A further bias in determining dermatopharmacokinetics may originate from imprecise repetitive application of the tape on the skin being stripped. The use of a thin template to delimit the skin area is a simple way to keep the tape stripping area constant and unchanged during the entire tape stripping procedure.

35.2.2.3 Dissimilar Removal

A major problem in dermatopharmacokinetic characterization is that the removal of corneocytes can be dissimilar. Macroscopic skin furrows, which are commonly present, may lead to such a dissimilar removal of corneocytes yielding cell layers that originate from various depths after SC tape stripping.[14] As a result, a chemical may appear to have penetrated into deeper layers of the SC, whereas in fact the penetration restricted to the top layers. To overcome the problems caused by furrows, it has been suggested to slightly stretch the skin and thereby reduce the furrow depth before carrying out tape stripping. The change in corneocytes cohesiveness after product application may be another cause of dissimilar removal of SC.[2] Dissimilar SC removal might be limited by using less adhesive tapes or applying moderate pressure and shortened tape application time. However, the question whether such adaptations of tape stripping procedure do indeed improve the homogeneity of removed SC layers needs to be further investigated.

35.2.2.4 Time of Tape Stripping Procedure

A further factor influencing dermatopharmacokinetics is the duration of tape stripping procedure. Redistribution of the solute in the SC may occur when tape stripping procedure is performed over a long period of time relative to its lag time for diffusion across SC.[15] Furthermore, lateral spreading of the solute from neighboring, noninvolved skin into the stripped area may happen under certain circumstances.[16]

35.2.3 Estimation of SC Amount Removed by Tape Stripping

As described earlier, the amount of SC removed by tape stripping is highly variable and depends on the way the tape stripping procedure is performed as well as on SC characteristics and conditions.

As a consequence, the amount of SC removed by tape stripping is not proportional to the number of strips removed and has to be determined using accurate and reliable methods.

35.2.3.1 Gravimetric Method

Today, weighing is the preferred method to measure the amount of SC removed on a tape strip.[17] Tapes are weighed before and after stripping and the amount of SC is given by weight difference. High precision balances are needed since a very low amount of SC is removed per square centimeter of tape. However, weighing is time consuming and may be biased by water absorption or desorption during weighing procedure before and after stripping.[17] Furthermore, after topical product application, the weighing of SC is only reliable to some extent since the tape strips may not only contain SC but also applied vehicle and solute.

35.2.3.2 Colorimetric Methods Based on Protein Determination

As an alternative to weighing, a simple colorimetric method based on a commercially available protein assay similar to the Lowry assay was recently proposed.[18] Besides the Lowry assay, total protein content can also be determined using the Bradford assay. Briefly, the total protein assay was carried out by immersing SC containing tapes in a sodium hydroxide solution in order to extract the soluble SC protein fraction (SC is mainly composed of corneocytes filled with keratins) and neutralizing the solution with hydrochloric acid (the protein assay is not compatible with acidic conditions). This method was shown to be accurate and reproducible making it possible to determine even very small amounts of SC adhering to a single tape strip. Furthermore, with the exception of protein containing products and some other compounds interfering with the Lowry or Bradford assay, the uptake of product ingredients into the SC after topical application does not interfere with this colorimetric method. In addition, water content of the SC tape strip has no influence on the colorimetric assay. This method is particularly adapted for hydrophilic solutes, which are chemically stable under alkaline conditions (e.g., hydroxy acids), since they may be easily extracted from the SC adhering to tape strips and can thus be analyzed in parallel. However, this method is less convenient for hydrophobic compounds and obviously for those, which are not stable under the conditions of SC extraction. In such a case, tape strips have to be divided into two parts; one used for SC protein determination and the other for solute analysis.

Another method consists of a spectroscopic measurement of Comassie brilliant blue stained SC protein directly on the tape.[17] In contrast with the colorimetric method described earlier, this method does not require any SC extraction procedure prior to protein assay. However, it has been shown to be variable and not appropriate for quantitative determination since the absorbance of colored SC proteins is negligible as compared to light scattering of the SC material adhering to tape strips.

35.2.3.3 Spectroscopic Methods

Recently, a method based on the evaluation of SC amount adhering on tape strips using UV/VIS-spectroscopy as measured at 430 nm was reported.[19] Unlike colorimetric methods, this technique did not require any previous treatment or staining of SC. SC determination was performed directly on the tape (Tesa Film no. 5529, Beiersdorf, Germany) using a double-beam UV/VIS-spectrophotometer, modified in order to obtain a 1 cm $\times$ 1 cm light beam. The reference beam chamber contained an unused tape. The absorbance at 430 nm originated from light reflection, scattering, and diffraction by corneocyte aggregates on the tape and thus was directly related to SC weight removed by tape stripping. Except products absorbing in the wavelength range of corneocyte absorbance, the uptake of vehicle ingredients and solute into the SC after topical application did not interfere. However, at the present state of knowledge of this method, it cannot be excluded that

differences in SC moisture content as well as factors related to topical product application might have an impact on light reflection, scattering, and diffraction properties of corneocytes aggregates and thus on the spectroscopic measurement. Moreover, as discussed by the authors, the accuracy of this spectroscopic method was particularly sensitive to the occurrence of stacked corneocytes adhering to the tape.

35.3 TAPE STRIPPING IN DERMATOPHARMACOKINETIC STUDIES

As described in the draft guidance set up during an AAPS/FDA workshop, the dermatopharmacokinetic evaluation of a topical drug consists in any measurement of drug content in the skin, which can be determined continuously or at least intermittently for a period of time, and, which may include measurement of SC drug concentration over time in humans.[6] Two topical preparations that produce comparable SC drug concentration versus time curves may be bioequivalent just as two preparations for oral administration are deemed bioequivalent if they produce comparable plasma concentration versus time curves. The successful application of dermatopharmacokinetics thus lies on the assumption that SC concentration versus time curves are closely related to the concentration versus time curves of the drug in nucleated epidermis, hair follicles, sebaceous glands, and dermis, at least for drugs targeting these skin compartments instead of SC.

In order to establish dermatopharmacokinetics according to the FDA draft guidelines, the major part of applied drug absorbed in SC must be removed by applying a sufficient number of sequential tape strips. For that purpose, the draft guidelines recommend a protocol in which at least ten successive tape strips are collected (first and eventually second strip being discarded since they represent unabsorbed drug at skin surface) on a single skin site.[6] The SC absorbed drug is then extracted from combined tapes and analyzed using an appropriate analytical method. FDA draft guidelines recommend expressing the results as the amount of drug per area of adhesive tape. When applying this protocol and ensuring that the majority of drug was removed, the amount of SC recovered by tape stripping would not be relevant. Consequently, no recommendations regarding the important issue of standardizing the amount of SC removed by tape stripping was given. However, such a protocol is only applicable when comparing test to reference product in the same individual assuming that intra-site variability on the test site (e.g., forearm) is negligible. Furthermore, there is large evidence that much more than ten strips should be necessary to remove the entire SC[20] so that the major part of drug contained in the related tissue could be recovered, namely in the case of prolonged product application when steady-state of penetration was reached. In fact, it is not advisable to set up a given number of tape strips for removing the entire SC. It was recently demonstrated that a constant number of strips removed different fractions of SC from one individual to another[21] (and most likely also from site-to-site[22]) even when standardized tape stripping conditions (tape type, pressure, time, removal) were used. For instance, the absolute thickness of intact SC on the ventral forearm may vary from 5 to 20 μm in healthy adults.[21] And, the amount of SC removed by tape stripping after product application may vary considerably according to the preparation applied. Therefore, a given number of tape strips neither removes the same amount of SC, nor the same relative percentage of the total barrier function in different individuals. Consequently, it is not correct to relate the amount of absorbed drug to the tape strip number or to pooled tape strips.

Drug content in SC is not homogeneous but rather forms a gradient through skin.[23] In addition to the tape stripping protocol as outlined by the FDA, dermatopharmacokinetic studies may therefore include the assessment of the drug concentration profile within the SC.[24] For this purpose, the uptake of a drug into the SC is expressed as the amount of drug per mass or volume of SC removed (corresponding to drug concentration in SC) versus SC depth. In order to allow for inter-individual as well as inter-site comparison, SC depth should be normalized with respect to the total SC thickness.[21,25] To normalize data, the cumulative amount of SC removed for each individual is divided by the

corresponding total SC thickness. The total SC thickness may be determined by measuring TEWL in combination with tape stripping assuming a constant SC density of 1 $g\,cm^{-3}$ across SC as well as a uniform SC removal.[26] This measurement may be performed in a skin area adjoining that on which the product is applied.

35.4 TAPE STRIPPING FOR MEASURING SC COHESION

Tape stripping can be further used to investigate intercorneocyte cohesion within the SC by quantifying the amount of SC removed.[11] When using standardized tape stripping conditions, the more SC removed, the smaller the SC cohesion. For instance, tape stripping in combination with a protein assay to accurately quantify SC removal proved to be a sensitive method in detecting keratolytic efficacy of salicylic acid preparations within hours of application.[27] This method may also be useful to measure the influence of moisturizers or other skin care preparations on SC cohesion.

35.5 SUMMARY AND CONCLUSION

The application of tape stripping technique is well-established in dermatopharmacological research and the technique is appreciated as one of the most useful method to remove SC allowing investigation of its structure, properties, and functions. However, despite apparent simplicity, the tape stripping technique entails several technical problems and care has to be taken to avoid misleading conclusions when interpreting data. For instance, results given as a function of tape strip number or pooled tape strips have to be interpreted with care, since the amount of SC removed by tape stripping may be highly variable and may depend on numerous factors related to tape stripping procedure and SC properties. Therefore, SC removal by tape stripping should be determined using accurate and reliable methods. Today, weighing is the most commonly used method for such a purpose. But, due to possible artifacts associated with weighing procedure, other methods such as a colorimetric and a spectroscopic assay were recently proposed to improve the reliability of data obtained on SC amounts removed by tape stripping. However, both methods need further validation before considering that they provide an adequate and more accurate alternative than weighing.

Tape stripping is also used to assess both drug uptake and clearance from the SC making it a particularly powerful method for the evaluation of bioequivalence of topical drug products using a dermatopharmacokinetic approach as described by the FDA. However, implementing the technical recommendations mentioned in this review to avoid problems associated with tape stripping technique and subsequent proper validation are paramount requisites to make tape stripping a viable method for bioequivalence evaluation of topically applied dermatological drug products and use it as a standard technique to pursue the development of safe and effective generic topical products. In addition, recent studies indicate that tape stripping in combination with an accurate method to quantify SC removal is a reliable and sensitive method to measure keratolytic efficacy.

Taken together, sufficient background information exists to begin to permit to the use of stripping method to define the role of individual agents and combinations thereof on so called dry skin syndrome.

REFERENCES

1. Pinkus, H., Examination of the epidermis by the strip method of removing horny layers. *J. Invest. Dermatol.* 16, 1951, 383–386.
2. Surber, C., Schwarb, F.P., and Smith, E.W., Tape-stripping technique, in *Percutaneous Absorption: Drugs — Cosmetics — Mechanisms — Methodology*. Bronaugh, R.L. and Maibach, H.I. (Eds.), 3rd edn. *Drugs Pharm. Sci.* 97, 1999, 395–409.

3. Vickers, C.F.H., Existence of reservoir in the stratum corneum. *Arch. Dermatol.* 88, 1963, 20–23.
4. Rougier, A., Dupuis, D., Lotte, C., Roguet, R., and Schaefer, H., *In vivo* correlation between stratum corneum reservoir function and percutaneous absorption. *J. Invest. Dermatol.* 81, 1983, 275–278.
5. Lücker, P., Nowak, H., Stüttgen, G., and Werner, G., Penetrationskinetik eines Tritium-markierten 9α-Fluor-16-methylen-prednisolonesters nach epicutaner Applikation beim Menschen. *Arzneim Forsch./Drug Res.* 18, 1968, 27–29.
6. Shah, V.P., Flynn, G.L., Yacobi, A., Maibach, H.I., Bon, C., Fleischer, N.M., Franz, T.J., Kaplan, S.A., Kawamoto, J., Lesko, L.J., Marty, J.P., Pershing, L.K., Schaefer, H., Sequeira, J.A., Shrivastava, S.P., Wilkin, J., and Williams, R.L., Bioequivalence of topical dermatological dosage forms — methods of evaluation of bioequivalence. *Pharm. Res.* 15, 1998, 167–171.
7. Howes, D., Guy, R., Hadgraft, J., Heylings, J., Hoeck, U., Kemper, F., Maibach, H., Marty, J.P., Merk, H., Parra, J., Rekkas, D., Rondelli, I., Schaefer, H., Täuber, U., and Verbiese, N., Methods for assessing percutaneous absorption. The report and recommendations of ECVAM workshop 13. *ATLA* 24, 1996, 81–106.
8. Tsai, J.C., Weiner, N.D., Flynn, G.L., and Ferry, J., Properties of adhesive tapes used for stratum corneum stripping. *Int. J. Pharm.* 72, 1991, 227–231.
9. Löffler, H., Dreher, F., and Maibach, H.I., Stratum corneum adhesive tape stripping: influence of anatomical site, duration and removal. *Br. J. Dermatol.* 151, 2004, 746–752.
10. Tokumura, F., Ohyama, K., Fujisawa, H., Suzuki, M., and Nukatsuka, H., Time-dependent changes in dermal peeling force of adhesive tapes. *Skin Res. Technol.* 5, 1999, 33–36.
11. King, C.S., Barton, S.P., Nicholls, S., and Marks, R., The change in properties of the stratum corneum as a function of depth. *Br. J. Dermatol.* 100, 1979, 165–172.
12. Tsai, J.C., Cappel, M.J., Weiner, N.D., Flynn, G.L., and Ferry, J., Solvent effects on the harvesting of stratum corneum from hairless mouse skin through adhesive tape stripping *in vitro*. *Int. J. Pharm.* 68, 1991, 127–133.
13. Tokumura, F., Ohyama, K., Fujisawa, H., and Nukatsuka, H., Seasonal variation in adhesive tape stripping of the skin. *Skin Res. Technol.* 5, 1999, 208–212.
14. Van der Molen, R.G., Spies, F., Van't Noordende, J.M., Boelsma, E., Mommaas, A.M., and Koerten, H.K., Tape stripping of human stratum corneum yields cell layers that originate from various depths because of furrows in the skin. *Arch. Dermatol. Res.* 289, 1997, 514–518.
15. Reddy, M.B., Stinchcomb, A.L., Guy, R.H., and Bunge, A.L., Determining dermal absorption parameters *in vivo* from tape strip data. *Pharm. Res.* 19, 2002, 292–298.
16. Ashworth, J., Watson, W.S., and Finlay, A.Y., The lateral spread of clobetasol 17-propionate in the stratum corneum *in vivo*. *Br. J. Dermatol.* 119, 1988, 351–358.
17. Marttin, E., Neelissen-Subnel, M.T.A., De Haan, F.H.N., and Boddé, H.E., A critical comparison of methods to quantify stratum corneum removed by tape stripping. *Skin Pharmacol.* 9, 1996, 69–77.
18. Dreher, F., Arens, A., Hostynek, J.J., Mudumba, S., Ademola, J., and Maibach, H.I., Colorimetric method for quantifying human *stratum corneum* removed by adhesive-tape-stripping. *Acta Dermatol. Venerol. (Stockh.)* 78, 1998, 186–189.
19. Weigmann, H.J., Lademann, J., Meffert, H., Schaefer, H., and Sterry, W., Determination of the horny layer profile by tape stripping in combination with optical spectroscopy in the visible range as a prerequisite to quantify percutaneous absorption. *Skin Pharmacol. Appl. Skin. Physiol.* 12, 1999, 34–45.
20. Reed, J.T., Ghadially, R., and Elias, P.M., Skin type, but neither race nor gender, influence epidermal permeability barrier function. *Arch. Dermatol.* 131, 1995, 1134–1138.
21. Kalia, Y.N., Alberti, I., Sekkat, N., Curdy, C., Naik, A., and Guy, R.H., Normalization of stratum corneum barrier function and transepidermal water loss *in vivo*. *Pharm. Res.* 17, 2000, 1148–1150.
22. Ya-Xian, Z., Suetake, T., and Tagami, H., Number of cell layers of the stratum corneum in normal skin — relationship to the anatomical location on the body, age, sex and physical parameters. *Arch. Dermatol. Res.* 291, 1999, 555–559.
23. Watkinson, A.C., Bunge, A.L., Hadgraft, J., and Naik, A., Computer simulation of penetrant-depth profiles in the stratum corneum. *Int. J. Pharm.* 87, 1992, 175–182.
24. Stinchcomb, A.L., Pirot, F., Touraille, G.D., Bunge, A.L., and Guy, R.H., Chemical uptake into human stratum corneum *in vivo* from volatile and non-volatile solvents. *Pharm. Res.* 16, 1999, 1288–1293.

25. Pirot, F., Kalia, Y.N., Stinchcomb, A.L., Keating, G., Bunge, A., and Guy, R.H., Characterization of the permeability barrier of human skin *in vivo*. *Proc. Natl Acad. Sci. USA* 94, 1997, 1562–1567.
26. Kalia, Y.N., Pirot, F., and Guy, R.H., Homogeneous transport in a heterogeneous membrane: water diffusion across human stratum corneum *in vivo*. *Biophys. J.* 71, 1996, 2692–2700.
27. Bashir, S.J., Dreher, F., Chew, A.L., Zhai, H., Levin, C., Stern, R., and Maibach, H.I., Cutaneous bioassay of salicylic acid as a keratolytic. *Int. J. Pharm.* 292, 2005, 187–194.

36 XLRS Squamometry Revisited

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CONTENTS

In its most widely appreciated context, the stratum corneum (SC) exhibits an important barrier function extending to protection from ultraviolet light, oxidants, micro-organisms, and toxic xenobiotics. It also protects from loss of water and electrolytes from the body. The SC can also be viewed as a highly specialized structure showing perpetual renewal keeping ideally a steady state in its structure and thickness. It is structurally and biochemically diverse. It possesses a limited form of metabolic activity. It also acts as a unique sophisticated biosensor that signals the underlying epidermis to respond to external stresses.[1,2]

Corneocytes are about 1 μm thick and have a mean area of approximately 1000 μm^2. However, the surface area is dependent upon age, anatomical location, and conditions that influence epidermal proliferation such as chemical irritation and UV irradiation.[3] Corneocyte size increases with age. This is sometimes assumed to be related to the increased transit time within the SC. On most body sites, the SC consists typically of 12 to 16 layers of flattened corneocytes.

Each corneocyte corresponds to a water-insoluble protein complex made primarily of a highly organized keratin microfibrillar matrix. This structure is encapsulated in a protein and lipid-enriched shell. This cornified cell envelope shows differences in maturation among corneocytes. Specific probes allow to distinguish two distinct types of cornified cell envelopes, which have been called "fragile" and "rigid," or "immature" and "mature."[2,4,5]

In some instances, the SC homeostasis is altered. Indeed, the SC representing the interface between the epidermis and the environment is the repository of many biological events that have occurred below it in previous days. It is also affected by diverse and repeated external threats. The genetic background, nutritional status, and the intervention of some physical agents, drugs, cosmetics, toiletries, and other chemical xenobiotics represent other major modulators of the SC structure. Knowledge about the fine SC structure is crucial in many aspects of the dermocosmetic science, particularly when dealing with xerosis and related effects of emollients and squamolytic agents.[6–8]

36.1 XEROSIS ASSESSMENT AND SAMPLING

The formation, composition, structure, and function of human SC have been scrutinized by intense research over the past few decades. Clinical assessments of the SC aspect are based on ordinal grading scales. They often suffer from vague and overlapping definitions.[9] The subjective clinical assessment based on visual and tactile scoring may suffer from variability by inconsistencies from grade to grade and also from poor reproducibility. Variations in the environmental conditions may jeopardize subjective grading systems since hydration swells the outer SC and often camouflages low-grade scaling and dryness.

Investigators were also striving relentless to provide more precise evaluation tools. Bioinstrumentation plays an ever-increasing role reducing grading variation and yielding better distinction between competing drugs and cosmetic formulations.[6,7,8,10–12] One of the ancillary method designed to study the outer SC layers relied on tape stripping. The use of casual sticky tapes often proved to be unreliable for quantitative assessments because there was considerable variation in the adhesion of different batches to the SC.[13] Currently, the standard operating procedures in many laboratories rely on two other sampling methods, namely the cyanoacrylate skin surface stripping (CSSS) and stripping with adhesive-coated discs (SACD).[6,7,14,15]

Groundbreaking work using the SACD method has won international recognition for fundamental research, clinical experimentation, and applied dermocosmetology. The sampling device for SACD is one of the crystal clear pressure-sensitive adhesive-coated discs (D-Squame®, Cuderm Corporation, Dallas, TX; Corneofix®, C+K Electronics, Cologne, Germany; Corneodisc L'Oréal, Paris, France). This specific material provides the required rigidity and adhesion to uniformly sample a fixed area of skin surface. After peeling off the protective seal, the disc is applied to the skin surface using a gauge spring dynamometer to ensure a calibrated pressure usually chosen in the range 100 to 250 g/cm^2. Both the pressure and the time of application of the disc influence the amount of SC removed.[6,7,13,14,16] A short-time (5 sec) SACD procedure removes less corneocytes from the stratum disjunctum than a longer time (1 h) application. This is due to occlusion modifying the SC hydration and cohesion between corneocytes.[9,17–19] A seasonal variation in the amount of harvested SC is possible.[20]

If greasy products have been applied to the skin before sampling, the SACD procedure is impaired giving rise to unreliable data. When such a pitfall is suspected oily product substantivity can be checked before SACD sampling using a Sebumeter SM 810 (C+K Electronics, Cologne, Germany). Prior application of lipid solvents also alters the SC harvesting.[21] In general, however, sampling errors for SACD are expected to be reduced by delipidizing the skin with one application of ether:acetone (1:1). By contrast, repeated applications of ethanol solutions and other solvents significantly alter the physiological desquamation process and reduce the amount of corneocytes collected.[14]

The SACD method is used for different purposes. The most popular goals deal with the evaluation of skin scaliness and internal cohesiveness of the superficial SC. For several years, the challenge of analytical evaluations of SACD allowed exploring various aspects of the structure and biological dynamics of the SC.

36.2 SACD AND SCALINESS

The SC continually renews itself, imperceptibly casting off corneocytes from its stratum disjunctum. The most important adhesive forces holding keratinocytes together come from the desmosomes. During these cells move toward the skin surface, desmosomes are modified into corneosomes (also called corneodesmosomes) in a process following a programed protease-mediated destruction.[8,18,22,23] Desquamation results in the degradation of nonperipheral corneosomes first at the stratum compactum–disjunctum interface and finally the peripheral corneosomes are degraded in the upper layers of the SC. This process leads to loss of corneocytes from the surface of the skin by discrete frictional forces.

Xerosis is the term dermatologists use to describe corneocyte aggregation clinically featuring rough, flaky, and scaly skin. Such a condition, is otherwise known to the laity as dry skin.[7,24,25] In dry flaky skin conditions corneosomes are not degraded efficiently and corneocytes accumulate on the skin surface.[19,26] Thus, failure to degrade corneosomes timely and correctly is the key factor responsible for most flaking conditions. During the xerotic process, corneocytes remain attached to each other with uneven strength until physical forces cause whole rafts of cells to detach partially from the skin. The reduced corneodesmolysis that occurs in xerotic skin disorders is due to reductions in the levels and activities of SC proteases together with elevated levels of corneosomal glycoproteins in the superficial layers of the SC. Additionally, increased levels of fragile corneocytes are associated with reduced transglutaminase activity and corneocyte envelope cross-linking events.[4] The recently introduced silicon image sensing (SIS) technology shows the low water content of the SC in these cases.[27–30]

The SACD can be employed to sample and quantify loose corneocytes and squames from the skin surface. Such a procedure is only valid for conditions where the intercorneocyte bonds are weaker than the SACD adhesion to the SC.[14] In fact, tiny scales found in the common type of facial dry skin, dandruff, and seborrheic dermatitis come off quite easily.[31–42] By contrast, thick compact adherent scales found in ichthyosis and severe winter xerosis of the lower legs[12,31] are not reliably harvested using SACD. This represents a classical pitfall of the method.[14]

When the sampling procedure of SACD is correct, the amount of collected SC can be weighed with confidence.[7,43,44] However, it is time-consuming and often subject to artifacts due to adsorption or desorption of water taking place in the SACD sample.[45] An indirect assessment of the amount in harvested corneocytes was designed by measuring the attenuation of transmitted light using photometry.[46] There may be a bias in such a procedure because a given amount of corneocytes either stacked in clumps or joint in a thin sheet exhibits different optical properties.[7] The same limitation applies to the measure of light reflectance when the sample is placed upon a dark background.[7] The quantitative and qualitative aspects of the scale pattern are probably better appreciated by means of image analysis of the dry SACD samples.[14,47–53] Texture analysis of SACD is another sophisticated method.[54] A series of other innovative methods have also been proposed to provide information about the amount of SC present in SACD.[45,55–58]

Contrasting with the aforementioned assessments, the SC can be studied after staining the harvested SACD. Many dyes are available and their choice depends on the goal of the study. As an example, a mixture of rhodamine B and methylene blue conveniently decorates the corneocytes for microscopic examination. Another quantitative assay is based on the Lowry's reaction of proteins with an alkaline copper tartrate solution and Folin reagent followed by spectrophotometric measurement of the reaction product with maximum absorbance at 750 nm.[59]

A wealth of applications have benefited from staining with a mixture of toluidine blue and basic fuschin in 30% ethanol at pH 3.4. Samples are stained for 1 min and gently dipped into water in order to rinse off the excess of dyes. This step is critical as it is important for not loosing corneocytes from SACD.[7,14] As such, samples are suitable to microscopic examination and reflectance colorimetry. This latter assessment is coined squamometry.[14,31] Basically, the stained SACD samples are placed over a hole cut out of either a glass or plastic slide, which is then placed onto a white reference tile. The color of the specimens is measured by reflectance colorimetry. The most convenient colorimetric parameter is represented by the squamometry index (SQMI) corresponding to the Chroma C^* value, which combines the values of red and blue chromacities following: Chroma $C^* = (a^{*2} + b^{*2})^{1/2}$.

Squamometry is currently used for four main purposes. Accordingly, different names were coined and defined[14]:

- *Squamometry X* refers to the assessment of xerosis and any other scaly condition.
- *Squamometry L* refers to the assessment of the intercorneocyte cohesiveness altered by light, particularly in the ultraviolet range.
- *Squamometry R* refers to the dynamic aspect of the SC renewal.

- *Squamometry S* refers to the assessment of the effect of surfactants on the corneocyte integrity.

36.3 SQUAMOMETRY X

Squamometry X corresponds to the analytical evaluation of xerosis using SACD samplings.[6,7,14,31] A linear correlation exists between the amount of harvested orthokeratotic SC and SQMI. Data are also influenced by the presence of parakeratotic cells and serum deposits as found in spongiotic dermatoses such as atopic dermatitis, irritant or allergic contact dermatitis, seborrheic dermatitis and psoriasis.[32,36]

The efficacy of squamolytic agents, emollients, and other SC biomodifyers is conveniently evaluated using squamometry X.[7,14,39] In general, it is appropriate to take SACD at entry in a given clinical study, as well as after two and four weeks of treatment, which is then stopped. After a two-week post-treatment period corresponding to the regression phase, late SACD samples are collected. SQMI values of the successive samplings show the kinetics of improvement followed by the post-treatment regression.

The efficacy of corticosteroids in inflammatory dermatoses is also conveniently evaluated by the same approach. SQMI values decrease when the effects of inflammation disappear from the SC.[32,35,36]

Squamometry X is particularly gratifying in the assessment of both dandruff severity and efficacy of its treatment.[6,7,14,33,34,37,38,42] Similarly, seborrheic dermatitis can be evaluated noninvasively.[40,41] In these related scalp conditions, some correlation can be established between SQMI and the load in *Malassezia* spp.[33,37,38,40,41,60]

36.4 SQUAMOMETRY L

Ultraviolet irradiation alters the biology of the epidermis. One of the consequences of an acute exposure is altered maturation and desquamation of the SC. The kinetics of the modifications in the SC cohesiveness can be assessed using squamometry L.[14,61] Similarly, the effect of any topical intervention for controlling such a process can be quantified.[62]

36.5 SQUAMOMETRY R

Once the SC stained *in vivo* by an appropriate dye, it is possible to assess the rate of corneocyte renewal in the two following weeks.[63] One of the SC marker is dihydroxyacetone (DHA). The brown color of the SCAD sample can be measured by reflectance colorimetry after placing the DHA–SCAD sample on a white reference tile. After the DHA–SACD colorimetric evaluation, the same samples are stained in a second *ex vivo* step using toluidine blue-basic fuschin dyes to derive SQMI. The latter value serves to assess the amount of collected corneocytes. The squamometry R index is the ratio between the Chroma C^* of DHA–SACD and SQMI. Performing iterative SACD samplings over two weeks shows the kinetics of fading DHA skin color, thus representing an indirect estimation of the desquamation rate.

36.6 SQUAMOMETRY S

The SC is subjected to various environmental chemical threats. Squamometry S was designed to test the effect of surfactants on the SC *in vivo*.[6,7,14,64] Several studies have confirmed the reliability of such a method in predicting irritation by selected xenobiotics[61,65–70] and protection afforded against them by diverse agents.[62,71] As such, squamometry S appears to be a suitable method for evaluating

TABLE 36.1
Ordinal Scales for the Microscopic Assessment of Squamometry S (From Reference 14)

Score	Predominant pattern
Staining intensity	
0	No staining
1	Discrete between corneocytes
2	Spotted on corneocytes
3	Strong in single and clustered corneocytes
4	Strong in most corneocytes
Intercorneocyte loosening	
0	Large and uniform sheet of corneocytes
1	Large clusters, few single corneocytes
2	Small clusters, some single corneocytes
3	Disrupted clusters, many single corneocytes
4	Single/torn corneocytes

the efficacy of barrier substances when minimally damaged conditions are chosen, such as with semi-open applications of the offending product.[71]

Squamometry S is somewhat reminiscent of the *ex vivo* corneosurfametry bioassay although the information is not similar but complementary.[64] Squamometry S entails the application of surfactants or other chemicals onto the skin. Whatever risk exists is small but not zero, all practical steps reducing the risk to the smallest acceptable level must be considered. Hence, the potentially offending agents remain in place for a short period of time only, usually ranging from 15 to 90 min. Such an *in vivo* application can be repeated for instance every second hour when the xenobiotic is weakly aggressive to the skin.[64] At the issue of a single or repeated insult(s), SACD are collected and submitted to SQMI measurements. In addition, microscopic examination is warranted in order to categorize the patterns of corneocyte confluence and intensity of staining.[7,14,34,65,67] Indeed, with increasing aggression, corneocytes may show both increased dye binding and loosening. This is due to the variable combination of protein denaturation, corneosome disruption, and corneocyte lysis. A trained microscopist is endowed to score SACD according to an ordinal scale (Table 36.1) in order to avoid as much as possible inter-observer variations. It is unfortunate that the grading systems differ among research groups.[34,67] Hopefully, image analysis is superior in sensitivity and objectivity to discriminate differences between SACD samples. The most informative values are the percentage of the SACD area occupied by corneocytes and the distribution in size of the corneocyte aggregates relative to that of a single cell. The staining intensity relies on the evaluation of grey levels on a 1 to 8 scale. Such a method is sensitive enough to detect subclinical irritation well before any increase in erythema and transepidermal water loss can be detected.

36.7 CONCLUSION

The SC is not a dead tissue of little importance. SACD is becoming a time-honored method for studying this dynamic and heterogeneous structure. There is strong circumstantial evidence indicating that squamometry test in its XLRS variants affords valuable information. It is a simple, highly reproducible, and sensitive method. In many instances changes induced by physiological variations and environmental threats, and improvement brought by cosmetics and other SC biomodifyers are finely demonstrated by this method. Further experience with squamometry will probably continue to define the relative strength and weakness of the method and perhaps design new variants of it.

REFERENCES

1. Elias, P.M. Stratum corneum architecture, metabolic activity and interactivity with subjacent cell layers. *Exp. Dermatol.* **5**, 191–201 (1996).
2. Harding, C.R. The stratum corneum: structure and function in health and disease. *Dermatol. Ther.* **17**, 6–15 (2004).
3. Marks, R. and Barton, S.P. The significance of the size and shape of corneocytes. In: *Stratum corneum*. Marks, R. and Plewig. G. (Eds.). Springer-Verlag, Berlin, pp. 161–170 (1983).
4. Hirao, T., Denda, M. and Takahashi, M. Identification of immature cornified envelopes in the barrier-impaired epidermis by characterization of their hydrophobicity and antigenicities of the components. *Exp. Dermatol.* **10**, 35–44 (2001).
5. Harding, C.R., Long, S., Richardson, J. et al. The cornified cell envelope: an important marker of stratum corneum maturation in healthy and dry skin. *Int. J. Cosmet. Sci.* **25**, 157–167 (2003).
6. Piérard, G.E. and Piérard-Franchimont, C. Drugs and cosmetics evaluations with skin strippings. In: *Dermatologic research techniques*. Maibach, H. (Ed.). CRC Press, Boca Raton, pp. 133–148, (1996).
7. Piérard, G.E. EEMCO guidance for the assessment of dry skin (xerosis) and ichthyosis: evaluation by stratum corneum strippings. *Skin Res. Technol.* **2**, 3–11 (1996).
8. Rawlings, A.V. Trends in stratum corneum research and the management of dry skin conditions. *Int. J. Cosmet. Sci.* **25**, 63–95 (2003).
9. Serup, J. EEMCO guidance for the assessment of dry skin (xerosis) and ichthyosis: clinical scoring systems. *Skin Res. Technol*. **1**, 109–114 (1995).
10. Lodén, M. Biophysical methods of providing objective documentation of the effects of moisturizing creams. *Skin Res. Technol.* **1**, 101–108 (1995).
11. Fischer, T.W., Wigger-Alberti, W., and Elsner, P. Assessment of "dry skin": current bioengineering methods and tests designs. *Skin Pharmacol. Appl. Skin Physiol.* **14**, 183–195 (2001).
12. Piérard-Franchimont, C., Petit, L., and Piérard, G.E. Skin surface patterns of xerotic legs: the flexural and accretive types. *Int. J. Cosmet. Sci.* **23**, 121–126 (2001).
13. Tsai, J.C., Weiner, N.D., Flynn, G.L., and Ferry, J.P. Properties of adhesive tapes used for stratum corneum stripping. *Int. J. Pharm.* **72**, 227–231 (1991).
14. Piérard-Franchimont, C., Henry, F., and Piérard, G.E. The SACD method and the XLRS squamometry tests revisited. *Int. J. Cosmet. Sci.* **22**, 437–446 (2000).
15. Piérard-Franchimont, C. and Piérard, G.E. Cyanoacrylate skin surface strippings for visualising the stratum corneum structure and dynamics. in: *Measuring the skin*. *Non invasive investigations, physiology, normal constants*. par, Agache, P. and Humbert, P. (Eds.). Springer-Verlag, Berlin 2004.
16. Tokumura, F., Ohyama, K., Fujisawa, H., Suzuki, M., and Nukatsuka, H. Time-dependent changes in dermal peeling force of adhesive tapes. *Skin Res. Technol.* **5**, 33–36 (1999).
17. Rawlings, A., Harding, C., Watkinson, A., et al. The effect of glycerol and humidity on desmosome degradation in stratum corneum. *Arch. Dermatol. Res.* **287**, 457–464 (1995).
18. Watkinson, A., Harding, C., Moore, A., and Coan, P. Water modulation of stratum corneum chymotryptic enzyme activity and desquamation. *Arch. Dermatol. Res.* **293**, 470–476 (2001).
19. Piérard, G.E., Goffin, V., Hermanns-Lê, T., and Piérard-Franchimont, C. Corneocyte desquamation. *Int. J. Mol. Med.* **6**, 217–221 (2000).
20. Tokumura, F., Ohyama, K., Fujisawa, H., and Nukatsuka, H. Seasonal variation in adhesive tape stripping of the skin. *Skin Res. Technol.* **5**, 208–212 (1999).
21. Tsai, J.C., Cappel, M.J., Weiner, N.D., Flynn, G.L., and Ferry, J. Solvent effects on the harvesting of stratum corneum from hairless mouse skin through adhesive tape stripping *in vitro*. *Int. J. Pharmacol.* **68**, 127–133 (1991).
22. Harding, C.R., Watkinson, A., and Rawlings, A.V. Dry skin, moisturization and corneodesmolysis. *Int. J. Cosmet. Sci.* **22**, 21–52 (2000).
23. Horikoshi, T. Proteinases involved in desquamation of human stratum corneum. *J. Jpn. Cosmet. Sci.* **24**, 319–328 (2000).
24. Piérard-Franchimont, C. and Piérard, G.E. Les xéroses: structure de la peau rêche. *Int. J. Cosmet. Sci.* **6**: 47–54 (1984).
25. Piérard, G.E. What do you mean by dry skin? *Dermatologica* **179**, 1–2 (1989).

26. Simon, M., Bernard, D., Minondo, A.M., Camus, C., Fiat, F., Corcuff, P., Schmidt, R., and Serre, G. Persistence of both peripheral and non-peripheral corneodesmosomes in the upper stratum corneum of winter xerosis skin versus only peripheral in normal skin. *J. Invest. Dermatol.* **116**, 23–30 (2001).
27. Lévêque, J.L. and Querleux, B. SkinChip®, a new tool for investigating the skin surface *in vivo*. *Skin Res. Technol.* **9**, 343–347 (2003).
28. Piérard, G.E. and Lévêque, J.L. What is SkinChip®? From silicon image sensor technology to SkinChip®. *Dermatology.* **208**, 291–292 (2004).
29. Uhoda, E., Piérard-Franchimont, C., Petit, L., and Piérard, G.E. The conundrum of skin pores in dermocosmetology. *Dermatology*, 210, 3–7, (2005).
30. Uhoda, E., Lévêque, J.L., and Piérard, G.E. Silicon image sensor technology for *in vivo* detection of surfactant-induced corneocyte swelling and drying. *Dermatology*, 210, 184–188, (2005).
31. Piérard, G.E., Piérard-Franchimont, C., Saint-Léger, D., and Kligman, A.M. Squamometry: the assessment of xerosis by colorimetry of D-squame adhesive discs. *J. Soc. Cosmet. Chem.* **47**, 297–305 (1992).
32. Piérard, G.E. and Piérard-Franchimont, C. Squamometry as an aid for rating severity of target lesions in atopic dermatitis. *Giorn. Int. Dermatol. Ped.* **6**, 125–128 (1994).
33. Arrese, J.E., Piérard-Franchimont, C., De Doncker, P., Heremans, A., Cauwenbergh, G., and Piérard, G.E. Effect of ketoconazole-medicated shampoo on squamometry and *Malassezia ovalis* load in pityriasis capitis. *Cutis* **58**, 235–237 (1996).
34. Goffin, V., Piérard-Franchimont, C., and Piérard, G.E. Antidandruff shampoos and the stratum corneum. *J. Dermatol. Sci.* **7**, 223–225 (1996).
35. Letawe, C., Piérard-Franchimont, C., and Piérard, G.E. Squamometry in rating the efficacy of topical corticosteroids in atopic dermatitis. *Eur. J. Clin. Pharmacol.* **51**, 253–258 (1996).
36. Piérard, G.E., Lachapelle, J.M., Frentz, G., Schopf, E., and Stolz, E. Hydrocortisone-17-butyrate topical emulsion (Locoid Crelo®) in psoriasis. *J. Eur. Acad. Dermatol. Vener.* **6**, 11–14 (1996).
37. Piérard, G.E., Arrese, J.E., Piérard-Franchimont, C., and De Doncker, P. Prolonged effects of antidandruff shampoos-time to recurrence of *Malassezia ovalis* colonisation of skin. *Int. J. Cosmet. Sci.* **19**, 111–117 (1997).
38. Piérard-Franchimont, C., Arrese, J.E., Durupt, G., Ries, G., Cauwenbergh, G., and Piérard, G.E. Correlation between *Malassezia* spp load and dandruff severity. *J. Mycol. Med.* **8**, 83–86 (1998).
39. Piérard-Franchimont, C., Goffin, V., and Piérard, G.E. Modulation of human stratum corneum properties by salicylic acid and all-*trans*-retinoic acid. *Skin Pharmacol. Appl. Skin Physiol.* **11**: 266–272 (1998).
40. Piérard-Franchimont, C., Willemaers, V., Fraiture, A.L., and Piérard, G.E. Squamometry in seborrheic dermatitis . *Int. J. Dermatol* . **38**, 712–715 (1999).
41. Piérard-Franchimont, C. and Piérard, G.E. A double-blind placebo-controlled study of ketoconazole + desonide gel combination in the treatment of facial seborrheic dermatitis. *Dermatology*, **204**, 344–347 (2002).
42. Piérard-Franchimont, C., Uhoda, E., Loussouarn, G., Saint Léger, D., and Piérard, G.E. Effect of residence time on the efficacy of anti-dandruff shampoos. *Int. J. Cosmet. Sci.* **25**, 267–271 (2003).
43. Lévêque, J.L., Corcuff, P., de Rigal, J., and Agache, P. *In vivo* studies of the evolution of physical properties of the human skin with age. *Int. J. Dermatol.* **23**, 322–329 (1984).
44. Weigmann, H.J., Lademann, J., Meffert, H., Schaefer, H., and Sterry, W. Determination of the horny layer profile by tape stripping in combination with optical spectroscopy in the visible range as a prerequisite to quantify percutaneous absorption. *Skin Pharmacol. Appl. Skin Physiol.* **12**, 34–45 (1999).
45. Martin, E., Neelissen-Subnel, M.T.A., De Haan, F.H.N., and Boddé, H.E. A critical comparison of methods to quantify stratum corneum removed by tape stripping. *Skin Pharmacol.* **9**, 69–77 (1996).
46. Serup, J., Winther, A., and Blichmann, C. A simple method for the study of scale pattern and effects of a moisturizer — qualitative and quantitative evaluation by D-Squame® tape compared with parameters of epidermal hydration. *Clin. Exp. Dermatol.* **14**, 277–283 (1989).
47. Prall, J.K., Theiler, R.F., Bowser, P.A., and Walsch, M. The effectiveness of cosmetic products in alleviating a range of dryness conditions as determined by clinical and instrumental techniques. *Int. J. Cosmet. Sci.* **8**, 159–164 (1986).

48. Kligman, A.M., Schatz, H., Manning, S., and Stoudemayer, T. Quantitative assessment of scaling in winter xerosis using image analysis of adhesive-coated disks (D-Squames). In: *Noninvasive methods for the quantification of skin fuctions.* Frosch, P.J. and Kligman, A.M. (Eds.). Springer-Verlag, Berlin, pp. 309–15 (1993).
49. Schatz, H., Kligman, A.M., Manning, S., and Stoudemayer, T. Quantification of dry (xerotic) skin by image analysis of scales removed by adhesive discs (D-Squames®). *J. Soc. Cosmet. Chem.* **44**, 53–63 (1993).
50. Miller, D.L. Sticky slides and tape techniques to harvest stratum corneum material. In: *Handbook of non-invasive methods and the skin*. Serup, J. and Jemec, G.B.E. (Eds.). CRC Press, Boca Raton, pp. 149–51 (1995).
51. Schatz, H., Altmeyer, P., and Kligman, A. Dry skin and scaling evaluted by D-Squames and image analysis. In: *Handbook of non-invasive methods and the skin*. Serup, J. and Jemec, G.B.E. (Eds.). CRC Press, Boca Raton, pp. 153–157 (1995).
52. Martin, E., Neelissen-Subnel, M.T.A., De Haan, F.H.N., and Bodde, H.E. A critical comparison of methods to quantify stratum corneum removed by tape stripping. *Skin Pharmacol.* **9**, 69–77 (1996).
53. Miller, D. D-Squame adhesive discs. In: *Bioengineering of the skin: skin surface imaging and analysis.* Wilhelm, K.P., Elsner, P., Berardesca, E., and Maibach, H.I. (Eds.). CRC Press, Boca Raton, pp. 39–46 (1997).
54. Afdel, K., Banouni, M., Soler, B., Petit, L., and Adhoute, H. Caractérisation de la rugosité tactile par l'analyse des textures des images de desquamation. *Nouv. Dermatol.* **18**, 328–330 (1999).
55. Henn, U., Surber, C., Schweitzer, A., and Bieli, E. D-Squame® adhesive tapes for standardized stratum corneum stripping. In: *Prediction of percutaneous penetration.* Brain, K.R., James, V.J., and Walters, K.A. (Eds.). STS Publishing, Cardiff, pp. 477–481 (1993).
56. Flamand, N., Justine, P., Bernaud, F., Rougier, A., and Gaetani, Q. *In vivo* distribution of free long-chain sphingoid bases in the human stratum corneum by high-performance liquid chromatographic analysis of strippings. *J. Chromatogr. B* **656**, 65–71 (1994).
57. Lodén, M., Boström, P., and Kneczke, M. Distribution and keratolytic effect of salicylic acid and urea in human skin. *Skin Pharmacol.* **8**, 173–178 (1995).
58. Rogers, J., Harding, C., Mayo, A., Banks, J., and Rawlings, A. Stratum corneum lipids: the effect of ageing and the seasons. *Arch. Dermatol. Res.* **288**, 765–770 (1996).
59. Dreher, F., Arens, A., Hostynek, J.J., Mudumba, S., Ademola, J., and Maibach, H.I. Colorimetric method for quantifying human stratum corneum removed by adhesive-tape-stripping. *Acta Derm. Venereol.* **78**, 186–189 (1998).
60. Piérard-Franchimont, C., Arrese, J.E., and Piérard, G.E. Immunohistochemical aspects of the link between *Malassezia ovalis* and seborrheic dermatitis. *J. Eur. Acad. Dermatol. Venereol.* **4**, 14–19 (1995).
61. Piérard, G.E. and Piérard-Franchimont, C. Squamometry in acute photodamage. *Skin Res. Technol.* **1**, 137–139 (1995).
62. Goffin, V., Henry, F., Piérard-Franchimont, C., and Piérard, G.E. Topical retinol and the stratum corneum response to an environmental threat. *Skin Pharmacol.* **10**, 85–89 (1997).
63. Piérard, G.E. and Piérard-Franchimont, C. Dihydroxyacetone test as a substitute for the dansyl chloride test. *Dermatology* **186**, 133–137 (1993).
64. Piérard, G.E., Goffin, V., and Piérard-Franchimont, C. Squamometry and corneosurfametry in rating interactions of cleansing products with stratum corneum. *J. Soc. Cosmet. Chem.* **45**, 269–277 (1994).
65. Paye, M. and Morrison, B.M. Non-visible skin irritation. Proceedings of the 4th world surfactant congress, Barcelona **3**, 42–51 (1996).
66. Goffin, V., Piérard, G.E., Henry, F., Letawe, C., and Maibach, H. Sodium hypochlorite, bleaching agents and the stratum corneum. *Ecotoxicol. Environ. Saf.* **37**, 199–202 (1997).
67. Charbonnier, V., Morrison, B.M., Paye, M., and Maibach, H.I. Open application assay in investigation of subclinical irritant dermatitis induced by sodium lauryl sulfate (SLS) in man: advantage of squamometry. *Skin Res. Technol.* **4**, 244–250 (1998).
68. Paye, M. and Cartiaux, Y. Squamometry: a tool to move from exaggerated to more and more realistic application conditions for comparing human skin compatibility of surfactant-based products. *Int. J. Cosmet. Sci.* **21**, 59–68 (1999).

69. Paye, M., Gomes, G., Zerweg, Ch., Piérard, G.E., and Grove, G.G. A hand immersion test under laboratory-controlled usage conditions: a need for sensitive and controlled assessment methods. *Contact Dermatitis* 40, 133–138 (1999).
70. Paye, M., Cartiaux, Y., Goffin, V., and Piérard, G.E. Hand and forearm skin: comparison of their respective responsiveness to sufactants. *Skin Res. Technol.* **7**, 78–83 (2001).
71. Shimizu, T. and Maibach, H.I. Squamometry: an evaluation method for a barrier protectant (tannic acid). *Contact Dermatitis* **40**, 189–191 (1999).

37 Methods for Testing Stratum Corneum Barrier Properties

Ludger Kolbe and Soeren Jaspers

CONTENTS

37.1 INTRODUCTION

Moisturizers are marketed as products that combat the signs of dry skin by delivering moisture to the skin. They are appreciated by the consumer for making the skin smooth and pliable. Since the discovery of Blank[1] that water has a plasticizing effect on the stratum corneum (SC), the main goal of formulators was to develop moisturizers that deliver more moisture. However, enhancement of hydration, softness, and smoothness are short-lived effects, lasting only for hours. Therefore, moisturizers need to be reapplied frequently. In short-term *in vivo* studies the improvement of skin mechanics or plasticity[2] and hydration parameters[3] have been analyzed following a single application of a moisturizer.

The traditional view of moisturizers is that they increase the moisture content of the skin by forming an occlusive, hydrophobic film on the surface of the SC, thereby trapping moisture in the underlying tissue. However, restoration of barrier function after disruption can be accelerated by the application of lipids, like free fatty acids, cholesterol, and nonphysiological lipids like the complex hydrocarbon mixture petrolatum.[4] Barrier repair can be measured within an hour after barrier disruption and leads to normalization of barrier function within hours to days, depending on the initial insult.

Consumers use moisturizers frequently, especially women use them often on twice daily basis for years or even decades. Therefore it is appropriate to ask for the consequences of this life-long treatment on the barrier function of the skin. Fortunately we do not see adverse reactions after

long-term use of moisturizers. Individuals identifying themselves as having sensitive skin will change their cosmetics as soon as they perceive itching, tingling, or other adverse effects. However there are positive consequences of moisturizers on barrier properties, evident only after long-term use, that is, twice daily application for several weeks. Such an improvement of barrier function occurs even in normal skin,[5] but skin conditions with impaired barrier function, like winter xerosis, represent an excellent model for studying barrier improvement by moisturizers. Moisturizers are well known for relieving the symptoms of dry, scaly, and itchy skin. The efficacy of moisturizers for the treatment of dry skin of the lower leg is usually assessed by the regression method,[6] estimating the time course for reduction of scaliness and reappearance after treatment. However, this method gives little information on barrier properties. Experience has taught us that products that are equivalent in removing scales may have different effects on the integrity of the horny layer.

The SC is not only a barrier preventing excessive diffusional water loss, it is also a mechanical and permeability barrier reducing the absorption of substances from the environment. All these barrier properties can be tested in order to gain a complete picture of the state of the horny layer.

37.2 TECHNIQUES

Over the years a panel of methods has been published for testing different properties of the SC barrier (Table 37.1). The combination of tests, employing the application of chemical probes to the skin and the measurement of skin responses with new bioengineering read-out systems allows to measure changes in barrier properties with high sensitivity and reliability. A subjective selection of skin barrier tests will be discussed further in this chapter.

37.2.1 Transepidermal Water Loss

Measurement of the transepidermal water loss (TEWL) is the standard method to determine SC barrier status. A disturbed skin barrier is characterized by high TEWL.[7] The measurement of the TEWL is based on the diffusion principle in an open chamber. The density gradient is measured

TABLE 37.1
Selection of Tests for the Investigation of Skin Barrier Function

Tests involving chemical probes
- Ammonium hydroxide blister test[30]
- Chloroform:methanol burning test[30]
- Dimethyl sulfoxide (DMSO) test[21]
- Erythema after sodium lauryl sulfate provocation assay[26]
- Lactic acid sting test[30]
- Nicotinate test[30]
- Repetitive irritation test[17]
- Sodium hydroxide erosion assay[11]

Bioengineering methods to measure skin responses to chemical probes
- Chromametry[30]
- Evaporimetry[30]
- Hydration measurements with various electrical devices[30]
- Laser Doppler imaging[30]
- Transepidermal water loss (TEWL)

indirectly by two pairs of sensors (temperature and relative humidity) inside a hollow cylinder and is analyzed by a microprocessor, integrated in the handle.

The TEWL measurement has been a valuable tool in barrier repair experiments where repair kinetics were followed by TEWL. However, there are some problems with this method when the efficacy of moisturizers is to be evaluated. Some ingredients of moisturizers like petrolatum are shown to be occlusive.[8] Therefore TEWL readings might reflect the occlusive effect of the cream, rather than an improved, less permeable SC. Measurements performed shortly after application of the moisturizer may represent surface water loss of residual emulsion water instead of TEWL. The TEWL measurements must be performed in an environmental chamber with constant temperature and humidity after an appropriate rest period in order to minimize the contribution of sweat gland activity.[9]

Recently, there was some significant improvement in the engineering of TEWL — Probes (Tewameter TM 300, Courage & Kazaka, Cologne, Germany). Preheating of the probe and usage of new integrated electronic sensors resulted in a significantly reduced measurement period and a considerably more stable signal. As a result of the new assembly the handle is efficiently thermally insulated against the electronics. This new engineering, linked with an accurate and skillful operation and used under constant ambient conditions leads to very significant results.

Newly, so called close chamber instruments become available. The AquaFlux device (Biox Systems Ltd, London, UK), for example, is sealed with a condenser that is maintained at a temperature of −13°C using a Peltier cooler.[10] This leads to significantly higher TEWL values compared to conventional open systems. The possible benefits and fields of application of these instruments remain to be examined in the near future.

37.2.2 Sodium Hydroxide Erosion Assay

This assay is a recently published modification of the alkali resistance test.[11] Sodium hydroxide (NaOH) is strongly keratolytic and rapidly introduces structural defects in the horny layer. Burckhardt introduced the alkali resistance test in 1947.[12] His goal was to develop a procedure that would enable the identification of individuals at increased risk of chemical injury to skin. In some European centers the alkali resistance test subsequently became an accepted screening procedure, in the United States the test never caught on. Researchers after Burckhardt largely found the alkali resistance test to be unreliable and irreproducible.[13,14] Attempts to improve the method failed[15] and therefore the alkali resistance test fell into disuse. However, NaOH has been used as an irritant in other tests.[16,17]

Burckhardt's original test involved application of a drop of 0.5 *N* NaOH under each of three glass blocks for periods of 10 min. The end-point was the time required for ten small vesicles and erosions to develop.

In an attempt to strengthen the reliability and sensitivity of Burckhardts method the procedure was changed substantially and became the erosion assay: Fifteen microliters of 1.0 *M* NaOH were applied to the test site and covered immediately by a circular plastic disc 1.3 cm in diameter to achieve uniform distribution. After one minute the solution was wiped off with a facial tissue; the test site was then gently rubbed with a cotton swab soaked in a solution of blue food coloring. Erosions stain deep blue. If no erosions developed after the first minute fresh solution was reapplied in a series of one minute intervals. Precautions have been introduced in order to avoid severe alkali necrosis, an occasional adverse effect of the original alkali resistance test, by recommending a less ambiguous end-point for the sodium hydroxide erosion assay. The appearance of the first erosion(s) was found to be both safer and more reliable than counting multiple erosions as Burckhardt did. The time in minutes required for the development of the first erosion is recorded as the erosion time. The usual size of an erosion is about 0.5 mm. Exposed sites were allowed to heal spontaneously without treatment or bandaging.

37.2.3 Dimethyl Sulphoxide (DMSO) Whealing Test

The use of Dimethyl sulphoxide (DMSO) in dermatology has a long history.[18,19] In concentrations up to 50% DMSO is used for topical treatment of amyloidosis,[20] but in concentrations of 90% and higher it is known to induce whealing and a flare reaction. The procedure described here is a modification of that described by Frosch et al.[21] for the identification of individuals with sensitive skin. The DMSO reaction can be assessed clinically or measured with the laser Doppler flowmeter.[22] The recent development of laser Doppler imagers[28] make it possible to measure the DMSO induced increase in blood flow with unparalleled accuracy. To elicit the reaction 15 μl of 90%, 95%, and 100% DMSO were applied to three adjacent sites and immediately covered with a circular plastic disc, 1.3 cm in diameter, spreading the solution evenly over the skin. The discs were removed after 15 min and the surface was wiped with tissue paper. Readings were made 5 min later (approximately the peak response) clinically according to the following scale: 0 = no reaction, 1 = discrete, follicular wheals, 2 = flat wheals, with partial convergence, 3 = confluent solid wheal.

The DMSO induced increased blood flow was measured using the moorLDI laser Doppler imager (Moor Instruments Ltd, Devon, UK). The instrument scans a low power laser beam in a raster pattern over the skin. Moving blood in the microvasculature causes a Doppler shift, which is processed to build up a color-coded image of cutaneous blood flow. The mean and standard deviation of the blood perfusion units in a region of interest was calculated.

37.2.4 Sodium Lauryl Sulfate (SLS) Irritation

The SLS is frequently used to induce experimental irritant dermatitis. The reaction is characterized by erythema, increased TEWL, and scaliness. Susceptibility to SLS irritation can be used as an assay for the prevention of irritant reaction by moisturizers.[23–25]

A vast number of different protocols for the induction of SLS irritancy can be found in the literature.[26] Concentrations spanning several order of magnitude have been used for single or multiple applications with exposure times ranging from minutes to days. In order to show the efficacy of moisturizers to prevent SLS irritation low concentrations should be used. With higher concentrations the individual differences between subjects tend to increase. The prevention of irritant dermatitis from a strong insult with high concentrations of SLS is probably beyond the capacity of bland moisturizers and more the domain of barrier creams.

37.3 DATA

37.3.1 Xerotic Leg Skin

37.3.1.1 Erosion Assay

On normal skin NaOH erosions first develop at hair follicles and orifices of sweat gland ducts, usually after 4 to 5 min. They are small and circular in appearance. However, erosions on xerotic leg skin look different, they often develop along cracks in the SC, revealing the weak spots of xerotic skin. Erosion times are significantly reduced, on severely dry skin erosions develop within the first minute.

In a small test group of only five subjects erosion times of the dry skin of the calves were determined before and after four weeks of treatment with either a water-in-oil cream or a 12% lactic acid lotion (Figure 37.1). The assay was performed on four adjacent spots per leg. Spot-to-spot variation was on average less than one minute. In the majority of subjects erosions formed within two minutes. Erosion times on the legs treated with the water-in-oil cream were strongly increased, whereas the opposite legs, treated with the lactic acid lotion showed only slight improvement. This is not unexpected since alpha-hydroxy acids are known for their keratolytic activity which will weaken the

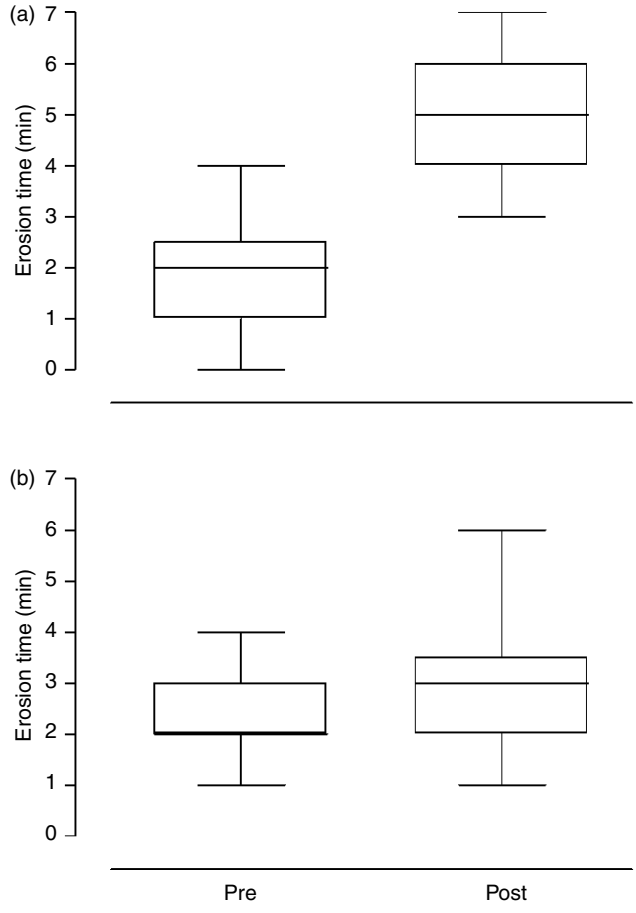

FIGURE 37.1 Effect of moisturizer treatment on sodium hydroxide erosion time of xerotic leg skin. (a) Marked improvement of alkali resistance after treatment with a water-in-oil emulsion for four weeks ($p < .0001$). (b) Slight but significant improvement after treatment with a lotion containing 12% lactic acid ($p < .01$). Erosion assays were performed before and after treatment on four adjacent spots per leg. Data shown as box plots. Statistical analysis was performed with the paired t-test.

horny layer. The improvement by moisturizers is probably not due to a protective moisturizer film in the outer layers of the SC, since there was no significant increase of erosion times after only a few applications. Erosion times gradually increase over the treatment period.

37.3.1.2 DMSO Reaction

The DMSO readily penetrates the SC of dry leg skin and induces an inflammatory response, characterized by a strong whealing reaction and increased cutaneous blood flow. Determination of blood perfusion units with a laser Doppler imager showed a dose dependent response (Figure 37.2). However, after six weeks of treatment with a water-in-oil emulsion the reaction was markedly reduced, only 1 out of 14 subjects responded slightly to 90% DMSO. Laser Doppler imaging is a reliable and sensitive method and the obtained images can be stored in the computer for further investigation (Figure 37.3). Nevertheless, a trained examiner will also get good results with clinical grading.

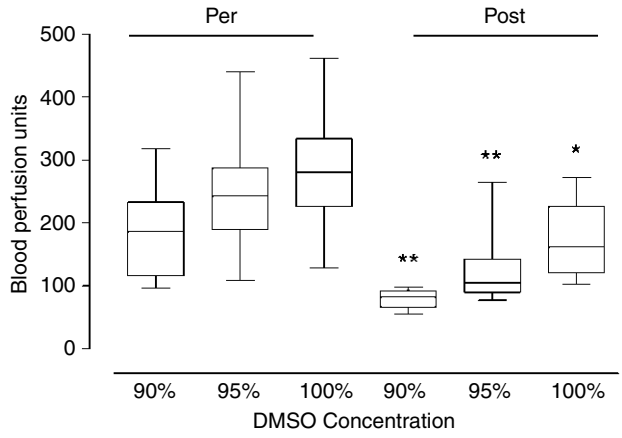

FIGURE 37.2 DMSO response of xerotic leg skin. (a) Dose dependent increase of DMSO induced cutaneous blood flow before on untreated skin. (b) After twice daily application of a water-in-oil emulsion for six weeks the response was markedly reduced. DMSO induced blood flow was measured with a laser Doppler imager. Data expressed as blood perfusion units. Statistical significance was determined using the paired *t*-test ($^{*}p > .05$, $^{**}p < .01$).

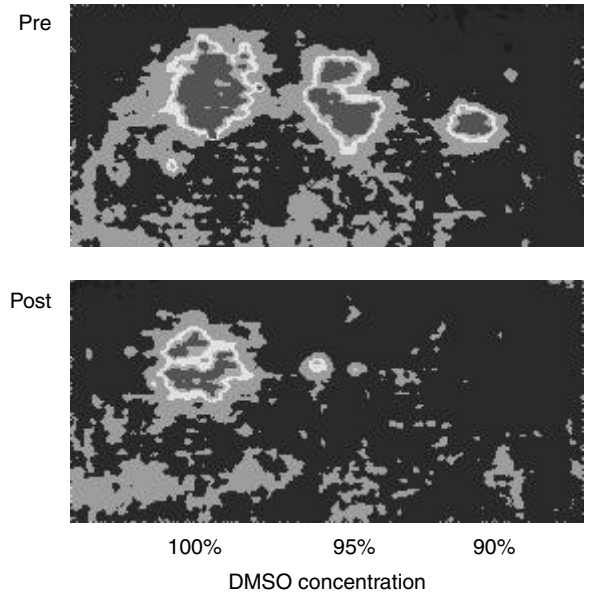

FIGURE 37.3 The laser Doppler imager generates a pseudo-colored image of blood flow. In this gray scale image the various shader of grey represent regions with increased blood flow. The dark background represents the baseline blood flow. At baseline DMSO evoked a dose dependent increase in cutaneous blood flow. After 6 weeks of treatment with a water-in-oil emulsion the DMSO response was markedly reduced.

37.3.2 Normal Volar Forearm Skin

At first glance it might seem a bizarre idea to improve a normal skin barrier. Can there be a better barrier than the normal barrier? However, we know there is considerable individual variation. Marie Lodén[5] demonstrated that indeed some moisturizers improve barrier properties of normal skin. Sensitive skin is a multidimensional phenomenon but at least in part a weak SC barrier contributes

to the problem.[27,28] Moisturizers formulated for individuals with sensitive skin accordingly should be aimed at improving the barrier function.

37.3.2.1 SLS Irritation

Susceptibility to SLS irritancy was analyzed before and after treatment with a water-in-oil emulsion for four weeks. Patches with SLS ranging from 0.025 to 0.1% were applied to the volar forearm of nine subjects for 24 h. At 48 h SLS induced erythema was measured with the Minolta Chromameter and the laser Doppler imager from Moor Instruments (Figure 37.4). The laser Doppler imager revealed a significant lower vascular response to SLS at all concentrations after treatment. There was also a decrease of background blood flow, measured at adjacent unchallenged skin. This probably reflects a subclinical response of the supplying and draining blood vessels in larger area surrounding the exposure sites, which was also reduced by the moisturizer. No significant differences were found

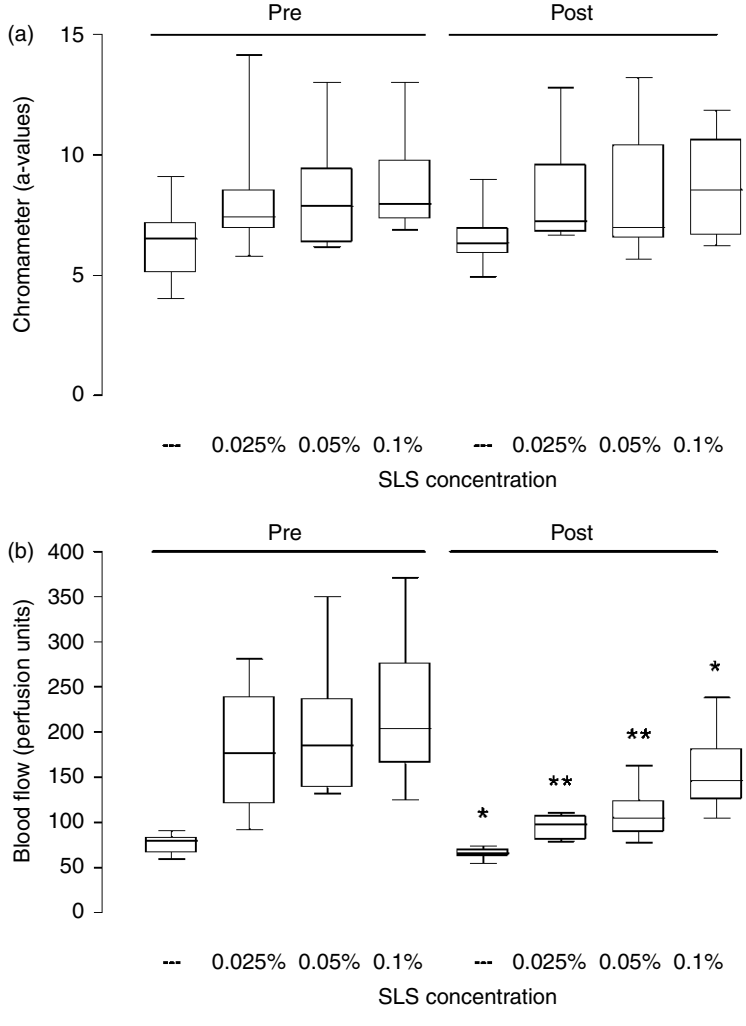

FIGURE 37.4 SLS irritation and moisturizer treatment. (a) No significant differences in skin color (a-values) were detectable with the Chromameter. (b) The same sites as in (a) scanned with the laser Doppler imager showed marked reduction of erythema. Volar forearms were treated with a water-in-oil emulsion twice daily for four weeks. Statistical significance was determined using the paired *t*-test ($^*p > .05$, $^{**}p < .01$).

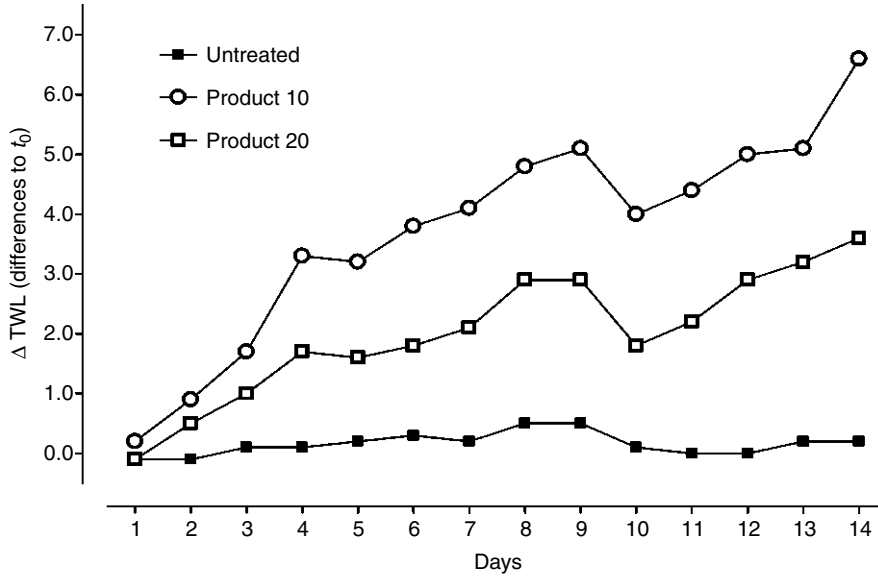

FIGURE 37.5 TEWL after harsh washing. Washing of forearm skin leads to barrier damage characterized by increased TEWL. The improved TEWL probes allow to discriminate between products with different content of surfactants. Barrier damage was more pronounced on areas treated with higher concentration of surfactants. Repair is fast, stop of treatment for one day (day 4 and 9) leads to reduced TEWL readings the next day.

with the Chromameter but DMSO test and erosion assay demonstrated a significant improvement of barrier function (data not shown).

37.3.2.2 TEWL

In this study a washing test on the inner forearm was performed over a period of 14 days. Two products (10 and 20) containing different amounts of surfactants were used for this test (Figure 37.5). TEWL was determined daily using the improved TEWL probe (Tewameter TM 300). It gradually increased over time showing a stronger increase with higher content of surfactant (product 20). The decrease in TEWL between day 4 and 5 and day 9 and 10 is a result of a stop of treatment between these measurements, respectively. The trend of the untreated area is considerably stable, also the run of the curves and the discrimination of the treated sites are very clear and meaningful.

37.4 CONCLUSION

The TEWL measurement is an indispensable method to evaluate barrier function. Particularly the new improved TEWL probes will bring us more reliable data than ever before. But not always is improvement of barrier properties reflected by reduced TEWL. Especially on normal skin where TEWL is already low the slight additional reduction after moisturizer treatment might not reach statistical significance. Furthermore reduction of TEWL might reflect the occlusivity of the product rather than a less permeable horny layer. Hence, additional information must be obtained with other methods. Product occlusivity might interfere with other techniques as well, but by using a set of different methods the problem can be minimized. One can argue that it makes no difference whether a moisturizer is protective because of an improved SC or because the product forms an occlusive layer. However, these are two different mechanisms, which should be investigated separately. The evaluation of protective product layers is a valid approach for the evaluation of barrier creams,

in short-term studies,[29,30] but in long-term studies with moisturizers improvement of the structural integrity of the horny layer itself should be evaluated.

The DMSO, NaOH, and SLS are useful chemical probes because of the different properties of the substances and the different mechanisms by which they induce irritation. Due to its amphiphilic nature DMSO rapidly penetrates the SC and induces whealing and erythema. The aqueous NaOH solution dissolves the keratin layers, thereby introducing structural defects in the horny layer. The mechanism by which an aqueous solution of SLS induces irritation is still unknown, but SLS penetrates the barrier like DMSO, although much slower. Combined, tests with these and other chemical probes provide valuable information on the barrier properties of the SC.

REFERENCES

1. Blank, I.H., Factors which influence the water content of the stratum corneum, *J. Invest. Dermatol.*, 18, 433, 1952.
2. Jemec, G.B.E. and Wulf, H.C., The plasticizing effect of moisturisers on human skin *in vivo*: a measure of moisturising potency? *Skin Res. Technol.*, 4, 88, 1998.
3. Blichmann, C.W., Serup, J., and Winther, A., Effect of single application of a moisturizer: evaporation of emulsion water, skin surface temperature, electrical conductance, electrical capacitance, and skin surface (emulsion) lipids, *Acta Derm. Venereol.*, 69, 327, 1989.
4. Man, M.-Q., Brown, B.E., Wu-Pong, S., Feingold, K.R., and Elias, P.M., Exogenous nonphysiologic versus physiologic lipids: divergent mechanisms for correction of permeability barrier dysfunction, *Arch. Dermatol.*, 129, 809, 1995.
5. Lodén, M., Urea-containing moisturizers influence barrier properties of normal skin, *Arch. Dermatol. Res.*, 288, 103, 1996.
6. Kligman, A.M., Regression method for assessing the efficacy of moisturizers, *Cosmet. Toiletries*, 93, 27, 1978.
7. Shahidullah, M., Raffle, E.J., Rimmer, A.R., and Frain-Bell, W., Transepidermal water loss in patients with dermatitis, *Br. J. Dermatol.*, 81, 722, 1969.
8. Rietschel, R.L., A method to evaluate skin moisturizers *in vivo*, *J. Invest. Dermatol.*, 70, 152, 1978.
9. Pinnagoda, J., Tupker, R.A., Agner, T., and Serup, J., Guidelines for transepidermal water loss (TEWL) measurement, *Contact Dermatitis*, 22, 164, 1990.
10. Imhof, R.E., Berg, E.P., Chilcott, R.P., Ciortea, L.I., and Pascut, F.C. New instrument for measuring water vapour flux density from arbitrary surfaces. *IFSCC Magazine*, 5, 297, 2002.
11. Kolbe, L., Kligman, A.M., and Stoudemayer, T., The sodium hydroxide erosion assay: a revision of the alkali resistance test, *Arch. Dermatol. Res.*, 290, 382, 1998.
12. Burckhardt, W., Neuere Untersuchungen über die Alkaliempfindlichkeit der Haut, *Dermatologica*, 94, 73, 1947.
13. Björnberg, A., Low alkali resistance and slow alkali neutralization of the eczematous subject, *Dematologica*, 194, 90, 1974.
14. Schnyder, U.W., Gloor, M., and Taugner, M., Über die sozialmedizinische Bedeutung von Alkaliresistenz, Alkalineutralisation und Hautoberflächenlipide bei Neurodermitis atopica und Ichtyosis vulgaris, *Berufsdermatosen*, 25, 101, 1977.
15. Ummenhofer, B., Zur Methodik der Alkaliresistenzprüfung, *Dermatosen*, 28, 104, 1980.
16. Wilhelm, K.P., Pasche, F., Surber, C., and Maibach, H.I., Sodium hydroxide- duced subclinical irritation, *Acta Derm. Venereol.*, 70, 463, 1990.
17. Frosch, P.J. and Kurte, A., Efficacy of skin barrier creams (IV). The repetitive irritation test (RIT) with a set of 4 standard irritants, *Contact Dermatitis*, 31, 161, 1994.
18. Kligman, A.M., Topical pharmacology and toxicology of dimethyl sulphoxide — Part 1, *JAMA*, 193, 796, 1965.
19. Kligman, A.M., Topical pharmacology and toxicology of dimethyl sulphoxide — Part 2, *JAMA*, 193, 923, 1965.
20. Özkaya-Bayazit, E., Baykal, C., and Kavak, A., Lokale DMSO-Behandlung der makulösen und papulösen Amyloidose, *Hautarzt*, 48, 31, 1997.

21. Frosch, P.J., Duncan S., and Kligman, A.M., Cutaneous biometrics. I. The response of human skin to dimethyl sulphoxide, *Br. J. Dermatol.*, 102, 263, 1980.
22. Agner, T. and Serup, J., Quantification of the DMSO-response: A test for assessment of sensitive skin, *Clin. Exp. Dermatol.*, 14, 214, 1989.
23. Lodén, M. and Andersson, A.-C., Effect of topically applied lipids on surfactant-irritated skin, *Br. J. Dermatol.*, 134, 215, 1996.
24. Ramsing, D.W. and Agner, T., Preventive and therapeutic effects of a moisturizer, *Acta Derm. Venereol.*, 77, 335, 1997.
25. Lodén, M., Barrier recovery and influence of irritant stimuli in skin treated with a moisturizing cream, *Contact Dermatitis*, 36, 256, 1997.
26. Tupker, R.A., Willis, C., Berardesca, E., Lee, C.H., Fartasch, M., Agner, T., and Serup, J., Guidelines on sodium lauryl sulfate (SLS) exposure tests. A report from the Standardization Group of the European Society of Contact Dermatitis, *Contact Dermatitis*, 37, 53, 1997.
27. Draelos, Z.D., Sensitive skin: Perception, evaluation, and treatment, *Am. J. Contact Dermatitis*, 8, 67, 1997.
28. Morizot, F., Le Fur, I., and Tschachler, E., Sensitive skin, definitions, prevalence and possible causes, *Cosmet. Toiletries*, 113, 59, 1998.
29. Wigger-Alberti, W. and Elsner, P., Petrolatum prevents irritation in a human cumulative exposure model *in vivo*, *Dermatology*, 194, 247, 1997.
30. Frosch, P.J., Kurte, A., and Pilz, B., Efficacy of skin barrier creams (III). The repetitive irritation test (RIT) in humans, *Contact Dermatitis*, 29, 113, 1993.