Functional Analyses of the Eyelid Skin Constituting the Most
Soft and Smooth Area on the Face: Contribution of its Remarkably
Large Superficial Corneocytes to Effective Water-holding Capacity
of the Stratum Corneum

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Functional analyses of the eyelid skin constituting the most soft and smooth area on the face: contribution of its remarkably large superficial corneocytes to effective water-holding capacity of the stratum corneum

Walaion Pratchyapruit¹, Katsuko Kikuchi¹, Pimonpun Gritiyarangasan¹, Setsuya Aiba² and Hachiro Tagami²

¹Institute of Dermatology, Bangkok, Thailand and ²Department of Dermatology, Tohoku University School of Medicine, Japan

Background/purpose: The eyelid constitutes a unique area on the face because of its soft, smooth and thin skin distinct from that of other facial portions. Its softness facilitates their easy compliance to blinking movement, which is indispensable to protect the wet surface of the eyeball. Moreover, the skin of the eyelid does not show any prominent follicular orifices or an oily appearance even in adults. Despite such uniqueness, its bioophysical characteristics have remained unclear as compared with other facial skin.

Methods: We conducted non-invasive instrumental measurements on the skin of the upper eyelid of 22 healthy Japanese adults in comparison with those of the adjacent facial skin, i.e., the cheek and nose. Additionally, we examined 10 adult patients with atopic dermatitis (AD) whose facial skin remained clinically free from skin lesions for at least 2 months.

Results: The eyelid skin showed high transepidermal water loss like other facial skin. Its skin surface hydration state was as high as that of the adjacent skin, despite the fact that the eyelid skin revealed extremely low amounts of surface lipids unlike its neighboring skin regions. However, in contrast to small corneocytes found in other facial areas, the corneocytes of the eyelid skin displayed a significantly larger surface size, suggesting that slow turnover of its stratum corneum (SC) takes place to allow sufficient maturation of the corneocytes, enabling them to exert efficient water-binding capacity. Its pH tended to be higher than that of the adjacent skin. Its superficial blood flow was significantly higher than that of others, although skin color assessment showed lower color values for redness than those of the other facial skin sites. In clinically non-lesional skin of adult AD patients, we found increased blood circulation and a higher parameter for redness, suggesting the presence of invasive mild inflammation in the dermis even long after subsidence of visible inflammatory changes.

Conclusion: Our present biophysical findings suggest that, although the eyelid skin is poor in surface lipids, its extra-ordinary large superficial corneocytes play an important role in maintaining sufficient hydration state of its skin surface to keep it soft and flexible, enabling its good compliance with the blinking movement.

Key words: atopic dermatitis – corneocytes – hydration – non-invasive instrumental measurements – stratum corneum

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The skin serves as a vital protective barrier between the living tissues and environment. This barrier function actually depends on the presence of thin stratum corneum (SC) on its surface. Moreover, healthy skin surface remains soft and smooth to comply readily with any kinds of bodily movement by its water-holding capacity of the SC without producing any scales, fissures or cracks that are observed in various types of pathologic skin. The skin is not uniform all over the body but has great divergences in its functional properties as well as in its structural components according to anatomical location. In contrast to the palmoplantar skin, the facial skin is remarkably smooth and oily, being packed with small skin areas that show distinct functional properties from each other (1, 2). Among them, the eyelid constitutes a unique area that must comply smoothly with the quick blinking movement to maintain the wet surface of the eyeball. The eyelid skin is also unique clinically in that, despite its anatomical closeness to the cheek and forehead, they are seldom affected by the dermatoses such as acne vulgaris and
seborrheic dermatitis whose development is because of the presence of actively functioning sebaceous glands or by melasma that depends on the localized presence of actively functioning melanocytes. In contrast, it is one of the predilection sites for contact dermatitis and atopic dermatitis (AD) that develop under the influence of various environmental insults probably because of its thin SC even compared to that of adjacent facial portions (1). Based on these characteristics, it is reasonable to presume that the eyelid skin may display functional properties distinct from those of the neighboring facial skin (3, 4). However, there has been little information about them. In the present study we conducted non-invasive, biophysical measurements on the upper eyelid skin to characterize its functional properties in adults by comparing it with the neighboring cheek and nose skin.

Subjects and Methods

Twenty-two healthy Japanese adults consisting of seven males and 15 females with ages ranging from 20 to 40 years (mean 27 ± 6 years) participated in this study after giving informed consent. All the subjects washed their faces with a liquid soap 20 min before the start of instrumental measurements which were performed in a temperature- and humidity-controlled room (21 °C and 50% relative humidity). An acclimatization period of at least 15 min was placed before the start of the measurements.

In addition, 10 adult AD patients, four males and six females aged between 20 and 32 years (mean 25 ± 4.3 years), participated in the study. They were unaccompanied by any visible skin lesions on their faces for at least 2 months without application of any topical medications to their faces for prior 2 weeks.

From July to August 2004, we conducted the instrumental measurements on their upper eyelids that were kept in an eye-shut position, tip of the nose and mid-porition of the cheeks as reported before (2). After taking a photograph, we measured skin color, TEWL, superficial skin blood flow, skin surface lipids, skin pH and corneocyte surface area. TEWL was measured with a portable device using a closed chamber system (Model H 4300-S; Nikkiso-YSI Co. Ltd, Tokyo, Japan). The corresponding value of an open chamber system was obtained from computing with the following equation: 1.42 × (the recorded value with the present instrument) + 1.892 (5, 6).

High-frequency conductance, a parameter for the hydration state of the superficial portion of the SC, was determined with a 3.5-MHz high-frequency impedance meter (Skincon-200, IBF, Hamamatsu, Japan) (7–9). The upper eyelid was divided into five parts, and the measuring probe was applied first to the skin next to the inner canthus, then moved laterally along the upper eyelid margin toward the fifth spot. From these five measurements, two outlying values were cut-off and the middle three values were used to obtain the average.

The size of superficial corneocytes was estimated by tape stripping with Subada Checker® (Kanebo, Tokyo, Japan) after staining with hematoxylin and eosin. Photograph of corneocytes were taken under a light microscope, being processed by a computer to collect the surface areas of 50 corneocytes from each slide to perform image analysis (IPLab® Scatalytics Inc., Fairfax, VA, USA) (2).

Skin surface lipids were assessed with a Sebumeter® SM810 (Courage+Khazaka, Köln, Germany) (10) on the skin at the temporal side of the eyelid adjacent to the orbital rim in order to apply sufficient force on the underlying skin without causing discomfort to the eyebrow.

The skin surface pH was measured with a pH meter® (pH 900, Courage+Khazaka) which provided the data in μg/cm² (11).

Blood flow in the sub-papillary vascular plexus was evaluated with a Laser-Doppler flowmeter (ALFZ1N®, Advance Co. Ltd, Tokyo, Japan) (12). We obtained the mean value from five selected stable values.

The light reflected perpendicular to the skin was collected for a tri-stimulus color analysis at 450, 560, and 600 nm, using the L*a*b* system by a colorimeter (Minolta CR 300 Chromameter, Osaka, Japan) where L*, a*, b* are attributed to the skin surface reflectance, red vs. green scale and yellow vs. blue scale, respectively (13).

Statistical analysis
To compare the differences between the male and female and between the healthy individuals and AD patients, we used independent t-test. One-way ANOVA (Bonferroni’s) was used to compare the means of measured data prepared from three
different anatomical sites. The level of significance was set at 0.05.

Results

Measurements in healthy adults

All the mean values ± standard deviations (SD) obtained from the biophysical measurements of the eyelid skin in healthy adults are listed in Table 1, together with those of the tip of the nose and cheek.

TEWL

The TEWL values measured on the upper eyelids tended to be higher than those of the cheek but lower than those of the nose. However, there were no statistically significant differences between them.

TEWL values of the eyelid showed a significantly positive correlation with those of the cheeks ($r = 0.716$, $P = 0.001$).

High-frequency conductance

High-frequency conductance, the parameter for the skin surface hydration state, tended to be higher on the skin of the eyelid than those on the nose and cheeks, but there was no statistically significant difference.

Size of superficial corneocytes

The size of superficial corneocytes from the eyelid skin with the mean value of 632.3 ± 64.9 $\mu m^2$ was exceedingly larger than that of the nose (523.3 ± 57.2 $\mu m^2$) or that of the cheeks (591.7 ± 72.0 $\mu m^2$) (Table 1, Fig. 1a–c). They showed a negative correlation with TEWL values, which was found to be statistically significant ($r = -0.502$, $P < 0.05$) (Fig. 2).
Skin surface lipids
Extremely high amounts of skin surface lipids were found on the nasal tip. In contrast, remarkably low amounts, less than one-tenth of the former in average, were revealed on the eyelid. There was a statistically significant, positive correlation between the amounts of skin surface lipids and skin surface hydration state of the eyelid ($r = 0.515$, $P < 0.05$) (Fig. 3).

pH
The eyelid skin surface tended to show higher pH values than the nose and cheeks. The pH values revealed a clear inverse correlation with high-frequency conductance values on the eyelids ($r = -0.742$, $P < 0.001$) as noted on other facial portions (Fig. 4).

Skin blood flow
Skin blood flow evaluated with laser doppler flowmeter on the eyelid was significantly higher than that on the nose ($P < 0.001$) or that on the cheek ($P < 0.001$).

Skin color
We found statistically significant lower $L^*$, $a^*$ and $b^*$ values on the eyelids than those on the nose and cheeks.

Differences between male and female subjects
There was no difference between the sexes for the following parameters, i.e. TEWL, high-frequency conductance, cell surface area of corneocytes, skin surface lipids, pH, skin blood flow, and skin color except the $b^*$ value, for which the males showed significantly lower values than the females on the eyelid ($P < 0.05$) (data not shown).

Non-lesional skin of atopic dermatitis patients
The non-lesional eyelid skin of the AD patients displayed mostly similar biophysical findings to those of normal individuals for TEWL, skin surface hydration state, skin surface pH and skin blood flow. Their corneocytes were also larger than those of the nose and the cheeks. However, microscopically their surfaces were less wrinkled and their lateral margins were found to attach more closely to each other than those from the nose and cheeks (data not shown). The AD patients demonstrated significantly lower surface lipid values on the eyelid ($P < 0.05$) and on the cheek ($P < 0.05$, data not shown) and marginally lower values on the nose than the healthy individuals ($P < 0.1$, data not shown). They revealed significantly lower $L^*$ values ($P < 0.01$) but higher $a^*$ values on the eyelid ($P < 0.05$) than the normal individuals (Table 2).

Discussion
The eyelid skin is known to have unique architectural characteristics to carry out its special
function to protect the eyeball (3, 4). As mentioned above, it is essential for them to show extreme softness and flexibility to comply with the quick blinking movement. Including its SC (1), the eyelid skin is one of the thinnest in the body. The peripheral vascular arcade of the upper eyelid lies just above the upper border of the tarsus in the upper eyelids. Thus, they are composed of thin, highly moveable and well-vascularized skin but they are vulnerable to various environmental insults to easily develop various types of dermatitis.

Moreover, although its nasal side shows fine hairs and more sebaceous glands than the temporal side (3), their activities are extremely lower than those on other facial locations as clearly demonstrated in our present study. The eyelid skin neither displays a shiny, oily appearance nor prominent follicular orifices even in adults. Under ultraviolet A irradiation, orange-red fluorescence is demonstrable at the orifices of sebaceous follicles of the adult facial skin because of porphyrin production by Propionibacterium acne, which depends upon the sebum secretion (14). However, this fluorescence was also extremely rare on the eyelid skin as compared with the neighboring facial skin (data not shown). Although the meibomian glands are tarsal sebaceous glands, their ducts open only at the lid margin, not onto the skin surface, to produce a superficial lipid layer of the tears (4). Therefore, the lipids measured on the upper eyelid skin are mostly derived from its functionally poor, small sebaceous glands.

The softness of the skin surface depends on the hydration state of the SC (15). Sebum is known to play an important role in increasing the water content of the SC on the scalp and face (16, 17). In fact, we found that the nasal skin showed the highest hydration state among various facial skin regions because of its high sebum secretion as reported before (2). Therefore, it is noteworthy that the SC hydration state of the eyelid was still comparable with or even better than that of other facial portions, despite its extremely low skin surface lipids. From such a viewpoint, the SC of the eyelid is thought to have special functional properties to exert its uniquely high water-holding capacity that effectively compensates the low amounts of skin surface lipids.

Our previous study demonstrated that, similar to the genital skin, the eyelid skin is characterized by its uniquely thin SC (18). Such thin SC is thought to facilitate water supply to the superficial layers of SC from the fully hydrated underlying viable skin tissues as exemplified by the high TEWL values. Most of all, however, we demonstrated the presence of remarkably large-sized corneocytes in the eyelid skin as compared with those on other facial areas in the present study. In general, the sizes of the skin surface corneocytes can be used as a parameter of the SC turnover speed; there is an inverse correlation between them (19). The corneocytes of the facial skin are much smaller than those on other portions of the body, reflecting much quicker SC turnover time on the face than on other bodily locations (20). Therefore, the present findings of extremely large corneocytes on the eyelid skin suggest its much slower turnover time as compared to that of the surrounding facial skin, also reflecting that of the underlying epidermis. When the proliferation and differentiation of the epidermal keratinocytes proceed at a slow and
steady pace as noted in normal skin, they can produce functionally efficient, mature corneocytes in the skin surface. As a result, there develop a sufficiently protective barrier function and efficient water-holding capacity, in contrast to the inflamed skin in which functionally deficient SC is always generated (21, 22). However, the high TEWL values demonstrated on the eyelid in spite of the presence of the exceedingly large corneocytes indicates that they are effective to produce sufficient water-holding capacity for the SC but that they cannot provide sufficiently high barrier function because of its small number of SC cell layers.

The pH value of the skin surface is always mildly acidic under the influence of deamination of filaggrin-derived histidine, phospholipid hydrolysis and sodium proton membrane transporter (23). Our present study revealed that the pH values on the eyelids tended to be higher than those of the neighboring skin. Such an increase in pH is observed on the facial skin of aged individuals who tend to show slow SC turnover time than the young (21, 22). At this point, it is not clear why pH values showed a negative correlation with the skin surface hydration state on the eyelid.

Finally, the present study displayed a richer blood supply in the eyelid skin than in other facial skin at a statistically significant level (P<0.01), which may be related to its unique ability to comply with the blinking movement. Nevertheless, our color assessment revealed lower redness values in the skin in the eyelid skin than in the cheek and nose skin. In a similar fashion, it showed lower L* and b* values for the eyelid skin than others. The significance of these color differences is not clear at this point except for its lesser influence of sun exposure than the other facial portions.

In the present investigation, although we studied clinically non-lesional skin of adult patients with AD, it showed lower lightness and higher redness parameters, suggesting the influence of invisible mild inflammation in the dermis even long after subsidence of clinically visible inflammatory changes.

In conclusion, our present findings demonstrated that, although the skin of the eyelid is covered with low skin surface lipids unlike other facial skin, its thin SC shows high water-holding capacity to produce soft and flexible skin because of its composition of fully maturated corneocytes. This enables the skin of the eyelid to promptly adapt to constant blinking movement to protect the eye.

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