Epidermal Permeability Barrier Recovery Is Delayed in Vitiligo-Involved Sites

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Key Words
Vitiligo · Stratum corneum · Barrier function · Hydration

Abstract
Background/Objectives: Prior studies have demonstrated that both the skin surface pH and epidermal permeability barrier function vary with skin pigmentation types. Although melanin deficiency is the main feature of vitiligo, alterations in cutaneous biophysical properties in vitiligo have not yet been well defined. In the present study, stratum corneum (SC) hydration, the skin surface pH and epidermal permeability barrier function in vitiligo were evaluated. Methods: A total of 30 volunteers with vitiligo comprising 19 males and 11 females aged 13–51 years (mean age: 27.91 ± 2.06 years) were enrolled in this study. The skin surface pH, SC hydration, melanin/erythema index and transepidermal water loss (TEWL) were measured by respective probes connected to a Courage-Khazaka MPA5. SC integrity was determined by measuring the TEWL following each D-Squame application. The barrier recovery rate was assessed at 5 h following barrier disruption by repeated tape stripping. Results: In addition to SC hydration, both melanin and erythema index were significantly lower in vitiligo lesions than in contralateral, nonlesional sites, while no difference in skin surface pH between vitiligo-involved and uninvolved areas was observed. In addition, neither the basal TEWL nor SC integrity in the involved areas differed significantly from that in the uninvolved areas. However, barrier recovery in vitiligo-involved sites was significantly delayed in comparison with uninvolved sites (40.83 ± 5.39% vs. 58.30 ± 4.71%; t = 2.441; p < 0.02). Conclusion: Barrier recovery following tape stripping of the SC is delayed in vitiligo. Therefore, improvement in epidermal permeability barrier function may be an important unrecognized factor to be considered in treating patients with vitiligo.

Introduction
Skin pigmentation is primarily determined by the quantity of melanin [1], while multiple cutaneous functions are associated with skin pigmentation. For example, darker skin is less susceptible to Candida albicans infection [2]. In addition, following sodium lauryl sulfate treatment, darker skin is more sensitive to stinging sensation and displays a lesser increase in transepidermal water loss (TEWL) [3, 4]. Moreover, there is a significant difference in the transcutaneous penetration of nicotine between highly and lightly pigmented skin [5]. Furthermore, the severity of photodamage differs between highly and lightly pigmented skin [6], and the onset of skin aging is delayed in darker skin compared with lighter skin [7]. In addition, lighter skin becomes dryer than darker skin upon sun exposure [8], while the skin electric...
resistance is higher in darker skin, too [9]. However, regarding the epidermal permeability barrier function in darker versus lighter skin, results are inconclusive. Some studies have shown that both basal TEWL and percent increase in TEWL are higher in darker skin following tape stripping [10–12]. In contrast, Singh et al. [13] reported that the basal TEWL was lower in darker skin. However, Reed et al. [14] reported that the basal TEWL in darker skin did not differ from that in lighter skin, but rather that stratum corneum (SC) integrity was significantly lower in lighter skin than in darker skin. Interestingly, barrier recovery is reportedly faster in darker skin as compared with lighter skin [14]. Although the basal skin surface pH is higher in black Africans than in Caucasians [15], it is significantly lower in darker-skinned individuals following tape stripping [12]. Nevertheless, these data all indicate that cutaneous functions vary with pigmentation.

Vitiligo is generally viewed as an autoimmune disorder. In support of this, histological studies have revealed increased cutaneous lymphocyte and macrophage infiltration in vitiligo-involved sites [16, 17], while helper T cells are decreased in patients with vitiligo [18, 19]. Moreover, cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α are increased in both vitiligo-involved skin sites and patients’ serum [20–22]. Accordingly, immunosuppressors such as tacrolimus improve vitiligo [23–25], and imiquimod, an immunostimulator, could induce vitiligo [26–29]. Vitiligo is clinically featured by depigmentation and characterized by melanin deficiency [30]. Although the primary function of melanin is to protect the skin from UV damage [31–33], melanin also plays an important role in regulating multiple cutaneous functions. Firstly, melanin participates in the regulation of cutaneous immunity. For instance, synthetic melanin suppressed the production of TNF, IL-1β, IL-6 and IL-10 by lipopolysaccharide-stimulated monocytes [34]. In normal human monocytes, herbal melanin induces TNF-α, IL-6 and vascular endothelial growth factor (VEGF) mRNA expression [35]. Melanin also exhibits an antiviral activity in human lymphoblastoid cells [36–38]. Secondly, sodium/hydrogen (Na+/H+) exchangers, key regulators of pH [39], are expressed on melanocytes and colocalize with melanosomes; melanosomes are also acidic [40, 41]. Recent studies have demonstrated that the skin surface pH is lower in darker-than in lighter-skinned individuals [42]. Thirdly, alterations in keratinocyte proliferation have been observed in vitiligo. Keratinocytes from involved vitiligo skin have a shorter life span [43], while decreased levels of stem cell factor and increased apoptosis were observed in keratinocytes from vitiligo [44, 45]. Furthermore, the SC is thicker in vitiligo-involved sites than that in uninvolved sites [46]. These noted changes in skin surface pH, cytokine levels, SC thickness, keratinocyte proliferation and cytokine expression all have a significant impact on cutaneous biophysical properties such as epidermal permeability barrier function and SC integrity [14, 47–53]. However, the changes in skin biophysical properties including epidermal permeability barrier function in vitiligo remain largely unknown.

In the present study, the skin surface pH, SC hydration and epidermal permeability barrier function are assessed in vitiligo-involved and contralateral (uninvolved) skin.

### Materials and Methods

#### Subjects

A total of 30 Chinese volunteers with stable vitiligo vulgaris, 19 males and 11 females aged 13–51 years, were enrolled in this study (table 1). Previous studies showed that skin response to UV radiation is influenced by skin pigmentation [54]. To eliminate any UV-induced changes in SC function in vitiligo-involved and uninvolved sites, only patients with vitiligo in non-sun-exposed sites were enrolled in the present study. All subjects were new patients with skin types III or IV (Fitzpatrick classification), and were without any topical or systemic medication for vitiligo. Their vitiligo was diagnosed by a vitiligo specialist at the vitiligo clinic of the Dalian Skin Disease Hospital, PR China. A clinical examination showed no sign of inflammation in both lesions and contralateral uninvolved sites. No skin care products had been applied to the measured sites 24 h prior to the measurements, and the measured sites had not been washed with soaps or surfactants for at least 2 h prior to the study.

#### Measured Sites and Methods

The basal TEWL, skin surface pH, capacitance and melanin/erythema were measured on both vitiligo-involved and contralateral uninvolved sites by respective probes connected to a Courage-Khazaka MPA5 (Courage Khazaka electronic GmbH, Köln, Germany) [55]. The skin surface pH was also measured by a

### Table 1. Characteristics of subjects

<table>
<thead>
<tr>
<th>Number</th>
<th>Age, years</th>
<th>Involved sites, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>11</td>
<td>36.82 ± 3.51</td>
</tr>
<tr>
<td>Male</td>
<td>19</td>
<td>22.79 ± 1.68</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>27.90 ± 2.06</td>
</tr>
</tbody>
</table>

Values denote means ± SEM unless stated otherwise.
Barrier Recovery Is Delayed in Vitiligo

**Results**

**Basal SC Biophysical Properties**

Since the primary clinical feature of vitiligo is skin depigmentation, we first quantified the pigmentation in the vitiligo lesions. As seen in figure 1a, the skin melanin index was significantly lower in vitiligo-involved sites than in uninvolved sites (melanin index: 60.93 ± 4.95 vs. 203.20 ± 11.64; t = 11.24; p < 0.0001). In addition, the erythema index was also significantly lower in vitiligo-involved sites than in uninvolved sites (erythema index: 235.50 ± 19.79 vs. 329.00 ± 13.86; t = 3.872; p < 0.0003). These results demonstrate that both melanin and erythema indexes decreased in vitiligo-involved sites.

We next assessed SC hydration, determined by measuring SC capacitance. As seen in figure 1b, SC hydration was significantly diminished in vitiligo-involved sites as PH905 probe immediately after 5 consecutive D-Squame applications. The barrier recovery rate was assessed at 5 h after barrier perturbation by repeated D-Squame applications [56]. For the SC integrity assay, the TEWL was measured by a TM300 probe after each D-Squame application [51, 57]. All subjects rested at 24–26°C, with a relative humidity of 50–55%, for 30 min before measurements were taken. All studies were completed between January and May 2009 (i.e. late winter and spring in northern China).

**Statistics**

Data are expressed as means ± SEM. The GraphPad Prism 4 software was used for all statistical analyses. The paired two-tailed Student t test was used to determine the significance for the skin surface pH before and after 5 D-Squame applications. The unpaired two-tailed Student t test with Welch’s correction was used to determine the significance between vitiligo-involved and uninvolved sites. The paired two-tailed Student t test was used to determine the significance in skin surface pH before and after 5 D-Squame applications.

This study was performed under human research protocols that were approved by the Human Research Subcommittee of the Dalian Skin Disease Hospital (DSDH122008).
compared with contralateral uninvolved sites (capacitance: 94.67 ± 2.26 vs. 116.70 ± 3.15; t = 5.684; p < 0.0001).

Since skin pigmentation affects the skin surface pH [12, 15, 29], we next determined if there was a difference in skin surface pH between vitiligo-involved and contralateral uninvolved sites. As seen in figure 1c, we observed no significant differences in basal skin surface pH between vitiligo-involved and contralateral uninvolved sites. However, the skin surface pH in vitiligo-involved sites increased significantly after 5 D-Squame applications (pH 5.55 ± 0.079 vs. 5.67 ± 0.057; t = 3.597; p < 0.002), whereas in uninvolved sites, it was not elevated significantly following 5 D-Squame applications (pH 5.55 ± 0.059 vs. 5.63 ± 0.047; t = 1.958; not significant).

Epidermal Permeability Barrier Function

The present study demonstrated that the basal TEWL in vitiligo-involved sites did not differ significantly from that in uninvolved sites (10.71 ± 0.94 vs. 9.57 ± 1.10 g/m²/h; t = 0.789; not significant) (fig. 1d). However, barrier recovery was significantly delayed in vitiligo-involved sites in comparison with contralateral uninvolved sites (40.83 ± 5.39 vs. 58.30 ± 4.71%; t = 2.441; p < 0.02) (fig. 2). These results suggest that an alteration in epidermal permeability barrier homeostasis occurs in vitiligo-involved sites.

SC Integrity

As shown in figure 3, there was no difference in SC integrity between vitiligo-involved and contralateral uninvolved sites following up to 10 D-Squame applications. This result indicates that melanin deficiency does not significantly alter SC integrity in vitiligo.

Discussion

Melanocytes have been thought to function primarily as melanin producers, to generate a natural protectant against UV damage. Although studies demonstrated that photodamage is reduced in vitiligo skin [58], highly pigmented skin requires more UV radiation than lightly pigmented skin to produce erythema [59]. Melanin content negatively correlates with the amount of DNA damage induced by UV radiation [60–63]. The ratio of the minimum erythema dose between skin type V (darkly pigmented) and skin types I/II (lightly pigmented) is close to the ratio of pigment in these skin types [64]. In addition, patients with pigmented skin have a reduced risk for melanoma [65, 66]. Recent studies have demonstrated that SC function is also influenced by pigmentation [42]. Although the present study does not show a difference in basal TEWL between vitiligo-involved and uninvolved sites, we have found that barrier recovery is significantly delayed in vitiligo-involved sites. This finding is consis-
tent with the reports of others that the barrier recovery rates in type V/VI skin are higher than that in type I/II or II/III skin [12, 14, 42]. Although the mechanisms underlying this abnormal barrier function in vitiligo-involved sites are not clear, it is well known that SC lipids play an important role in permeability barrier homeostasis [67]. Pertinently, darker individuals have more epidermal lipids, especially sterols, than lighter subjects [68]. Moreover, a significantly lower expression of granulocyte-macrophage-colony-stimulating factor is evident in vitiligo-involved sites [21]. Granulocyte-macrophage-colony-stimulating factor stimulates keratinocyte proliferation and VEGF transcription [69, 70], and a deficiency in epidermal VEGF delays barrier recovery [71]. Thus, the alteration in cytokine expression in vitiligo-involved sites could account, at least in part, for the noted alterations in barrier function. Furthermore, epidermal calcium plays a key role in regulating epidermal permeability barrier homeostasis [72]. Keratinocytes from vitiligo-involved sites demonstrate defects in calcium uptake and distribution [73]. Therefore, a defective calcium distribution could represent another mechanism accounting for the abnormal barrier function in vitiligo-involved sites.

Data regarding variations in SC integrity with skin pigmentation are controversial. For example, Berardesca et al. [12] reported that the TEWL was higher in black African-American than in Caucasian skin following 3 or 6 tape strippings, while Reed et al. [14] reported that darker skin (type V/VI) required more tape strippings to generate equivalent barrier dysfunction. In contrast, the present study does not show differences in SC integrity following up to 10 D-Squame applications. These differing results may be due to the different study population involved. In the present study, we compared SC integrity in vitiligo-involved and contralateral uninvolved sites on the same subjects, while the other studies compared the integrity between different races, which is known to affect cutaneous function [14, 42, 74].

Regarding the skin surface pH in darkly and lightly pigmented skin, the results are also inconclusive [12, 15, 42]. Recent studies have demonstrated that the skin surface pH is lower in darker skin, which is attributable to accelerated barrier recovery [42]. However, the present study does not reveal any differences in skin surface pH between vitiligo-involved and contralateral uninvolved sites, which is inconsistent with previous findings in darker and lighter skin [12, 15]. Again, these distinct findings regarding the skin surface pH could be due to differences in the studied subjects’ race(s) and geographic location. Our results are generated from subjects of the same race living in the same geographic location/environment. In other studies, the subjects were of different races living in different geographic locations/environments [42]. Both the living environment and geographic location have a significant impact on cutaneous function including SC properties [75–78].

Taken together, the results from the present study further suggest that race and living environment are at least partial determinants of the skin surface pH. Thus, it is worthwhile to note that the subjects enrolled in the present study are of the same race and skin type, and that they live in the same geographic location. In addition, the changes in SC properties were compared between vitiligo-involved and uninvolved sites on the same subjects. These factors were able to minimize any variation imposed by differences in race, environment and/or individuals, in contrast to previous studies [12, 15, 42].

The present study also demonstrates that SC hydration is diminished in vitiligo-involved sites compared with contralateral uninvolved sites. The diminished SC hydration in vitiligo-involved sites might result from defective sweat and sebaceous gland functions, which are known to influence SC hydration [79, 80]. In addition to sebaceous gland degeneration, sweat gland degeneration has been reported in vitiligo-involved sites [81]. Additionally, a lower erythema index in vitiligo-involved sites is also evident in the present study. Although the mechanism for this finding is unknown, it is possible that the lower melanin content in vitiligo interferes with erythema measurements since the levels of melanin and erythema can affect each other when measured by a reflectance spectrometer [82].

In summary, in the present study we have demonstrated that a defective permeability barrier function exists in vitiligo-involved sites, and that melanin does not play a crucial role in regulating the skin surface pH in vitiligo; therefore, in the treatment of vitiligo, attention should be given to improving the barrier function.

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References


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