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Structural and functional differences in barrier properties of African American, Caucasian and East Asian skin

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ABSTRACT

Background: Differences in structural and functional skin characteristics have been linked with ethnical background. But racial differences in skin have not been thoroughly investigated by objective methods and the data are often contradictory.
Objectives: This study was undertaken to compare skin barrier-related parameters of the stratum corneum on African American, Caucasian and East Asian skin by objective measurements.
Methods: Baseline values of trans epidermal water loss were collected on the face. Consecutive stratum corneum D-squame® tape strippings were collected on the panelist's ventral forearm and face to evaluate skin barrier strength and cohesion. Stratum corneum ceramides, maturation, measured as the transglutaminase-mediated cross-linking of stratum corneum proteins, and stratum corneum trypsin like enzyme activity were measured on the D-squame® tape strippings.
Results: East Asian and to some extent Caucasian skin was characterized by low maturation and relatively weak skin barrier. African American skin was characterized by low ceramide levels and high protein cohesion in the uppermost layers of the stratum corneum. These data can be interpreted in terms of the high prevalence of xerosis in black skin and increased skin sensitivity in East Asian skin.
Conclusion: These results demonstrate that skin properties at the level of the stratum corneum vary considerably among these ethnic groups. This contributes to an improved understanding of physiological differences between these study populations.

1. Introduction

The most obvious differences in skin of different ethnicities are related to pigmentation [1]. While research into the genetic basis underlying normal variation in the pigmentary traits of skin, hair and eye color has made significant progress over the last few years [2], the structural and functional differences in ethnic skin are less well characterized. Several publications have reported on ethnic differences in skin physiology, often with conflicting results [3–7].

The barrier function is responsible for not only the control of the trans epidermal water loss (TEWL), but also for the prevention of the penetration of toxic, sensitizing or irritant substances into the living layers of the skin. It is therefore evident that skin reactivity to external factors is greatly affected by the quality of the skin barrier. When reviewing the available literature on differences in skin permeability, reactivity and barrier function; the data have often been inconclusive [8]. Several studies report that TEWL is higher in black skin compared with Caucasian skin [9], while better stratum corneum (SC) integrity and cohesion [10] as well as decreased penetration has been reported in black skin [11]. Sensitive skin, as perceived by an individual, also known as reactive or irritable skin, can be described as a skin that reacts more rapidly to a challenge such as cold, wind, or a cosmetic product via an irritation response [12,13]. In this perspective, Asian skin was reported to be more reactive than the skin of Caucasians, which was more reactive than the skin of African Americans [14]. African Americans were recently reported to display a reduced neurosensory response to capsaicin compared with East Asians, Hispanics and Caucasians [15].

There is a requirement to better understand the differences in skin properties among ethnic groups. It was decided to undertake a large scale clinical study that involved human subjects representing different racial groups according to the guidelines and definitions provided by United States Office of Management and Budget [16]. Panelists were classified as African Americans,

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Caucasians and East Asians. The subjects were living in the same geographical area in order to exclude differences due to environmental exposure. The focus of this study was on skin properties related to the barrier function of the SC, based on various objective measurements.

From previous experience [17], we know that the measurement of baseline values of TEWL describes a steady state situation that yields only partial information about the SC barrier. More information on the functionality of the barrier can be obtained when an additional challenge and response to that challenge is evaluated. We therefore measured the skin barrier strength and cohesion [18]. Skin barrier function depends on the total architecture of the SC, which includes epidermal lipids such as ceramides [19,20], the protein compartment and its maturation [21], as well as the proteolytic enzymes involved in the desquamation process [22]. Measurements related to these three compositional aspects were taken on the SC of these subjects and were included in the study.

A thorough characterization of the skin's barrier function and structural elements of the SC is expected to increase the level of understanding about ethnic related differences in skin barrier properties and thereby help to target the needs in different ethnic skin types better.

2. Materials and methods

2.1. Clinical procedure

All aspects of this study conformed to the Code of Federal Regulations, Title 21 Section 50. After informed consent, panelists were allowed to participate in the study. A total of 73 African Americans, 119 Caucasians and 149 East Asians were chosen for the study. The East Asian population was first-generation immigrants to New York, from China, Japan and Korea. The Caucasian population included the local Caucasian population in the New York tri state area as well as a subset of Anglo Saxons living in the UK. African Americans, defined as people with origins in any of the black racial groups of Africa, were selected from the Tri State area.

All subjects were females between the ages of 18 and 45, in normal health, with no evidence of acute or chronic disease or dermatologic problems. The subjects reported having a regular menstrual cycle of 25–30 days. Additional demographic characteristics are summarized in Table 1. The test area was the subjects' full face and arms. The outermost layers of the SC from the upper inner arms (the side of the biceps) were sampled by non-invasive successive D-squame® tape stripping. The D-squame discs (22 mm diameter, standard, CuDerm Corporation, Texas) were placed on the test site and using a spring loaded device, an even pressure was exerted on the sites, after which they were removed using forceps. The same process was repeated to collect consecutive tape strippings. Sampling was performed from spring to late summer. The level of cis-urocanic acid in the SC is a marker for recent sun exposure [23] and was used to eliminate highly UV-exposed skin samples from the data set (data not shown). The strips were stored at −80 °C until further analysis.

Table 1
Demographic data on the subject population.

Ethnicity	*N*	Pigment type	Age ± SD
African Americans	73	IV–VI	35.1 ± 7.5
Caucasians	119	II–III	36.0 ± 6.0
East Asians	149	III–IV	30.2 ± 5.8

2.2. Measurement of the barrier function

The measurement of skin barrier function was based on previously published methods [17,24]. The subjects were allowed to equilibrate for at least 30 min prior to testing in a controlled environment of 20–21 °C and 25% relative humidity. The subjects were settled in a relaxed inclined position and they were not allowed to converse or become excited. Baseline trans epidermal water loss (TEWL) was recorded on the right and left facial cheek area with an evaporimeter (Dermalab Cotex Tech, 9560 Hadsund, Denmark) that was set to a total measurement time of 45 s and a 15 s data acquisition period. TEWL was measured at three adjacent sites and expressed as grams per square meter per hour.

Baseline TEWL data were supplemented with barrier strength data obtained after a disruptive insult. To measure the barrier strength, the sites were disrupted by a minimally invasive, non-painful method using a sticky tape (Tuck: Tesa Tuck Inc., New Rochelle, NY). Starting from the top of the cheek, the tape was removed by gently pulling in a downward direction parallel to the skin [25]. The skin was stripped in increments of three and TEWL measurements were obtained after every third stripping. Damage to skin barrier was described in terms of increase in water loss. A TEWL value of 18 $g/m^2/h$ was set as the threshold value for barrier disruption. The barrier strength (number of strippings required to reach this TEWL value) was calculated using a linear equation [17].

2.3. Measurement of cohesion in the uppermost layers of the SC (total amount of protein per tape)

The measurement of total protein content on the SC D-squame tape strippings was determined as the amount of amino acids after complete acid hydrolysis. Proteins were totally hydrolyzed into amino acids at 120 °C for 20 h with excess of HCl 6N. After neutralization, hydrolysates were transferred to 96-well plates and derivatized with orthophtalaldehyde (OPA) in alkaline medium (0.5 M borate buffer pH 9.5) in the presence of N-Acetyl-Cystein (NAC). The amount of resulting isoindole derivatives was quantified by UV absorption (335 nm) from which the protein content was calculated.

2.4. Measurement of SC ceramide content

Stratum corneum samples collected on D-squame tape strippings were extracted with methanol and analyzed for the presence of sphingoid bases before and after acidic hydrolysis. Sphingoid bases were quantified by reverse-phase HPLC with fluorescence detection (λ_{ex} 340 nm/λ_{em} 445 nm) after automated pre-column derivatization with ortho-phtaldialdehyde and mercapto-propionic acid in 0.5 M borate buffer pH 9.5. The total amount of sphingoid bases (after hydrolysis) minus the free sphingoid bases (before hydrolysis) corresponded to the amount of sphingoid bases that was originally bound in ceramides. This procedure was used to quantify the C18 phytosphingosine based ceramides as a representative fraction of the total amount of ceramides present in the SC samples.

2.5. Measurement of the maturation index

Stratum corneum samples collected on D-squame tape strippings were derivatized with dansyl chloride (0.2 mg/ml acetone) in 0.2 M carbonate buffer pH 9 for 30 min at room temperature. Free amino acids, N-terminal amino acids and the free Nε-amine groups of lysine present in the SC proteins were dansylated. After complete acid hydrolysis in 6N HCl at 120 °C for 16 h, the amount of Nε-dansyl lysine was quantified by reverse-phase HPLC with fluorescence detection (λ_{ex} 340 nm/λ_{em} 520 nm). The total protein