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Ethnic Differences in Skin Physiology, Hair Follicle Morphology and Follicular Penetration

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Key Words

Cyanoacrylate skin surface biopsy · Hair follicle morphology · Caucasian · Asian · African · Follicular penetration

Abstract

Background/Aims: Inconsistent evidence is available that different ethnic groups exhibit differences in skin physiological parameters. Recently, variations in hair follicle morphology have been described, although the influence of such variations on the follicular penetration process has not been investigated until now. ***Methods:*** The aim of the present study was thus to investigate skin physiological parameters, follicle morphology and the penetration process in different ethnic groups. ***Results and Conclusion:*** Whereas no significant differences with regard to skin physiological parameters could be observed, morphological analysis of the hair follicles revealed, inter alia, that Caucasians had significantly larger terminal hair follicles than Asians and Africans. The surface of the hair follicle infundibulum was shown to be 3% in Caucasians, 2.6% in Africans and 2.4% in Asians. The investigations into penetration revealed no significant differences after a 30-min penetration time, whereas after 24 h, the Asian volunteers presented significantly larger amounts of sodium fluorescein in the hair follicles and the stratum corneum, which may be explained by cultural habits.

Introduction

Although the majority of the world's population has colored skin, most of the studies on skin characteristics and penetration have been performed on Caucasian skin. Whereas variations in skin coloration are evident, differences in skin structure and function are less obvious and need detailed examination.

Whilst several studies have already endeavored to investigate structural changes [1], penetration studies on different ethnic groups are rare and especially the follicular penetration pathway, which has recently been demonstrated to contribute significantly to the total penetration process, has hardly been studied until now.

Structural analysis of the stratum corneum, e.g., has revealed differences concerning the number of corneocyte layers and, in this context, conflicting results concerning the desquamation rate have been reported [2–4]. Controversial results have been published as well on the lipid content of the stratum corneum. Weigand et al. [5] suggested a higher stratum corneum lipid content in black skin, whereas Hellemans et al. [6] reported similar stratum corneum ceramide levels in Asians and Caucasians with lower levels in Africans. Similar inconsistencies were, among others, also found for the stratum corneum barrier function [7, 8], the stratum corneum water content [4, 9] and skin resistance to chemical irritation [10, 11].

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As structural differences in the skin might influence the penetration behavior of topically applied substances, penetration studies have been performed on different ethnic groups, although the results are heterogeneous too. Kompaore et al. [12], for instance, observed more rapid penetration of methyl nicotinate through the skin barrier in Asians and Caucasians than in Africans, which is in concordance with several other studies, whereas Guy et al. [13] found no differences in the penetration of the same substance.

To our knowledge, only the follicular penetration pathway has not been investigated in different ethnic groups although the follicular pathway continuously gains in importance [14, 15]. The hair follicles are the appendages of the skin and represent complex and dynamic three-dimensional structures interrupting an otherwise highly potent skin barrier. The hair follicle is considered to be an interesting therapeutic target site as it is connected with the sebaceous gland, surrounded by a dense network of capillaries and dendritic cells and host stem cells [16]. Therefore, several research groups are currently working intensively on improving the delivery of substances into the hair follicle. Several methods are now available to assess the follicular penetration quantitatively, such as the differential stripping method recently developed by Teichmann et al. [17].

As not only the hair color but also the hair structure is phenotypically completely different among diverse ethnic groups [18], it can be speculated that the follicle morphology and the associated follicular penetration process might also vary in different ethnic groups. Hair follicle morphology has already been investigated by different research groups. Mangelsdorf et al. [19] revealed that Caucasians have significantly more hair follicles in the forehead region than Africans and Asians, and an increased percentage of hair follicle orifices on the skin surface in the forehead region and the calf. Additionally, Caucasians displayed increased follicular infundibulum volumes.

Considering the results of these previous investigations, we hypothesized that due to differences in follicular morphology, variations in the follicular penetration process can be expected as well in different ethnic groups. In order to overcome the interindividual differences, we determined the skin physiological parameters and the hair follicle morphology of the calf and scalp region of Caucasian, Asian and African volunteers before performing the penetration studies. Therefore, the differential stripping method was utilized for a selective analysis of the penetration and storage of an aqueous sodium fluorescein solution into the hair follicle and the stratum corneum.

Material and Methods

Volunteers

The study was conducted on 18 healthy male volunteers of three different ethnic origins (Caucasians, Asians and Africans) not suffering from any skin diseases. The 6 Caucasian volunteers had a mean age of 28.1 ± 4.3 years and were of phototype II–III according to the Fitzpatrick classification; the 6 African volunteers were 27.3 ± 3.2 years old and were of phototype V–VI and the 6 Asian volunteers were 23.5 ±1.6 years old and of phototype IV. The volunteers investigated lived in Germany at the time of the investigation and had lived in Europe for at least 2 years. Before initiation of the study, the volunteers had given their informed written consent. The study had been approved by the local Ethics Committee of the Charité – Universitätsmedizin, Berlin, Germany. The volunteers had to complete a standardized questionnaire regarding eating, smoking habits, lifestyle and alcohol consumption and were not allowed to use any skin care products on the days of the investigation.

Study Design

The study consisted of three parts, always comparing the results of the three ethnic groups. Study protocol A involved the determination of physiological skin parameters; study protocol B was aimed at determining hair follicle morphology on the calf and scalp, and study protocol C investigated the inter- and intracellular penetration on the calf region.

Study Protocol A – Determination of Physiological Skin Parameters

The physiological skin parameters were investigated mainly on the volar forearm and included the determination of (a) the transepidermal water loss (TEWL) using the Tewameter TM 210 as well as (b) skin hydration, (c) the pH and (d) the sebum content of the skin. All corresponding measuring devices were from Courage and Khazaka Electronic GmbH, Cologne, Germany.

Additionally, the skin roughness (e) was determined using the device Primos 4.0 (GF Messtechnik GmbH, Teltow, Germany) by creating three-dimensional images of the skin surface structure as described previously [20].

The antioxidative status of the skin was determined as well. It was recently shown that carotenoids could serve as marker substances for the complete antioxidative status of the human skin [21]. Therefore, the skin levels of two antioxidant substances, lycopene and β-carotene (f), were determined using resonance Raman spectroscopic measurements as described in detail elsewhere [22]. The lycopene and β-carotene concentrations were determined only on the palm, as it is known that deep melanin pigmentation most likely interferes with the penetration of the laser beam. Therefore, measurements are routinely performed on the palm of the hand, where pigmentation is usually quite light even in darkly pigmented individuals [23].

As the final skin parameter, melanin distribution (g) was investigated on the volar forearm utilizing the confocal laser scanning microscope VivaScope ® 1500 [24]. Since the refraction index of melanin is relatively higher than that of the cytoplasm, structures with high contrast contain more melanin.

Study Protocol B – Determination of Follicular Morphology

The hair follicle morphology was investigated according to the method described in detail by Otberg et al. [25]. Briefly, cyanoacrylate skin surface biopsies were taken from the calf region and the scalp of each volunteer. Therefore, 4 × 4 cm areas were marked and shaved in the occipital region and the calf. Subsequently, the skin regions were rinsed with water and dried with paper towels. Adjacently, cyanoacrylate glue (Uhu Sekundenkleber flüssig, Uhu GmbH, Bühl, Germany) was applied homogeneously onto the marked skin areas, covered with a plastic foil and then pressed onto the skin using a roller. After 2 min, the glue had polymerized and could be removed together with the foil and the follicular contents, which represent casts of the follicular infundibulum.

The cyanoacrylate surface biopsies were then analyzed using microscopy (Olympus, BX60M system microscope, Hamburg, Germany). The follicular casts were counted in a marked area of 1 cm^2. The diameters of the follicular orifices and hair shafts were measured directly. The percentage of orifices of the skin surface was determined by adding all calculated circle areas of the follicular orifices in the labeled biopsy area.

For analyzing the volume and surface of the follicular infundibula, a special three-dimensional extended focal image was generated using software program analysis (Soft Imaging System GmbH SIS, Münster, Germany). The program could also be utilized to measure the length, height and diameter of the follicular casts. Then every infundibular cast was divided into truncated cones of which the volume was calculated with the formula

$$V_n = \pi/12 h_n(d^2_1 + d^2_2 + d_1 d_2) \quad (1)$$

where h is the height of the truncated cone and d is the diameter of the covers.

The complete infundibular volume was then calculated by adding all single volumes and subtracting the hair shaft volumes (V_{hs}) which was calculated as follows:

$$V_{hsn} = \pi/4 d^2 h_n \quad (2)$$

where h is the height of the whole infundibulum and d the hair shaft diameter.

For determining the surface of the follicular infundibula, the curved surface (A) of the truncated cones was calculated as follows:

$$A_n = \pi s(d_1/2 + d_2/2) \quad (3)$$

and

$$s^2 = (d_1/2 - d_2/2)^2 + h_n \quad (4)$$

where h is the height of the truncated cone and d is the diameter of the covers.

The above-mentioned parameters were determined for each terminal and vellus hair follicle separately. In order to determine the total volume and surface of the hair follicles, the density of the vellus hair follicles (D_v) and terminal hair follicles (D_t) was multiplied by the follicular volume (V_v and V_t) or the surface (A_v and A_t), respectively.

$$V = D_v V_v + D_t V_t \quad (5)$$
$$A = D_v A_v + D_t A_t \quad (6)$$

Study Protocol C – Investigation of Follicular Penetration by Means of Differential Stripping

In the frame of study protocol C, the follicular penetration of sodium fluorescein was investigated over a time period of 96 h. Skin sampling was performed 30 min, 24 h and 96 h after topical application of sodium fluorescein. Therefore, in the calf region of each volunteer, 3 adjacent skin areas (A–C) of 4 × 4 cm were marked using a permanent marker. The hairs were removed with scissors and the skin was rinsed with water and carefully dried with paper towels.

Subsequently, 2 mg/cm^2 of an aqueous solution containing 2% sodium fluorescein was applied onto the marked skin area. Skin sampling was performed from skin area A 30 min after application, from skin area B 24 h and from skin area C 96 h after application, using the method of differential stripping as described previously by Teichmann et al. [17].

Briefly, differential stripping combines the tape stripping method with the cyanoacrylate skin surface biopsy and affords selective determination of intercellular and follicular penetration in vivo. Primarily, the stratum corneum is removed by tape stripping. Therefore, an adhesive film (teas No. 5529, Beiersdorf, Hamburg, Germany) was used to remove the stratum corneum layer by layer. Each tape strip was pressed onto the skin with a roller to minimize the influence of skin furrows and wrinkles [26]. The procedure was repeated until no more sodium fluorescein was detectable in the stratum corneum. A corresponding control was performed utilizing a laser scanning microscope (LSM 2000, Carl Zeiss, Jena, Germany). A cyanoacrylate skin surface biopsy was removed as described above.

For quantification, the tape strips and the cyanoacrylate skin surface biopsies were dissolved separately in ethanol (Uvasol, Merck, Darmstadt, Germany) using an ultrasonic bath (Sonorex Super RK 102H, Bandelin Electronic, Berlin, Germany). The concentration of sodium fluorescein was determined by means of a fluorescence spectrometer (Luminescent LS 50B, PerkinElmer, Überlingen, Germany). After excitation of the sodium fluorescein at 450 nm, the fluorescence signal was measured using a spectral filter covering the wavelength between 480 and 600 nm. The maximum fluorescence signal was detected at a wavelength of 510 nm. The intensity of the fluorescence signal was used as a measure of the concentration of the fluorescent dye. The intensity is given in relative units.

Statistical Analysis

For statistical analysis, the Kruskal-Wallis and the Mann-Whitney tests were applied (SPSS 16.0). Data are expressed as means ± SD with $p < 0.05$ considered significant.

Results

The questionnaire revealed no relevant differences concerning dietary habits and alcohol consumption. With regard to smoking, 4 Caucasians were smokers, whereas the Asians and Africans were all nonsmokers. Whereas Caucasians and Africans, without exception, usually wore trousers, the Asian volunteers wore shorts, as the investigations were performed in summer.

Table 1. Physiological skin parameters of Caucasians, Africans and Asians given as mean values ± standard deviations

	Caucasian	African	Asian	Statistical evaluation (p)
TEWL, g/m^{2}h	11.9 ± 1.2	13 ± 2.5	14.3 ± 3.1	>0.05
Skin moisture, rel. units	79.6 ± 10.1	75 ± 6.5	69.5 ± 10.7	>0.05
pH	5.2 ± 0.3	5.4 ± 0.7	5 ± 0.2	>0.05
Sebum excretion, μg/cm^2	0.7 ± 0.8	1.8 ± 1.3	0.8 ± 0.9	>0.05
Skin roughness, μm	26.8 ± 4.9	22.5 ± 5.9	26 ± 2.6	>0.05
β-Carotene, nmol/g	0.368 ± 0.175	0.412 ± 0.232	0.479 ± 0.24	>0.05
Lycopene, nmol/g	0.511 ± 0.163	0.711 ± 0.277	0.457 ± 0.372	>0.05

Study Protocol A – Determination of Physiological Skin Parameters

The investigations revealed no statistically significant differences for TEWL, skin moisture, pH, sebum excretion, skin roughness, and β-carotene and lycopene concentrations.

The mean ± SD of these physiological skin parameters are summarized in table 1.

The investigations concerning the melanin distribution revealed a different distribution of the melanin in all ethnic groups. The African volunteers exhibited melanin in all epidermal layers, with increased amounts especially in the stratum corneum and stratum basale.

Study Protocol B – Determination of Follicular Morphology

The morphological results for the scalp region and the calf region are summarized in tables 2 and 3, respectively.

The analysis of the terminal hair shaft diameter on the scalp revealed significantly larger diameters in Asians compared with Africans and Caucasians ($p < 0.05$), whereas the analysis of the terminal hair shaft diameter on the calf revealed no statistically significant differences ($p > 0.05$). The vellus hair shaft diameter on the scalp and the calf showed similar diameters in all ethnic groups; only the Africans had a significantly larger diameter in the calf region compared with the Caucasians ($p < 0.05$).

As the cyanoacrylate skin surface biopsy was incomplete when removed from the scalp skin, and only 70–80% of the hair follicles could be removed, follicular density was only determined on the calf. For terminal hairs, no significant differences could be detected among the different ethnic groups. Vellus hair follicle density was significantly higher in Asians than in Africans and Caucasians ($p < 0.05$). The proportion of terminal to vellus hair follicles was therefore 2:1 in Caucasians, 1.9:1 in Africans and 1:1 in Asians.

The percentage of follicular orifice surface of the skin surface in the calf region was calculated by multiplying the follicular density per square centimeter by the follicular orifice surface and yielded comparable results in all ethnic groups.

The volume of the follicular infundibulum was significantly different among the ethnic groups only for the terminal hair follicles on the scalp. For the vellus hair follicles on the scalp and the terminal and vellus hair follicles of the calf region, no significant differences were found.

The follicular volume per square centimeter skin surface was calculated too as it represents the follicular reservoir that is important for penetration. The results are illustrated in figure 1. The calculations revealed no statistically significant differences among the ethnic groups.

In contrast, the surface of the terminal follicular infundibulum on the scalp, which represents the penetration surface, was the largest in Caucasians ($p < 0.05$) (fig. 2).

For the vellus follicular infundibulum of the scalp as well as for the terminal and vellus follicular infundibulum of the calf, no significant differences were obtained. The surface of the terminal follicular infundibulum per square centimeter skin surface was also calculated for the calf region. Here, the Caucasians exhibited a significantly larger follicular surface than the Asians ($p < 0.05$). For the vellus hair follicles, the largest follicular surface per square centimeter skin surface was found in the Asians compared with the Caucasians ($p < 0.05$) and Africans. In figure 3, the total follicular infundibulum surface per square centimeter skin surface is depicted.

Table 2. Hair follicle morphology of Caucasians, Africans and Asians in the scalp region, given as mean values ± standard deviations

	Caucasians		Africans		Asians	
	THF	VHF	THF	VHF	THF	VHF
Hair shaft diameter, μm	67 ± 9*	20 ± 3	62 ± 4**	20 ± 2	78 ± 10*, **	22 ± 3
Follicular orifice diameter, μm	259 ± 40	109 ± 23	214 ± 21	94 ± 7	216 ± 30	101 ± 16
Hair follicle density/cm^2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Percentage of follicular orifice on skin surface, %	n.d.		n.d.		n.d.	
Volume of follicular infundibulum, mm^3	0.017 ± 0.008*, **	0.001 ± 0.001	0.010 ± 0.003*	0.001 ± 0.0003	0.009 ± 0.003**	0.0012 ± 0.0007
Volume of follicular infundibulum, mm^3/cm^2 skin surface	n.d.		n.d.		n.d.	
Surface of follicular infundibulum, mm^2	0.42 ± 0.12*, **	0.06 ± 0.016	0.29 ± 0.05*	0.06 ± 0.01	0.31 ± 0.06**	0.07 ± 0.03
Surface of follicular infundibulum, mm^3/cm^2 skin surface	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Percentage of follicular infundibulum surface on skin surface, %	n.d.		n.d.		n.d.	

THF = Terminal hair follicles; VHF = vellus hair follicles; n.d. = not determinable, * or ** = $p < 0.05$.

Table 3. Hair follicle morphology of Caucasians, Africans and Asians in the calf region, given as mean values ± standard deviations

	Caucasians		Africans		Asians	
	THF	VHF	THF	VHF	THF	VHF
Hair shaft diameter, μm	60 ± 9	18 ± 2*	55 ± 5	22 ± 3*	57 ± 13	20 ± 3
Follicular orifice diameter, μm	218 ± 32	105 ± 20	203 ± 11	114 ± 20	208 ± 22	111 ± 20
Hair follicle density/cm^2	9.7 ± 1.9	4.7 ± 1*	8.8 ± 1.5	4.8 ± 2.2*	8 ± 1.8	7.8 ± 1.9*
Percentage of follicular orifice on skin surface, %	0.4 ± 0.06		0.33 ± 0.03		0.35 ± 0.05	
Volume of follicular infundibulum, mm^3	0.0093 ± 0.0024	0.0027 ± 0.0015	0.0083 ± 0.0017	0.0015 ± 0.0007	0.0075 ± 0.0018	0.0016 ± 0.0006
Volume of follicular infundibulum, mm^3/cm^2 skin surface	0.104 ± 0.02		0.084 ± 0.02		0.073 ± 0.02	
Surface of follicular infundibulum, mm^2	0.28 ± 0.052	0.064 ± 0.018	0.26 ± 0.039	0.072 ± 0.016	0.23 ± 0.044	0.077 ± 0.008
Surface of follicular infundibulum, mm^3/cm^2 skin surface	2.69 ± 0.5*	0.3 ± 0.15**	2.27 ± 0.5	0.36 ± 0.19	1.77 ± 0.4*	0.61 ± 0.2**
Percentage of follicular infundibulum surface on skin surface, %	2.99 ± 0.5		2.63 ± 0.5		2.38 ± 0.4	

*, ** $p < 0.05$.

Table 4. Concentration of sodium fluorescein (relative units) that has penetrated into the stratum corneum (SC) and hair follicle (HF) 30 min, 24 and 96 h after topical application

	30 min		24 h		96 h	
	SC	HF	SC	HF	SC	HF
Caucasians	470 ± 59	7.03 ± 1.85	29 ± 16*	1.05 ± 0.73**	2.2 ± 1.6*	0.43 ± 0.22
Africans	467 ± 99	5.05 ± 1.9	30 ± 15*	0.88 ± 0.3**	2.4 ± 2.1*	0.37 ± 0.06
Asians	449 ± 45	6.2 ± 1.15	60.8 ± 26*	2.15 ± 0.9**	8.5 ± 6*	0.55 ± 0.2

*, ** $p < 0.05$.

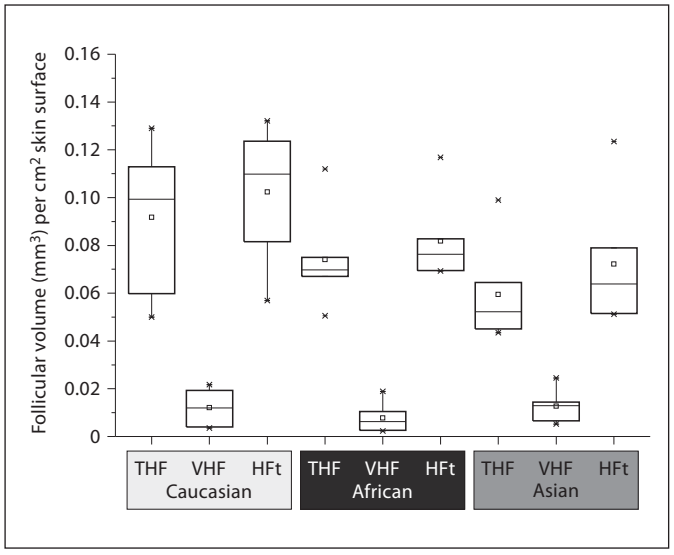

Fig. 1. Follicular volume (cubic millimeters) per square centimeter skin surface for terminal hair follicles (THF), vellus hair follicles (VHF) and all hair follicles (HFt).

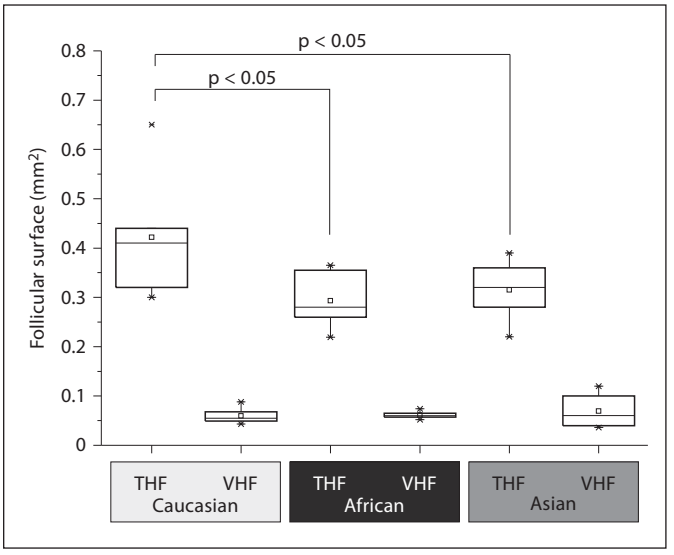

Fig. 2. Follicular surface in square millimeters for terminal hair follicles (THF) and vellus hair follicles (VHF).

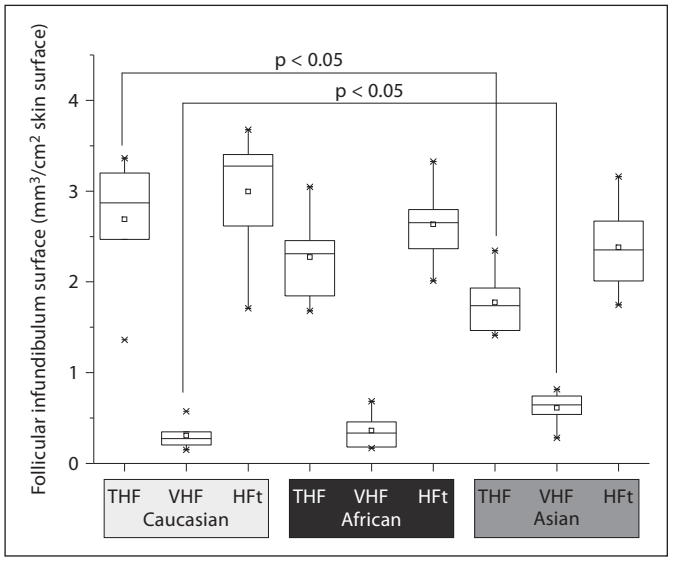

Fig. 3. Follicular infundibulum surface (cubic millimeters) per square centimeter skin surface for terminal hair follicles (THF), vellus hair follicles (VHF) and all hair follicles (HFt).

Study Protocol C – Investigation of Follicular Penetration by Means of Differential Stripping

The penetration of sodium fluorescein into the stratum corneum and the hair follicle was determined 30 min, 24 and 96 h after topical application. The results are summarized in table 4.

After a 30-min penetration time, no significant differences were detected among the different ethnic groups. After 24 h, significantly higher sodium fluorescein concentrations were detected in the stratum corneum of the Asian volunteers compared with the African volunteers ($p < 0.05$). Likewise after 96 h, the sodium fluorescein concentration in the stratum corneum was significantly higher in Asians than in Caucasians and Africans ($p < 0.05$).

After 30 min and 96 h, similar results were observed for follicular penetration among all ethnic groups. A significantly higher sodium fluorescein concentration was detected in Asians compared with Caucasians and Africans only after 24 h ($p < 0.05$).

This means that in Caucasians 98.2 ± 0.4% of the sodium fluorescein penetrated into the stratum corneum and 1.8 ± 0.4% into the hair follicle. In Africans, the proportion was 99 ± 0.2% and 1 ± 0.2%, and in Asians 98.6 ± 0.3% and 1.4 ± 0.3%.

Discussion

In summary, no statistically significant differences in skin physiological parameters were found in the present study, whereas data reported in the literature are often inconsistent. For TEWL, e.g., which is the total amount of water vapor lost through the skin under nonsweating

conditions, some authors reported no differences in TEWL in different ethnic groups [27, 28]; several other studies, however, have demonstrated that Africans and Asians have a higher TEWL than Caucasians [8, 12]. Similar tendencies could likewise be observed in Asians and Africans in the present study; however, the differences did not reach statistical significance. Also, the stratum corneum water content was shown to be slightly but not significantly lower in both these groups, whereas data in the literature are again inconsistent. For pH and sebum excretion, no statistically significant differences could be observed, which is in concordance with the results reported in the literature [27, 28].

The measurement of skin roughness likewise revealed no statistically significant differences; in contrast, interindividual differences were much more pronounced. Explanations for the lack of differences might be the young age of the volunteers, gender and the skin site investigated, as previous studies could show that Caucasian women had significantly deeper wrinkles in the face than Asian women [29, 30].

The concentration of carotenoids was shown to be comparable in the skin of the palm of individuals of all ethnic groups, which can be explained by the strong influence of nutrition and lifestyle on the carotenoid concentration in human skin [31, 32]. Moreover, measurements were performed during the summer months where a 'seasonal increase' in carotenoids in the skin has to be considered [31], which may be explained by increased consumption of fruit and vegetables as well as increased sweating during these months, due to the fact that carotenoids are transported to the skin surface via sweat [33] and then again penetrate as topically applied substances [34]. To our knowledge, the antioxidant status of the skin in different ethnic groups has not been described previously. For serum concentrations, however, differences among ethnic groups have been reported, with lower concentrations of most antioxidants and also lower oxidative DNA damage levels in African Americans than in whites [35]. Investigations on skin should be repeated in a higher number of volunteers of comparable lifestyle and nutritional habits in order to detect genuine ethnic differences.

Melanin distribution was investigated with laser scanning microscopy and was shown to differ among the ethnic groups as reported previously [36, 37]. Whereas in Africans, increased melanin amounts exist in all epidermal layers, in Caucasians and Asians, smaller amounts of melanin were found to be mainly located in the superficial epidermis.

The investigation of the hair and follicle morphology revealed partially significant differences. As concerns the hair shaft diameter, the Asian volunteers showed a significantly larger mean terminal hair diameter on the scalp than the Caucasian and African volunteers, although interindividual differences were large, which is in concordance with previous data [18]. In contrast, the African volunteers had significantly larger vellus hair shaft diameters in the calf region than the Caucasians whereas all other vellus hair shaft diameters were comparable in agreement with the data obtained by Mangelsdorf et al. [19]

The hair follicle density could only be determined on the cyanoacrylate skin surface biopsies removed from the calf region, as the cyanoacrylate skin surface biopsies removed from the scalp were incomplete, probably due to the strong anchoring of the hair in the follicle and, therefore, were not suitable for determination of the density. However, the hair follicle density of the scalp in different ethnic groups has been described by many investigators. The results revealed that the hair density on the scalp varies among individuals and additionally depends on the area selected, but they clearly indicate a higher density in Caucasians than in Asians and Africans [38–40]. In this context, Mangelsdorf et al. [19] and Otberg et al. [25] investigated the follicular density on further body regions of Asians, Africans and Caucasians, and observed significant differences only for the forehead region, again with increased density in Caucasians. The present study additionally revealed a significantly increased vellus hair density in Asians in the calf region; however, the overall follicle density was not significantly different as reported previously [19].

The hair follicle represents an invagination of the epidermis and thus increases the skin surface for the penetration of topically applied substances. The percentage of hair follicle orifices on the skin surface, the volume of the follicular infundibulum per square centimeter skin and the follicular infundibulum surface per square centimeter skin are important parameters to estimate the percentage of hair follicles participating in the penetration process.

Whereas the percentage of hair follicle orifices on the skin surface has long been estimated to be around 0.1%, recent investigations revealed that this percentage strongly depends on the body site and can represent up to 1.28% in the forehead region [25]. For the calf region, the percentage was measured to be 0.4% in Caucasians, which confirms the data of Otberg et al. [25]. The results in Asians and Africans were similar.

The volume of the follicular infundibulum constitutes an additional reservoir for topically applied substances. The relevant reservoir of the stratum corneum has recently been estimated to be 1 mm^3/cm^2 [41]. The follicular volume depends on follicle density and size. In the forehead region, it was calculated to be 0.19 mm^3/cm^2, i.e. nearly one fifth of the stratum corneum reservoir [25]. Certainly, this volume is not completely open for the incorporation of topically applied substances but is filled with sebum and desquamated cells [17].

Mangelsdorf et al. [19] compared the follicular reservoir of several body sites of three ethnic groups and found an increased reservoir in most body sites in Caucasians compared with Asians and Africans. Comparable results were obtained in the present study for the calf and scalp regions, although only the latter was statistically significant.

The hair follicle is lined with epithelial cells which contribute to the penetration surface. Thus, the surface area of the follicular infundibulum represents a further important parameter to estimate follicular penetration. The Caucasians showed a partially significantly larger infundibulum surface on the calf and the scalp than the other ethnic groups.

The hair follicle is now considered an irrefutable penetration pathway for topically applied substances. In the last decade, several methods were developed to investigate follicular penetration selectively [17, 42, 43]. In the present study, the method of differential stripping has been utilized to differentiate the penetration of an aqueous sodium fluorescein solution into the stratum corneum and the hair follicle in different ethnic groups. As the hair follicle represents a long-term reservoir for topically applied substances [44], the reservoir capacity of the stratum corneum and the hair follicle were investigated as well over 96 h. After a 30-min penetration time, comparable concentrations of sodium fluorescein were detected in the stratum corneum and hair follicles in all ethnic groups. These results correlate with the data on follicular volume, and likewise did not differ among the ethnic groups, although the Caucasians showed slightly increased values for follicular volume and penetration, which could again correlate to the significantly increased follicular surface. The percentage of intercellular (stratum corneum) and follicular penetration was shown to be 98.2% versus 1.8% for Caucasians. Teichmann et al. [17] reported a mean follicular penetration percentage of 5% for sodium fluorescein in a previous study. This slight difference could be the result of either the partially large interindividual differences, also observed in the present study, or the different localizations on which the investigations had been performed. Whereas the probes for the present study were removed from the calf, Teichmann et al. [17] used the skin of the back. Although the skin of the back and of the calf is known to have similar follicular reservoir sizes, i.e. 0.11 and 0.1 mm^3/cm^2, respectively (table 3), the aspect of closed and open hair follicles has to be considered as well. Whereas it has been reported recently that only about 75% of the hair follicles are open for penetration on the upper forearm [45], this might be different in other body regions. Whereas follicular penetration in different ethnic groups has not previously been investigated to our knowledge, Lotte et al. [46] studied the penetration of different substances into the stratum corneum by tape stripping in Caucasians, Asians and Africans and, likewise, found no significant differences.

After 24 and 96 h, the Asian volunteers showed increased concentrations of sodium fluorescein in both the stratum corneum and the hair follicles. As their results were comparable to those of the other ethnic groups after 30 min, it appears that the Asians had an increased storage capacity. This might be explained by their cultural habits. Whereas Caucasians and Africans, without exception, usually wore trousers, the Asian volunteers were dressed in shorts, as the investigations were performed during the summertime. This could have led to reduced sweating in Asians and removal of topically applied substances by the trousers in Caucasians and Africans.

In general, the weather conditions might have additionally influenced the results. As the experiments were performed during the summertime with temperatures around 28°C, increased sweat production might have led to a wash-out effect on the stratum corneum and upper follicular reservoirs. Although, as far as we know, there are no indications that increased sweat production diminishes penetration, there are indications for heat-induced sebum production in rat and porcine skin [47], leading to depletion of the sebaceous glands and blocking of the hair follicle, on the one hand, and depletion of the infundibulum from already absorbed substances on the other hand.

The results of the present study indicate that although structural and morphological differences with regard to skin and hair follicle morphology exist among different ethnic groups, their influence on the penetration process seems to be of minor importance. In fact, it seems to be important to take into account the cul-

tural habits and weather conditions, which might influence storage capacity. Nevertheless, it will be of interest to perform penetration studies on larger numbers of volunteers and on other body sites of interest such as the scalp region.

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