Variation in the activities of late stage filaggrin processing enzymes, calpain-1 and bleomycin hydrolase, together with pyrrolidone carboxylic acid levels, corneocyte phenotypes and plasmin activities in non-sun exposed and sun-exposed facial stratum corneum of different ethnicities
Abstract

Background

Knowledge of the ethnic differences and effects of photodamage on the relative amounts of natural moisturizing factor (NMF) together with filaggrin (FLG) processing enzymes in facial stratum corneum (SC) is limited. Our aim was to characterize the activities of calpain-1 (C-1), bleomycin hydrolase (BH) and the levels of pyrrolidone carboxylic acid (PCA) as a marker for total NMF levels and to relate them to plasmin activities and corneocyte maturation.

Methods

Enzyme activities, PCA levels and corneocyte maturation were determined from facial tape stripplings of photoexposed cheek and photoprotected post-auricular areas (PA) of healthy Caucasian (C), Black African (BA) and Albino African (AA) female subjects living in South Africa.

Results

PCA concentration levels were of the order AA > BA > C subjects and the highest activities of BH were present in the AA subjects. BH activities were greater on the photoexposed sites for the BA and C subjects but they were only numerically elevated in the AA subjects. Photoprotected sites had an increase in C-1 activity in pigmented groups (C and BA) whereas in the AA subjects the opposite was measured. Plasmin activities were greater on the cheek compared with the PA site for the AA and C subjects but the activity was low in the BA subjects. In both test sites, the AA, but not the BA and C subjects, had smaller, parakeratotic and less mature corneocytes.

Conclusion
Variation in PCA levels has been found for different ethnic groups in this study (AA > BA > C subjects). The values in the AA subjects are surprising as one might expect that the lack of pigmentation, and thereby increased photodamage, might lead to lower levels. Increased BH, but not C1 activity, was observed in the AA subjects indicating that BH is associated with PCA production to a greater extent. Surprisingly, corneocyte maturation is still impaired with elevated PCA levels in AA subjects. The higher levels of plasmin and BH activities on the cheeks, especially for AA and C subjects, suggest that they can be used as markers for epidermal photodamage.
Introduction

The epidermis is the primary barrier of the body to solar radiation which is mainly provided by melanocytes that transfer melanin into keratinocytes to protect their nuclear DNA from irradiation [1, 2]. However, the protective role of the SC in photoprotection is often underestimated [3]. In this respect, a study comparing subjects with vitiligo and normally-pigmented skin showed that photoprotection is a property of SC in both groups [4] and that a thicker SC acts as a physical barrier to light in an attempt to compensate for the absence of pigmentation and it becomes thicker [2, 5]. In support of this Thomson et al. found that photoprotection in Albino African (AA) skin was similar to minimally pigmented European skin [2]. Moreover, in the most recent report on facial skin pigmentation and photodamage that dissects the relationship of SC basal transepidermal water loss (TEWL), barrier integrity and epidermal barrier repair to SC thickness, it was shown that although AA SC was thicker it was less protective in terms of basal TEWL and skin hydration [6]. These studies offer an insight into the compensation mechanisms that the skin develops in these subjects but the biochemical differences in their thickened SC have not been fully evaluated.

In other studies the superior facial barrier function exhibited by African Americans was shown to be due to be related to a more complete maturation of forearm corneocytes even though their associated levels of SC ceramides were low [7]. However, these corneocyte maturation results are inconsistent with those of Hirao et al. who found no differences on facial SC [8]. Nevertheless, corneocyte surface area is reported to be similar between different ethnicities despite differences in intracellular cohesion [9]. Additionally, one study shows Asian skin has lower NMF.
levels compared with African American and Caucasian skin [10]. As it has previously been shown that pigmentation/ethnicity has no effect on facial barrier skin function we were interested if an ethnic difference in NMF production and corneocyte maturation is present in Black African (BA), Caucasian (C) and AA subjects [6].

Filaggrin (FLG) is a major protein found in the SC and is involved in its barrier and moisturisation properties [11]. The final degradation of FLG gives rise to a group of hygroscopic molecules in the upper SC (stratum disjunctum) responsible for effective SC hydration and its water-holding capacity [12] together with SC plasticisation [13]. These hygroscopic protein breakdown products (free amino acids (FAA) and associated products) together with lactate, urea, inorganic ions and sugars are collectively termed natural moisturizing factor (NMF) [14]. Urocanic acid (UCA) and pyrrolidone carboxylic acid (PCA), two transformed FLG breakdown products are often used as markers for NMF and together with FAA have been used to assess SC moisturisation in different dry skin types [15-18]. Their production by the enzymatic processing of profilaggrin/FLG has been of recent interest with several groups examining the role of calpain -1 (C-1) and bleomycin hydrolase (BH) in the later stages of FLG degradation [17, 19-25].

Takeda et al. first identified BH as an aminoacyl hydrolase in rat and human SC to be involved in the keratinization process [19, 20]. Schwartz et al. also demonstrated that BH was essential for epidermal integrity [21]. Later Kamata et al. identified BH in the upper layers of SC as a late stage NMF generating enzyme by monitoring its citrulline-releasing activity [22].
In contrast, C-1 is a heterodimeric calcium-activated cysteine protease comprised of a large subunit (80kDa) and a small subunit (30kDa). C-1 is known to be present in human and porcine epidermis [23] and has been reported to play an important role in the generation of NMF [24] [25]. It also has a direct role in epidermal differentiation by activation of epidermal transglutaminases [26]. Thus, C-1 is involved not only in the processing of profilaggrin to FLG but also corneocyte maturation [24, 27]. In fact, C-1 inhibitors have been shown to reduce corneocyte envelope (CE) formation by inhibiting the proteolytic processing of transglutaminases [28].

Kamata et al. [29] identified the sequence of FLG degradation by these two enzymes and determined their relationship to peptidyl arginine deiminases (PADs) together with caspase-14. In this sequence of events, arginine residues in FLG monomers are deiminated by PADs and although caspase-14 participates in the processing of deiminated FLG to limited fragments, C-1 degrades them to smaller peptides. BH then eventually degrades these peptides into FAA. However, recent studies have demonstrated that BH but not C-1 activities that are decreased in the SC of aged and sensitive skin, are associated with lower NMF levels and elevated TEWL [30, 31]. Additionally, BH was found to be decreased in parakeratotic but increased in hyperkeratotic, skin disorders [31-33]. Most recently Son et al. has shown that BH and NMF levels but not C-1 and filaggrin expression was decreased in dry volar forearm SC [30].
The induction of CE maturity by transglutaminases is key for barrier function and their phenotypes are assessed microscopically through Nile red lipid staining and involucrin antigen binding [8]. There is a specific distribution pattern of more rigid/polygonal shaped, mature CEs in the superficial layers of SC, whereas, fragile/asymmetrical immature CEs are found predominantly in the deeper layers of SC. Increased levels of immature CEs are known to occur in barrier compromised and dry skin conditions [8, 34, 35]. Moreover, as there appears to be an inverse relationship between FLG degradation and CE maturation we were interested to examine CE phenotypes in different ethnic groups together with the activities of FLG degrading enzymes (C1 and BH) and NMF levels [18].

In normal epidermis, plasminogen is expressed mostly in the basal cell layer of the epidermis but it is expressed suprabasally in compromised skin conditions [36, 37]. Indeed, elevated SC plasmin levels are associated with elevated TEWL [38]. To date there has been no investigation of differences in the activities of this enzyme in the SC of different ethnic groups.

The present study was therefore designed to characterize the ethnic differences in the activities of the FLG processing enzymes (C1 and BH) and the levels of NMF in facial regions of 3 ethnic groups (C, BA and AA) living in the same region with varying degrees of pigmentation and photodamage. Moreover, as there are few studies examining the effect of photodamage on facial SC, we chose to examine cheek and post-auricular skin testing sites in the three different ethnic groups [6, 39, 40]. Also to bring some clarity to the discussion on corneocyte maturity we also
included this measure in our study and plasmin activity was chosen as a marker of barrier damage [8, 34-38].

Materials and methods

Standard D-Squame® tapes, a D-Squame® pressure applicator and D-Squame® disc racks were obtained from Cuderm Corporation (Dallas, US). The Orbital incubator SI50 was purchased from Stuart Scientific (Staffordshire, UK) and the infrared densitometer, model SquameScan™ 850A was obtained from Heiland electronic (Wetzlar, Germany). Sodium dodecyl sulphate (SDS) ACS reagent> 99.0% (436143), ethylene diamine tetra-acetic acid, Triton X-100, acetic acid and Nile red were obtained from Sigma-Aldrich (Dorset, UK). High performance liquid chromatography (HPLC) analytical grade water and acetonitrile were obtained from Fisher Scientific (Hertfordshire, UK). A Waters Symmetry® C_{18} HPLC column (5 µm, 150 x 4.60 mm) (Waters, Milford MA, US) was used for the determination of AMC and a Synergi HPLC column (4 µm POLAR-RP-80A, 150 x 4.60 mm) (Phenomenex, USA) for the determination of PCA. DL-dithiothreitol, trifluoro acetic acid and Tris-HCl (0.1M) were purchased from Fluka (Buchs, Switzerland). Dimethylsulphoxide (DMSO) was obtained from VWR International Ltd (Bedford, UK) and phosphate buffered saline (PBS) tablets were obtained from Oxoid (Hampshire, UK). The primary monoclonal antibody, anti-human involucrin (clone SY5) was purchased from Cambridge Scientific Products (Watertown, MA, USA). The rabbit anti-mouse IgG labelled with fluorescein isothiocyanate (FITC) was purchased from Abcam (Cambridge, UK). Aminomethyl coumarin (AMC) and all fluorogenic peptide substrates were donated by DSM Nutritional Products Ltd. (Kaiseraugst, Switzerland). A water bath was purchased from Benchmark Scientific (New Jersey,
USA) and a Canon 70D 20.2 megapixel camera was obtained from Canon USA Inc. (New York, USA). IMAGEJ™ software was downloaded from the; National Institutes of Health website (Bethesda, MD, US).

Study subjects

The study was a cross-sectional study and was approved by the School of Health Care Sciences Research and Ethics committee (SREC) together with the Medunsa Campus Research and Ethics Committee (MREC) and was conducted in accordance with the Declaration of Helsinki Principles. Written informed consent was obtained from all participants before enrolment. 60 healthy female volunteers, living in Pretoria, South Africa participated in this observational study which took place from the end of November to early December 2013. There were three age-matched groups composed of 20 AA (40.3 ± 2.9 years old), BA (38.2 ± 2.3 years old, Fitzpatrick skin phototype V) and C (44.6 ± 3.1 years old, Fitzpatrick skin phototype II/III) subjects each. The subjects did not apply any dermatological or cosmetic products to their faces for 3 days before expert grading and evaluation of their facial skin. For the 3-day conditioning phase subjects cleansed the face with distilled water in the morning as well as in the afternoon. Before tape stripping the skin was cleaned by gently swabbing with a cotton pad soaked in distilled water at ambient temperature, allowed to dry for 20 min and then acclimatized for 30 min at 21±1°C and 35± 10% relative humidity.
Sample collection and SC protein evaluation

D-Squame® disks with a diameter of 2.2 cm and an area of 3.8 cm² were placed on the right cheek (3 cm vertically beneath the outer edge of the eye) and post-auricular area area opposite to right earlobe). D-Squames were applied with 225g cm⁻² of pressure with a pressure device for 5 sec and then removed by a single stroke movement. In order to minimize variation, the procedure was conducted by the same technician for all volunteers throughout the study. The interval between the tape strippings was 20±5 seconds [41]. The SC protein content of the tape strippings was quantified by infrared absorption measurements at 850 nm with a SquameScan™ 850A. This non-destructive method of SC protein quantification permits the further use of tape stripping’s for biochemical assays and enables normalisation of the SC protein content [42,43]. For SC protein quantification, the following equation was used [42]:

\[ \text{C}_{\text{protein}}[\mu\text{g cm}^{-2}] = 1.366 \times \text{Absorption [%]} - 1.557 \quad \text{Equation 1} \]

Tape strips 1, 4 and 6 were used for the biochemical and morphological studies.

PCA quantification

The fourth tapestrip was used for PCA quantification. Tapes were transferred into a 1.5 ml Eppendorf tubes and extracted at 1000 rpm in 750 µl, 0.1 mol L⁻¹ KOH solutions for 2 h at 32°C with continuous shaking. The alkaline extracts were neutralized with 4.5 µL of perchloric acid (12 M), shaken again for 2 h and filtered through a 0.2 µm membrane. HPLC with UV detection at 210 nm was used to analyse the PCA content of the tape stripping extracts, at room temperature. The volume of injection was 10 µL and the flow rate 0.4 mL min⁻¹. The mobile phase
consisted of 20 mmol L\(^{-1}\) ammonium formate, containing 1.5 mmol L\(^{-1}\) tetrabutylammonium hydroxide and 3% acetonitrile at pH 7.3 [44]. The retention time was 7.5 min. The total SC protein determined by infrared densitometry was used to normalize the PCA amounts.

**Protease activities**

Protease activities were quantified using the methods previously reported [38, 45-47] and the sixth tape strip was used for analysis. Tapes were transferred into 1.5 ml Eppendorf tubes and extracted for 25 min at 25°C and 1000 rpm in 750 µl of 0.1 M Tris–HCl and 0.5% Triton X-100 buffer at pH 7.5. The extracts (250 µl) were combined with 1.25 µl fluorogenic peptide substrates, namely for BH (H-Cit-AMC), C-1 (Suc-Leu-Leu-Val-Tyr-AMC) and for plasmin (MeOSuc-Ala-Phe-Lys-AMC). The mixtures were shaken at 1000 rpm at 37°C. The reaction was stopped after 2 hours by adding 250 µl of acetic acid (1%). The wavelength for emission was 442 nm and for excitation it was 354 nm. The elution was conducted using reverse phase HPLC with a mobile phase of 75% water, 25% acetonitrile, 0.01% trifluoroacetic acid (TFA). The flow rate was 1 mL min\(^{-1}\), the injection volume 20 µl and the retention time of AMC was 6.5 minutes. The total SC protein determined by infrared densitometry was used to normalize activity values for the proteases.

**Corneocyte maturity and surface area measurement**

Corneocyte maturity was assessed by differential Nile red and immunostaining of involucrin for the first tape strip, using a modification of the method described by Mohammed *et al.* [45]. Tapes were transferred to Eppendorf tubes and extracted
using 750 µl of dissociation buffer (2% SDS, 20 mmol L\(^{-1}\) DL-dithiothreitol, 5 mmol L\(^{-1}\) EDTA, 0.1 M Tris HCl buffer adjusted to pH 8). Using a dry water bath, the tubes were heated at 75°C for 10 minutes, centrifuged and washed with dissociation buffer (3 times). The corneocytes were separated as pellets, which were then suspended in dissociation buffer. The suspension was applied on a microscopic slide and dried. Primary monoclonal antibody was added and incubated overnight at 4°C in a humidity chamber. The next day FITC-labelled 1:50 rabbit polyclonal to mouse antibody IgG (H&L) was added and incubated for 1 hour in the dark. The slides were rinsed with PBS and incubated with Nile red for 10 min. The slides were subsequently rinsed with PBS and covered with a slide cover. Fluorescence was measured with a fluorescence microscope equipped with a Canon 70D, 20.2 megapixel camera (2.85 pixels µm\(^{-1}\)). IMAGEJ™ image analysis software was used to analyse the red pixels obtained from the Nile red stained cells and the green pixels from the immunostained cells. The ratio of red:green pixels corresponds to the extent of corneocyte maturity [45]. Nile red stained images of corneocytes were used to measure corneocyte envelope (CE) surface area. The percentage of corneocytes with nuclei in a sample was quantified by the ratio of total number of cells/CE to the total number of nuclei counted. The Nile red stained cell images were further processed using IMAGEJ™ to analyse the cell surface area.

**Statistics**

All data were collected in Microsoft Excel 2011 and expressed as mean ± SEM, with differences between means analysed with SPSS software version 22 (IBM®, New York, USA). For all the parameters two body sites were first analysed together and
later analysis was performed for each site separately. The data were also pooled between ethnic groups (subjects with pigmentation = BA and C) and AA to investigate structural differences between facial areas. Pair wise group comparisons were performed using the Student’s paired t-test (normally distributed), analysis of variance (ANOVA) and a non-parametric Mann-Whitney U test with a two-tailed test of significance. Differences where p<0.05 were considered to be statistically significant.

Results

PCA measurements

AA subjects had a significantly higher amount of PCA compared with the BA and C subjects (Figure 2). In all subject groups the differences between the photoprotected post-auricular test sites were not statistically significant. The amount of SC removed and the SC depth probed was similar in all three groups and no significant differences were observed for cumulative tape stripping for tapes 1 to 4 and tapes 1 to 6 (data not shown).

Protease activity measurements

AA subjects had the highest BH activity in both sites compared with the BA and C subjects. The BH activity was significantly higher on photoexposed areas for BA and C subjects (Figure 3). Interestingly, there was no significant difference in BH activity between BA and C subjects in the photoprotected sites. The AA subjects had the highest C-1 activity in the cheeks but had significantly lower activity in the PA site in comparison with the BA and C subjects. In the subject groups with normal
pigmentation, C-1 activity is lower in the BA subjects. Photoprotected sites show elevated activity of the enzyme in all three ethnicities. (Figure 4). The AA and C subjects had significantly higher plasmin activity on their cheeks, whereas it was only numerically elevated for the BA subjects (Figure 5).

**CE maturity**

Differential Nile red and involucrin immunostaining is shown in Figure 6. Differences in the maturity of corneocytes were observed for the different ethnic groups. Corneocyte maturity was significantly lower for the AA subjects compared with the other two groups. The subjects with normal pigmentations (BA and C) showed no significant difference in the corneocyte maturity ratio. In addition, the photoprotected sites generally had more mature CEs than the cheeks in all three groups but this was only significant for the C cohort (Figure 7). A similar trend was observed between values for corneocyte surface area and ethnicity. The AA subjects had corneocytes with the smallest surface areas compared with the other two groups and there was no significant difference in corneocyte surface area between the two groups with normal pigmentation (Figure 8). A 10-fold higher value for corneocytes with nuclei was observed for the AA group compared with the other groups irrespective of facial site. There was no difference in the percentage of cells with nuclei observed between the groups with normal pigmentation in both sites (Figure 9).
Discussion and Conclusion

Ethnic differences in SC properties have been reported previously, particularly for barrier function [6-10, 48-50]. However, there is a paucity of data on the differences in NMF levels, activities of BH, C-1 and plasmin together with corneocyte maturation in different ethnic groups.

We found that the SC of AA subjects contained higher PCA levels irrespective of sun exposure. This was surprising, as previously Voegeli et al. had reported that this group of subjects had the lowest skin hydration values and the highest facial dryness and roughness compared to the other groups [6]. Moreover, for other anatomic sites increased skin dryness is usually associated with reduced NMF levels [15, 30]. This suggests that the increased PCA levels are not compensating for the reduced skin hydration and that other SC abnormalities are responsible for this [6]. Nevertheless, an increased NMF level in BA subjects is also associated with increased hydration compared with C subjects [6]. Both of these findings of increased SC NMF levels may be related to the increased SC thickness of the AA and BA subjects relative to a thinner SC of C subjects for the photoexposed site [6]. FLG may simply have a greater length of time to be completely degraded in these subjects. Degradation of FLG has been reported to be compromised on photodamaged facial and forearm skin sites [18, 51]. Nevertheless, the levels of PCA in the BA and C subjects were not different which is consistent with earlier reports [10].

UV radiation also causes significant changes in the mechanical properties of the SC [52]. The corneocyte intercellular junctions are the gate-keepers of barrier function and any damage to them causes mechanical challenges to SC function. Rupture of
the corneodesmosomes occurs at very low strains and under conditions of low humidity [53-55]. As the SC is thicker and drier in the AA subjects increased levels of NMF may be required to plasticise keratin to ensure that corneodesmosomal rupture does not occur prematurely. Jokura et al. highlighted the important role of NMF as a plasticizer of the SC rather than simply being a humectant [13].

To understand better the generation of NMF in the SC in these three ethnic groups we studied the activities of BH and C-1. The active forms of these proteases are mainly localized in the upper layers of the SC and their activity was shown by others to be critical in the final stages of FLG degradation [19-22, 24, 27]. Increased activity of BH was observed in UV-exposed photodamaged sites of all subject groups but the highest activities were observed in the SC of the AA subjects. An increase in activity of BH is associated with higher PCA concentrations in both sites studied. Nevertheless, the differences in the activities of C-1 in SC for the various groups were more complex. For the pigmented subjects higher activities were observed in the post-auricular sites compared to the cheek. In contrast, the activity profile of BH and concentration of PCA was observed highest in cheeks. However, despite having the highest level of NMF on both pre- and post-auricular sites the activity of C-1 was minimal on the post-auricular site in the AA subjects and was comparable on the pre-auricular testing site versus the post-auricular site of C subjects suggesting that the activity of this enzyme is not rate limiting on the face in these subjects. These results contrast with those previously reported that show decreased BH but not C-1 mass levels in forearm dry skin [30].
Equally, although not examined previously for its effects on FLG degradation, plasmin activities were elevated with increased PCA levels. Photodamage is expected to cause a feedback mechanism to upregulate the proteases involved in FLG degradation thereby improving barrier activity by producing more NMF. Although we did not quantify UCA levels, they are expected to be elevated in the SC of AA subjects much like that of PCA, and UCA is known to protect against UV irradiation [56]. Nevertheless, the increased SC thickness and NMF levels in AA subjects may be a mechanism to protect against UV irradiation when melanin is absent. These findings are also, in line with reports of hyperkeratosis in patients with vitiligo offering increased photoprotection [4].

Generally, more mature corneocytes were observed on the post-auricular site but this only reached statistical significance in the C subjects. Increased quantities of premature corneocytes observed in the AA group on both testing sites are indicative of poor barrier function and delayed desquamation [18]. Indications of improper keratinocyte differentiation and SC maturation were also observed in the AA subjects where corneocytes retained nuclei (parakeratosis). However, there was no differences in CE maturity between the BA and C subjects consistent with the facial study of Hirao et al. but inconsistent with the forearm study of Muizzuddin et al. [7, 8]. This may reflect the variation in subjects, climatic conditions (adaptation), and the different methodologies used but is most likely as a result of different body sites tested. The reduction in CE maturation is probably related to the higher incidence of dry skin, which is associated with decreased transglutaminase activity, in the AA subjects and the cheeks of the other two cohorts [6, 35].
Upon comparison between the previously reported skin bio-instrumental data [6] for this study with our biochemical results, the higher water loss and inferior skin capacitance in the AA subjects may be an outcome of poor SC maturation. It appears that the increased SC thickness in the AA subjects does not compensate for elevated TEWL levels. Equally, increased levels of PCA in the AA cohort also do not compensate for the inferior skin hydration. These results highlight the vital importance of corneocyte maturation for a competent skin barrier. Nevertheless, the thickened SC in AA subjects may be a compensation mechanism for the absence of skin pigmentation, providing vital UV protection. It may be considered as an epigenetically-driven alternative adaption to melanogenesis but it has a negative impact on barrier function.

In conclusion, ethnic differences in levels of NMF, activities of BH, C-1 & plasmin and corneocyte maturation occur. Considering the different groups, the AA cohort always had higher activities of BH and plasmin, higher PCA levels and more immature corneocytes compared with the other two ethnic groups. However, the difference in values for C-1 activities is more complex. In addition, this study also demonstrates that photodamage of the cheek causes an increase in the activities of BH and plasmin. Equally, on the sun-exposed site the AA group had the highest C-1 activities but demonstrated the lowest C-1 activity on the photoprotected site. These results suggest that C-1 is not the rate-limiting factor for the processing of FLG but BH is highly associated with the final steps in NMF formation. Moreover, it is suggested that both elevated plasmin and BH activities are indicators of epidermal photodamage.
References


Figure 1: Schematic representation of NMF generation and the plasminogen pathway. The keratohyalin granules store profilaggrin which is dephosphorylated by phosphatases and the proteolytically cleaved to produce free filaggrin (FLG). FLG monomers bind to keratin filaments causing them to aggregate to form keratin tonofilaments in lower layers of the SC. FLG undergoes further degradation by caspase-14, C-1 and BH to form free amino acids and eventually PCA and UCA. These terminal breakdown products of FLG together with lactate, urea, inorganic ions and sugars are collectively termed NMF. The cornified envelope (CE) is shown as dotted lines.
Figure 2: PCA levels of photoexposed cheek and photoprotected post-auricular test sites. Data are mean ± SEM, n = 20 per group, * p < 0.01, ** p < 0.001, n.s. not significant.
Figure 3: BH activity of photoexposed cheek and photoprotected post-auricular test sites. Data are mean ± SEM, n = 20 per group, * p < 0.01, ** p < 0.001, n.s. not significant.
Figure 4: C-1 activity of photoexposed cheek and photoprotected post-auricular test sites. Data are mean ± SEM, n = 20 per group, * p < 0.01, ** p < 0.001, n.s. not significant.
Figure 5: Plasmin activity of photoexposed cheek and photoprotected post-auricular test sites. Data are mean ± SEM, n = 20 per group, ** p < 0.001, n.s. not significant.
Figure 6: Differential immunostaining of corneocytes from cheek (tape stripping 1), Nile red staining (left column) and involucrin antibody immunostaining (right column). The nuclei inside the corneocytes (dark stained) and CE as loosely bound aggregates (green) in involucrin immunostaining, (2.85 pixels/µm), scale, bar = 100 µm.
Figure 7: Maturity of corneocytes originating from photoexposed cheek and photoprotected post-auricular test sites. Data are mean ± SEM, n = 20 per group, ** p < 0.001, n.s. not significant.
Figure 8: Corneocyte surface area between photoexposed and photoprotected sites. Data are mean ±
SEM, ** p < 0.001, * p < 0.01, n.s. not significant.
Figure 9: Percentage of nucleated cells between photoexposed and photoprotected sites in three different ethnicities. Data are mean ± SEM, ** p < 0.001, * p < 0.01, n.s. not significant.