**A proof-of-principle study comparing barrier function and cell morphology in face and body skin**

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**Objective:**

The purpose of this work was to investigate *in vivo,* the differences between two anatomical skin sites, facial cheeks and volar forearms, as a function of consecutive tape stripping. This article investigates the changes in corneocyte size as a function of depth after tape stripping facial cheeks and volar forearms. Skin barrier function measured as trans epidermal water loss (TEWL) and corneocyte size were determined as a function of stratum corneum (SC) depth at both anatomical sites. The current investigation compares the changes in cheek and arms corneocyte size with depth and correlates these changes with the TEWL barrier function after tape stripping challenge. To our knowledge this is the first published comparison based on these parameters. The initial (24 hours post stripping) and long-term SC barrier recovery (4 weeks post stripping) in facial skin after tape stripping challenge were reported previously (Gorcea et all, 2012, 2013).

**Methods:** TEWL measurements were used to analyze *in vivo* the forearm and facial skin sites before and during the tape stripping challenge. Optical microscopy and image analysis techniques were employed to characterize corneocyte size as a function of skin depth (tape strip number) for both anatomical sites.

**Results:** TEWL increased significantly from baseline with sequential tape stripping in both skin sites. The volar forearms required approximately three times as many tapes to "damage" the SC barrier (arbitrarily defined as twice baseline TEWL) compared to facial cheeks demonstrating significant differences in barrier properties between cheeks and forearms ($p<0.05$). Corneocyte size decreased significantly with depth for both sites ($p<0.001$). Facial corneocytes were significantly smaller compared to the corneocytes from the forearms.

**Conclusions:** The results demonstrate several key differences between facial and body skin stratum corneum organization and barrier function, and relate these changes to differences in corneocyte size, morphology and SC thickness (number of cell layers).

**Key words**: **Cheeks, forearms, SC barrier damage, TEWL, corneocyte size**

## Introduction

There are significant differences in SC barrier across the body, which reflect the unique function of each anatomical location (Tagami, 2008). Facial cheek and volar forearms are the two anatomical skin sites where most therapeutic and cosmetic treatments are applied. In vivo measurements of TEWL demonstrate significant variations between facial and body skin sites reflecting differences in thickness, maturity of corneocytes and the degree of structural integrity of the SC (Tagami, 2010). It has been reported that facial SC has less cell layers (10 ±2) than the forearms (16 ±4) (Kobayashi and Tagami, 2004) and a higher TEWL compared to forearms (Ya, Xian, 1999, Breterniz 2007, Machado et all, 2010). This indicates that the poorer barrier function of facial skin is due to its thinner structure and faster cell turnover which does not allow proper maturation of the corneocytes (Hirao et al., 2001; Kashibushi et al., 2002). It is reported that corneocyte size is a consequence of enzymatic activity in stratum corneum, cell renewal and proliferation (Hirao at al., 2001; Kashibuchi at all., 2002). Facial SC is thinner with smaller corneocyte size, faster proliferation and desquamation rate than forearm SC (Marrakchi and Maiback, 2007).

In the past, ATR-FTIR spectroscopic in vivo studies demonstrated regional variations in the molecular composition of the SC. The IR peak intensities of SC water and lipids varied as function of anatomical site, the forearm skin sites contained lower lipid content compared to face and at the surface of the SC more ordered lipids were found in the forearms compared to face (Brancaleon, at all, 2001). There are few published studies comparing the barrier function, organization and composition of facial and forearm skin after an external challenge. This report describes a pilot proof of principle small study which addresses some of the questions regarding the differences between facial cheeks and volar forearms in terms of their stratum corneum barrier response to the tape stripping challenges.

## Methods

### Volunteer recruitment

Five healthy panelists, 3 females and 2 males (30-54 years old, mean 43 years old, Caucasians) participated in this study. Each volunteer signed a participant consent form. The research protocol was conducted according to Camden and Islington Research Ethics Committee approval (Reference 06/Q0511/26). Subjects were instructed not to apply any cosmetic product to their faces and forearms or to shave for 24 hours prior to the study.

### Tape Stripping

Adhesive tape strips (3M™ polyester tape blue plastic core 8901, MN, US) were used for the *in vivo* study. Tape stripping experiments were performed on the center part of the facial cheeks (left side), and the center part of volar forearms (left side), with no previously applied moisturizing treatment or washing regimen. Each tape strip was firmly pressed on the skin using a D-Squame pressure device (CuDerm Corporation, Dallas TX, USA). Tape strips were collected until TEWL values increased 100% from baseline, which for cheeks required 5 or 6 tapes depending on the individual. In the case of volar forearms, 15-20 tapes were required until TEWL values increased 100% from baseline. Tape stripping and TEWL measurements were performed at a temperature of 21±1°C and a controlled relative humidity of 30 ± 10 %. Each panelist was left in the room for 15-20 minutes before measurements to equilibrate with the environmental conditions and minimize the activity of sweat glands.

### TEWL

TEWL was measured on the exact same site that was tape stripped on the left cheek and left volar forearms of 5 volunteers. TEWL was measured with a closed chamber condenser instrument (AquaFlux AF -200, Biox, London, UK). The TEWL measurements were performed at baseline, and after each tape strip was removed. Each skin site (cheek and forearm) was left for one minute to allow SC water diffusion to equilibrate. Data were processed with the AquaFlux software v.6.2 (Biox, London, U.K.). The results were expressed as the TEWL absolute value (g $m^{-2}h^{-1}$) as a function of tape strip number.

### Corneocyte Surface Area

Corneocytes were collected from all tapes. Six tapes were collected from each volunteer for the facial cheeks, while for the volar forearms twenty tapes were collected. The tapes were immersed in hexane (HPLC grade) followed by sonication for 15-20 minutes. The methodology was described in detail in our previous work (Gorcea at all, 2013). Corneocytes were analyzed using optical microscopy with bright field microscope at 20X magnification (Olympus BX-50, Olympus Corporation, PA, USA) equipped with a digital camera with software (Spot Advance, Spot Flex, vs 4.6, USA). Image analysis was used to calculate the corneocyte surface area for cheeks and forearms. A mean value of the corneocytes surface area was calculated for facial cheeks from 150 measured corneocytes for each tape layer from 5 volunteers (30 corneocytes per tape per each volunteer).

## Statistical analysis

Statistical analysis was carried out with Sigma Stat (Systat Softare Inc San Jose, CA, USA). Data values of TEWL, % barrier damage and corneocytes size are presented as means and standard deviation (SD). In the case of two groups comparision a student t-test was calculated. The significance level was set at $p < 0.05$.

### Results and discussions

### TEWL: Cheeks and Volar Forearms

It was reported previously, that facial cheek SC is thinner than other anatomical sites (Tagami, 2010). Thus, fewer tape strips are required to increase baseline TEWL and damage the skin barrier. In the current work, SC barrier was considered damaged when TEWL reached twice the baseline. Facial cheek requires only 5 to 6 tapes to reach twice the TEWL from baseline, thus to damage the skin barrier. TEWL measurements were conducted before tape stripping and after each tape strip was removed.

Figure 1 illustrates the mean absolute values of TEWL in facial cheeks and arms as a function of SC depth from five subjects. The average baseline TEWL in volar forearms (10.45 $g/m^2/h$) is significantly lower than the average baseline TEWL in facial cheeks (19.63$g/m^2/h$) ($p<0.05$). This reflects the poorer barrier function in facial skin compared to arms. In the case of forearms, each panelist reached the damaged barrier endpoint after a different number of tapes (between 18-20 tapes). Thus, the TEWL after the last tape was considered as the measurement point where each panelist had a “damaged” skin barrier.This is different than the cheek sites where all panelists reached the damaged barrier endpoint at tape 6. This is due to the differences in SC thickness between the skin sites, volar forearms being reported to have thicker SC compared to the cheek area (Ya- Xian et al., 1999). Clearly SC thickness and the number of cell layers play an important role in barrier damage differences between cheeks and arms. It is noted that the absolute values of TEWL are significant different at baseline as facial cheeks have significantly larger TEWL at baseline (19.63 g/m2/h) compared to arms TEWL (10.45 g/m2/h). Another important factor to consider is the difference in cell cohesion between the two anatomical sites which could contribute to a different response to the tape stripping stress.

In both skin sites, the TEWL increased after successive tape stripping, thus damaging barrier function. In both skin sites tape stripping stress damaged SC barrier function, however cheek barrier function damaged faster compared to arms proving significant differences in terms of SC barrier damage between the two anatomical skin sites.

### Barrier Damage: Cheeks and Volar Forearms

The mean percent barrier damage after tape stripping for cheeks and arms is plotted in Figure 2. The percent barrier damage was calculated individually for each volunteer after each tape strip, and afterwards the mean percent barrier damage was calculated (n=5; mean ±SD). The SC barrier was considered damaged when TEWL increased 100 % from baseline.

The mean percent barrier damage in facial cheeks for all subjects increased significantly from baseline with each tape stripping ($p<0.05$).

The mean percent of barrier damage from baseline in facial cheeks was 171.77% ± 39.83, while the barrier damage for the arms was 86.47 % ± 20.58, consistent with our previously reported results (Gorcea at all, 2013). The percent barrier damage for the volar forearms is significantly lower compared to the facial cheeks ($p<0.05$). This is an indication that these two anatomical sites are very different in terms of their response to tape stripping. In cheeks, the barrier damage was induced after tape 6, while for arms occurred between tapes 18-20. The volar forearms required approximately three times more tapes to damage the SC barrier (twice baseline TEWL) compared to facial cheeks demonstrating significant differences in barrier properties between cheeks and forearms ($p<0.05$).

## Corneocyte size depth profile: facial cheeks and volar forearms

### Corneocyte size in cheeks

Figure 3 shows changes with depth in the corneocyte surface area in facial cheek and volar forearms sampled by successive tape stripping. The average surface area of corneocytes from facial cheeks was calculated from 150 measured corneocytes for each tape for 5 volunteers (30 corneocytes per tape per each volunteer). In this study, the average corneocyte size (surface area) at the surface of human facial cheeks from the 5 volunteers was 754.68 ± 126 $\mu m^2$**.** This is in line with the literature (Corcuff and Leveque, 1988; Kashibuchi et al., 2002; Kobayashi and Tagami, 2004; Voegeli et al., 2007a; Tagami, 2008; Machado et al., 2010). The average corneocyte size in facial cheek decreased with depth and was significantly smaller with each succesive tape compared to the baseline ($p<0.05$).

To correlate changes in corneocyte size with depth and barrier function, TEWL measurements were conducted before and after each tape strip. TEWL increased with tape stripping and is significantly higher compared to the baseline from tape 2 until tape 6 ($p<0.05$) as reported earlier in this work.

Previously it was reported that skin anatomical sites (such as facial skin) with higher TEWL values at baseline are generally associated with smaller corneocytes (Rougier, et al., 1988; Kabayashy and Tagami, 2008; Machado et al., 2010). Our study results support this fact. TEWL values followed an inverse trend with corneocyte size as it was previously reported in our early work (Gorecea at all, 2013). This study demonstrates that corneocyte size decreased with SC depth, while TEWL increased as a result of tape stripping in both skin sites.

**Corneocyte size in volar forearms**

As is shown in Figure 3, the average corneocyte size for the forearms was 1000.18 ± 164 $\mu m^2$ at baseline, which agrees with the literature (Plewig et al., 1970; Corcuff and Leveque, 1988; Rougier et al., 1988; Voegeli et al., 2007b; Machado et al., 2010; Mohammed et al; 2011). The average surface area of corneocytes harvested from volar forearms was calculated from 150 measured corneocytes for each tape for 5 volunteers (30 corneocytes per tape per each volunteer). The average corneocyte size in the volar forearms decreased significantly compared to the baseline, after each tape strip ($p<0.05$). After 20 tapes the corneocyte size was 700.40 ±122 $\mu m^2$ (which is similar to the corneocyte size reported for the surface layers of the facial cheeks). These results show that it takes about 3 times more tapes for volar forearms to reach the same degree of damage as facial cheeks. This is due to the difference in SC thickness between the two skin sites.

The current work proves a significant decrease in corneocyte size with depth in human facial cheeks and in volar forearm. The results are in line with the previously reported literature (Rougier at al., 1988; Ya-Xian et all., 1999; Machado et al., 2010, Gorcea at all, 2013). In both skin sites, the TEWL increased after successive tape stripping, thus damaging barrier function. In both skin sites tape stripping stress damaged SC barrier function, however cheek barrier function damaged faster compared to arms proving significant differences in terms of SC barrier damage between the two anatomical skin sites. For both skin sites, as the number of cell layers decreases with tape stripping, corneocyte size decreases progressively and significantly (Figure 3), while TEWL increases significantly with SC depth (Figure 1). Corneocyte size for cheeks and arms are significantly smaller from tape 1 (surface of SC) compared to the last tape ($p<0.001$).

## Corneocyte Morphology

In terms of their morphology, the corneocytes collected from the facial cheeks of all the subjects, appeared mostly flat with pentagonal, hexagonal or irregular forms. Some cells had rough surfaces, while others appeared smooth. The morphological profile of the corneocytes from facial cheeks was similar at all depths for each tape strip. Morphologically, the corneocytes collected from the volar forearms of all volunteers were similar to the facial cheeks, having mostly irregular shapes. The morphological profile of the corneocytes from volar forearms was similar at all depths for each tape strip. It is known that corneocyte size is a consequence of enzymatic activity in stratum corneum, cell renewal and proliferation (Hirao at al., 2001; Kashibuchi at all., 2002).

The changes in corneocyte surface area with depth in facial cheek and volar arms of a representative volunteer (43 years old, female) are illustrated in Figure 4. The upper images represent the corneocytes collected from the cheek, while the lower images represent the corneocytes obtained from the arms. All corneocytes images were processed via image analysis. At baseline the size of the corneocytes in facial cheeks were significantly smaller than the forearms ($p<0.05$). Corneocyte size decreased with depth for both sites. Changes in the cell size from tape 1 (considered baseline) until tape 6 are evident. For this subject, the corneocyte surface area decreased from 780±12 $\mu m^2$ at baseline to 623±20 $\mu m^2$ after tape 6.

Figure 4 shows the differences in depth between the corneocyte size from the volar forearms for several representative tapes.The images were taken from the same volunteer (43 years old, female) as for the cheeks, and processed via image analysis. The corneocyte size decreased with depth in forearms. Due to the large number of tapes collected from the volar forearm of this representative volunteer corneocytes images from only a few tapes were selected to capture the changes in cell size with depth (e.g. tape 1, 2, 5, 10, 15 and 20). Corneocyte size for this volunteer decreased form 1100±12 $\mu m^2$ at baseline to 650±20 $\mu m^2$ after tape 20.

## Conclusions

The results demonstrate several key differences between facial and body skin stratum corneum organization and barrier function, and relate these changes to differences in corneocyte morphology, size with depth and SC thickness (number of cell layers) and corneocytes maturation. As it was reported in our previous work, sebum lipids may play a role in barrier function after tape stripping stress especially in facial cheeks (Gorcea at all., 2012).

However, cheek SC cannot provide a robust barrier function, in part, due to the small corneocytes and a thinner SC containing fewer number of cell layers. This study demonstrated a significant decrease in corneocyte size with depth in human facial cheeks, as less mature corneocytes are stripped from the deeper layers of SC. The volar forearms followed the same trend. This work proves that at SC surface, the number of cell layers of cheeks was statistical significant smaller compared to arms demonstrating thinner SC in cheeks. With SC depth, the number of cell layers of cheeks (thus SC thickness) decreased significantly compared to arms for each corresponding tape. For both skin sites, as the number of cell layers decreases with depth, corneocyte size decreases significantly, while TEWL increases significantly with SC depth indicating damage of SC barrier function. However, SC barrier damage was significantly more pronounced in cheeks than arms in part due to thinner SC in cheeks, smaller number of cell layers and smaller corneocyte size. It is important to note that SC sebum lipids and intercellular lamellar lipids organization play an important role in the recovery of SC cheek and arm barrier function, facial cheek SC demonstrating faster recovery after stress promoting accelerated barrier repair for the intercellular lipids and sebum lipids (Gorcea at all, 2013).

The current work demonstrates that by combining *in vivo* TEWL measurements, successive tape stripping and corneocyte characterization via image analysis it is possible to investigate SC barrier function at the phenomenological, compositional, and organizational level in facial cheek and forearms. Our study results prove that the differences between facial SC and body skin are significant in terms of skin's barrier response to tape stripping challenges. Such differences between facial SC and body (arms) skin are substantial most likely due to skin's ability to respond to stress and environmental changes. Such differences in SC barrier function between face and arms may help customize the design of therapeutic or cosmetic topical treatments to address specific skin conditions and characteristics specifics for each site. These findings may be important in understanding skin permeability and barrier function in healthy skin and compromised skin conditions.

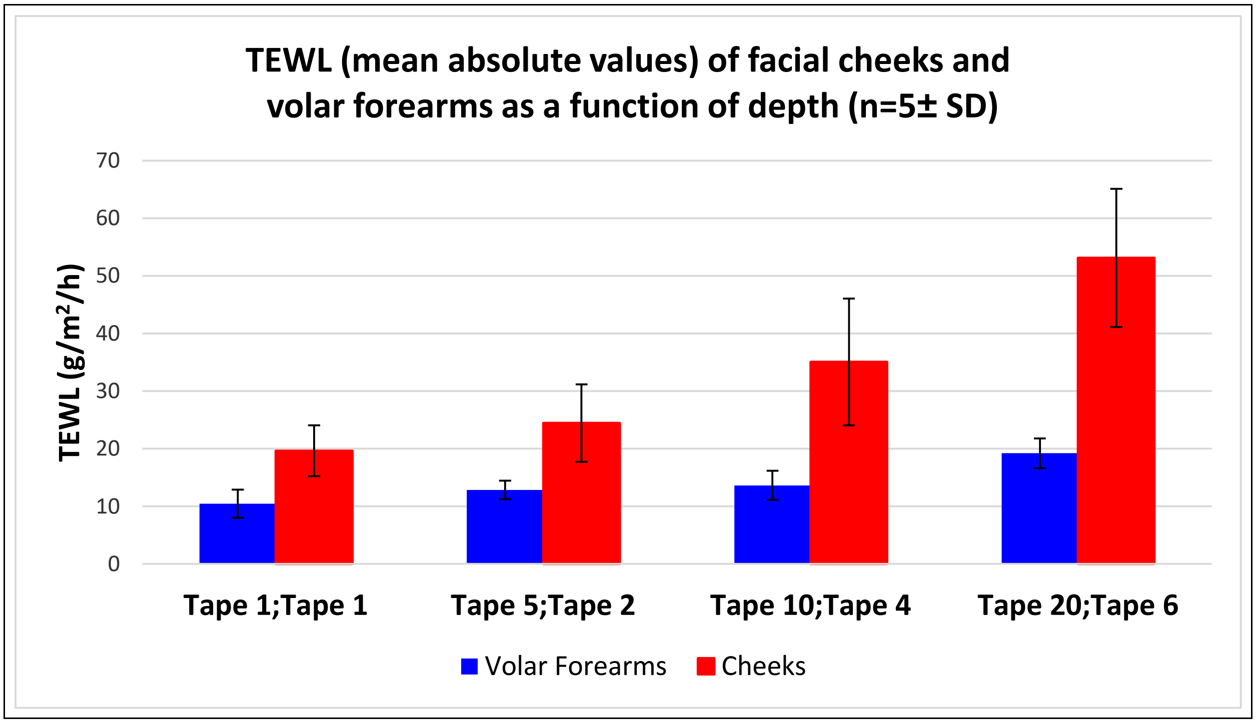


Figure 1. TEWL (mean absolute values) in human facial cheeks and volar forearms as a function of depth from 5 panelists (mean± SD)

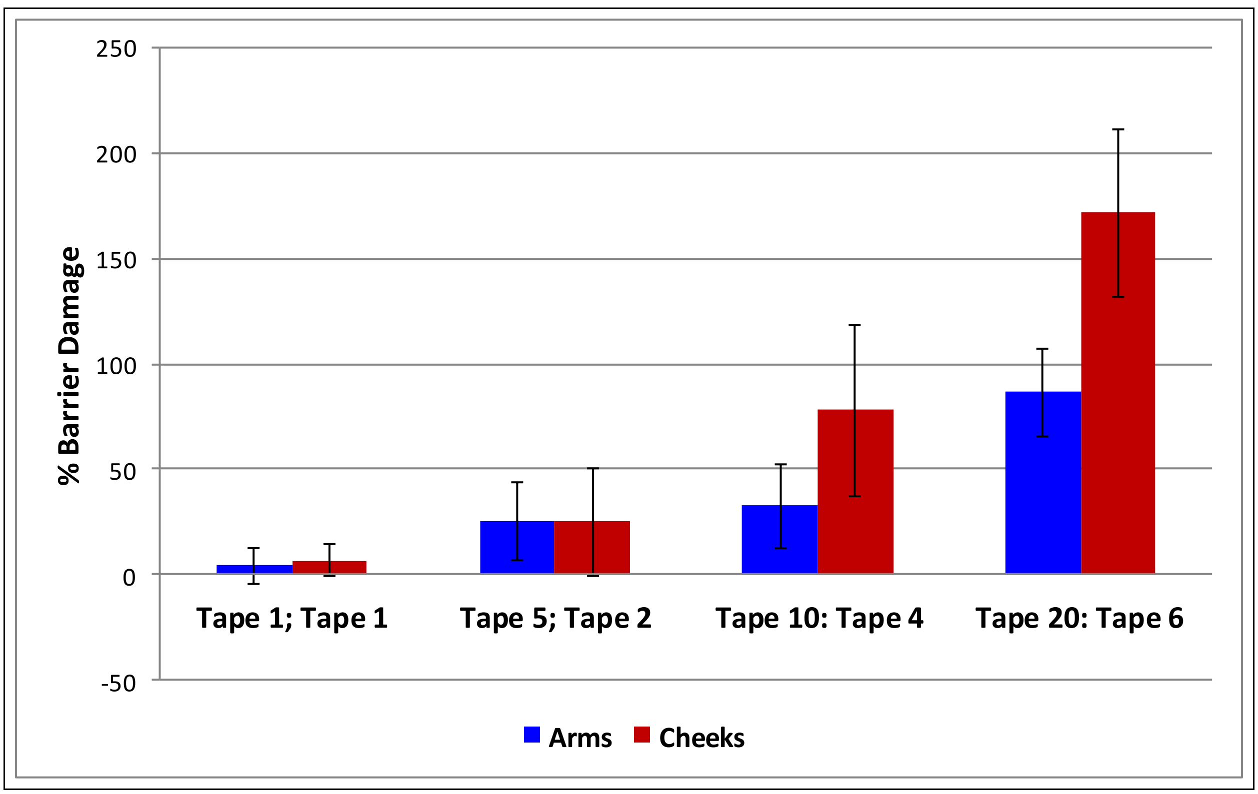


Figure 2. Percent barrier damage in facial cheeks and volar forearms (n=5; mean ±SD).

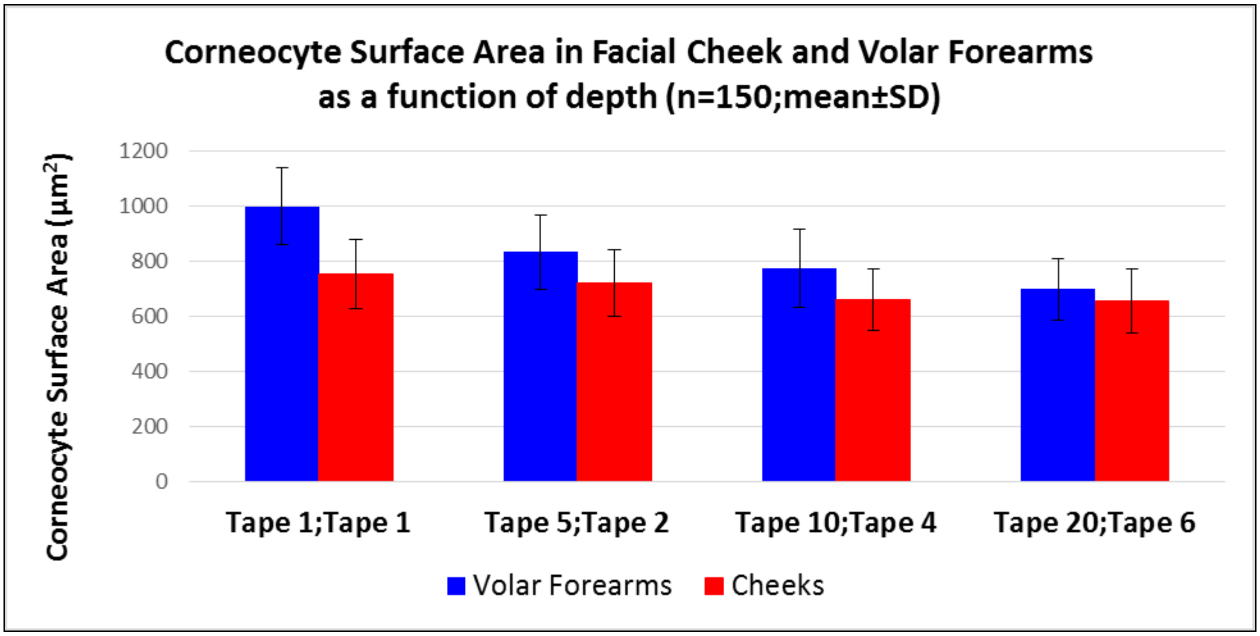


Figure 3. Corneocyte surface area (n=150; mean + SD) in human facial cheeks and volar forearms as a function of depth from 5 panelists.

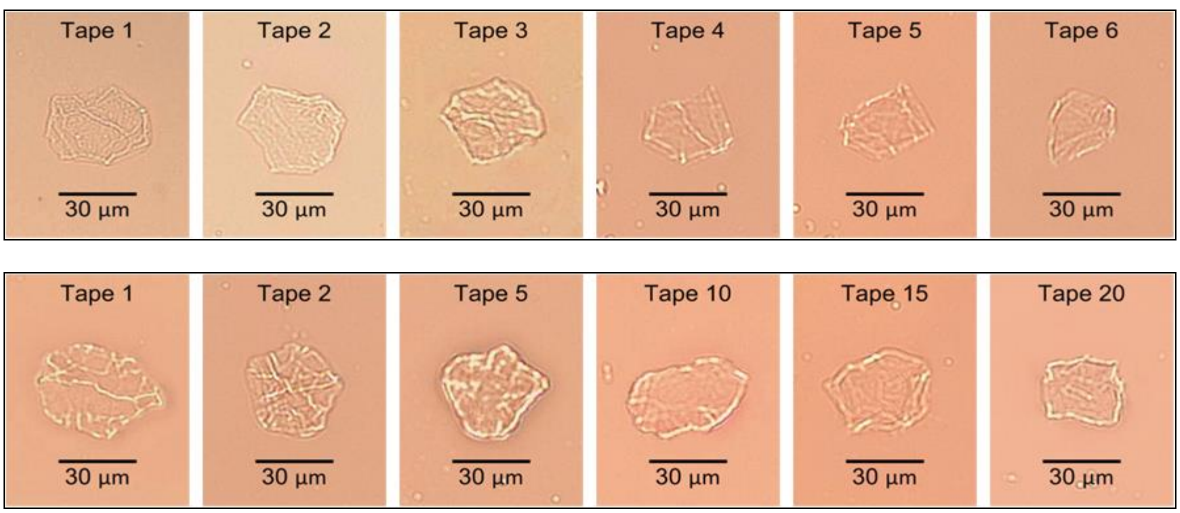


Figure 4. Changes with depth in the corneocyte size in facial cheek (upper images) and volar forearms (lower images) from a single volunteer (43 years old)

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