Characterisation of superficial corneocytes in skin areas of the face exposed to prolonged usage of respirators by healthcare professionals during COVID-19 pandemic

Evora, Ana; Abiakam, Nkemjika; Jayabal, Hemalatha; R Worsley, Peter; Zhang, Zhibing; Johnson, Simon; Adams, Mike; L Bader, Dan

DOI:
10.1016/j.jtv.2023.02.007

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):
Evora, A, Abiakam, N, Jayabal, H, R Worsley, P, Zhang, Z, Johnson, S, Adams, M & L Bader, D 2023, 'Characterisation of superficial corneocytes in skin areas of the face exposed to prolonged usage of respirators by healthcare professionals during COVID-19 pandemic', *Journal of Tissue Viability*, vol. 32, no. 2, pp. 305-313. https://doi.org/10.1016/j.jtv.2023.02.007

Journal of Tissue Viability xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Tissue Viability

journal homepage: www.elsevier.com/locate/jtv

Characterisation of superficial corneocytes in skin areas of the face exposed to prolonged usage of respirators by healthcare professionals during COVID-19 pandemic

Ana S Évora [a,*], Nkemjika Abiakam [b], Hemalatha Jayabal [b], Peter R Worsley [b], Zhibing Zhang [a], Simon A Johnson [a], Michael J Adams [a], Dan L Bader [b]

[a] School of Chemical Engineering, University of Birmingham, Birmingham, UK
[b] School of Health Sciences, University of Southampton, Southampton, UK

ARTICLE INFO

Keywords:
Personal protective equipment (PPE)
COVID-19
Corneocytes
Desmoglein-1
Cornified envelope
Corneodesmosomes

ABSTRACT

Introduction: During the COVID-19 pandemic healthcare workers (HCWs) have used respiratory protective equipment for prolonged periods, which has been associated with detrimental effects on the underlying skin. The present study aims to evaluate changes in the main cells (corneocytes) of the stratum corneum (SC) following prolonged and consecutive use of respirators.

Methods: 17 HCWs who wore respirators daily during routine hospital practice were recruited to a longitudinal cohort study. Corneocytes were collected via tape stripping from a negative control site (area outside the respirator) and from the cheek which was in contact with the device. Corneocytes were sampled on three occasions and analysed for the level of positive-involucrin cornified envelopes (CEs) and the amount of desmoglein-1 (Dsg1), as indirect measurements of immature CEs and corneodesmosomes (CDs), respectively. These were compared to biophysical measurements (Transepidermal water loss, TEWL, and SC hydration) at the same investigation sites.

Results: A large degree of inter-subject variability was observed, with maximum coefficients of variation of 43% and 30% for the level of immature CEs and Dsg1, respectively. Although it was observed that there was not an effect of prolonged respirator usage on the properties of corneocytes, the level of CDs was greater at the cheek than the negative control site ($p < 0.05$). Furthermore, low levels of immature CEs correlated with greater TEWL values after prolonged respirator application ($p < 0.01$). It was also noted that a smaller proportion of immature CEs and CDs was associated with a reduced incidence of self-reported skin adverse reactions ($p < 0.001$).

Conclusions: This is the first study that investigated changes in corneocyte properties in the context of prolonged mechanical loading following respirator application. Although differences were not recorded over time, the levels of CDs and immature CEs were consistently higher in the loaded cheek compared to the negative control site and were positively correlated with a greater number of self-reported skin adverse reactions. Further studies are required to evaluate the role of corneocyte characteristics in the evaluation of both healthy and damaged skin sites.

1. Introduction

To undertake clinical commitments during the COVID-19 pandemic, healthcare workers (HCWs) were required to wear personal protective equipment (PPE) in order to reduce the transmission of the infection in different care settings [1]. In the context of PPE, respiratory protective equipment (RPE) is particularly essential for avoiding airborne particle transmission, involving a filtration level of 95–99% via a respirator that is tightly fastened to the face in order to create a seal. However, the prolonged use of such devices can affect skin health, resulting in a

Abbreviations: BMI, Body mass index; CD, Corneodesmosome; CE, Cornified envelope; CV, Coefficient of variation; Dsg1, Desmoglein-1; HCW, Healthcare worker; INV, Involucrin; PPE, Personal protective equipment; PU, Pressure ulcer; RPE, Respiratory protective equipment; SC, Stratum corneum; TEWL, Transepidermal water loss.

* Corresponding author.
E-mail address: a.s.m.m.evora@bham.ac.uk (A. S Évora).

https://doi.org/10.1016/j.jtv.2023.02.007
Received 6 November 2022; Received in revised form 27 January 2023; Accepted 10 February 2023
Available online 11 February 2023

variety of problems ranging from erythema, acne, dermatitis, and skin breakdown in the form of pressure ulcers (PUs) [2,3]. These adverse reactions are the result of non-uniform pressure, shear, and frictional forces at the skin–respirator interface [4], together with changes in the microclimate, which includes increased moisture and temperature from excess sweating due to mental and physical stress [5]. Indeed, moisture is known to increase skin friction [6,7] and has been attributed to an increase in the contact area due to its plasticising action [6,7] that reduces the stiffness and strength of the skin layers [8,9]. This is a direct result of compression of the surface topographical features of skin, which may also become smoother with increasing hydration [8,9].

Han et al. analysed the biophysical responses to medical mask and respirator application, showing consistent lower values of skin hydration in those regions of interest (~ 56-62 a.u.) compared to uncovered areas (~ 68–79 a.u.) [10]. In a cohort of 17 HCWs, a recent study by Abiakam et al. evaluated various biophysical skin parameters as well as the levels of certain inflammatory cytokines in sites exposed to prolonged respirator application [11]. The main finding included the consistent upregulation in Transepidermal water loss (TEWL) response particularly at the bridge of the nose, which coincided with high interface pressures at that location [12].

Although biophysical and biochemical markers are usually assessed in studies pertaining to the integrity of skin [13,14], the effects of mechanical insults on the stratum corneum (SC) and its relationship to its main cells, the corneocytes, have been limited. Indeed, the SC represents the primary skin barrier that is exposed to friction, pressure, and shear, and corneocytes represent the building blocks of the SC, which impart stiffness and create the skin barrier function [15–17]. Moreover, these cells are known to undergo a complex process of maturation [18], the so-called cornification process, which includes the cross-linking of certain precursor proteins e.g. involucrin and loricrin, and the covalent attachment of lipids to produce a rigid and hydrophobic cornified envelope (CE) structure [19]. In addition, a gradual degradation of corneodesmosomes (CDs) occurs in the central region of the cell, originating a residual honeycomb formation of cell junctions. It is this honeycomb pattern that is believed to underpin the barrier function resulting in lower values of TEWL when compared to the inner SC or body locations where this maturation is impaired or not achieved e.g., palms of hands and soles of feet [15,16,20]. The honeycomb feature of a mature SC results in improved flexibility of the whole layer by attenuating mechanical insults, such as bending or flexing, and allowing minimal relative sliding of the outer SC over the inner SC [21]. Corneodesmosomes are usually observed indirectly by immunostaining of desmoglein 1 (Dsg1) [15,16], which is a cadherin-type cell-cell adhesion molecule found in stratified epithelial desmosomes, expressed in the suprabasal layer of the epidermis [16].

The complex mechanisms leading to SC maturation and desquamation depend on intrinsic [22,23] and extrinsic factors [24–26] and may influence the way in which skin responds to the mechanical insults. Therefore, there is a need to evaluate the effects of the RPE that are related to mechanical and thermal insults on the SC and its relationship to its main cells.

The present paper presents a complementary methodology and analysis of a previously published study on the biophysical and biochemical changes in skin health in HCWs during the COVID-19 pandemic [11]. It is focused on establishing the characteristics of superficial corneocytes, namely CE maturation and corneodesmosome distribution, in areas exposed to prolonged mechanical loading. Changes will be compared to the biophysical skin responses and perceived skin damage in the same cohort of HCWs to evaluate the role of corneocytes in skin surface integrity for understanding the role of corneocytes in skin surface integrity.

2. Materials and methods

2.1. Participants and study protocol

The study was conducted as previously published by Abiakam et al. [11]. To review briefly, a convenience sample of HCWs were recruited from specific departments treating COVID-19 patients in a UK University Hospital Trust. Participants were included if they were aged over 18 years, wore FFP2/3 respirators daily while attending to clinical commitments and were on duty for at least three consecutive shifts per week. Except for those using N95 masks, all participants were fit tested according to standardised procedures (CITE fit2fit guideline) [27]. Those presenting with broken skin were excluded from the study. The study was approved by the UK Health Research Authority committee (IRAS 285764) and written informed consent was obtained from participants prior to commencing the study.

The study was conducted during the second wave of the COVID-19 pandemic in the UK (December 2020 to March 2021). Anatomical locations on the face, namely an area outside the perimeter of respirator application (site A) and either the right or left the cheek (site C) were investigated (Fig. 1a). The assessment of biophysical skin parameters at these sites was performed as described previously in Abiakam et al. [11]. In summary, TEWL, (Tewameter, Courage + Khazaka electronic GmbH, Germany), stratum corneum hydration (Corneometer, Courage + Khazaka electronic GmbH, Germany) and erythema was collected in the indicated order. This was followed by the sampling of skin surface sebum using Sebutapes, while corneocytes were the last samples to be collected. Biophysical skin parameters and sebum collection were performed pre- and post-respirator application. The skin response to respirator application (TEWL and SC hydration) was calculated as the ratio between pre- and post-shift biophysical skin values (normalized values), as previously described and presented in Abiakam et al. [11].

Corneocytes were collected in three distinct sessions after the working shift of the HCW (Fig. 1b). During the test session, each participant acclimatized in an indoor environment and the skin on their face was dried with paper towels (Tork®, Bedfordshire, UK) prior to measurements. Corneocyte collection was only performed post-respirator application. This was done to avoid the possibility that the act of tape stripping immediately prior to wearing RPE makes the skin more susceptible to respirator-induced damage.

All test sessions were conducted in a temperature and humidity-controlled laboratory (room temperature of 22.5 ± 0.7 °C and relative humidity of 42 ± 6%). Data collection was performed following established and published patterns to minimise interferences [28].

Two cell collection sites with three distinct data collection sessions were used as highlighted in Fig. 1.

- Session 1: participant first day of respirator usage following return to work after a period of absence (minimum of 24 h).
- Session 2: second consecutive day of respirator usage in a given working week.
- Session 3: third consecutive day of respirator usage in a given working week.

2.2. Collection and isolation of corneocytes

At the end of each shift, and following the biophysical assessment procedures, corneocytes were collected from the two sites (Fig. 1a), after sebum collection using commercially available Sebutapes (CuDerm, Dallas, TX, USA) as described in our published study on the analysis of the same subjects [11]. The cells were sampled via tape stripping (Sellotape, UK) by pressing the tape gently onto the skin with gloved hands and gently removing by peeling the tape. Each tape strip was cut in half and used for the assessment of CE maturation and indirect visualization of CDs by the immunostaining of a CD protein – desmoglein-1 (Dsg1).

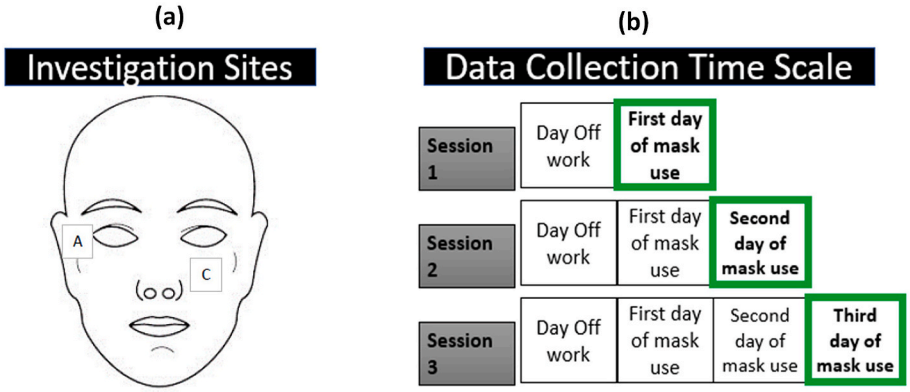

Fig. 1. (a) Investigation sites for the analysis of the properties of corneocytes. A - area outside the perimeter of respirator application (negative control); C – left cheek. **(b)** Scheme of sample collection. After a day off work, tape stripping was performed after one, two or three days of consecutive respirator usage. Corneocytes were collected after assessment of biophysical skin parameters and sebum collection. Corneocyte collection was only performed post-respirator application to avoid the possibility that the act of tape stripping immediately prior to wearing RPE could make the skin more susceptible to respirator-induced damage. TEWL and stratum corneum hydration were measured pre- and post-respirator application. The periods between consecutive test sessions varied for practical reasons, ranging between 1 and 8 weeks. The corresponding analysis depicts the number of consecutive days wearing a FFP2 or FFP3 masks, denoted as test sessions.

2.3. CE maturity assay: CE extraction and immunostaining for involucrin and Nile red staining for lipids

Cornified envelopes were isolated from the tape using an established methodology [29]. Briefly, half of each tape was extracted with 750 mL of dissociation buffer containing 100 mM Tris-HCl pH 8.0, 5 mM EDTA (ethylenediaminetetraacetic acid), 2% SDS (sodium dodecyl sulphate) and 20 mM DL-dithiothreitol (Sigma Aldrich Dorset, UK). Tapes were extracted in the dissociation buffer for 10 min at 75 °C and centrifuged at room temperature for 10 min at 5000 g. The extracted CEs were washed (three times) in washing buffer: 20 mM Tris-HCl pH 9.0, 5 mM EDTA, 0.2% SDS and 10 mM DL-dithiothreitol and suspended in 1 × PBS buffer (Sigma Aldrich Dorset, UK).

Extracted CEs were transferred onto a Polysine-coated microscope slide (5 μL, VWR international Ltd, Leicestershire, UK) for the immunostaining protocol, as previously described [29]. This included an overnight incubation in a humidity chamber at 4 °C in the primary monoclonal antibody (1:100, mouse anti-human involucrin SY5, ABCAM, Cambridge, UK). The antibody solution was washed with PBS three times for 5 min before adding the secondary antibody Alexa-Fluor 488-labeled goat anti-mouse IgG antibody (1:200, ABCAM, Cambridge, UK) for 60 min at room temperature (in the dark). The slides were washed with 1 × PBS (three times for 5 min) and mounted with 20 μg/mL Nile red (Sigma Aldrich, Dorset, UK) in 75% glycerol solution.

2.4. Immunostaining for Dsg1

The immunostaining protocol for Dsg1 was adapted from Ref. [16]. Corneocytes attached to the tape were washed with 1 × PBS (10 min) and incubated with a P23 mouse monoclonal antibody against the extracellular domain of Dsg1 (Progen, Heidelberg, Germany) at 4 °C overnight. This was followed by incubation with an Alexa-Fluor 488-labeled goat anti-mouse IgG antibody (1:200, ABCAM, Cambridge, UK), for 60 min at room temperature in the dark. The samples were mounted with anti-fade fluorescence mounting medium (ABCAM, Cambridge, UK).

2.5. Image and data analysis

Five non-overlapping images were taken in total for each CE and each Dsg1 sample with a field of view of 720 × 580 μm. The fluorescence images were acquired at a 10 × objective magnification and analysed using a Leica DMRBE microscope (Leica Microsystems, USA) equipped with PL-Fluotar 5×/0.12 and 10 × /0.30 lenses mounted with a CoolLED pE-300 series blue-illumination source at the wavelength of 460 nm and with a Motic Pro 252 microscope camera. Image quality control was performed using a QC pipeline [30] on the open-source software Cell Profiler (http://cellprofiler.org/). Images were analysed using Image J® version 1.53a (National Institutes of Health, Bethesda, MD, USA).

CE maturation was evaluated using a sequential approach (Fig. 2a). After application of a Gaussian filter, images were converted to either an 8-bit map (for total cell number count) or divided in RGB channels (to count cells staining for Alexa-Fluor 488, i.e., positive to involucrin). The Huang threshold followed by Watershed command was applied to define CE borders and the cells with a surface area of 300–2000 μm^2 were counted based on the reported average values [18].

$$\%\ \mathrm{INV}+ = \frac{\text{number of INV} + \text{cells}}{\text{total number of cells}} \times 100$$

For the distribution of Dsg1, the ratio of pixels expressing Dsg1 to the total area in pixels was counted in two random regions of interest (ROIs) of 70 × 70 μm (see yellow boxes in Fig. 2b) that contained corneocytes, as previously established and described in Refs. [15,16]. Images were, first, transformed in 8-bit maps and a Huang threshold was applied. This was followed by the Watershed command and using "Analyse Particle" command to count number of pixels (Fig. 2b).

2.6. Data analysis

The median of five repeat images for each body site and session was calculated for each participant and imported into IBM® SPSS® Statistics (version 27) for analysis and assessed for normality using probability plots and the Shapiro-Wilk test, which revealed a non-normal distribution across participants. Accordingly, the Friedman test was employed to investigate whether the challenges to the skin were able to induce temporal variations in cell properties. It was followed by the Wilcoxon Signed Ranks Test to investigate whether there were differences in cell properties between the control site and the cheek at the different time points. Tests were considered statistically significant at a 5% level ($p < 0.05$).

The Spearman's correlational analysis was employed to investigate the relationships between the properties of corneocytes and biophysical parameters as presented in Abiakam et al. [11] as well as for self-reported adverse reactions and body mass index BMI. A rank-sum was used for the properties of skin cells and biophysical markers, by summing the ranks of corneocyte properties and of the pre-respirator application values of TEWL and SC hydration to study the relationship between barrier function and cell properties in unloaded skin. Additionally, a rank sum of normalized TEWL values (post/pre-respirator application ratio) at the cheek was used to correlate the effect of respirator usage with corneocyte properties. Self-reported skin adverse

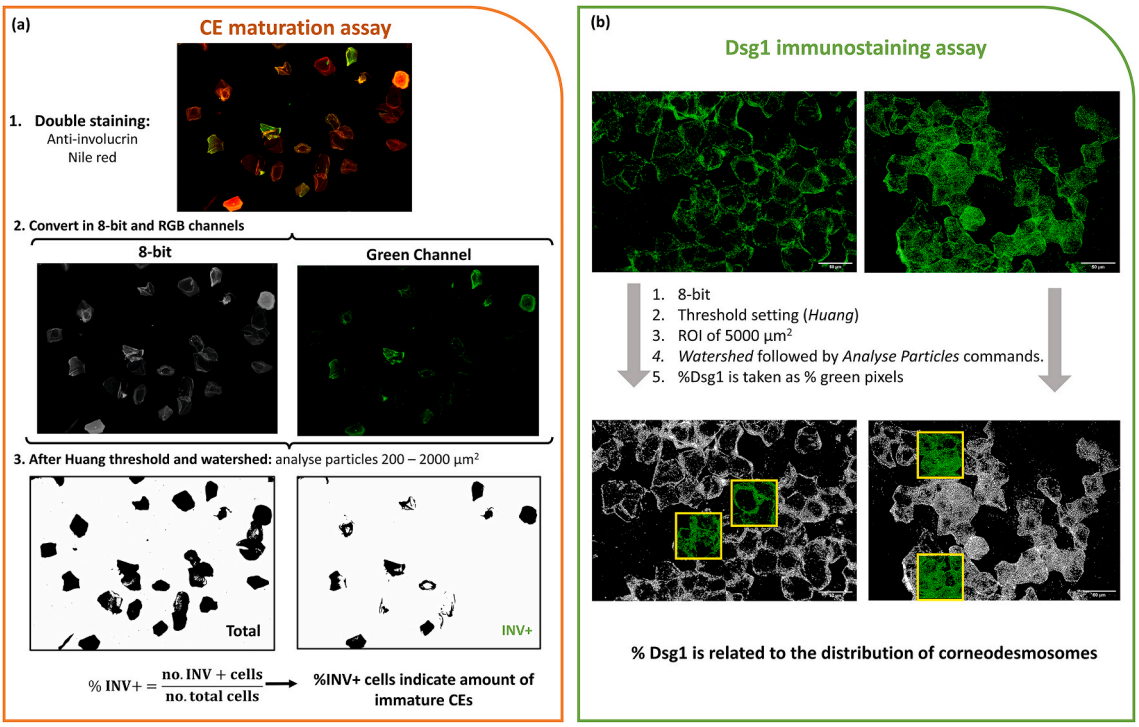

Fig. 2. Scheme of image analysis procedures. For each sample, 5 images were analysed for each assay. **(a)** Design of maturation assay image analysis. 8-bit and RGB (green) channel are used to count the total number of cells and those staining with Alexa-fluor 488 (INV+), respectively. The Huang threshold is applied to the images, followed by watershed segmentation. The ratio between the number of green/total cells is calculated to obtain % of immature cells (% INV+). **(b)** Design of Dsg1 immunostaining assay image analysis. Raw images of Alexa-fluor fluorescence are transformed in 8-bit images. Huang threshold followed by watershed command were applied, and two ROIs are analysed using the "Analyse particles" command: the percentage of green pixels against the total number of pixels in the two ROIs are taken as the percentage of Dsg1 in each image. Image scale bar = 50 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

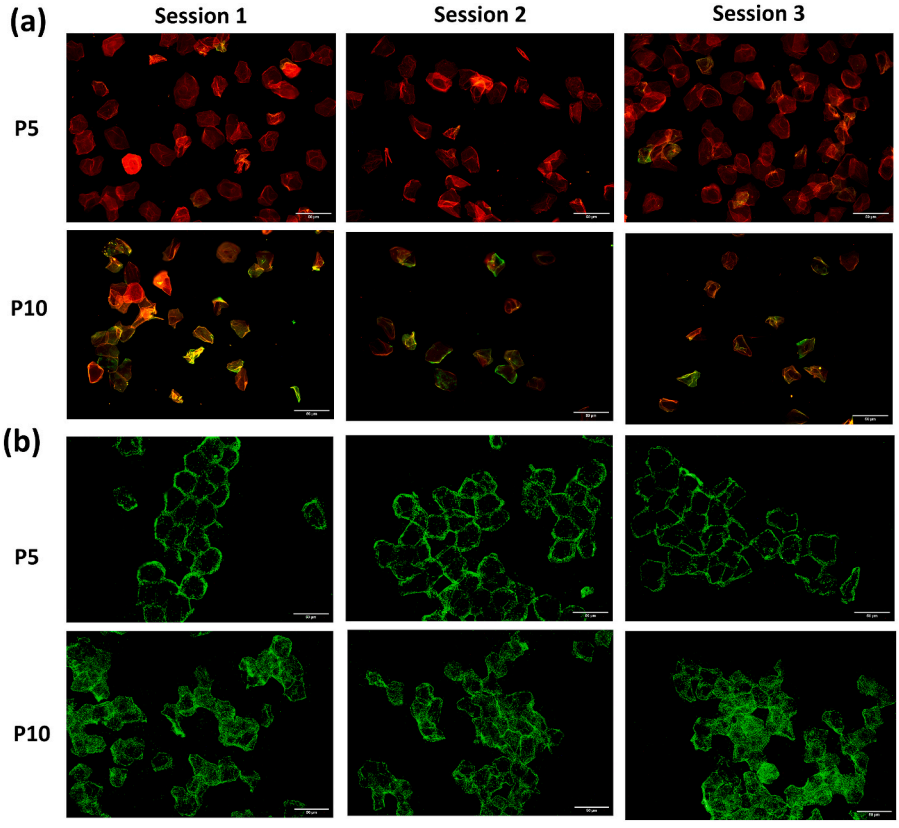

Fig. 3. Representative immunostaining images showcasing the case of low (P5) and high (P10) levels of INV+ CEs **(a)** and Dsg1 **(b)** at the cheek. **(a)** Low level of CE maturity is represented by a high number of CEs positive for involucrin (median = 31, 35, 54% on sessions 1, 2 and 3, respectively for P10), while high level of maturity is characterized by hydrophobic CEs (median = 17, 23 and 8% for sessions 1, 2 and 3 respectively for P5). **(b)** Honeycomb pattern of Dsg1 is observed for P5 (median = 22, 12, 15% Dsg1 on sessions 1, 2 and 3, respectively), while CDs are present ubiquitously over the cell surface for P10 (median = 46, 45 and 39% Dsg1 on sessions 1, 2 and 3 respectively). P5 – participant 5; P10 – participant 10. Scale bar = 50 µm.

reactions were scored (0 – no reactions, 4 – maximum reported reactions) and plotted against the two corneocyte properties.

3. Results

The study recruited 17 HCWs (15 females and 2 males) who used N95 or FFP3 during established clinical shift patterns as described in Abiakam et al. [11]. The age of the participants ranged between 22 and 61 years (mean ± SD was 33 ± 11 years), and the BMI was 25.1 ± 5.4 kg/m^2. Participants shift length ranged from 8 to 12 h and breaks ranged from 1 to 4 per shift, being consistent for each participant, as they followed an established working pattern.

3.1. Cheek presented higher levels of immature CEs and Dsg1 compared to the control site

Representative immunostaining images for two participants, P5 and P10 corresponding to individuals with low and high levels of immature CEs and Dsg1, respectively, are shown on Fig. 3, for the cheek at each session.

The properties of corneocytes across the three sessions for each participant are shown in Fig. 4. The median levels of immature CEs based on five repeat images ranged from 11 to 50%, while the median levels of Dsg1 ranged from 12 to 56% at the two sites across the sessions (Table 1). The inter-subject variability was measured between participants for the three sessions, and the maximum coefficient of variation (CV) for the level of immature CEs was found to be 33% and 43% for the cheek and the control site, respectively. Similar values were found for the level of Dsg1, with maximum values of 30% at both sites.

Although there was a variability between participants, a positive correlation ($r = 0.81$, $p < 0.001$) was found between the two anatomical locations, i.e., participants with higher levels of Dsg1 at the control site, also generally presented higher values at the cheek. The same positive trend was found for the level of CE maturity, although it was not statistically significant ($r = 0.47$, $p = 0.061$).

When considering the median values of the cohort, significant differences were not found between the test sessions for either of the anatomical locations. However, differences were identified between anatomical sites for each session (Table 1), with higher levels of Dsg1 ($p < 0.05$ for each session) in the loaded cheek compared to the control. Similar trends were also observed for the level immature CEs, although statistical significance was only achieved at session 2.

3.2. High levels of mature CEs and low levels of Dsg1 correlate with increased SC hydration for unloaded skin

A correlational analysis of the rank-sums across test sessions was employed to investigate the relationship between the SC structure and the skin barrier function at the two facial locations of unloaded skin (Fig. 4), i.e., by investigating the relationship between SC cell properties and pre-respirator application skin biophysical parameters. Indeed, corneocytes were only collected post-respirator application, but considering that these are dead cells, any biochemical alteration to their maturation properties would not be expected to occur during a single shift, since these can take several days to manifest [31].

The correlations between the properties of corneocytes and TEWL were not statistically significant (Fig. 5 a and c), with the exception of a positive correlation in the values of Dsg1 at the cheek ($p < 0.05$). However, when SC hydration values were correlated to both the levels of immature CEs and Dsg1 a significant negative relationship was evident at both the control and cheek sites (Fig. 4b and d), i.e., an increasingly hydrated SC correlates with lower levels of immature CEs and corneodesmosomes.

3.3. Low levels of immature CEs correlate with barrier function disruption after prolonged respirator usage

In our previous analysis of the same subjects, we found changes in the barrier function of the cheek after each test session [11]. Indeed, 2-fold-changes were recorded in 24% and 19% of the participants in test sessions 2 and 3, respectively, measured as the ratio post/pre-respirator application for TEWL. Consequently, a correlational analysis was employed between the rank-sums (of all three sessions) of both % INV+ and % Dsg1 and the skin response to insult measured as the normalized (pre-to post-respirator application) value of TEWL (Fig. 6).

Interestingly, a negative correlation ($p < 0.01$) was observed between the number of immature CEs and the TEWL response to respirator application (Fig. 6a). By contrast, the corresponding negative correlation with %Dsg1 was not statistically significant (Fig. 6b).

3.4. A higher level of self-reported skin adverse reactions correlate with high level of immature CEs and Dsg1

Finally, the relationship between the properties of the corneocytes and skin adverse reactions reported by the participants (Table 2) was

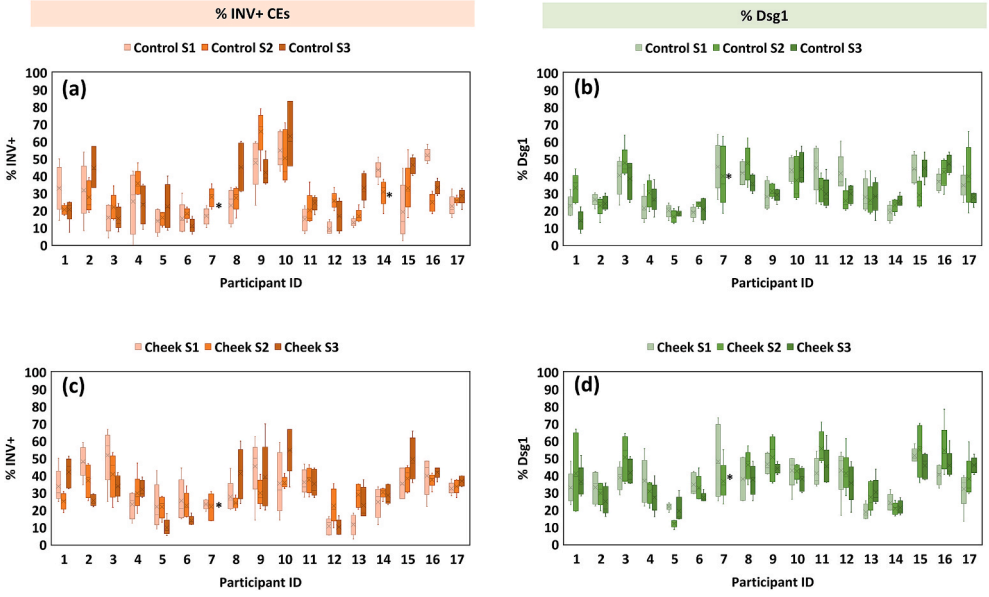

Fig. 4. (a) and **(c)** Percentage of immature CEs in the negative control site and cheek expressed as the percentage of cells positive for involucrin. **(b)** and **(d)** Percentage of Dsg1 puncta stained at the surface of corneocytes from the negative control site and cheek. All data are shown as box-plots. The box boundaries indicate the 25th and 75th percentiles, while the whiskers represent the 10th and 90th percentile. The mean is shown by the cross (×) and the median by the line (−). (* indicates missing data).

Table 1
Summary of corneocyte properties of the 17 HCWs cohort at each of the three test sessions.

Parameter (%)	Session 1					Session 2					Session 3				
	Control		Cheek			Control		Cheek			Control		Cheek		
	Median	Range	Median	Range	p-value	Median	Range	Median	Range	p-value	Median	Range	Median	Range	p-value
INV+	24.3	11–39	29.9	11–50	0.100	25.9	16–36	29.9	22–38	0.028*	26.2	11–46	33.9	10–49	0.496
Dsg1	35.7	19–45	37.9	16–48	0.015*	31.5	16–48	41.1	12–56	0.025*	28.7	14–47	36.9	19–46	0.023*

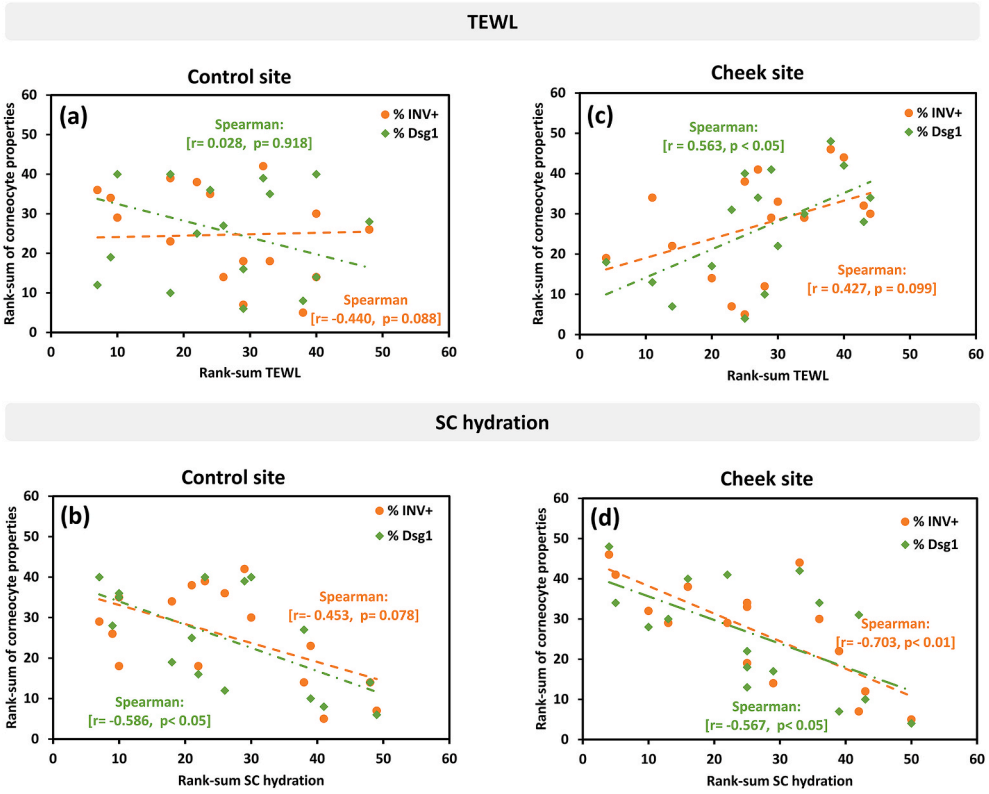

Fig. 5. Correlations between the rank-sums of the properties of corneocytes (% INV+ and % Dsg1) and biophysical parameters, TEWL (**a** and **c**) and SC hydration (**b** and **d**) measured pre-respirator application across test sessions for the control site (**a** and **b**) and the cheek (**c** and **d**).

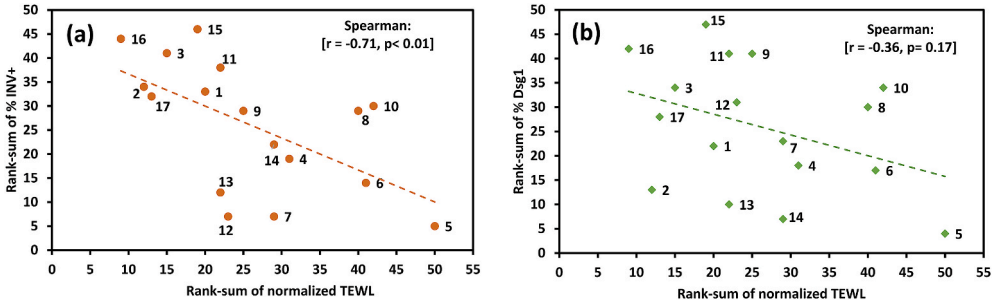

Fig. 6. Relationship between the rank-sum of **(a)** %INV+ and **(b)** % Dsg1 on the rank-sum of normalized TEWL (i.e., rank-sum of the ratio pre/post-respirator application) at the cheek.

investigated. The reactions were graded from 0 to 4, where 0 corresponded to no adverse reaction and 4 to reports of a combination of dry skin, spots, itchiness, and rashes (i.e., one point per reaction). The categorical score of skin adverse reactions was plotted as a function of the rank-sum of values of % INV+ and Dsg1 of the loaded site of the cheek, as shown in Fig. 7.

The analysis revealed that each of the 7 participants reporting 2 or more skin adverse reactions, presented a high levels of immature CEs (rank sum % INV+ across sessions was above 25), while the majority of these (6 out of 7) also presented high levels of Dsg1 (rank sum above 30). By contrast, those with fewer self-reported skin reactions had a lower rank of immature CEs and Dsg1. In fact, when performing a correlational analysis (Spearman coefficient), a positive correlation was found for the rank-sum of immature CEs ($r = 0.797$, $p < 0.001$) and for %Dsg1 ($r = 0.743$, $p < 0.001$).

Table 2
Adverse reactions to the skin reported by the participants following respirator usage [11].

Participant ID	Adverse reactions	Score
1	spots, dry skin	2
2	itchiness, excessive sweating	1
3	spots, itchiness	2
4	none	0
5	spots, lumps	1
6	none	0
7	spots, itchiness	2
8	excessive sweating	0
9	spots, dry skin	2
10	spots, dry skin, excessive sweating, headache	2
11	itchiness, spots, excessive sweating	2
12	none	0
13	spots	1
14	none	0
15	dry skin, rashes, spots, itchiness	4
16	dry skin, rashes, spots, itchiness, excessive sweating	4
17	dry skin	1

4. Discussion

The extensive use of personal protective equipment (PPE) during the outbreak of the coronavirus pandemic led to ubiquitous reports of adverse skin reactions in HCWs [2,3,5]. In our published study on the analysis of the same subjects we demonstrated how the integration of biophysical and biochemical markers could provide a comprehensive analysis of changes in local skin health following respirator usage [11]. However, there is still a limited understanding of the actual mechanisms in the skin leading to those changes. Therefore, the primary aim of this complementary study was to investigate the properties of superficial corneocytes in the context of prolonged respirator application to broaden the knowledge of skin surface physiology. While an effect of respirator usage was not observed for corneocyte maturity and CD distribution over time, the level of CDs was higher in the skin site at the loaded cheek site compared to an area outside the perimeter of the respirator, representing a negative control site, for all three sessions. Furthermore, a greater number of self-reported skin adverse reactions was shown to correspond with a greater number of immature CEs and CDs at the cell surface (Fig. 7).

In the present study, the properties of the corneocytes were observed to vary between individuals. In the context of CE maturity, the maximum coefficients of variation (CVs) between participants (considering the three sessions analysed) of 33% and 43% were found for the cheek and the control site, respectively. Equally high CVs have been observed previously by Guneri et al. [29] for cells of the cheek and post-auricular areas (54 and 27%, respectively). Similar inter-subject variability was also found for the level of Dsg1, with a maximum CV between participants of 30% at both sites. However, such variability did not appear to arise from intrinsic factors such as age or BMI, since no significant correlations between these factors were observed (see supplementary information). In fact, this was expected considering the age range of the cohort, which was between 21 and 41 years (with two participants aged 57 and 61). A prominent effect of age is expected when comparing adult and aged skin [32,33].

When analysing the temporal effects of respirator usage on the properties of superficial cells, there were no statistically significant differences between the test sessions. However, the level of Dsg1 was higher for the loaded cheek site compared to the unloaded control site ($p < 0.05$ for all 3 test sessions). Furthermore, similar trends existed for the level of immature CEs, although the difference was only statistically significant for session 2 (Table 1). This could be due to a temporal adaptation of the cheek to respirator application involving mechanical loading or be caused by the stripping of superficial SC layers due to rubbing of respirator against the skin. The current study was performed during the second wave of COVID-19 in the UK, when respirators had been mandated in the clinical settings for over 12 months, and hence adaptation of the SC may have occurred. Indeed, the disruption of the SC has been observed in *in vitro* histological studies, following 24 h of severe loading (200 mmHg) [34]. Recently, Caggiari et al. observed high interface pressures at the nasal bridge (>70 mmHg) for four different respirators [4]. However, there has not yet been a study that has investigated the effects of persistent loading on the physiology of the SC. Alternatively, this difference between anatomical sites could suggest that spatially close anatomical regions can be differently regulated, as suggested by Guneri et al. [29].

Previously, TEWL and SC hydration have been correlated with SC cohesion, associated with the pattern and number of CDs [15,16]. However, such observations have been mainly used to correlate different anatomical sites [35], and in tape stripping procedures in which the outer layers of skin are removed to compare SC properties at different depths [16,29]. This motivated the present correlational analysis between the biophysical markers and the properties of corneocytes to further investigate the role of corneocytes in the skin barrier function. When correlating the integrated rank-sum of cell properties with the biophysical markers measured prior to the HCW shift, a statistically significant association was evident for SC hydration at both sites (Fig. 5c and d), and for TEWL at the cheek (Fig. 5b). This supports the assertion that the skin barrier function depends on a mature SC, characterized by a honeycomb distribution of CDs at the cell's edge and rigid and hydrophobic CEs [15,16,36].

However, this relationship was not observed with the values of biophysical markers measured after respirator application. Participants with a high TEWL ratio pre-to post respirator application, tended to have lower levels of immature CEs ($p < 0.01$). Although seemingly contradictory, this observation may pertain to information underlying the different mechanisms and functions of the SC. It is hypothesised that while a mature SC, with a honeycomb pattern of CDs and hydrophobic CEs, may be a requirement for a well-maintained barrier function, with lower TEWL values, it may be detrimental when exposed to mechanical insults such as respirator application. This would be consistent with the properties of the anatomical regions, such as the palm of the hand or

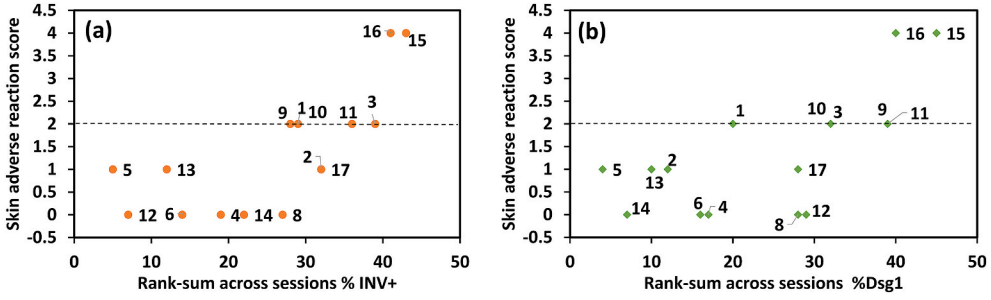

Fig. 7. Relationship between the score of self-reported skin reactions, scored from 0 to 4 types of skin adverse reactions registered, and the rank-sum of % INV+ **(a)**, % Dsg1 **(b)** at the cheek.

plantar region of the foot, which are adapted to grip and load-bearing functions, presenting an immature SC [37]. Future studies should focus on understanding this apparent dichotomy between load-bearing and barrier functions, particularly by comparing glabrous and non-glabrous skin.

When analysing the trends between self-reported adverse skin reactions and the studied cell properties, it was observed that participants reporting dry skin, spots, itchiness, rashes, and excessive sweating usually presented greater levels of immature CEs and Dsg1 (Fig. 6). This indicates that a mature SC is associated with less adverse reactions after prolonged use of respirators, which seems to contradict the relationships found with biophysical markers. However, it must be emphasised that such reactions may be induced by different mechanisms than those provoking changes in TEWL, and consequently a direct comparison and causal associations cannot be made. As an example, itchiness associated with dry skin may be related to the properties of corneocytes, such as an interrupted corneodesmosome remodelling as occurs in ichthyosis vulgaris [22].

This study was limited by the relatively small HCW cohort from a single UK acute care provider, and necessarily most participants were female of white (Caucasian) ethnicity [11]. These factors and the small variations in BMI and age of the participants limit the generalisability of the findings. Also, it should be emphasised that the study was conducted over varying time periods (1–8 weeks), which might have impacted on the properties of superficial corneocytes, since it is known that the upper SC characteristics vary with both external and seasonal conditions [38]. Furthermore, future studies should focus on the effect of respirator application causing potential rubbing of the most superficial layers of the SC, which could explain a lack of mature SC registered at the cheek.

Although biophysical markers can provide insights on the status of the skin barrier function, they may be insufficient to understand the mechanisms leading to the loss of skin integrity in the context of mechanical insults. In future studies, the mechanical properties of corneocytes could also be measured using nanoindentation, to better understand the role of these cells in the mechanical resistance of skin. Superficial corneocytes, which are easily collected by tape stripping, are an interesting candidate for a skin integrity biomarker. The present work confirms previous claims relating barrier function with SC structure [15,16], but raises questions concerning the role of this layer in adapting to prolonged pressure and shear insults. Future studies should focus on the role of corneocytes in skin surface integrity and challenge the relationships between barrier function and mechanical resistance.

5. Conclusions

The current study examined two surface properties of corneocytes, namely, the levels of immature CEs and Dsg1, at two skin sites on the face of healthcare workers following prolonged wearing of respiratory protective equipment during routine clinical shifts. While there was minimal effect of respirator usage on corneocyte maturity and CD distribution over time, the levels of CDs and immature CEs were consistently higher in the loaded cheek site compared to the negative unloaded control site. Furthermore, participants with a greater number of self-reported skin adverse reactions presented a larger number of immature CEs and larger level of Dsg1. These results contrasted with the relationships observed between corneocyte properties and biophysical parameters, such as skin barrier function measured by TEWL. These findings raise questions about the role of corneocytes in maintaining skin integrity. Further investigation is necessary to determine if the properties of corneocytes associated with a healthy barrier function are detrimental when the SC is subjected to pressure, shear, and frictional forces.

Declaration of competing interest

The authors have no conflicts of interest.

Acknowledgments

The authors wish to thank all the healthcare professionals who engaged with the study for their commitment even in these unprecedented times.

This work was supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 811965 (Project STINTS - Skin Tissue Integrity under Shear).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtv.2023.02.007.

References

[1] Smith LE, Serfioti D, Weston D, Greenberg N, Rubin GJ. Adherence to protective measures among healthcare workers in the UK: a cross-sectional study. Emerg Med J 2022;39:100.
[2] Yuan X, Xi H, Le Y, Xu H, Wang J, Meng X, et al. Online survey on healthcare skin reactions for wearing medical-grade protective equipment against COVID-19 in Hubei Province, China. PLoS One 2021;16:e0250869.
[3] Abiakam N, Worsley P, Jayabal H, Mitchell K, Jones M, Fletcher J, et al. Personal protective equipment related skin reactions in healthcare professionals during COVID-19. Int Wound J 2021;18:312–22.
[4] Caggiari S, Bader DL, Foxell F, Pipe N, Couch S, Turner A, et al. Biomechanical and physiological evaluation of respiratory protective equipment application. Med Devices (Auckl) 2022;15:241–52.
[5] Peko L, Ovadia-Blechman Z, Hoffer O, Gefen A. Physiological measurements of facial skin response under personal protective equipment. Mech Behav Biomed Mater 2021;120:104566.
[6] Adams MJ, Briscoe BJ, Johnson SA. Friction and lubrication of human skin. Tribol Lett 2007;26:239–53.
[7] Comaish S, Bottoms E. The skin and friction: deviations from Amonton's laws, and the effects of hydration and lubrication. Br J Dermatol 1971;84:37–43.
[8] Park A, Baddiel C. On the elastic properties of stratum. J Soc Cosmet Chem 1972;23:471–9.
[9] Dąbrowska AK, Adlhart C, Spano F, Rotaru G-M, Derler S, Zhai L, et al. In vivo confirmation of hydration-induced changes in human-skin thickness, roughness and interaction with the environment. Biointerphases 2016;11:031015.
[10] Han HS, Shin SH, Park JW, Li K, Kim BJ, Yoo KH. Changes in skin characteristics after using respiratory protective equipment (medical masks and respirators) in the COVID-19 pandemic among healthcare workers. Contact Dermatitis 2021;85:225–32.
[11] Abiakam N, Jayabal H, Mitchell K, Bader D, Worsley P. Biophysical and biochemical changes in skin health of healthcare professionals using respirators during COVID-19 pandemic. Skin Res Technol 2023;29:e13239.
[12] Worsley PR, Prudden G, Gower G, Bader DL. Investigating the effects of strap tension during non-invasive ventilation mask application: a combined biomechanical and biomarker approach. Med Devices (Auckl) 2016;9:409–17.
[13] Hua W, Zuo Y, Wan R, Xiong L, Tang J, Zou L, et al. Short-term skin reactions following use of N95 respirators and medical masks. Contact Dermatitis 2020;83:115–21.
[14] Park S-R, Han J, Yeon YM, Kang NY, Kim E. Effect of face mask on skin characteristics changes during the COVID-19 pandemic. Skin Res Technol 2021;27:554–9.
[15] Goto H, Tada A, Ibe A, Kitajima Y. Basket-weave structure in the stratum corneum is an important factor for maintaining the physiological properties of human skin as studied using reconstructed human epidermis and tape stripping of human cheek skin. Br J Dermatol 2020;182:364–72.
[16] Naoe Y, Hata T, Tanigawa K, Kimura H, Masunaga T. Bidimensional analysis of desmoglein 1 distribution on the outermost corneocytes provides the structural and functional information of the stratum corneum. J Dermatol Sci 2010;57:192–8.
[17] Boyle CJ, Plotczyk M, Villalta Sergi F, Patel S, Hettiaratchy S, Masouros Spyros D, et al. Morphology and composition play distinct and complementary roles in the tolerance of plantar skin to mechanical load. Sci Adv 2019;5(10):eaay0244.
[18] Évora AS, Adams MJ, Johnson SA, Zhang Z. Corneocytes: relationship between structural and biomechanical properties. Skin Pharmacol Physiol 2021;34:146–61.
[19] Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 2005;6:328–40.
[20] Taylor NA, Machado-Moreira CA. Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans. Extreme Physiol Med 2013;2:4.
[21] Kitajima Y. Implications of normal and disordered remodeling dynamics of corneodesmosomes in stratum corneum. Dermatol Sin 2015;33:58–63.
[22] Mori S, Shiraishi A, Epplen K, Butcher D, Murase D, Yasuda Y, et al. Characterization of skin function associated with obesity and specific correlation to local/systemic parameters in American women. Lipids Health Dis 2017;16:214.
[23] Boireau-Adamezyk E, Baillet-Guffroy A, Stamatas GN. Age-dependent changes in stratum corneum barrier function. Skin Res Technol 2014;20:409–15.

[24] Vyumvuhore R, Tfayli A, Duplan H, Delalleau A, Manfait M, Baillet-Guffroy A. Effects of atmospheric relative humidity on Stratum Corneum structure at the molecular level: ex vivo Raman spectroscopy analysis. Analyst 2013;138:4103–11.
[25] Lipsky ZW, German GK. Ultraviolet light degrades the mechanical and structural properties of human stratum corneum. J Mech Behav Biomed Mater 2019;100:103391.
[26] Katagiri C, Sato J, Nomura J, Denda M. Changes in environmental humidity affect the water-holding property of the stratum corneum and its free amino acid content, and the expression of filaggrin in the epidermis of hairless mice. J Dermatol Sci 2003;31:29–35.
[27] Health and Safety Executive (HSE). Fit2Fit' mission statement. 2021. https://www.fit2fit.org/. [Accessed 16 May 2022].
[28] Kottner J, Ludriksone L, Garcia Bartels N, Blume-Peytavi U. Do repeated skin barrier measurements influence each other's results? An explorative study. Skin Pharmacol Physiol 2014;27:90–6.
[29] Guneri D, Voegeli R, Gurgul SJ, Munday MR, Lane ME, Rawlings AV. A new approach to assess the effect of photodamage on corneocyte envelope maturity using combined hydrophobicity and mechanical fragility assays. Int J Cosmet Sci 2018;40:207–16.
[30] Bray M-A, Carpenter AE. Quality control for high-throughput imaging experiments using machine learning in Cellprofiler. In: Johnston PA, Trask OJ, editors. High content screening: a powerful approach to systems cell biology and phenotypic drug discovery. New York, NY: Springer New York; 2018. p. 89–112.
[31] Guneri D, Voegeli R, Doppler S, et al. The importance of 12R-lipoxygenase and transglutaminase activities in the hydration-dependent ex vivo maturation of corneocyte envelopes. Int J Cosmet Sci 2019;41:563–78.
[32] Darlenski R, Berardesca E, Fluhr JW. Corneocyte size and cell renewal: effects of aging and sex hormones. In: Farage MA, Miller KW, Maibach HI, editors. Textbook of aging skin. Berlin, Heidelberg: Springer Berlin Heidelberg; 2017. p. 397–404.
[33] Gorzelanny C, Goerge T, Schnaeker E, Thomas K, Luger T, Schneider S. Atomic force microscopy as an innovative tool for nanoanalysis of native stratum corneum. Exp Dermatol 2006;15:387–91.
[34] Bronneberg D. Biochemical markers for early detection of superficial pressure ulcers. PhD Thesis. Technische Universiteit Eindhoven; 2007.
[35] Mohammed D, Matts PJ, Hadgraft J, Lane ME. Variation of stratum corneum biophysical and molecular properties with anatomic site. AAPS J 2012;14:806–12.
[36] Kunii T, Hirao T, Kikuchi K, Tagami H. Stratum corneum lipid profile and maturation pattern of corneocytes in the outermost layer of fresh scars: the presence of immature corneocytes plays a much more important role in the barrier dysfunction than do changes in intercellular lipids. Br J Dermatol 2003;149:749–56.
[37] Poonja P. Skin barrier changes in T1DM and T2DM. Int J Diabetes Clin Res 2022;9:156.
[38] Kikuchi K, Kobayashi H, Le Fur I, Tschachler E, Tagami H. The winter season affects more severely the facial skin than the forearm skin: comparative biophysical studies conducted in the same Japanese females in later summer and winter. Exog Dermatol 2002;1:32–8.