



## OPEN Mask wearing impacts skin barrier function and microbiome profile in sensitive skin

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Mask-wearing behavior, common in the post-COVID-19 era, raises concerns for sensitive skin. This split-face study investigated mask-related changes in skin barrier function and microbiome composition among 30 female volunteers with sensitive skin and assessed the mitigating effects of a moisturizer containing biological lipids and probiotics. Skin physiological indicators (transepidermal water loss, erythema index, stratum corneum hydration, pH, temperature) of masked and unmasked areas were collected at baseline, after three hours of mask-wearing, post-tape stripping, and after 24 h, respectively. Microbiome samples collected from the masked areas before and after wearing a medical mask were analyzed with bioinformatics methods. Mask-wearing significantly weakened barrier function in both masked and adjacent unmasked areas, while reducing bacterial diversity. It was also associated with an increase in *Cutibacterium* ( $P = 0.110$ ) and decreases in *Streptococcus* ( $P = 0.032$ ) and *Prevotella* ( $P = 0.026$ ) abundance. Moisturizer application prior to mask-wearing significantly reduced transepidermal water loss and erythema (both  $P < 0.001$ ) and further improved erythema after 24 h ( $P = 0.048$ ). These findings demonstrate that mask-wearing can disrupt the skin barrier and microbiome in individuals with sensitive skin and applying a moisturizer beforehand can mitigate mask-related discomforts by aiding barrier repair and reducing sensitivity.

**Keywords** Sensitive skin, Medical mask, Skin microbiome, Skin barrier, Dermocosmetics

As we navigate a post-COVID-19 world, self-awareness of wearing medical masks for disease prevention is becoming the new normal<sup>1,2</sup>. However, such mask-wearing habits also present an extra burden for individuals with sensitive skin. Sensitive skin (SS), characterized by heightened reactivity to environmental stimuli, often leads to discomfort and barrier dysfunction<sup>3</sup> and is an increasing concern among Chinese consumers. An earlier study has shown a prevalence of mild-to-severe SS as high as 39.04% in major Chinese cities<sup>4</sup>, with a rising trend alongside increased mask-wearing habits in recent years<sup>5</sup>. While multiple studies have investigated the influence of masking on the skin barrier function, a deeper understanding of its effects on sensitive skin requires specific clinical studies combined with an exploration of pathological mechanisms.

Compromised skin barrier, characterized by increased transepidermal water loss (TEWL) and sensitivity to external stimuli, is common in both sensitive skin and long-term masked skin<sup>6</sup>, which might in turn exacerbate underlying inflammation, hinder optimal hydration and increase vulnerability to pathogens, further contributing to skin sensitivity<sup>7</sup>. The occlusive microenvironment created by the medical mask can disrupt the skin barrier by altering the normal physiological profile and may lead to transient redness and even persistent erythema<sup>8,9</sup>. The importance of skin microbiome is also gaining recognition in sensitive skin research<sup>10,11</sup>. The balance of commensal bacteria, fungi, and other microorganisms plays a vital role in protecting against pathogens and maintaining physiological functions. Mask-wearing has been shown to alter the microbiome by significantly reducing beta diversity<sup>12</sup>, potentially exacerbating skin issues in individuals with SS who already have a compromised barrier function.

This study aims to evaluate the impact of mask-wearing on the skin microbiome and barrier function in women with sensitive skin under the real-world context of the COVID-19 pandemic. Additionally, we investigate whether applying a clinically proven moisturizer before mask-wearing can mitigate these adverse effects by accelerating skin barrier repair. Through understanding these mechanisms, we propose to generate new evidence for clinical recommendations regarding additional skincare practices for individuals with sensitive skin who tend to wear a mask as habitual protection.

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## Results

### Baseline characteristics

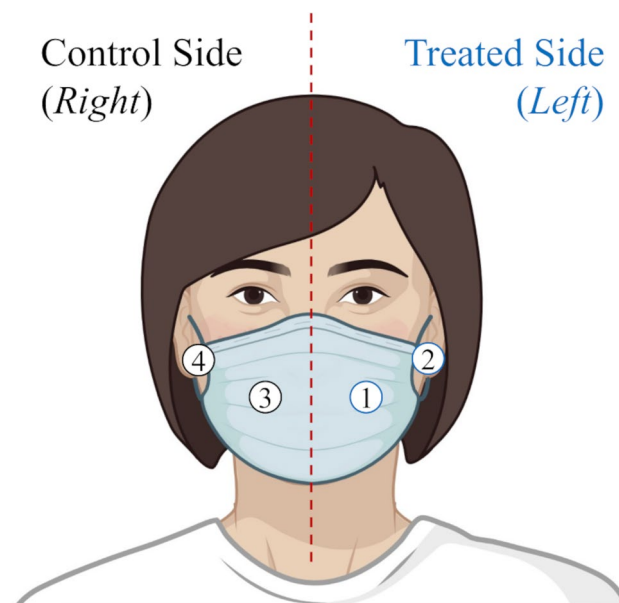
A total of 30 female volunteers with sensitive skin were enrolled in January 2021 for the study. All volunteers reported sensitivity to external stimuli on their facial skin and were tested positive during the lactic acid sting test (LAST) screening (mean LAST score: 4.14, range from 3 to 6). To reduce confounding factors, baseline values of skin physiological indicators, TEWL, erythema index (EI), stratum corneum hydration (SCH), pH and temperature, were tested on four sites including masked and unmasked areas on both sides of the face (Fig. 1). There was no statistically significant difference in the baseline values of skin barrier-related indicators (TEWL, EI and SCH) between the control side and the treated side (all  $P > 0.10$ , Table 1). However, these three indicators showed significant differences between the areas inside and outside the mask at baseline. Skin pH and temperature showed high heterogeneity, with significant differences in baseline levels among the four sampling sites (all  $P < 0.001$ ). According to investigator clinical gradings, participants showed mild erythema (median: 1, Q1-Q3: 1) and no visible scaling (median: 0, Q1-Q3: 0) in the masked areas at baseline.

### Skin barrier function changes in sensitive skin after three hours of mask-wearing

Wearing a medical mask for three hours (T1) significantly increased TEWL, EI, SCH, and temperature in the masked areas of sensitive skin compared to baseline, while pH significantly decreased (all  $P < 0.01$ , Fig. 2). Among all indicators, mask-wearing most significantly influenced the hydration level of stratum corneum, which increased from  $22.39 \pm 7.11$  to  $32.73 \pm 6.77$  on average. Furthermore, mask-wearing also negatively affected the barrier function of the adjacent, unmasked skin. Although increases in TEWL, EI, and SCH were observed in the unmasked areas, the rates of increase were still significantly lower compared to the masked areas (e.g., TEWL:  $13.92 \pm 2.87$  vs.  $18.96 \pm 4.40$ ,  $P = 0.002$ ). Similar trends were observed for pH and temperature in both masked and unmasked areas. Specifically, pH decreased by 0.53 and 0.54, respectively ( $P = 0.868$ ), while temperature increased by  $0.97^\circ\text{C}$  and  $0.83^\circ\text{C}$ , respectively ( $P = 0.155$ ).

### Microbiome changes in sensitive skin after mask-wearing

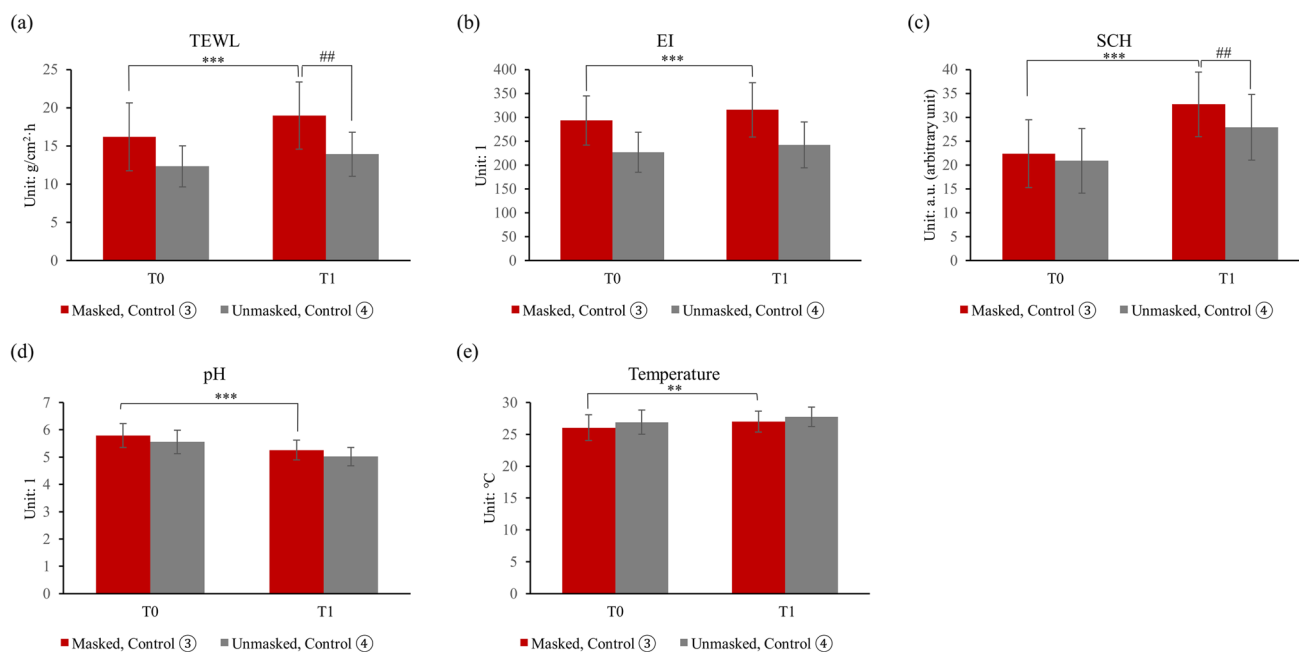
16 S rRNA gene sequencing analysis was performed on microbial samples collected before and after wearing a mask. We identified 2,128 OTUs with species annotations through comparison with the SILVA138 database (overall species mapping shown in Supplementary Fig. 1). Alpha diversity analysis, using the Wilcoxon rank-sum test, revealed no significant change in species richness after three hours of mask-wearing (Fig. 3a). However, the Shannon index indicated a significant decrease in bacterial diversity after masking ( $P = 0.013$ , Fig. 3b). Beta diversity was assessed to investigate within-group variability. Both principal coordinate analysis of UniFrac distances (UniFrac-PCoA, Fig. 3c) and non-metric multi-dimensional scaling (NMDS, Fig. 3d) demonstrated some overlap between the bacterial microbiota after three hours of mask-wearing and its baseline. This overlap suggests a degree of similarity in the overall microbiome on the facial skin of individuals with sensitive skin.



**Figure 1.** Schematic representation of the four facial sampling sites and group settings. Transepidermal water loss (TEWL), erythema index (EI), stratum corneum hydration (SCH), pH and surface temperature were measured on a  $0.8\text{ cm} \times 0.8\text{ cm}$  area at the center of all four sites (①: Masked, Treated side with moisturizer; ②: Unmasked, Treated side with moisturizer; ③: Masked, Control side with no moisturizer; ④: Unmasked, Control side with no moisturizer) at all timepoints (T0: baseline; T1: 3 h after masking; T2: immediately after tape-stripping; T3: 24 h self-recovery). Microbiome sampling was taken only on masked areas ③ at T0 and T1. Created with *BioRender.com*.

Indicators	Treated Side	Control Side	P-value
<b>TEWL</b>			
Masked	17.03 ± 5.29	16.17 ± 4.44	0.495
Unmasked	11.94 ± 2.39	12.34 ± 2.69	0.544
<b>P-value</b>	<0.001*	<0.001*	/
<b>EI</b>			
Masked	291.18 ± 55.51	293.41 ± 51.65	0.872
Unmasked	233.29 ± 47.12	226.90 ± 41.98	0.581
<b>P-value</b>	<0.001*	<0.001*	/
<b>SCH</b>			
Masked	22.68 ± 7.21	22.39 ± 7.11	0.876
Unmasked	20.66 ± 6.48	20.88 ± 6.73	0.897
<b>P-value</b>	0.041*	0.092	/
<b>pH</b>			
Masked	6.07 ± 0.44	5.79 ± 0.44	<0.001*
Unmasked	5.79 ± 0.42	5.56 ± 0.43	<0.001*
<b>P-value</b>	<0.001*	<0.001*	/
<b>Temperature</b>			
Masked	22.84 ± 2.63	26.04 ± 2.02	<0.001*
Unmasked	24.70 ± 2.23	26.92 ± 1.88	<0.001*
<b>P-value</b>	<0.001*	<0.001*	/

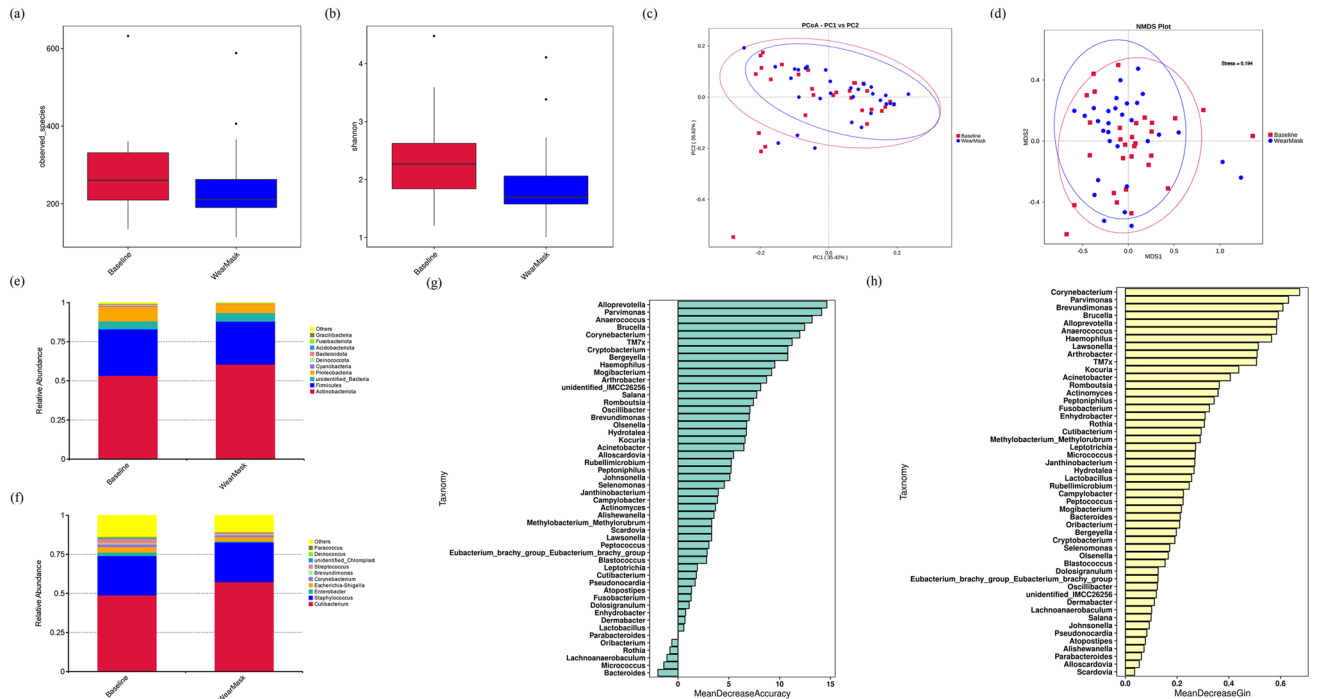
**Table 1.** Baseline measurements of four sampling sites. TEWL: transepidermal water loss, EI: erythema index, SCH: stratum corneum hydration. Paired t-test, \*:  $P < 0.05$ .



**Figure 2.** Effect of mask-wearing on skin physiological indicators. (masked vs. unmasked areas, ③: Masked, Control side with no moisturizer; ④: Unmasked, Control side with no moisturizer). Absolute measurements of (a) transepidermal water loss (TEWL), (b) erythema index (EI), (c) stratum corneum hydration (SCH), (d) pH and (e) temperature are shown at T0 and T1. T0: baseline before masking, T1: 3 h after masking. Paired t-test, \*\*\*: masked T0 vs. masked T1,  $P < 0.001$ ; #: masked T1 vs. unmasked T1,  $P < 0.01$ .

We suppose that a longer duration of mask-wearing might lead to a more significant separation in the skin microbiome composition.

Despite this overlap, mask-wearing led to specific shifts in taxonomic composition. At the phylum level, the abundance of Bacteroidota was significantly lower after mask-wearing ( $P = 0.018$ , Fig. 3e). Notably, at the genus level, mask-wearing was associated with an increase in *Cutibacterium* ( $P = 0.110$ ) and decreases in *Enterobacter*



**Figure 3.** Changes in the microbiome before and after wearing a mask (sampling site ③: Masked, Control side with no moisturizer). Box plots of observed species (a) and Shannon index (b) differences between groups. (c) Principal Coordinate Analysis (PCoA) based on Weighted UniFrac distances. (d) Non-Metric Multi-Dimensional Scaling (NMDS) analysis at the OTU level. Bar plot of relative species abundance at phylum (e) and genus (f) levels. Variable importance ranked by Mean Decrease Accuracy (g) and Mean Decrease Gini (h).

( $P=0.355$ ), *Streptococcus* ( $P=0.032$ ), and *Prevotella* ( $P=0.026$ ), as shown in Fig. 3f. Furthermore, we employed a machine learning model and correlation analysis to identify potential microbial biomarkers associated with responses to mask-wearing. Cross-validation of two Random Forest methods consistently ranked *Parvimonas*, *Alloprevotella*, and *Corynebacterium* as having the highest importance (Fig. 3g-h). Correlation analysis between skin physiological indicators and the top ten most abundant species revealed distinct associations. *Streptococcus*, *Enterobacter*, and *Escherichia-Shigella* were positively correlated with temperature before masking. In contrast, *Paracoccus* was negatively correlated with SCH before masking, while *Deinococcus* was negatively correlated with pH both before and after masking (all  $P < 0.05$ , Supplementary Fig. 2).

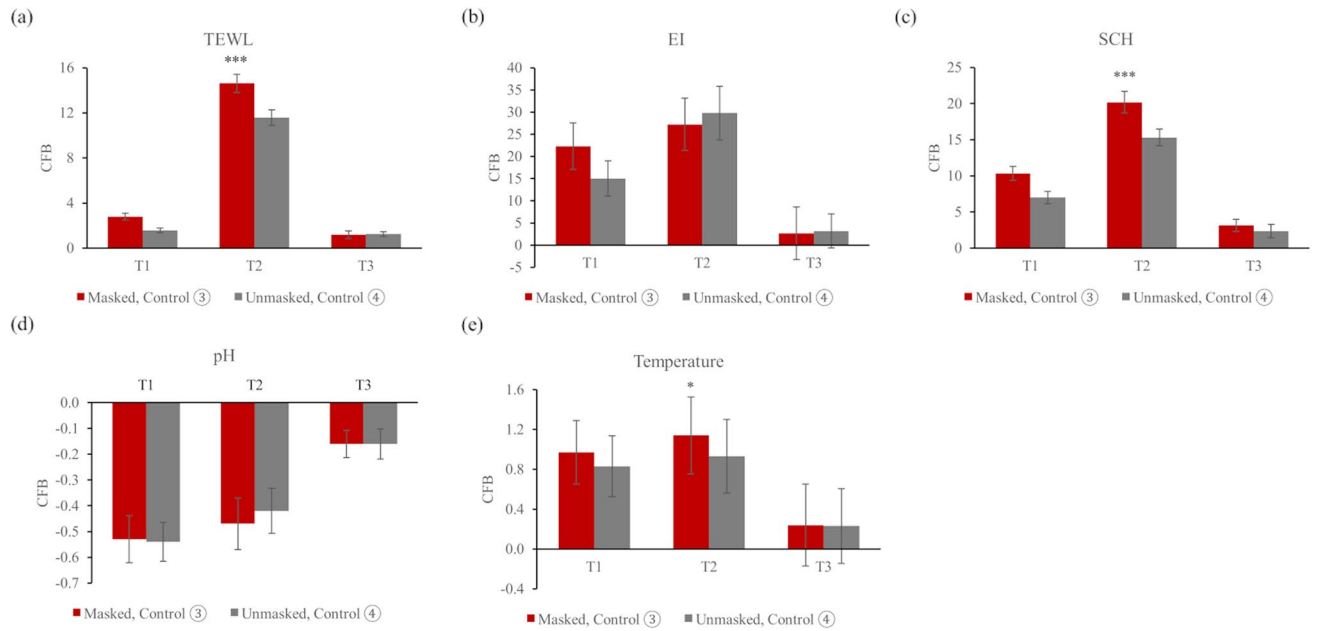
### Effect of mask-wearing on skin tolerance to acute barrier damage

Tape-stripping model was adopted to simulate acute barrier damage. TEWL and SCH increased significantly compared to the previous timepoint (TEWL: +62.44%, SCH: +30.06%, both  $P < 0.001$ ), while the increases in EI, pH and temperature did not reach statistical significance (all  $P > 0.200$ ). The mask microenvironment caused faster increases in TEWL, SCH and temperature in masked areas compared to unmasked areas (all  $P < 0.05$ , Fig. 4). Following tape-stripping, erythema increased similarly in both masked and unmasked areas ( $P=0.686$ , Fig. 4b). However, the absolute value of erythema was still significantly higher inside the mask compared to outside ( $320.66 \pm 53.04$  vs.  $256.69 \pm 50.95$ ,  $P < 0.001$ ). The pH of masked and unmasked areas showed an increasing trend than the previous timepoint, but the differences were not statistically significant ( $P=0.173$ , Fig. 4d).

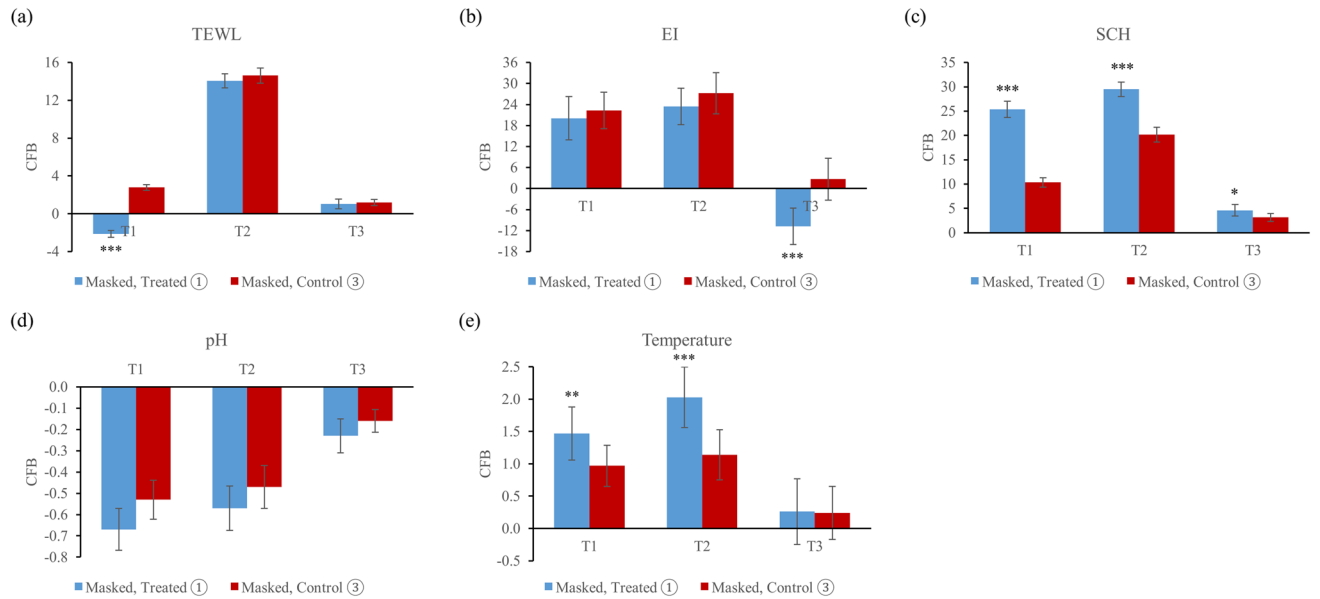
After 24 h of rest at home, all skin physiological indicators of the subjects gradually returned to baseline levels, with no significant difference between masked and unmasked areas. There was no significant difference in erythema and skin surface temperature in the masked area compared to baseline (EI:  $P=0.656$ ; temperature:  $P=0.556$ ). TEWL decreased significantly compared to T1 ( $P=0.002$ ) but remained elevated compared to baseline ( $P=0.001$ ). The change in stratum corneum water content showed a consistent trend with TEWL, remaining slightly above baseline levels ( $P < 0.001$ ). The pH decreased by  $0.53 \pm 0.50$  units after wearing the mask for 3 h, then slowly returned to near baseline levels, with a final difference of  $-0.16 \pm 0.29$  units ( $P=0.006$ ).

### Moisturizer application before wearing a mask improves mask-related discomforts and aids barrier repair

Subjects received sampled moisturizers to apply on the treated side. After wearing the mask for three hours, TEWL in the masked areas decreased on the treated side while increased on the control side ( $P < 0.001$ , Fig. 5a). SCH increased significantly on both sides, with the treated side showing an increase 2.45 times greater than the control side. The application of moisturizer also improved erythema after three hours of masking, with a slightly lower EI value on the treated side ( $P=0.428$ , Fig. 5b). After the tape-stripping induced barrier damage, the



**Figure 4.** Changes in skin physiological indicators at each timepoint (masked vs. unmasked areas, ③: Masked, Control side with no moisturizer; ④: Unmasked, Control side with no moisturizer). Change-from-baseline (CFB) measurements of (a) transepidermal water loss (TEWL), (b) erythema index (EI), (c) stratum corneum hydration (SCH), (d) pH and (e) temperature are shown at T1, T2 and T3. T1: 3 h after masking, T2: immediately after tape-stripping, T3: 24 h self-recovery. Paired t-test, \*:  $P < 0.05$ ; \*\*\*:  $P < 0.001$ .



**Figure 5.** Effect of moisturizer application before wearing a mask on skin physiological indicators inside the mask (treated vs. control areas, ①: Masked, Treated side with moisturizer; ③: Masked, Control side with no moisturizer). Change-from-baseline (CFB) measurements of (a) transepidermal water loss (TEWL), (b) erythema index (EI), (c) stratum corneum hydration (SCH), (d) pH and (e) temperature are shown at T1, T2 and T3. T1: 3 h after masking, T2: immediately after tape-stripping, T3: 24 h self-recovery. Paired t-test, \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ .

stratum corneum remained hydrated on the treated side, with a significantly higher SCH value than the control side ( $P < 0.001$ , Fig. 5c). This implies that prior application of moisturizer enhances the stratum corneum's tolerance to acute damage. Increases in TEWL, EI and pH after tape-stripping were all slightly lower on the treated side compared to the control side, but these differences were not statistically significant (all  $P > 0.200$ , Fig. 5d).

After 24 h of rest at home, erythema and hydration improved on the treated side. EI decreased further on the treated side, with a statistically significant improvement of  $-10.80 \pm 28.61$  units compared to baseline ( $P = 0.048$ ) and was significantly lower than the control side ( $P = 0.021$ ). Pre-masking application of moisturizer induced a sustained improvement in sensitive skin and mask-related erythema. Similarly, SCH on the treated side was significantly higher than the control side at the end of the study ( $P = 0.016$ ). Skin temperature returned to baseline, showing no statistically significant difference from baseline ( $P = 0.617$ , Fig. 5e), while TEWL decreased to a near-baseline level after 24 h ( $P = 0.052$ ). Furthermore, both investigator clinical assessments and participant subjective assessments confirmed improvements in various mask-related skin discomfort ratings at the end of the study. Specifically, investigator assessments showed that 90% of participants experienced only mild indentation in the treated area, 96.67% had no scaling, and 80% had no stinging. Self-reported outcomes showed improvement in erythema (86.21%) and no increase in perceived skin greasiness (93.10%). No adverse event related to the mask material or moisturizer used was observed during the study.

## Discussion

As a post-COVID-19 world embraces more daily mask-wearing habits, concerns arise about their enduring impact on skin health, especially for individuals with sensitive skin (SS). A recent cross-sectional survey by Zhao, et al.<sup>2</sup> suggests a more voluntary and prepared attitude towards medical mask use, particularly among high-income and well-educated individuals. However, previous studies have documented adverse effects of mask-wearing on otherwise healthy skin, with erythema, indentation marks, and itching being the most common complaints<sup>8,9</sup>. Masking could also exacerbate existing conditions like acne and rosacea, even prompting new clinical terms like “maskne”<sup>13–15</sup>. To generate specific clinical evidence for the Chinese SS population, we investigated the direct impact of mask-wearing on skin barrier function and microbiome composition. Additionally, we evaluated the potential of a clinically proven moisturizer to aid in barrier repair and mitigate patients' discomforts.

Our findings demonstrate that a moderate duration of mask-wearing could pose an extra burden to the skin barrier function in individuals with SS. Three hours of mask use, representing a general duration of wear, resulted in significant increases in TEWL, EI and SCH, indicating barrier disruption, which aligns with previous findings on post-masking physiological profiles<sup>16,17</sup>. Notably, the masking microenvironment also impacted adjacent unmasked areas, albeit to a lesser extent. We hypothesize that habitual long-term mask-wearing (approximately one year since the COVID-19 outbreak) led to a more fragile barrier function in the area covered by the mask compared with the uncovered area, which also implies the importance of extra skincare for unmasked areas as well. Moreover, mask-wearing reduced skin tolerance to acute barrier disruption, as evidenced by significantly higher increases in TEWL in masked areas compared to unmasked areas after the tape-stripping challenge and incomplete recovery even after 24 h. These observations suggest that although the airtight microenvironment is effective for respiratory virus prevention, mask-wearing could still impair the skin barrier function and also hinder its repair process, leading to cumulative skin damage for sensitive population. This is consistent with previous findings in both healthy individuals and SS populations, attributing mask-related barrier damage to the occlusive microenvironment and increased humidity and temperature<sup>18,19</sup>.

Interestingly, our study also revealed a significant decrease in bacterial diversity after mask-wearing in SS individuals. This aligns with emerging research linking SS phenotypes to alterations in microbiome composition<sup>20</sup>. Huang, et al.<sup>21</sup> investigated the microbiome variation during wearing masks earlier in 2020 and found that the diversity of the facial microbiome showed a decreasing trend after wearing masks. While the dominant bacterial phyla in our study (Actinobacteria, Firmicutes, and Proteobacteria) were consistent with previous reports<sup>12</sup>, variations in dominant genera suggest potential influences of sampling sites and bioinformatic methodology. In our study, mask-wearing was associated with an increase in *Cutibacterium* and decreases in *Streptococcus*, *Prevotella*, and *Enterobacter*, highlighting the microbiome's susceptibility to this altered microenvironment. Although these shifts in relative abundance provide evidence for mask-related dysbiosis, future studies incorporating more time-series data are needed to identify robust microbial biomarkers for targeted therapeutics.

Importantly, our study demonstrates that applying a moisturizer before mask-wearing can help mitigate the accompanied discomforts. The moisturizer-treated side showed reduced TEWL and erythema after mask-wearing and faster barrier recovery, indicating that preemptive skincare can enhance barrier resilience and reduce mask-related sensitivity. The moisturizer also proved good tolerance with no product-related adverse events reported during the study. These findings are consistent with previous clinical outcomes of dermocosmetic regimens, particularly those containing barrier-repairing actives, in soothing mask-related discomforts and sensitivity<sup>22–25</sup>. We hypothesize that the dermocosmetic formula used in our study effectively strengthened the skin barrier with lipid-replenishing agents like squalene, shea butter, and cetyl esters. Etyl esters could act on skin sensitivity by decreasing erythema and irritation related to neurogenic inflammation and hence improve the quality of life in rosacea patients<sup>22</sup>. Additionally, the probiotic *Vitreoscilla filiformis* extracts potentially modulate the skin microbiome, contributing to a more suitable microenvironment for recovery. In vitro studies also showed that *Vitreoscilla filiformis* extracts could strengthen the skin barrier by stimulating skin differentiation and tight junctions including claudin-1<sup>25</sup>. Given the study's limitations in terms of time and cost, future research could expand these preliminary findings to larger and more representative populations with sensitive skin. Long-term studies evaluating the real-world effects of mask-wearing and comprehensive interventions to protect skin health are also warranted<sup>26</sup>.



In conclusion, our findings highlight the significant impact of mask-wearing on skin physiology and microbiome in individuals with sensitive skin. Proactive skincare strategies including preemptive application of a barrier-repairing moisturizer offer protective benefits. These insights contribute to a better understanding of the adjunctive role of dermocosmetics in mitigating the adverse effects of necessary medical mask use in real-world settings.

## Methods

### Study design

All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the Ethics Committee of the First Hospital of Peking University (Approval No.: 2021057) and informed consent was obtained from all subjects. Female volunteers with self-reported sensitive skin (“I have responsive burning/stinging/flushing/itching on facial skin upon external stimuli”) were recruited, excluding individuals with facial atopic dermatitis, acne, or mask-covered area infections. Participants were then screened for skin sensitivity induced by 10% lactic acid solution using the lactic acid sting test (LAST)<sup>27</sup>. Compared with saline applied to the contralateral site, lactic acid-induced sting sensation was rated by the participants at 2.5 min and 5 min after application using a 4-point scale (0 ~ 3) separately. Total scores  $\geq 3$  (sum of scores at 2.5 min and 5 min, 0 ~ 6) were considered positive.

### Interventions

At the time of the study, local epidemic regulations in 2021 mandated mask-wearing in the public. While specific mask-wearing history was not collected, all participants had been wearing masks regularly since the COVID-19 outbreak. Disposable medical masks (three-layer non-woven fabric surgical mask, Borui, China), labeled as hypoallergenic, were provided by the investigators and the correct wearing procedure was instructed to ensure the mask margins were airtight. Participants were asked to only cleanse their faces with lukewarm water and commute to the study site without applying any skincare or makeup products. A commercially available moisturizer cream (Toleriane Ultra Light, La Roche-Posay, France) was used at the study site to investigate its benefits before mask-wearing. The key ingredients included glycerin, squalene, shea butter, cetyl esters and probiotic *Vitreoscilla filiformis* extracts, and the formulation had been tested clinically safe in sensitive skin populations.

Four timepoints were designed to evaluate the mask-wearing and moisturizer effects on four areas (Fig. 1). Baseline (T0): Upon arrival, participants took off their masks and rested for about one hour in a temperature- and humidity-controlled room<sup>17</sup>, as participants had to wear masks in their commute according to the epidemic regulations. Instrumental measurements and microbiome sampling were then taken and used as the baseline reference. After baseline measurements, one fingertip unit (FTU) of the test moisturizer was applied to the designated treated side of the face. Three hours after mask-wearing (T1): Participants then wore a new medical mask for three hours. After three hours, masks were removed and instrumental measurements and microbiome sampling were conducted again after a 15 min rest. Immediately after tape-stripping (T2): A standardized tape-stripping method<sup>27</sup> was adopted to simulate acute barrier damage on both sides of the face. Adhesive tape strips were applied seven times consecutively and removed with consistent pressure and speed over an area of approximately 0.8 cm $\times$ 0.8 cm at each designated site. Measurements were taken immediately after tape-stripping. 24 h post barrier damage (T3): Participants returned to the study site after 24 h and measurements were taken again on the four areas as described in T0.

### Skin physiological measurements

Skin barrier parameters including transepidermal water loss (TEWL), stratum corneum hydration (SCH), erythema index (EI), pH and surface temperature were measured at T0, T1, T2 and T3. A portable skin barrier measurement device (GPSkin Barrier, GPower, Korea) was used to measure TEWL, SCH and surface temperature. EI was measured indirectly by skin hemoglobin content using a Mexameter (MX18, Courage + Khazaka, Germany). pH was measured with a skin pH meter (pH900, Courage + Khazaka, Germany). All measurements were conducted as described in our previous study<sup>28</sup> and recorded as the average of three repeated tests.

### Microbiome sampling and 16 S rRNA sequencing

Sterile skin swabs were used to collect samples at T0 and T1 from marked areas inside the mask on the control side of the face. After swabbing the skin for 30s, swabs were stored at -70 °C to preserve DNA integrity for subsequent extraction and analysis<sup>12</sup>. Genomic DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen, Germany) and quantified with Nanodrop 2000 (Thermo Scientific, USA). Sequencing libraries were prepared by amplifying 16 S rRNA gene using the TruSeq DNA PCR-Free Library Preparation Kit (Illumina, USA), quantified with Q-PCR, and sequenced using NovaSeq 6000 (Novogene, China).

### Bioinformatics analysis

Sequencing data were processed using FLASH (v1.2.7)<sup>29</sup> to merge reads into raw tags, which were filtered to obtain high-quality clean tags. Operational taxonomic units (OTUs) clustering was performed using UPARSE (v7.0.1001)<sup>30</sup> with 97% sequence identity, and taxonomic annotation was assigned with the SILVA138 database<sup>31</sup>. Multiple sequence alignment was performed using MUSCLE (v3.8.31)<sup>32</sup>. Data were normalized to the lowest dataset size for subsequent alpha and beta diversity analyses, conducted using QIIME (v1.9.1)<sup>33</sup> and R software (v2.15.3). Alpha and beta diversity index differences were analyzed using paired t-test and false discovery rate (FDR) correction was applied to adjust for multiple comparisons. Correlation analysis between high-ranking species and physiological measurements was calculated with Spearman's correlation test. Heatmaps were generated using the pheatmap package in R. The significance level was set at  $P < 0.05$ .

## Subjective assessments

Investigator-rated clinical assessments in facial erythema, indentation, skin rashes, itching, scaling, stinging, burning and skin greasiness (0–3) were collected at all timepoints. Local tolerability was monitored throughout the study in case of severe mask- or moisturizer-related adverse events. Participants were also asked to report subjective ratings on erythema, indentation, and skin tightness on a scale of 0 to 3 at each timepoint.

## Statistical analysis

Statistical analyses were performed using SPSS 26.0 and R software. Normally distributed data (instrumental measurements, e.g., TEWL) were described as mean  $\pm$  standard deviation and analyzed using ANOVA for comparisons between treated and control sides and paired T-tests for masked and unmasked areas on the same side. Change from baseline (CFB, measurements at T1–T3 minus T0 values) was applied to instrumental measurements to minimize the impact of baseline differences and visualize mask effects (masked vs. unmasked areas) and moisturizer effects (treated vs. control side). Non-normally distributed data (subjective ratings, e.g., skin greasiness score) were described as median and interquartile range and analyzed using the Wilcoxon test. The significance level was set at  $P < 0.05$ .

## Data availability

The datasets generated and/or analyzed during the current study are available in the NCBI BioProject repository, PRJNA1164415.

Received: 22 July 2024; Accepted: 1 October 2024

Published online: 16 October 2024

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## Acknowledgements

The authors would like to thank La Roche-Posay China for donating the products for study use and Gloria (Yueqing) Niu and Alvin (Yuhao) Wei from L'Oréal China Research and Innovation for their assistance in literature retrieval and formulation information.

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Shaomin Zhong: Conceptualization, Methodology, Project Administration, Resources, Writing – Review & Editing. Yanyun Lai: Data Curation, Investigation, Methodology, Validation, Writing – Original Draft. Jun Na: Investigation, Methodology, Validation, Writing – Review & Editing. Yan Wu: Funding Acquisition, Supervision, Writing – Review & Editing. All authors reviewed the manuscript and approved the submission.

## Declarations

### Competing interests

The authors declare no competing interests.

### Additional information

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1038/s41598-024-75072-2>.

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