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ORIGINAL RESEARCH

The Characteristics of the Skin Physiological Parameters and Facial Microbiome of "Ideal Skin" in Shanghai Women

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Purpose: Everyone pursues perfect skin, but there exist significant differences between cultures, and no commonly accepted standards have been established. Therefore, our study attempted to define the "ideal skin" of oriental women and analyze the relationship between different skin physiological parameters and microbiomes.

Patients and Methods: Based on our customized grading standard, the VISIA CR photos of 111 young women aged from 18 to 25 in Shanghai were collected and scored by the severity of pores, acne, spots, and wrinkles. The volunteers were then divided into "ideal skin" (W1), "normal skin" (W2), and "undesirable skin" (W3) groups. The physiological parameters of facial skin were measured by non-invasive instrumental methods, and the skin microbiome was analyzed by 16S rRNA and ITS high-throughput sequencing.

Results: From "ideal skin" to "undesirable skin", the skin physiological parameters, α-diversity, and composition of the facial microbiome showed noticeable regular changes. Compared with the "normal skin" (W2) and "undesirable skin" (W3), the "ideal skin" (W1) group had lower sebum content, TEWL, melanin, hemoglobin, and roughness but higher hydration content and skin pH value. Furthermore, the Shannon index of skin bacteria was significantly increased in W1 ($P = 0.004$), suggesting that the ideal skin had higher species diversity. From W1 to W3, the species composition was changed significantly. The abundance of *Actinobacteria* was increased, while *Proteobacteria* and *Bacteroidetes* were decreased. Correspondingly, the abundances of lipophilic *Propionibacterium* and *Malassezia* were increased, while the abundances of *Stenotrophomonas, Pseudomonas, Ralstonia*, and *Streptococcus*, were significantly decreased. Additionally, Spearman correlation analysis revealed strong correlations between the physiological parameters and the microbiota. Notably, the Shannon index of skin bacteria was significantly positively correlated with skin hydration ($P = 0.03$) but negatively correlated with the abundance of *Cutibacterium* ($P = 0.000$), hemoglobin content ($P = 0.025$), and sebum content ($P = 0.5$). Therefore, the skin hydration content and the abundance of *Cutibacterium* played an important role in maintaining the α-diversity and skin homeostasis.

Conclusion: Ideal skin had better water-oil balance and barrier function, higher microbial diversity, and more reasonable species distribution. Therefore, daily skincare needs to control skin oil and maintain skin microecological balance to achieve ideal skin conditions for young women aged 18–25 years old.

Keywords: Shanghai women, ideal skin, definition, physiological parameters, skin microbiome

Introduction

In modern society, healthy skin and a beautiful appearance are considered the foundation of happiness. Beauty has been defined to be equal to happiness, life satisfaction, and a higher salary.[1–4] Moreover, beautiful are always considered superior, possessing characteristics such as higher intelligence, higher moral character, more interpersonal skills, and

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easier access to groups and families.[5–8] Therefore, as the zoologist Desmond Morris said, "flawless skin is the most universally desired human feature".[9]

However, flawless skin is difficult to obtain, like everything in the world. The skin covers the entire surface of the human body, and it is in direct contact with the external environment. Therefore, various skin problems inevitably occur under the combined influence of endogenous and exogenous factors.[10,11] For example, acne appears due to the increased sebum secretion caused by changes in hormone levels, and about 90% of people experience pain caused by acne.[12–14] Personal living habits, diet, sleep patterns, stress, and so on have led to the occurrence of acne.[15,16] Moreover, pore size is closely related to exuberant sebaceous gland secretion, skin aging, and increased hair follicle volume.[17,18] Enlarged pores can make facial skin look rough, loose, and lackluster.[17] In daily life, sunlight exposure can cause skin pigmentation and other problems. Facial pigmentation is not only related to sunlight exposure but also to hormone levels and genetics.[19,20] The appearance of wrinkles is inevitable in skin aging, resulting from the combined action of endogenous factors (natural aging) and exogenous factors.[21] As known, our skin is home to millions of bacteria, fungi, viruses and mites that compose the skin microbiota, which plays essential roles in protecting against invading pathogens, educating our immune system, and the breakdown of natural products.[22] When the microecological balance is disturbed, various skin problems, such as sensitive skin, acne, atopic dermatitis, and psoriasis, will occur accordingly.[23–27]

Many factors affect the skin condition, such as the above-mentioned pigmentation, acne, enlarged pores, and wrinkles. The existence of these problems will decline facial beauty and make it difficult to achieve a perfect state, which has become one of the problems that contemporary women are very concerned about. "Smooth and tender, delicate and transparent, moist and flawless, elastic and healthy skin" is in line with the interpretation of oriental women's ideal skin. In this era of pursuing youthful beauty, we are willing to invest more time and money in personal care to enhance facial characteristics, specifically to make us look younger, healthier, and more attractive. This has also promoted the successful development of the personal care industry, undoubtedly bringing challenges and opportunities to cosmetic research and development.

The pursuit of ideal skin has become the goal pursued by beauty, while what kind of skin belongs to perfect or ideal skin remains unclear. There are great differences between different cultures, and no commonly accepted standards have been established. Therefore, in our present study, we attempted to define the "ideal skin" of oriental women based on the understanding and aesthetics of oriental people for perfect skin. According to VISIA CR photos, female volunteers aged 18–25 in Shanghai (n=111) were divided into "ideal skin" (W1), "normal skin" (W2), and "undesirable skin" (W3) groups based on our customized scoring standard using pores, acne, spots, and wrinkles as evaluating indicators. We further analyzed the skin physiological parameters and facial microbiome of oriental women to obtain the characteristics of the ideal skin. This research would provide a scientific basis for skin care.

Materials and Methods

Volunteer Recruitment

This study was based on the investigation of the correlation between skin health status and skin and gut microbiota of qualified volunteers living in Shanghai (aged 18–60, n=494). Our research complied with the Declaration of Helsinki. Ethical approval was provided by the Institutional Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital South Campus (Shanghai Fengxian District Central Hospital, Approval No.: 2021-KY-15). Written informed consent was obtained from each participant prior to sample collection. The inclusion criteria included the following items: healthy adults with 18–60 years of age who live in Shanghai; being aware of the research purpose; read, understood and signed the informed consent forms. The exclusion criteria included the following items: received anti-inflammatory drugs or antibiotics orally or by injection within 3 months; facially applied anti-inflammatory drugs within 2 months; undergoing treatments for asthma or other chronic respiratory diseases; lactating or pregnant women; volunteers already enrolled in other clinical traits. All volunteers needed to finish the Self-Evaluation Questionnaire carefully, referring to questions about their skin condition, life habits, and physiology. All skin parameters and microbial samples were collected in July 2021.

Considering that skin physiological characteristics is regulated and influenced by hormone levels,[19] especially by estrogen level for women. Generally, estrogen secretion usually peaks at the age of 30, collagen secretion peaks at the age of 20, and overlapping peaks appear at the age of 25. Therefore, the age of 25 is considered the beginning of skin aging.[28,29] Therefore, we chose the age group of the ideal skin research group to be 18–25 years old. In order to minimize the influence of gender and age on skin biophysical parameters and skin microbes, female volunteers (n=111) aged 18–25 in Shanghai were selected as the research subjects.

Definition and Grouping of "Ideal Skin"

In general, the skin status was evaluated from aspects of acne, pores, spots, and wrinkles. Therefore, in our current study, the VISIA CR photos of volunteers were collected and scored using pores, acne, spots, and wrinkles as evaluating indicators. The estimated standard was slightly modified based on the pore score,[18] Fitzpatrick's wrinkle scale, acne grading standard, and the chloasma area and severity index (MASI) score.[30] The grading standard is shown in Table 1, and our customized scoring standard is illustrated in Figure 1. By integrating various skin statuses, we defined "ideal skin" was that "skin with almost no visible pores, no visible wrinkles, and no invisible acne and spots". Here, the skin color was not included in our study.

In our study, the VISIA CR photos of the volunteers were scored by a team of seven cosmetic specialists, who were uniformly trained according to our customized scoring standard (Figure 1 and Table 1), then the volunteers were divided into "ideal skin" group (W1), "normal skin" group (W2) and "undesirable skin" group (W3). The grouping and basic information of each group was shown in Table 2.

Collection of Skin Physiological Parameters and Skin Microbial Samples

The method for skin physiological parameters and microbial sample collection was according to Shao et.al,[31] described briefly as follows: all subjects were required not to use skin care products and cosmetics after washing their faces the night before the measurement and the day of the test. Volunteers were asked to stay in an environment of constant temperature and humidity (21±1°C and 50±5%) for 30 min, and an instrument was used to measure cheek-related parameters. The VISIA-CR facial image analyzer was used to take pictures of the front and side of the volunteer's face, and then Corneometer® CM 825, Tewameter® TM 300, Sebumeter® SM 815, Glossymeter® GL200, memetreter® MX 18, pH 905 and Cutometer dual MPA 580 were used to measure hydration, transdermal water loss (TEWL), sebum, roughness, melanin, hemoglobin, pH and elasticity (R2), respectively.

The microbial samples were collected in an area of 3*3 cm^2 on the left cheek of the volunteer, a sterile cotton swab with sterile collection solution containing 0.9% NaCl and 0.1% Tween-20 was used to repeatedly scrape at least 30 times, and then the samples were immediately stored at −80°C for subsequent DNA extraction.

DNA Extraction and PCR

Microbial genomic DNA was extracted using the Fast DNA® Spin Kit for Soil (MP Biomedicals, USA) according to the kit instructions. DNA quality, concentration, and purity were examined using 1% agarose gel electrophoresis and NanoDrop 2000 (Thermo Fisher Scientific, USA). The V3-V4 variable region in the bacterial 16S rRNA gene was

Table 1 Grading Standard of Facial Skin Status

Evaluation Index / Grade	Pores	Wrinkle	Acnes	Spots
0	No visible pores	No visible wrinkles	No visible acne	No visible spots
1	Visible skin pores of 0.1~0.3 mm^2	Fine wrinkles and slight indentations	1 or 2 acne	Spot content <10%
2	Pore size 0.3~0.6mm^2, no blackhead embedded	The wrinkles are clearly visible and the fold depth is 1–2 mm	Acne < 10	Spots content 10%~29%
3	Pore size 0.3~0.6mm^2 with blackhead embedded	The depth and number of wrinkles are significant, fold depth ≥3 mm	Acne ≥10, and the presence of papules nodules	Spots content >30%

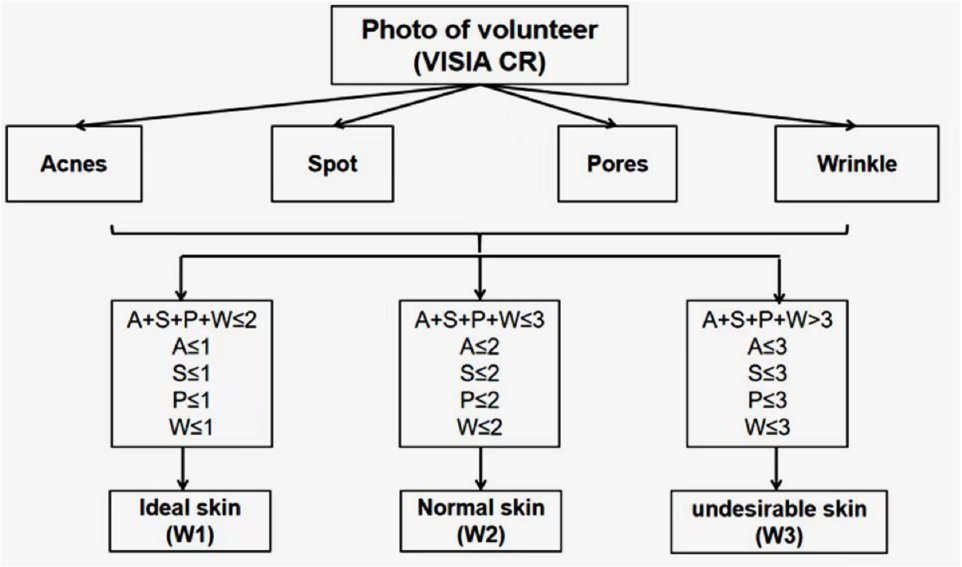

Figure 1 Customized scoring standard for "ideal skin" (W1), "normal skin" (W2) and "undesirable skin" (W3) groups; if acnes/spot/pores/wrinkles (A/S/P/W) gets three points, the total score, even if only have three points, is still in the undesirable skin group.

amplified by PCR using the forward primer 338F (5′-ACTCCTACGGAGGCAGCAG-3′) and the reward primer 806R (5′- GGACTACHVGGGTWTCTAAT-3′). Meanwhile, the fungal endogenous transcribed spacer (ITS1-ITS2) was amplified by PCR with the forward primer ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and the reward primer ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′). PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) according to the manufacturer's instructions.

Gene Sequencing and Data Processing

Library construction was performed using the NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific, USA). Sequencing was performed using MiseqPE300 (Illumination, USA). The raw data were uploaded to the NCBI SRA database (Accession Number: SRP330206). The raw sequencing data were quality-controlled using Fastp software (v0.19.6) and spliced using FLASH software (v1.2.11). The sequences were classified using Uparse software (v7.0.1090) and annotated according to the similarity of 97%, the Silva 16S rRNA and Unite ITS databases were aligned, and the alignment threshold was set to 0.7.

The raw data from high-throughput sequencing were collated and filtered, and the validated sequences were obtained for subsequent analysis. Approximately 2,861,229 (16S rRNA) and 4,282,096 (ITS) valid sequences were obtained. All samples were flattened out according to the minimum sequence number, and then, in turn, a total of bacteria co-clustered 9068 OTUs, belonging to 57 phyla, 1672 genera, and 3472 species. In addition, fungi co-clustered 2807 OTUs, belonging to 12 phyla, 554 genera, and 938 species.

Table 2 Basic Information of the Participants in Each Group

Characteristics	W1 (Ideal Skin)	W2 (Normal Skin)	W3 (Undesirable Skin)
Total number of subjects, n (percentage of total participants)	33 (29.7%)	38 (34.2%)	40 (36.1%)
Age (yr), mean ± SD	21.39±2.524	21.21±2.373	22.48±2.364
Skin problems no. (percentage of population in each group)			
Wrinkles	11 (33.33%)	19 (50%)	16 (40%)
Coarse pore	13 (39.39%)	18 (47.36%)	26 (65%)
Dryness	20 (60.6%)	17 (44.73%)	19 (47.5%)
Blackhead	23 (69.69%)	25 (65.78%)	29 (72.5%)
Acne scarring	16 (48.48%)	24 (63.15%)	32 (80%)
Spot	3 (9.09%)	3 (7.89%)	8 (20%)

Statistical Analysis

All data are represented as mean ± standard deviation (X±SD) unless otherwise indicated. The statistical significance level was 0.05 unless otherwise noted. SPSS 25.0 software was used for data analysis of skin physiological parameters. Non-parametric test and Kruskal–Wallis test were selected to analyze the differences between groups in skin physiological parameters. While non-parametric Wilcoxon rank-sum test was used to compare microbial diversity and composition between the two groups. To compare the β-diversity between two groups, unweighted distance metrics were used. Spearman rank correlation test was used to determine the Spearman correlation between the biophysical parameters and the microbiota. These data were analyzed on the online platform of MajorbioI-Sanger Cloud Platform (www.i-sanger.com).

Results

Volunteer Grouping

According to our customized scoring standard, 111 volunteers were divided into three groups as follows: "ideal skin" (W1), "normal skin" (W2), and "undesirable skin" (W3). The characteristics of each group are shown in Table 1, and the results indicated that the proportion of "ideal skin" accounted only for 29.7%, which was similar to our prediction. Obviously, most females had more or less skin problems, and their skin did not reach perfect status. Combined with the questionnaire, frequently concerning skin problems included wrinkles (52.58%), enlarged pores (51.94%), dryness (50.00%), blackheads (49.35%), acne scarring (43.87%), and pigmentation (24.52%). It was proved that the evaluation indicators we selected were reasonable. In addition, there was no significant difference in the average age of each group.

Comparison of Skin Physiological Parameters in Each Group

The cheek skin's physiological parameters of the three groups were shown in Figure 2. As we seen in Figure 2, from the "ideal skin" group to the "undesirable skin" group, namely from W1 to W3, sebum, TEWL, melanin and R2 showed a clear upward tendency ($P > 0.05$), roughness ($P < 0.05$) and hemoglobin ($P < 0.001$) were increased significantly, while for skin pH value, little changes ($P > 0.05$) were observed among the three groups. Compared with the "normal skin"

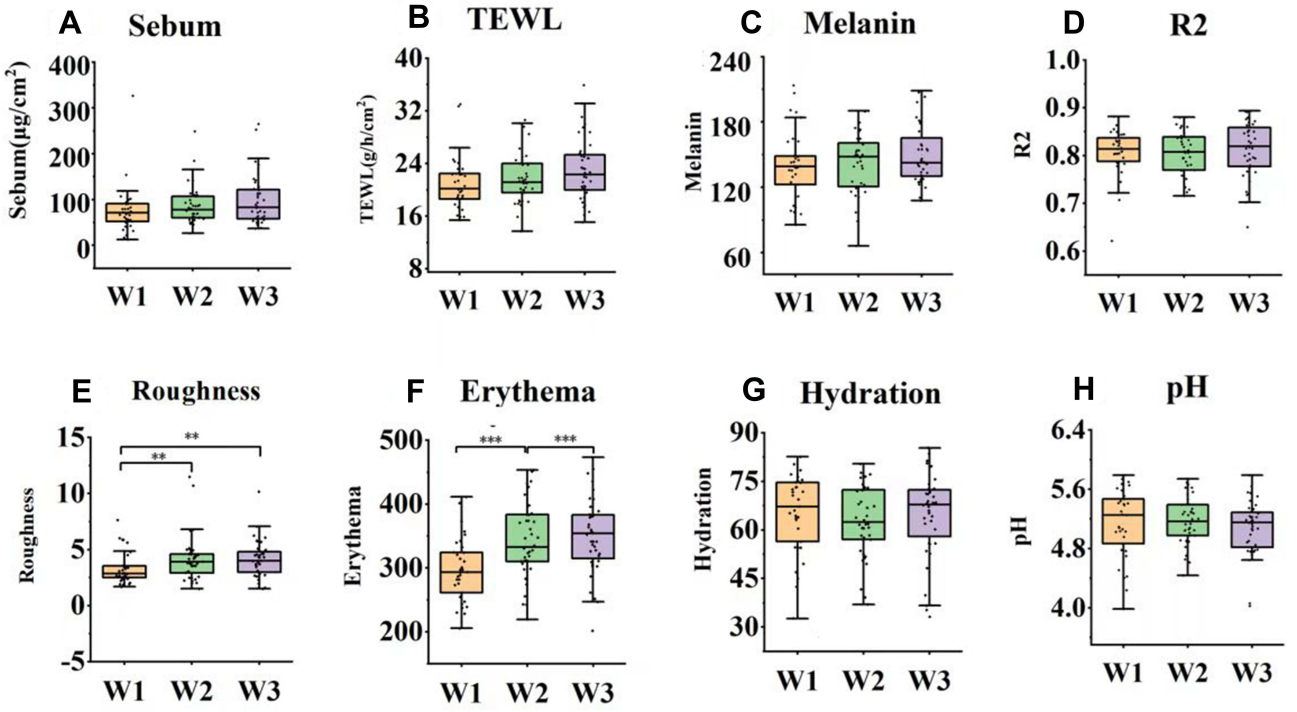

Figure 2 Comparison of physiological parameters of "ideal skin" (W1), "normal skin" (W2) and "undesirable skin" (W3). (**A**) Sebum, (**B**) TEWL, (**C**) Melanin, (**D**) R2, (**E**) Roughness, (**F**) Hemoglobin, (**G**) Hydration, (**H**) pH significant differences were shown in the figure, $^{**}P < 0.01$; $^{***}P < 0\ 0.001$.

(W2) and "undesirable skin" (W3) groups, the "ideal skin" group (W1) had higher skin hydration content and lower sebum content, TEWL, melanin, roughness and hemoglobin, showing better water-oil balance and skin barrier function.

Alpha Diversity Analysis

The α-diversity among group W1, W2 and W3 was analyzed by Wilcoxon rank-sum test. As it was shown in Table 3, the Shannon index of bacteria was significantly decreased from "ideal skin" to "undesirable skin" (from W1 to W3). The Shannon index of the W3 group was decreased, which was significantly different from that of W1 ($P = 0.0048$) and W2 ($P = 0.016$). However, the Ace and Chao indexes of bacteria revealed increased tendency, although no significant differences ($P > 0.05$). The Simpson index of the W3 group was increased significantly, which was different from W1 and W2 ($P < 0.05$). Coverage index was decreased in W1, which was significantly different from W2 ($P < 0.05$) and W3 ($P < 0.05$). It could be seen that from "ideal skin" to "undesirable skin" (from W1 to W3), the diversity of bacteria was decreased, the Shannon index and Ace index of fungi were also decreased, while there was no significant difference ($P > 0.05$), and the diversity of fungi was not obviously different. This finding suggested that the "ideal skin" had higher α-diversity and higher species diversity than normal and undesirable skin.

No Difference of β-Diversity Among Three Groups

Based on the unweighted_unifrac algorithm distance metrics, principal coordinate analysis (PCoA) was used to evaluate the β-diversity among three groups. The results showed that there was no significant difference in β-diversity between groups ($P > 0.05$) (data not shown), indicating that the overall species composition and relative abundance of bacteria and fungi among W1, W2 and W3 was similar.

Differences in Taxonomic Profiles of Skin Bacteria and Fungi Among Three Groups

The bacterial composition of the three groups was analyzed at different taxonomic levels. At the phylum level, the cheek bacteria of the three groups were mainly composed of *Actinobacteria, Proteobacteria, Firmicutes* and *Bacteroidetes*, accounting for more than 97% (Figure 3A). From the "ideal skin" group to the "undesirable skin" group, the abundance of *Actinobacteria* was increased significantly (Figure 3C, $P < 0.001$), the abundance of *Firmicutes* was increased (Figure 3E, $P > 0.05$), and the abundances of *Proteobacteria* (Figure 3D, $P < 0.05$) and *Bacteroidetes* (Figure 3F, $P < 0.05$) were significantly decreased. Compared with W1 and W2, Group W3 showed significant changes in species abundance, with a significant increase in *Actinobacteria* ($P = 0.01351$) and a pretty significant decrease in *Proteobacteria* ($P = 0.000003402$), while a significant decrease in *Bacteroidetes* ($P = 0.01425$) was observed. The fungi on the cheek skin of the three groups were mainly composed of *Ascomycota* and *Basidiomycota*, accounting for 90.2% (Figure 3B), while the difference analysis of species composition showed the difference was not significant. In other words, at the phylum level, the "ideal skin" group

Table 3 Alpha Diversity Index (Mean ± SD) of "Ideal Skin" (W1), "Normal Skin" (W2) and "Undesirable Skin" (W3)

Microbiota	α-Diversity Index	Ideal Skin (W1)	Normal Skin (W2)	Undesirable Skin (W3)	P-value
Bacteria	Shannon	1.50±0.74	1.59±1.10	1.05±0.80	0.004**
	Chao	274.80±163.31	443.80±488.50	446.96±542.39	0.265
	Sob	221.71±126.63	355±446.34	306.59±418.44	0.057
	Ace	287.77±171.51	476.29±502.29	546.41±566.33	0.058
	Simpson	0.49±0.22	0.46±0.22	0.63±0.23	0.005**
	Coverage	0.998±0.0017	0.997±0.0037	0.996±0.0056	0.757
Fungi	Shannon	3.37±0.50	3.15±0.61	3.17±0.46	0.97
	Chao	156.23±71.56	160.70±64.78	153.22±55.08	0.982
	Sob	133.32±63.34	135.65±59.66	128.64±54.51	0.873
	Ace	169.51±78.75	165.99±66.81	161.84±59.63	0.890
	Simpson	0.077±0.05	0.12±0.08	0.10±0.06	0.463
	Coverage	0.99950±0.0003	0.99951±0.0001	0.99948±0.0001	0.500

Note: **P < 0 0.01.

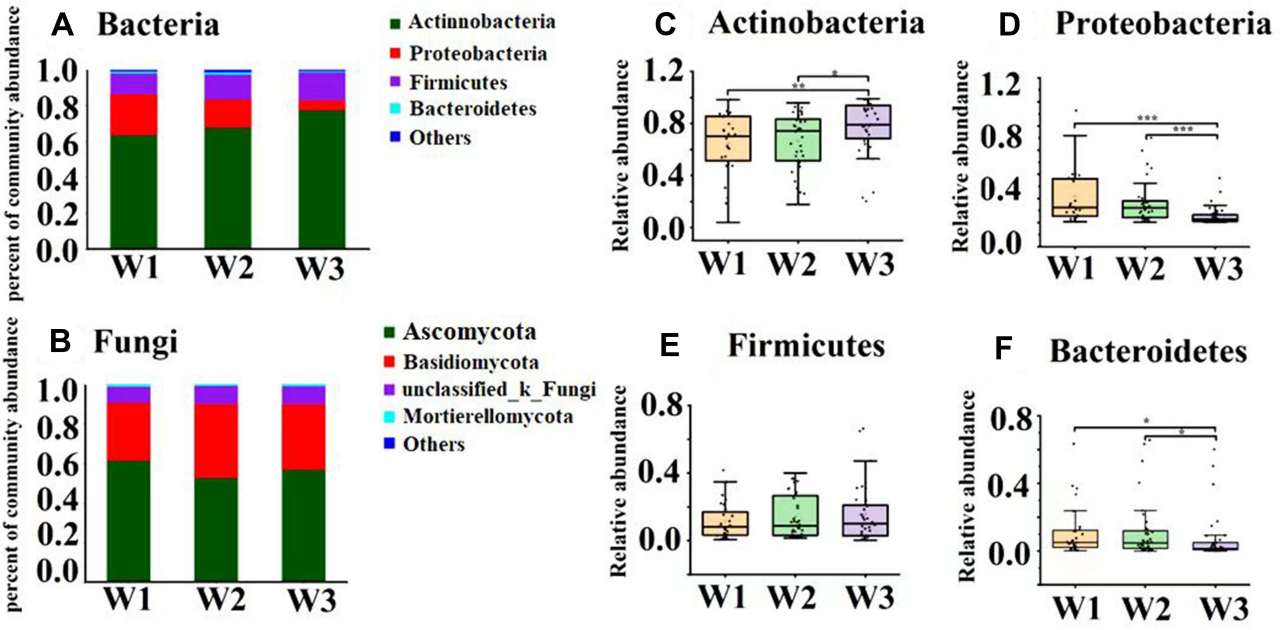

Figure 3 Differences of relative abundance of skin microbiota at the phylum level among "ideal skin" (W1), "normal skin" (W2) and "undesirable skin" (W3). (**A**) Bacterial abundance at the phylum level; (**B**) fungal abundance at the phylum level; (**C**) *Actinobacterial* phylum; (**D**) *Proteobacterial* phylum; (**E**) *Firmicutes* phylum; (**F**) *Bacteroidetes* phylum; Difference test method: Wilcoxon rank-sum test, **P* < 0 0.05; ***P* < 0.01; ****P* < 0 0.001.

contained more *Proteobacteria* and *Bacteroidetes* bacteria and relatively fewer *Actinobacteria* and *Firmicutes* bacteria than the "normal skin" and "undesirable skin" groups, while the fungal composition was not so significant.

At the genus level, the composition of cheek bacterial species was mainly composed of *Cutibacterium, Staphylococcus, Pseudomonas, Rhodococcus, Neisseriaceae, Streptococcus, Corynebacterium*, and *Stenotrophomonas* (Figure 4A). From the "ideal skin" group to the "undesirable skin" group, *Cutibacterium* was significantly increased (Figure 4C, P < 0.01) and *Staphylococcus* had a specific increasing trend (Figure 4D, P > 0.05), *Pseudomonas* (Figure 4E, P < 0.05), *Rhodococcus* (Figure 4F, P < 0.001), *Streptococcus* (Figure 4G, P < 0.01), *Ralstonia* (Figure 4I, P <0.001) and *Stenotrophomonas* (Figure 4H, P < 0.001) were significantly decreased. The species composition of cheek fungi was mainly composed of *Cutaneotrichosporon*, *Aspergillus*, *Cladosporium* and *Malassezia* (Figure 4B and 4J). The results of the between-group differential species analysis showed that the relative abundance of *Malassezia* was increased significantly ($P < 0.05$) from the "ideal group" (W1) to the "undesirable group" (W3). From the perspective of ideal skin, the abundance of *Cutibacterium*, *Staphylococcus*, and *Malassezia* in the "ideal skin" group was lower, however, the abundances of *Pseudomonas, Rhodococcus, Stenotrophomonas, Ralstonia* and *Streptococcus* were relatively high in the "ideal skin" group compared with the "normal skin" and "undesirable skin" groups.

Correlation Analysis of Skin Microbiota and Physiological Parameters

The Spearman correlation between skin physiological parameters (sebum, hydration, TEWL, roughness, R2, hemoglobin, melanin and pH) and the skin bacteria and fungi was analyzed and summarized into the Spearman correlation heatmap (Figure 5). As shown in Figure 5A, *Cutibacterium* was positively correlated with sebum content (r = 0.1202, $P = 0.24$) and hemoglobin (r = 0.1773, $P = 0.084$), and negatively correlated with hydration content (r = −0.3152, $P = 0.0018$). This finding was consistent with the trend analysis of the previous physiological parameters and bacterial flora, the sebum content was increased, and the relative abundance of *Cutibacterium* genus was also increased significantly from W1 to W3. Similarly, we saw a positive correlation between *Staphylococcus* and TEWL (r = 0.0613, $P < 0.05$), while hemoglobin was positively associated with *Stenotrophomonas* (r = −0.2094, $P < 0.05$), and it was negatively correlated with *Pseudomonas* (r = −0.2782, $P < 0.01$), *Ralstonia* (r = −0.2129, $P < 0.001$), and *Streptococcus* (r = 0.0519, $P < 0.001$). For fungi (Figure 5B), hemoglobin was significantly positively correlated with

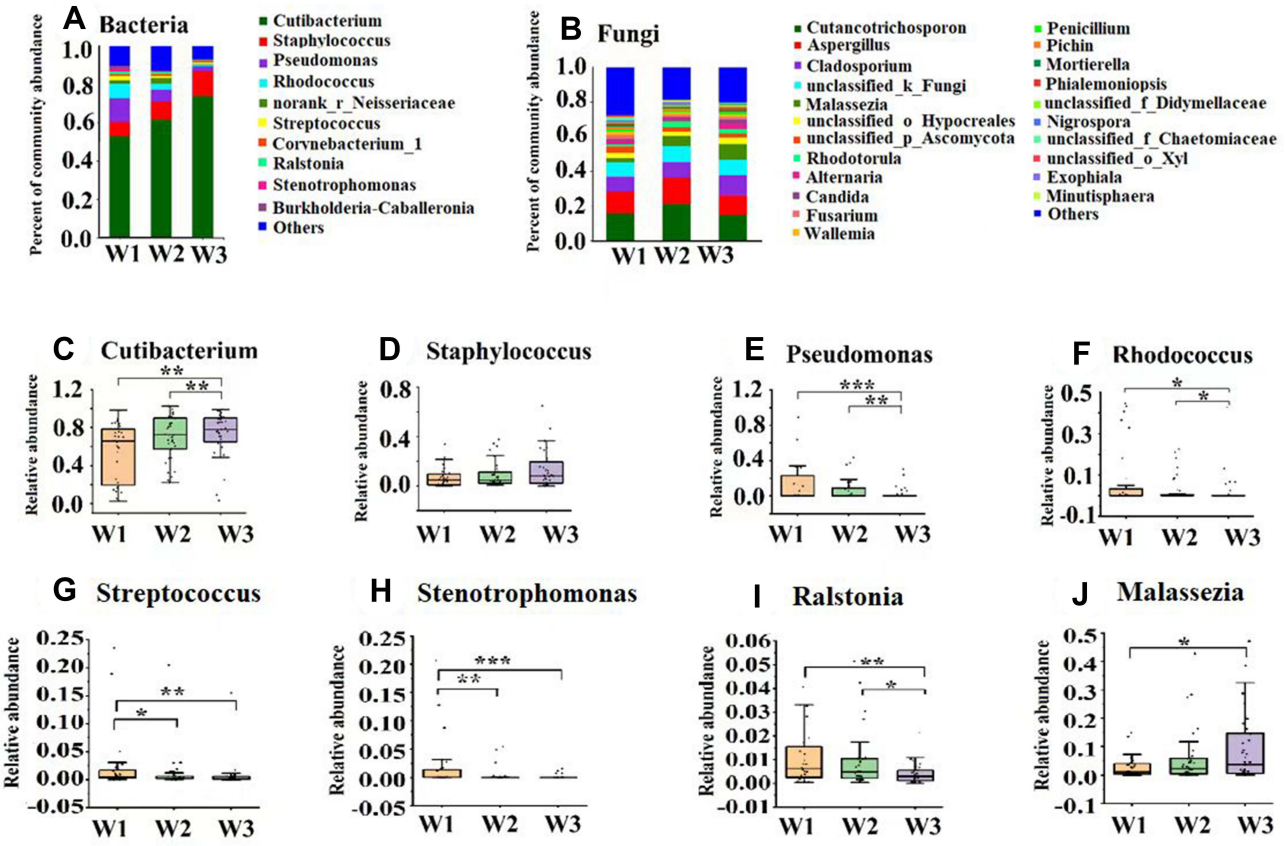

Figure 4 Differences of relative abundance of skin microbiota at the genus level among "ideal skin" (W1), "normal skin" (W2) and "undesirable skin" (W3). (**A**) Bacterial abundance at the genus level; (**B**) fungal abundance at the genus level; (**C**) *Cutibacterium* genus; (**D**) *Staphylococcus* genus; (**E**) *Pseudomonas* genus; (**F**) *Rhodococcus* genus; (**G**) *Streptococcus* genus; (**H**) *Stenotrophomonas* genus; (**I**) *Ralstonia* genus; (**J**) *Malassezia* genus. Difference test method: Wilcoxon rank-sum test, **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

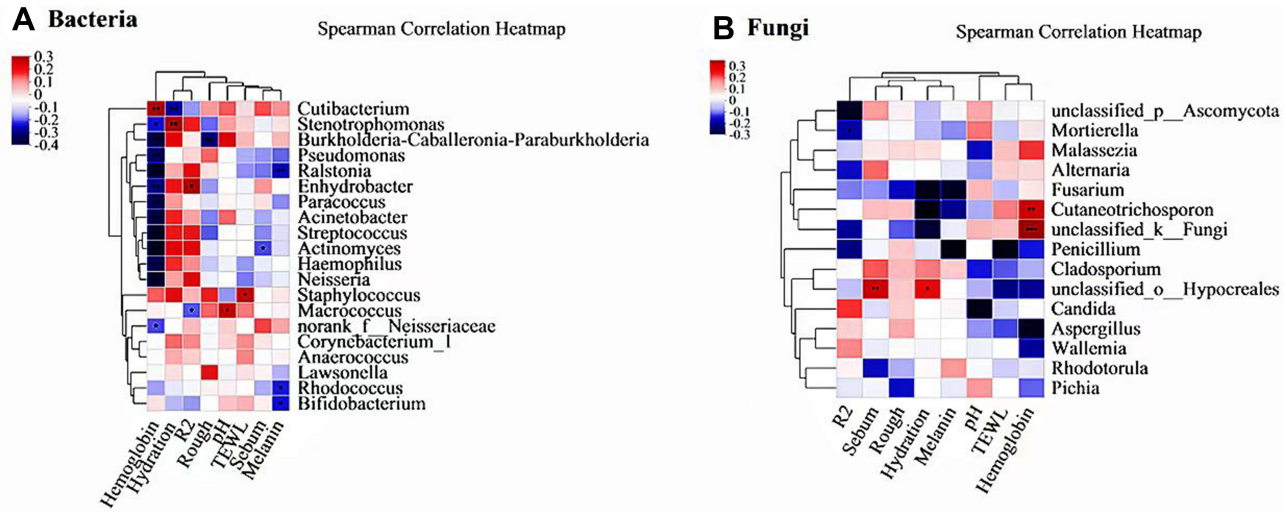

Figure 5 Spearman correlation analysis of (**A**) cheek bacteria, (**B**) fungi and physiological parameters. Difference test method: Wilcoxon rank-sum test, **P* < 0 0.05; ***P* < 0 0.01; ****P* < 0 0.001.

Cutaneotrichosporon (r = 0.2740, $P < 0.01$) and *unclassified_k_Fungi* (r = 0.1556, $P < 0.001$), while it was significantly negatively correlated with *Aspergillus* (r = −0.2099, $P < 0.01$). Moreover, *Malassezia* was positively correlated with hemoglobin (r = 0.2390, $P < 0.05$).

In addition, the correlation between physiological parameters and the Shannon index of bacteria was analyzed (Table 4). The Shannon index of bacteria was significantly positively correlated with hydration (r = 0.222 $P = 0.03$), but negatively correlation with sebum content (r = −0.069, $P = 0.5$) and hemoglobin content (r = −0.228, $P = 0.025$). Meanwhile, the Shannon index was significantly negatively with the abundance of *Cutibacterium* (r = −0.7303, $P = 0.000$), but significantly positively correlated with the abundances of *Streptococcus* (r = 0.4536, $P = 0.000$) and *Ralstonia* (r = 0.25, $P = 0.014$). Therefore, the skin hydration content and the abundance of *Cutibacterium* genus played an important role in maintaining the α-diversity and skin homeostasis.

Discussion

The skin is not only a "barometer" of the body's health, but also is endowed with more connotations. Generally, having good-looking and healthy skin means happier, better, and more accessible resources and opportunities. Therefore, in this era of pursuing youthful beauty, we are willing to spend more money on personal care, which promote the rapid development of the personal care industry. However, there is no standard for what kind of skin is perfect or ideal. Interestingly, beauty standards may vary between cultures, while different ethnic groups share a common attractiveness standard. Males are expected to be most sexually attracted to female skin free of lesions, eruptions, warts, moulds, cysts, tumors, acne, and hirsutism.[9] Based on the understanding and aesthetics of orientals for perfect skin, Our study was the first attempt to define the "ideal skin" of oriental women. Based on our customized scoring standard, female volunteers aged 18–25 in Shanghai (n=111) were divided into "ideal skin", "normal skin" and "undesirable skin" groups with pores, acne, spots and wrinkles as evaluation indicators, to analysis the skin physiological parameters and cheek microbial flora.

Our study found that skin physiological parameters presented regular changes from "ideal skin" to "undesirable skin", although the differences of some parameters were insignificant (Figure 2, P > 0.05). The "ideal skin" group had higher skin hydration content and lower sebum, TEWL, indicating that "ideal skin" had a reasonable water-oil balance and better skin barrier function. In contrast, in the "normal skin" and "undesirable skin" groups, decreased skin hydration content and increased TEWL reflected impaired skin barrier. Skin with low hydration content is more prone to wrinkled, scaly, or rough features, and severe cases may also experience cracking, redness, or itching.[32,33] Increased hemoglobin reflects increased blood flow in the blood vessels, with the potential for inflammation and pigmentation of the skin. The increase in sebum content is mainly related to the development of facial pores.[16] The increase of oil secretion leads to

Table 4 Spearman Correlation Analysis Between Skin Physiological Parameters, Microbiota and the Shannon Index of Bacteria

Parameter	Shannon Index of Bacteria	
	Rho	P-value
Hydration	0.222	0.03
Sebum	−0.069	0.50
Hemoglobin	−0.228	0.025
Cutibacterium	−0.73	0.000
Staphylococcus	0.056	0.58
Pseudomonas	0.119	0.24
Rhodococcus	0.068	0.51
Stenotrophomonas	−0.06	0.55
Ralstonia	0.25	0.014
Streptococcus	0.45	0.000
Malassezia	−0.14	0.16

enlarged pores, making faces look rough, slack, and lackluster. In the "normal skin" and "undesirable skin" groups, the skin roughness was increased significantly. From the "ideal skin" group to the "undesirable skin" group, the content of melanin was increased, and the skin was also prone to problems, such as pigmentation. Judging from the grouping criteria, most of the volunteers in the "undesirable skin" group had acne, pigmentation spots, and large pores, which was consistent with the changes in physiological parameters, such as increased sebum secretion and hemoglobin content. It was proved that different skin conditions have different physiological parameters. For example, skin aging includes chronological skin aging and photoaging,[34] the volunteers in our study ranged from 18–25 years, so the effects of chronological skin aging on skin physiological parameters and microorganisms can be excluded. Photoaging is mainly caused by ultraviolet radiation, the most immediate change caused by UV radiation is the accumulation of skin pigment, followed by the damage to skin extracellular matrix.[35,36]

Moreover, we were pleasantly surprised to find that the microbiota flora changed regularly from the "ideal skin" group to the "undesirable skin" group. Our data showed that the α-diversity of skin bacteria was more abundant in the "ideal skin" group, compared with the "normal skin" and "undesirable skin" groups, since the Chao index of species richness ($P > 0.05$) and the Shannon index of species diversity ($P < 0.01$) were significantly different. In studies of problematic skin, such as acne, atopic dermatitis, and dandruff, healthy groups have higher α-diversity compared with unhealthy groups, which is consistent with our findings.[37–40] A study compared the composition and diversity of microbes among 4 kinds of subjective skin types, and the sebum content and bacterial diversity of the facial skin both significantly differed among the 4 kinds of subjective skin types, and the relative abundance of *Propionibacterium* was significantly higher in the oily skin group than in the dry skin group.[41] Similarly, environmental pollution (air cleanliness) could also impact the flora since the α-diversity of the population would be higher in clean air.[42,43] Skin aging is associated with changes in cutaneous physiology including interactions with skin microbial community. It was indicated that the α-diversity was higher and a striking reduction in the relative abundance of the majority skin genus *Propionibacterium* of the older adults.[42] The Shannon index was significantly positively correlated with hydration but negatively correlated with sebum content and hemoglobin content (Table 4). Therefore, we could control the balance of water and oil to keep more high diversity of skin flora. The *ß*-diversity of the three groups did not show any difference, which might be related to the fact that our research subjects were all healthy people, and there was little difference in the composition of species.

Species composition was changed significantly from the "ideal skin" group to the "undesirable skin" group. The "ideal skin" group had lower in the abundance of *Cutibacterium*, *Staphylococcus*, and *Malassezia*, but relatively high in the abundances of *Pseudomonas*, *Rhodococcus*, *Stenotrophomonas*, *Ralstonia* and *Streptococcus* (Figure 4). Studies have shown that changes in species composition are often important factors in some diseases or skin conditions.[44] For example, *Propionibacterium acnes*, the primary strain of the genus *Propionibacterium* on the skin, is also known as sentinel bacteria, and its abundance changes have a significant impact on the flora and play an essential role in maintaining skin microecological homeostasis.[45] In our present study, the abundance of *Propionibacterium* was increased significantly from the "ideal skin" group to the "undesirable skin" group. Previous studies have found that the abundance of *Propionibacterium* is negatively correlated with the *α*-diversity.[46] Our study showed that the *α*-diversity of the "undesirable skin" group was decreased, suggesting that the abundance of *Propionibacterium* would affect the diversity of the entire flora in certain circumstances. In addition, a large number of studies have shown that in areas with high sebum secretion, *Propionibacterium* is the dominant bacteria, which can use sebum to promote its own reproduction, and the imbalance of *Propionibacterium acnes* can easily lead to the occurrence of acne.[47] Meanwhile, the abundance of *Malassezia* was significantly increased from the "ideal skin" group to the "undesirable skin" group ($P < 0.05$). Volunteers with acne and enlarged pores in the "undesirable skin" group accounted for most. There were a lot of *Malassezia, Propionibacterium acnes* and *Streptococcus* in the hair follicles of acne patients.[48] When the number of *Malassezia* was high, the skin was more prone to aging,[49] which was also consistent with our findings. A higher frequency of *Propionibacterium, Paracoccus* and *Corynebacterium* was also detected in sensitive skin, compared with non-sensitive skin.[33] Zheng et al[50] revealed that the abundance of *Malassezia* in the adolescent acne group was significantly higher compared with the healthy control group. Another research also demonstrated that there was also a close relationship between sensitive skin and its skin bacteria and fungi.[51] These studies support our findings that ideal and healthy skin

tended to have lower *P. acnes* and *Malassezia* abundances compared with the problematic skin. Human skin harbors a diverse milieu of commensals, including bacteria, fungi, viruses and mites, which live together as an intricate ecological community. In this study, we mainly focused on the impact of different skin states on the cutaneous bacterial, fungi abundance and diversity, but did not extend our research to viruses and skin mites. Indeed, viruses and mites also have important role for skin health.[52,53] For example, the *Demodex mites* are significantly increased in the skin of rosacea patients, causing sensitive skin.[54,55] Our research revealed that with the skin condition deteriorates, sebum content was significantly increased, so we could speculate that the abundance of mites would also increase, which might affect the microbial homeostasis and further the function of the skin. More studies are needed to reveal the effects of skin conditions on mites, and to get an insight into the association between skin condition and skin microecology.

There is a close correlation between the distribution and abundance of microflora and skin physiological parameters, especially sebum, skin hydration, TEWL and pH value.[56] Compared with the "ideal skin" group (W1), the "undesirable skin" group (W3) had increased sebum and decreased skin water content. The corresponding abundances of lipophilic *P. acnes* and *Malassezia* were significantly increased, while the abundances of bacteria preferring humid environments, such as *Stenotrophomonas*, *Pseudomonas*, *Ralstonia* and *Streptococcus*, were significantly reduced. It has demonstrated that *P. acnes* is significantly positively associated with sebum secretion, pore size and the number of porphyrin spots[46], which is consistent with our findings. Hemoglobin reflects vascular activity, and the increase in skin hemoglobin content often manifests as skin inflammation, which affects the appearance of the skin. The hemoglobin content in the "undesirable skin" group was significantly increased. Correlation analysis showed that the hemoglobin content was positively correlated with the abundance of *Cutibacterium* and *Malassezia*, while it was significantly negatively correlated with *Stenotrophomonas, Pseudomonas, Ralstonia, Streptococcus* and other strains that preferred humid environments. It has been found that the spot area is negatively correlated with *Propionibacterium*.[42] *Streptococcus* is negatively correlated with sebum content and positively correlated with skin hydration content,[16,49,56] which is consistent with our study. From the correlation analysis, TEWL was increased in the "undesirable skin" group, which was significantly positively correlated with the abundance of *Staphylococcus*. Increased *Staphylococcus* abundance was also associated with the impaired skin barrier. Song et al[57] have also found that TEWL was positively correlated with the abundance of *Staphylococcus* in sensitive skin, especially *Staphylococcus aureus*, which can easily cause damage to the skin barrier. It can be seen that there is a close relationship between the abundance of flora and the skin physiological parameters. The changes in the skin's physiological parameters affect the changes in bacterial abundance, and in turn, the changes in the metabolites further affect the function of the skin, which are interlinked and affect each other.

In our present study, we selected the volunteers in 18–25 years old, considered as the best age for skin. Although everyone wants to have ideal skin of 18–25 years old, we have to admit that in the natural law of skin aging, various care methods only slow down skin aging. Therefore, we should also follow the characteristics of skin at all ages to find the ideal skin state at that age, and adopt reasonable maintenance methods to make the skin look younger. In the further research, we will also pay attention to the "ideal skin" research of different age groups to provide a theoretical basis for scientific skincare for different age groups.

Conclusions

In our present study, we attempted to define the "ideal skin" of oriental women and obtain helpful information on the skin's physiological characteristics and the diversity and composition of the facial microbiome of ideal skin. The "ideal skin" group had a more appropriate water-oil balance, which was more suitable for the growth and reproduction of microorganisms. Therefore, it had higher microbial diversity and reasonable species composition. The skin changes of young women aged 18–25 were mainly reflected in physiological functions. The increase in skin oil would lead to changes in skin microecology, which might be one factor that further results in skin problems. Therefore, if young women aged 18–25 want to achieve the ideal skin condition, daily skincare needs to control skin oil and maintain skin microecological balance.

Data Sharing Statement

The sequence dataset has been deposited on the NCBI Sequence Reads Archive (SRA) Database (Accession Number: SRP330206).

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Disclosure

The authors declare no competing interests for this work.

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