CONTINUOUS IMPROVEMENT OF SKIN AGING APPEARANCE THROUGH A NEW FACIAL SERUM

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Abstract

Background:

Skin aging is in part characterized by loss of moisture, elasticity and firmness, rough texture, as well as skin tone dullness, which finally lead to the formation of fine lines and wrinkles. A typical clinical study for an anti-aging cosmetical product is no more than three months with only one targeted age group, which presents limitation to evaluate long term benefits as well as treatment response for different age groups.

Objective:
To fill this gap, a long-term clinical study, based on three age groups of 20-50 y.o subjects was designed to evaluate the efficacy and tolerance of a newly developed multi-benefit facial serum containing bifida ferment lysate, sphingomonas ferment extract, salicyloyl phytosphingosine, hydroxyethylpiperazine ethane sulfonic acid and papain.

Methods:
An open-label, single center clinical study was initiated, in which three age groups (Group 1: 20-29 years old, Group 2: 30-39 years old, Group 3: 40-50 years old) of Chinese women were recruited. Each age group contains 74 volunteers, who presented with mild to moderate signs of photoaging. A total of 222 volunteers enrolled for self-assessment while 196 volunteers for clinical evaluation. After a 3-week washout period, subjects were instructed to apply the investigational serum on full face twice a day in the morning and evening for nine months, from December 2020 to September 2021. Subjects were seen at baseline, 1 month, 2 months, 3 months, and 9 months after the first application. Clinical grading with a scale of 0-9 was performed on skin radiance, smoothness, and elasticity for all three age groups. In addition, age-specific attributes, for example, cheek pores were evaluated for subjects in Group1; underneath eye wrinkles were evaluated for subjects in Group2; crow's feet wrinkles were evaluated for subjects in Group3. Subject self-assessment questionnaire along with standardized photographs were taken at each visit for all the subjects.

Results:
182 subjects completed the 9-month clinical evaluation. For all the subjects, compare to clinical grading conducted at baseline, statistically significant ($p < 0.05$) improvement in skin radiance was observed with an improvement rate of 10.40%, 10.89%, 12.61% and 24.36% at 1, 2, 3, and 9 months, respectively. Significant improvement in clinical grading of skin smoothness was also noticed, with an improvement rate of 7.44%, 10.64%, 10.67%, and 33.64% at 1, 2, 3, and 9 months, respectively, compared to baseline. Similarly, statistically significant improvement in skin elasticity was found versus baseline, with an improvement rate of 11.67%, 10.77%, and 32.31% at 2, 3, and 9 months, respectively. Focusing on each age group, significantly better performance

based on clinical grading on cheek pore (12.85%) was seen in Group1, reduction of underneath eye wrinkles (49.16%) was seen in Group2, and reduction of crow's feet wrinkles (27.14%) was seen in Group3 after long-term use till 9 months, which were consistent with the favorable ratings from the self-assessment questionnaire. In addition, the investigational serum was well tolerated without any adverse event reported.

Conclusion:
The results suggest that this newly developed facial serum can continuously improve skin aging appearance over time with twice daily usage. Strong efficacy in improving age-specific skin attributes can also be delivered through the investigational product.

Key words:

Anti-aging, Long-term clinical study, Bifida ferment lysate, Sphingomonas ferment extract

Introduction.

In recent survey on Chinese women, skin aging related problems are their top conscious skin concerns. Although beauty procedure and device usage are increasing fast, the cosmetic products remain the first primary tool to address consumers skin anti-aging needs.

Skin aging is characterized such as loss of moisture, elasticity, firmness, and changing in rough skin texture, as well as skin tone dullness, which finally lead to the appearance of fine lines and wrinkles. The clinical evaluation is the classic methodology to evaluate cosmetic product performance. The visual skin aging signs comes from studies carried out in France, China, Japan, India, and United States on a total of 4000 women and men aged from 20 to 80 years, addressed to precise visual scales of facial aging (skin aging atlases), which allow to use for clinical evaluation of cosmetic or dermatological treatments. [1]

Varies of active ingredients were developed and many research indicated their positive benefits in-vitro and in-vivo, especially some probiotic bacterial fermentation extracts. For example, Bifidobacterium longum lysate can improve skin barrier function, inhibit the release of neuro-mediators involved in sensitivity phenomena.[2] Sphingomonas extract was showed to improve biomechanical skin properties and potential to delay intrinsic skin ageing process. [3] Those probiotic ingredients are suggested to be introduced into cosmetic product and to provide potential beneficial efficacy on sensitive skin and skin aging.

An innovative serum under a classic emulsified gel formulation system was recently developed, which contains 10% bifida ferment lysate and 0.3% sphingomonas ferment extract, as well as salicyloyl phytosphingosine, hydroxyethylpiperazine ethane sulfonic acid and papain. This technology is well appreciated by consumers for its light texture and fast absorbing characteristics.

A typical clinical evaluation for an anti-aging cosmetic product is usually short term from one month to three months, with only one targeted age group, which may have limitations in providing a holistic understanding of the product efficacy and across different age groups. Therefore, the purpose of the present nine-month clinical study, was to investigate the efficacy and tolerance of this newly developed multi-benefit facial serum across long term usage, and to explore age specific responses across a wide range of consumers.

Materials and Methods.

Subjects:

The research was carried out on a group of healthy volunteers, selected in accordance with the guidelines of the Helsinki Declaration, with subsequent additions and with approval of the Independent Ethics Committee (Approval No. SECCR2020-143-01)

222 Chinese females, 20-50 years of age, were enrolled in the trial, who covered all skin type from dry, normal, combination to oily skin, presented with mild to moderate signs of photoaging according to the skin aging atlas score and dermatologist scale grading, and self-claimed skin concerns such as lack of skin radiance, elasticity, translucent, skin dullness and roughness, appearance of fine line and wrinkles on the facial skin. The enrolled subjects were allocated into three age groups (Group 1: 20-29 years old, Group 2: 30-39 years old, Group 3: 40-50 years old).

After a 3-week washout period, enrolled subjects were instructed to apply the investigational serum on full face twice per day in the morning and evening for 9 months. In addition to serum, a moisturizer was provided with twice per day usage from washout period to the end of the study to ensure the consistency of treatment regimen throughout the study period. Subjects were instructed to use usual cleanser and sunscreen, and not allowed to use other skin care products and makeup products except eye make-ups and lipsticks.

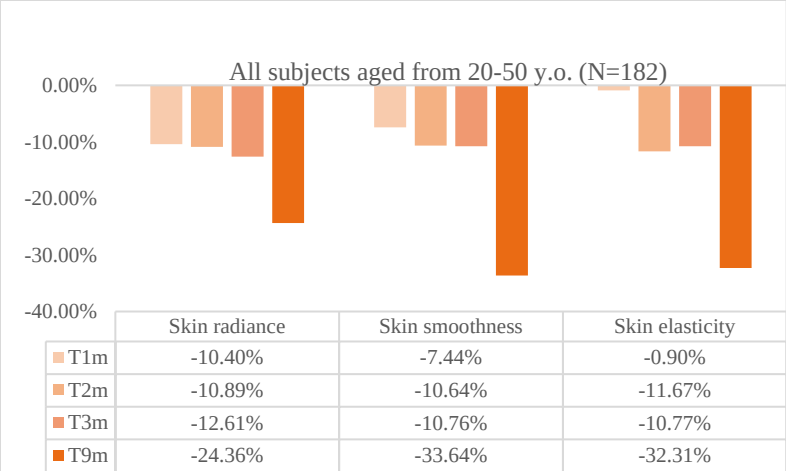
Clinical study design:
It was an open-label, single center clinical study which consisted with a 3-week washout period and 9-month application period from Dec 2020 to Sep 2021. Subjects were seen at clinical center at baseline, 1 month, 2 months, 3 months, 6 months, and 9 months after the first application.

Clinical expert grading with a scale of 0-9 was performed under same dermatologist on skin radiance, translucency, brightness, smoothness, softness, elasticity and firmness for all three age groups. In addition, age-specific attributes, for example, skin evenness, skin plumpness, and cheek pores were evaluated for subjects in Group1; underneath eye wrinkles and global appearance of fine lines were evaluated for subjects in Group2; global appearance of fine lines, global wrinkles and crow's feet wrinkles were evaluated for subjects in Group3. Subject self-assessment questionnaire along with standardized photographs were taken at each visit for all the subjects.

Statistical analysis:
Normality of residuals will be checked by the studentized residuals distribution. While the data distribution follows normal laws, ANOVA will be used to detect significant differences among time-points. If significant, Dunnett adjustment will be used for post-hoc comparison to detect significant pairs between T0 and others.

Results:
A total of 208 volunteers completed 9-month treatment of the study, all of them completed the questionnaire and 182 of them completed the clinical efficacy evaluation, which included 58, 63 and 61 subjects in each age group, respectively.

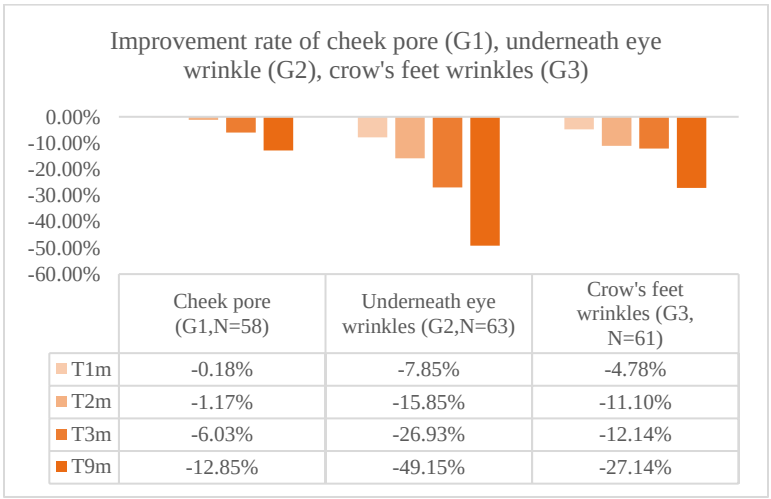
Clinical expert grading:
Statistical result showed that the treatment of investigational serum yielded significant improvements from baseline on skin radiance and skin smoothness in all subjects at 1, 2, 3 and 9 months after use, and the skin elasticity improved after 2 months use until 9 months. All the improvement rates are given in Figure 1. The clinical grading score of skin radiance at baseline, 1, 2, 3, and 9 months are 4.49±0.05, 4.02±0.04 ($p<0.05$), 4.00±0.04 ($p<0.05$), 3.92±0.04 ($p<0.05$) and 3.40±0.04 ($p<0.05$). The skin smoothness at baseline, 1, 2, 3, and 9 months are 4.80±0.06, 4.45±0.05($p<0.05$),4.29±0.06 ($p<0.05$), 4.29±0.06($p<0.05$) and 3.19±0.06($p<0.05$). And the skin elasticity of skin at baseline, 1, 2, 3, and 9 months are 4.29±0.05, 4.25±0.05 ($p=0.475$), 3.79±0.04 ($p<0.05$), 3.82±0.04 ($p<0.05$), and 2.90±0.05($p<0.05$).



	Skin radiance	Skin smoothness	Skin elasticity
T1m	-10.40%	-7.44%	-0.90%
T2m	-10.89%	-10.64%	-11.67%
T3m	-12.61%	-10.76%	-10.77%
T9m	-24.36%	-33.64%	-32.31%

Fig1. The improvement rates from baseline in clinical grading parameters of all subjects

In younger age group (Group 1), statistically significant reduction of cheek pore severity grading in average score were found at 3 and 9 months timepoint (1 month: 3.82 ± 0.09, p=0.913; 2 months:3.79 ± 0.08, p=0.480; 3 months:3.60 ± 0.09, $p<0.05$; 9 months: 3.34 ± 0.11, $p<0.05$) compared to baseline (3.83±0.09). In mid age group (Group 2), there was a statistically significant reduction of underneath eye wrinkles according to skin aging atlas at all timepoints (1 month: 2.09±0.10, $p<0.05$; 2 months: 1.90±0.09, $p<0.05$; 3 months: 1.65±0.09; $p<0.05$; 9 months: 1.15±0.09, $p<0.05$) compared to baseline (2.26±0.10). In elder age group (Group 3), the serum demonstrated statistically significant reduction of crow's feet wrinkles according to skin aging atlas at all timepoints (1 month: 2.84±0.11, p=0.047; 2 months: 2.65±0.10, $p<0.05$; 3 months: 2.62±0.10, $p<0.05$; 9 months: 2.17±0.12, $p<0.05$) compared to baseline (2.98±0.11). Figure 2 presented all the improvement rates. One example picture of those attributes before-and-after the usage were showed in Figure 3-5.



	Cheek pore (G1,N=58)	Underneath eye wrinkles (G2,N=63)	Crow's feet wrinkles (G3, N=61)
T1m	-0.18%	-7.85%	-4.78%
T2m	-1.17%	-15.85%	-11.10%
T3m	-6.03%	-26.93%	-12.14%
T9m	-12.85%	-49.15%	-27.14%

Fig2. The improvement rates compared to baseline: Skin cheek pores in Group 1, underneath eye wrinkles in Group 2 and crow's feet wrinkles in Group 3

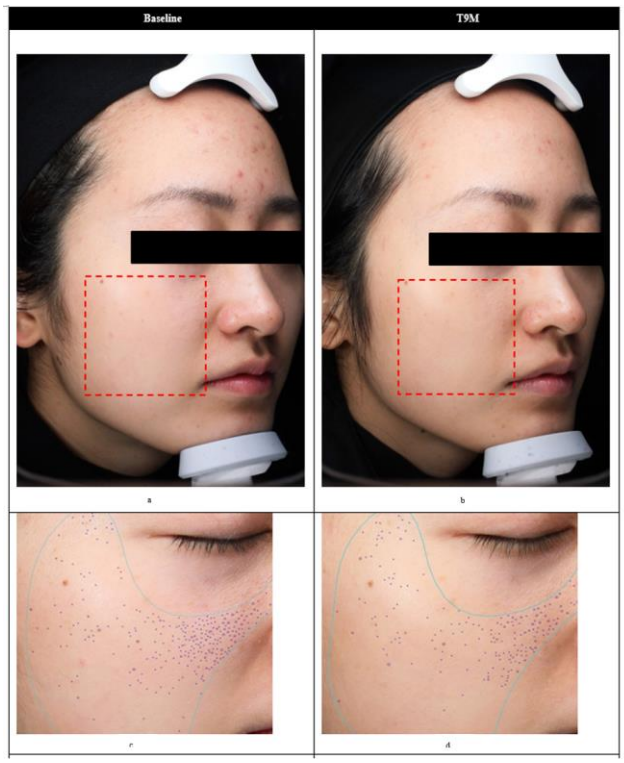


Fig 3: Representative example of skin cheek pore improvement in Group 1: a) right side face baseline b) right side face at 9 months c) cheek area baseline d) cheek area at 9 months

Fig 4: Representative example of underneath eye wrinkles improvement in Group 2: a) right side face baseline b) right side face at 9 months c) eye area baseline d) eye area at 9 months

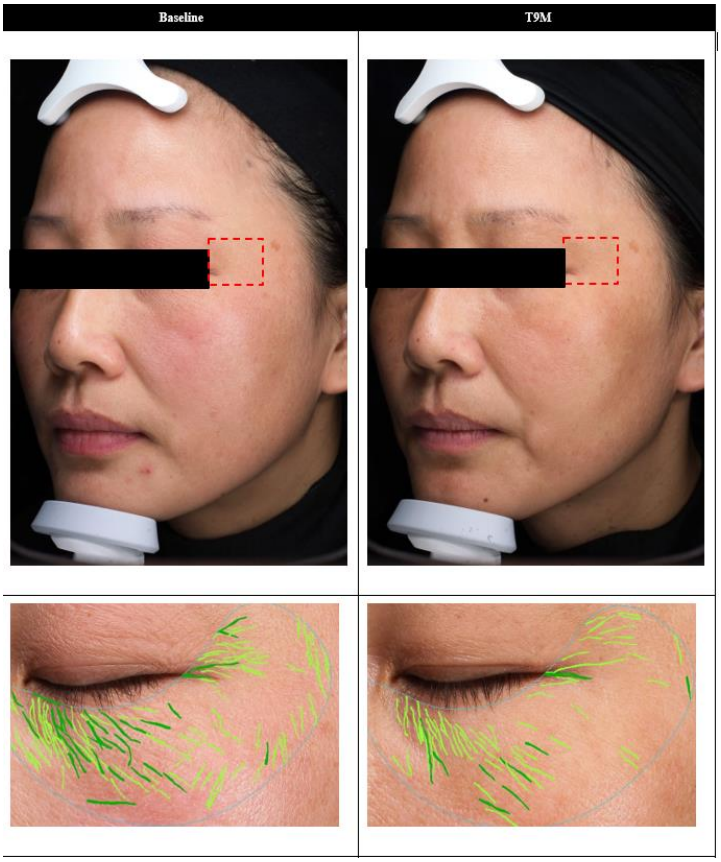


Fig 5: Representative example of crow's feet improvement in Group 3: a) left side face baseline b) left side face at 9 months c) eye area baseline d) eye area at 9 months

Tolerance

The investigational serum received good tolerance, there was no adverse event occurred in this study.

Self-assessment efficacy

208 subjects completed self-assessment questionnaire. Based on the percentage of top-2-boxes (T2B) from 5 scale, the investigational serum was appreciated for its skin quality improvement (soft, smooth, refined, elastic, healthy glow) and good hydration efficacy, younger look and skin stabilization with T2B above 85% by 9 months after use. Self-assessment demonstrated the efficacy of skin pore, finelines and wrinkles in each individual group were perceived by the subjects during the test period (data showed in Table 1).

All Subjects N=208	T1M	T2M	T3M	T9M
Skin feels softer	92.25%	91.55%	95.07%	95.07%
Skin feels smoother	92.96%	95.07%	92.96%	95.07%
Skin looks more radiant	84.51%	88.73%	85.92%	88.73%
Skin texture looks more refined	83.10%	88.03%	88.73%	90.14%
Skin feels more elastic	83.10%	88.03%	88.73%	92.25%

Skin looks with healthy glow	83.10%	88.03%	90.14%	92.25%
Skin quality feels improved	88.73%	91.55%	90.85%	93.66%
Skin feels moisturized	89.44%	90.85%	92.25%	93.66%
Skin looks younger	78.17%	80.28%	85.92%	88.73%
Skin feels more strengthened	73.94%	80.28%	85.21%	87.32%
Skin feels more stable	83.80%	90.85%	88.03%	90.14%
Skin look makes me more confident	84.51%	83.80%	87.32%	90.85%
Skin feels protected from external aggressions	/	/	/	87.50%
Group 1 N=66	**T1M**	**T2M**	**T3M**	**T9M**
Pores looks less visible	62.12%	62.12%	66.67%	83.33%
Group 2 N=69	**T1M**	**T2M**	**T3M**	**T9M**
Fine lines look less visible	76.81%	79.71%	82.61%	86.96%
Group 3 N=73	**T1M**	**T2M**	**T3M**	**T9M**
Wrinkles look less visible	72.60%	80.82%	82.19%	90.41%

Table 1: Subject self-assessment (T2B) after 9 months of the applications

Discussion:

In our study, the investigational serum was demonstrated good efficacy and tolerance over 9 months application and across the three age groups from 20 to 50 years old. A clearly accumulative improvement was observed for skin quality and aging-related signs with continuous use of the serum. For all the subjects, it was reported that clinical grading of skin radiance compared to baseline improved 10.40%, 10.89%, 12.61% and 24.76% at 1, 2, 3, and 9 months, respectively. Significant improvement in clinical grading of skin smoothness was also noticed, with an improvement rate of 7.44%, 10.64%, 10.67%, and 33.64% at 1, 2, 3, and 9 months, respectively, compared to baseline. Similarly, statistically significant improvement in skin elasticity was found versus baseline, with an improvement rate of 11.67%, 10.77%, and 32.31% at 2, 3, and 9 months, respectively. In each age group, for Group 1, there was a statistically significant reduction of cheek pore severity grading in average score at 3 and 9 months timepoint compared to baseline. The reduction rates were 6.03% and 12.85% at 3 and 9 months, respectively. For group 2, there was a statistically significant reduction of underneath eye wrinkles according to skin aging atlas at all timepoints compared to baseline. The reduction rates were 7.85%, 15.85%, 26.93%, and 49.16% at 1,2, 3, and 9 months, respectively. For group 3, there was a statistically significant reduction of crow's feet wrinkles according to skin aging atlas at all timepoints compared to baseline. The reduction rates were 4.78%, 11.10%, 12.14% and 27.14% at 1, 2, 3 and 9 months, respectively. There was no adverse reaction happened in our study.

Although many clinical studies have conducted to confirm cosmetic product treatment efficacy, hardly any last longer than 3 months. Similarly, seldom information is available for different age group. This study was designed to evaluate long term benefits within same city (Shanghai, China). Shanghai's climate usually shows very distinctive four seasons. 9 months observation period was organized at least to be able to cover the two peak periods of the year (winter and summer). From pervious study on Chinese women skin properties of various ages during winter and summer, it indicated that the season did induce slight changes in skin however concern more functional rather than structural properties, there is no seasonal alteration on wrinkles and sagging which is the most two aspects for aging skin, there was more obvious seasonal influence on skin tone which lighter in winter. [4] Therefore it was designed to include the clinical attributes on skin quality and aging related ones other than tone-related ones, such as skin smoothness, softness, elasticity, plumpness, radiance, cheek pores, finelines and wrinkles, etc. It was respected, for the properties related to deeper skin structure like elasticity and firmness, that take longer time that product reflect its efficacy, for instance, starting at 2 months after application in our study. With aging, skin structure

alters, that often seen as progressively reduction in the thickness of the epidermis, flattening of the dermal-epidermal junction, reduction of the number and diameter of the elastic fibers and the disorder of the bundles of collagen fibers. Therefore, on macro view of the skin, clinical aging sign severity goes up with age [1]. In our study, the baseline score of clinical grading generally reflects this trend although there is no significant difference probably due to small interval of age difference between age groups and less sample size. It is also interesting finding that the ratio of improvement is more obvious in the group which has higher severity, indicating if the skin problem is not obvious, the benefit from cosmetic or dermatological treatment will be less as well. That would be very important for designing relevant clinical attributes in a cosmeto-clinical study in terms of target consumer's age.

In the study, it was also demonstrated that the investigational serum can improve skin softness (35.16%), skin firmness (32.38%), skin plumpness (34.8%), skin translucent (27.64%) and skin brightness (24.34%) by 9 months application. And improvement of skin plumpness (34.8%) was seen in G1, reduction of fine lines (51.4%) was seen in Group2, and reduction of wrinkles (25.48%) was seen in Group3, which is clearly indicating strong multi-beneficial efficacy. The 10% bifida ferment lysate and 0.3% sphingomonas ferment extract may serve as anti-aging and skin stabilizing agents in the serum. In-vitro data showed bifida ferment lysate can increase the expression of K10 which is epidermal differentiation maker, directly inhibits neuron fibers by inhibiting neuromodulators release, improves epidemical skin barrier function repairing in sensitive skin subjects.[2] Sphingomonas ferment extract was reported significantly suppressed senescence associated with b-galactosidase activation. It also significantly inhibited the expression of cell cycle inhibitors (p21 and p16). [3] Associated with Bifidus ferment lysate, it can stimulate the expression of nidogen, which plays key role in Dermo-Epidermal Junction (DEJ) by helping Laminin 5 contact with Collagen IV. It showed to improve biomechanical skin properties and potential to delay intrinsic skin ageing process in vivo. [3] Both probiotics might play important roles to help the investigational serum deliver benefit to subjects.

The investigational serum yielded various visible improvement of skin condition by clinical grading and self-assessment, through 9-month study across three age groups. There is some limitation such as only one city was considered in this study, varies of cities all over China have different climate and environments, it may lead to different skin condition. The long-term clinical at multiple cities in different climates conditions may be further addressed to investigate the efficacy of cosmetic product.

Conclusions:

The results suggest that this newly developed facial serum can continuously improve skin aging appearance over time with twice daily usage. Strong efficacy in improving age-specific skin attributes can also be delivered through the investigational product.

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Conflict of Interest Statement.

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