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"Evaluation of anti-aging effect of polypeptide and bifida ferment lysate essence"

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1. Introduction

With the development of economy and the improvement of people's living standards, more and more consumers are concerned about skin aging. Skin aging is the accumulation of cellular and structural damage that occurs with age[1], Clinically, the main signs of skin aging include skin relaxation, abnormal pigmentation, and fine wrinkles[2]. Firmness is one of the signs of skin aging, and skin firmness is affected to some extent by the dermal extracellular matrix [3], which contains collagen, elastin and other components [4]. Young skin collagen fibers are rich and firm, and with aging, collagen fibers will be fragmented [5]. Reduced biosynthesis and increased degradation of collagen will lead to loss of skin elasticity [6], thinning dermal thickness and reduced skin firmness [7]. The appearance of aging skin can be improved by promoting dermal collagen synthesis and supplementing matrix [8]. Polypeptide has the advantages of good biocompatibility, good water solubility and low molecular weight, and also has the function of protecting and repairing damaged cells and promoting cell growth [9]. Bifida ferment lysate, with a variety of amino acids, proteins and various molecular mediators, and has the effect of regulating and balancing skin and regulating immune function. It is also a nutrient molecule in human skin cells [10]. In this study, the anti-aging efficacy of acetyl-hexapeptide-8 and Bifida ferment lysate compound essence was evaluated in multiple dimensions by in vitro experiment and human efficacy evaluation.

2. Materials and Methods

2.1Materials

Sample: The test sample is a complex essence containing acetyl hexapeptide-8 and Bifida ferment lysate, self-made by Qingdao Youdu Bioengineering Co., LTD., product lot number: YF220516.

Reagents and instruments: Type 6000 fetal bovine Serum, Gibco; C11995500BT high glucose DMEM medium, Gibco; 15140122 penicillin-streptomycin solution, Gibco Company; Trypsin-edta solution 25200-056, Gibco; ml057630 Human Collagen Type I (Col Ⅰ) Kit (96T), Shanghai Enzyme Link Company; CM088-5HP Recombinant TGF beta 1, Chamot Corporation. VISIA CR, Canfield, USA; Dermalab Series Skin Lab Combo, Cortex Company, Denmark.

2.2 Methods

2.2.1 In vitro test

Cell survival test: L929 fibroblasts were used to test cytotoxicity. The well-grown L929 fibroblasts were inoculated into 96-well culture plates and cultured overnight at 37℃ and 5%CO_2. The culture medium was abandoned and samples with different concentrations of 0.10%, 0.50%, 1.0% and 5.0% were added, respectively. The cell control group was added with serum-free DMEM culture medium and cultured for 24h. The absorption medium was washed twice with PBS, then 100 μL MTT (1.0 $g \cdot L^{-1}$) solution was added, and cultured in 37℃, 5%CO_2 environment for 4 h. After the solution was discarded, 150 μL DMSO was added, and placed at 37℃ for 10 min, the absorbance value of each hole was determined at 490 nm wavelength. Cell survival rate (%) = (OD sample -OD blank control)/(OD cell control -OD blank control) ×100%.

Type I collagen content test: After stable passage for two times, the cryopreserved cells were inoculated on 96-well plates and cultured overnight in 5%CO_2 incubator at 37℃. The medium in the 96-well plates was discarded. According to the results of cytotoxicity test, test samples with appropriate concentration and 100μg/ml TGF-β_1 were selected as positive controls for type I collagen content determination. Blank control group complete culture medium group. Three parallel groups were set up in each group to carry out drug administration. After the completion of administration, the 96-well plates were placed in a CO_2 incubator for (24±2) h. After incubation, the test was conducted according to the method of using the human collagen type I ELISA kit, and the light absorption value of each sample hole was measured at 450 nm with an enzyme marker. The absorbance OD value is the horizontal coordinate (X), the corresponding standard concentration of the substance to be measured is the longitudinal coordinate (Y), and the corresponding curve is made. The content of the substance to be measured in the sample can be converted from the standard curve to the corresponding concentration according to its OD value. The up-regulation rate of type I collagen is

calculated as follows: up-regulation rate (%) = (T/C-1) ×100%. In the formula: T is the average content of type I collagen of the subject; C is the mean value of collagen content of type I in blank control.

2.2.2 Human efficacy test

According to the Declaration of Helsinki, the subjects were aware of the details of this test and voluntarily participated in it and signed the informed consent form (Cosmetic Safety Technical Specifications (2022 Edition)).

Inclusion criteria: Subjects aged 35-55 years old, male or female; The subject's facial skin appears loose and dull; The subject is willing to cooperate with the test as required during the test.

Exclusion criteria: have received or are receiving medication or physical therapy for skin disease or systemic disease within 3 months; Use other anti-wrinkle and whitening products one month before the test; Pregnant or lactating women [11].

A self-controlled before and after trial was used. Subjects used the sample on their faces twice a day in the morning and evening according to the sample label information, and stopped using the same efficacy products during the test. The test lasted for 4 weeks. Facial skin elasticity data, facial skin brightness data and VISIA facial photography images were collected at baseline (D0), day 14 (D14) and day 28 (D28), respectively. The ambient temperature is 21℃ ~ 23℃ and the relative humidity is 40% ~ 60%.

Skin elasticity: Dermalab Series Skin Lab Combo is used to obtain facial skin elasticity data. Skin elasticity parameters include skin elasticity value (VE value), skin Young's elastic modulus (E value) and skin retraction time (R value). Each test area is measured 3 times and the average value is taken. The higher the VE and E values, the better the skin elasticity. The R value refers to the retraction time, and the lower the R value, the better the skin elasticity.

Skin brightness: Dermalab Series Skin Lab Combo is used to obtain facial skin brightness data. The skin brightness parameter is the L* value. The higher the L* value, the better the skin brightness.

Skin texture test: VISIA CR facial image analysis system was used to capture the subjects' front, right and left face images, and the subjects' front skin texture feature count was used to evaluate the improvement of facial texture. The lower the skin texture feature count was, the better the skin texture improvement was.

2.3 Statistical Analysis

SPSS26.0 statistical software was used for data analysis, the test results were described by mean± standard deviation, and t test was used for statistical analysis. $P<0.05$ indicated that the difference was statistically significant.

3. Results

3.1 In vitro evaluation

3.1.1 Cell survival rate

The cell survival rate of samples tested at different concentrations was as shown in the results, among which the cell survival rate of 0.1%, 0.5% and 1% samples was 104.85%, 107.55% and 102.07%, respectively, and the cell survival rate was greater than 90%. As shown in Figure 1.

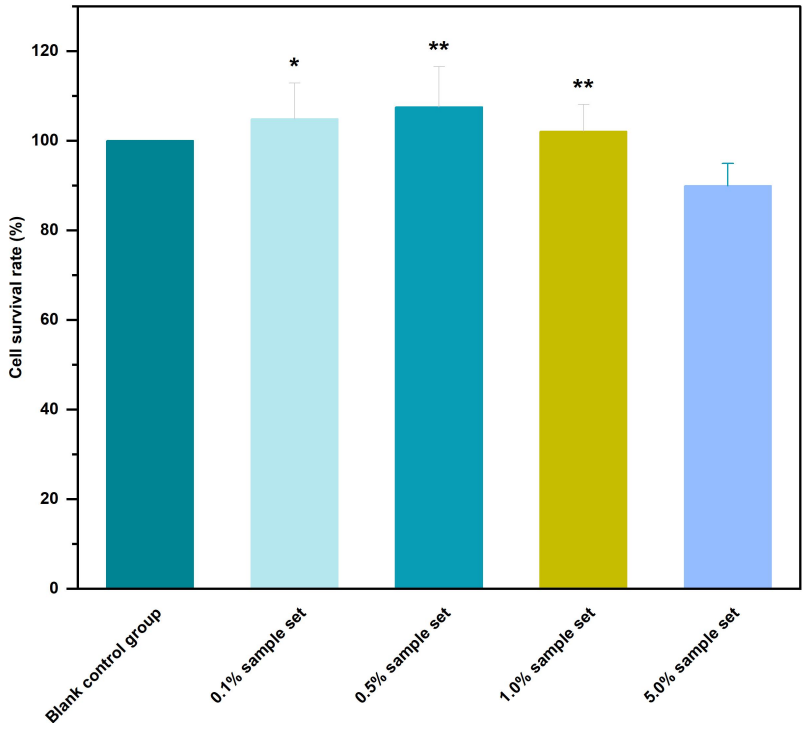


Figure 1.Experimental results of the influence of test samples on cell survival rate

3.1.2 Type I collagen content

The results showed that compared with the blank control group, the content of type I collagen in 0.1% sample group, 0.5% sample group and 1.0% sample group had a significant increase ($P<0.05$), and the up-regulation rate was 27.72%, 28.94% and 36.27%,respectively, indicating that the sample could promote the synthesis of type I collagen at this concentration. As shown in Table 1.

Table 1. Content and up-regulation rate of type I collagen

Group	Type I collagen content	Type I collagen

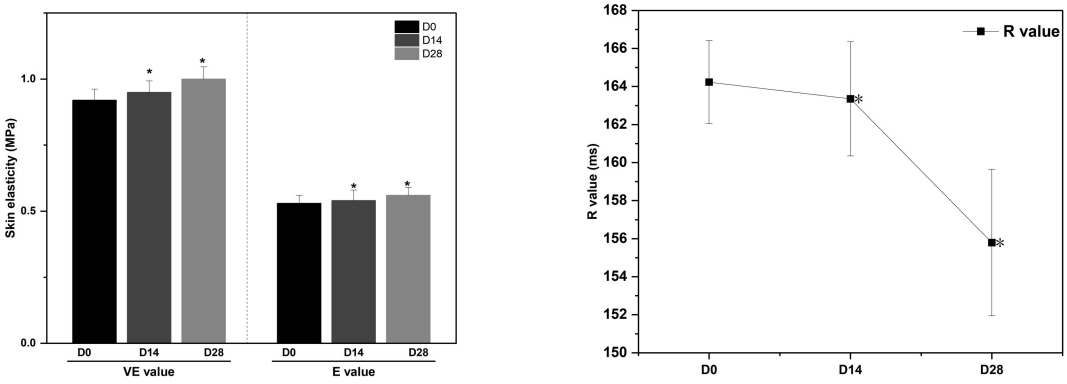
	（pg/ml）	up-regulation rate
Blank control group	397.78±16.05	-
Positive control group	412.24±22.76	25.76%
0.1% sample set	508.05±64.43	27.72%
0.5% sample set	512.89±15.44	28.94%
1.0% sample group	542.06±41.28	36.27%

3.2 Evaluation of human efficacy

A total of 30 female subjects aged 35 to 50 were screened in this study, and 30 cases were valid. Before using the product, there was no significant difference in the comparison of various index data of the left and right facial skin of the test subjects (*P*>0.05), excluding the influence of the initial state of the left and right facial skin.

3.2.1 Skin elasticity

Skin elasticity was tested on subjects at D0, D14 and D28, and the results of skin viscoelasticity value (VE value), skin Young's elastic modulus (E value) and skin retraction time (R value) were shown in Figure 2. After 28 days, VE value increased to 1.00±0.05, an increase of 7.89% (*P*< 0.05), E value increased to 0.56±0.03, a significant increase of 4.55% (*P*< 0.05), R value decreased to 155.79±3.86, a significant decreaseof 5.14% (*P*< 0.05). It shows that the sample can significantly improve skin elasticity.

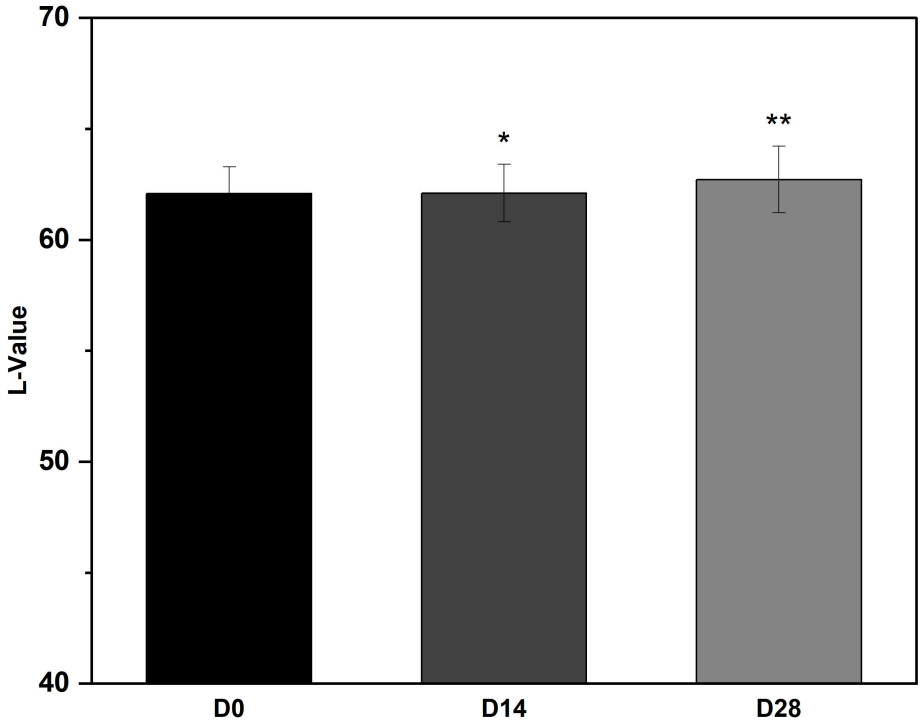


(**a**) (**b**)

Figure 2. (a) VE value and E value of skin elasticity at different time points; (b) R value of skin elasticity at different time points.

3.2.2 Skin brightness

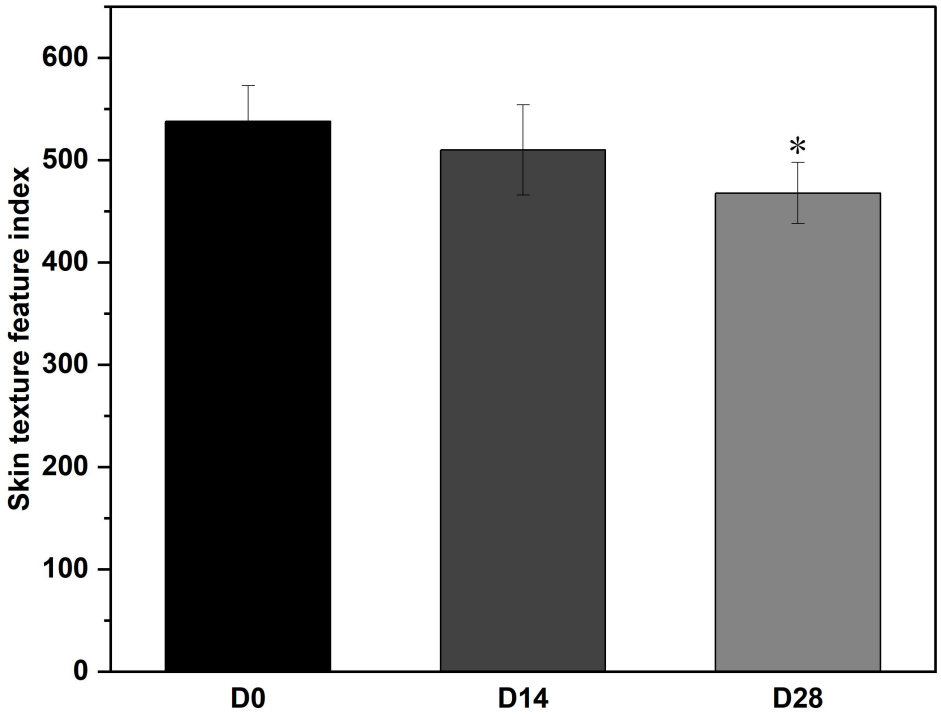
The skin colorimetric value L* value of volunteers was detected at D0, D14 and D28, and the L* value after D28 was increased to 72.71±1.50, a significant increase of 1.01% (P< 0.01), as shown in Figure 3.



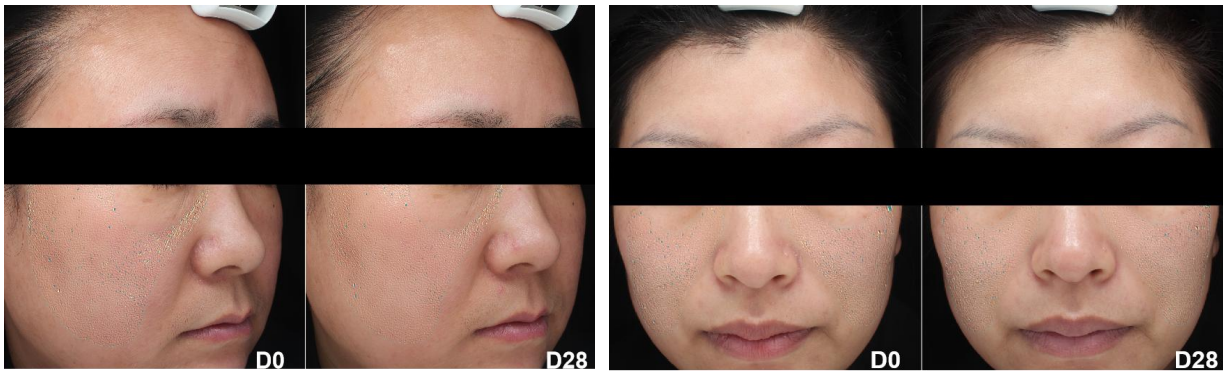
Figuer.3 Skin texture feature index at different time points

3.2.3 Skin texture

Facial texture was analyzed according to VISIA images, and the count of skin texture features was shown in Figure 4. The results showed that the skin texture characteristic index of D28 was significantly lower than that of D0, with a value of 467.88±48.21, and the improvement rate of skin texture characteristic index was 13.03% (P< 0.05). In different measurement periods, typical cases of VISIA texture images were shown inFigure 5.



Figuer.4 Skin texture feature index at different time points



(**a**) (**b**)

Figuer.5 (a)Comparison of skin texture at different times of subject1 (b) Comparison of skin texture at different times of subject2

4. Discussion

Peptide is a kind of compound formed by amino acids linked by peptide bond, and it is a protein fragment with biological function. Due to the diversity of amino acids and the high degree of freedom of the spatial conformation of the peptide chain, peptides have a variety of biological functions, which can be divided into signal peptides, carrier peptides, enzyme inhibiting peptides, neurotransmitter inhibiting peptides, such as acetylhexapeptide-8, mainly interfere with the formation of SNARE complex and the release of acetylcholine. Thus, it has the effect of blocking nerves similar to botox, but does not bring the side effects of botox. It also has the function of promoting collagen synthesis and adipose tissue regeneration, which is mainly used to delay skin aging and anti-wrinkle [13].

The acetyl hexapeptide-8 in the test samples in this study can soothe muscles and improve facial aging. The Bifida ferment lysate is rich in various nutrients and can cooperatively improve facial skin.

In vitro experiments, type I collagen is one of the main components of the dermal extracellular matrix. The up-regulation rate of type I collagen content in the blank and test sample groups was compared to evaluate whether the tested substance has efficacy in promoting collagen synthesis [14]. The test samples with 0.1%, 0.5% and 1.0% concentration had a significant effect on the production of collagen type I in human fibroblasts, indicating that the test samples had a good effect on promoting cell regeneration and extracellular matrix synthesis.

Human experiments showed that skin elasticity (E value, R value and VE value) after using test sample D28 were significantly improved compared with D0, indicating that the sample can significantly improve skin elasticity. With skin aging, extracellular matrix changes and dermal collagen fibers decrease, resulting in rough skin. After using the test sample for 28 days, the count of skin texture features decreased significantly, indicating that the test sample could refine pores and improve skin roughness. Another sign of skin aging is dullness. After 28 days of testing, the brightness of the skin can be significantly improved, indicating that the product can improve the dullness of the skin.

5.Conclusion

In summary, the sample can promote the generation of type I collagen, significantly improve the elasticity of facial skin, enhance skin brightness, improve skin texture, help to improve skin aging, and have a good application prospect in anti-aging.

6. References.

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