**The Effect and Analysis of Compound Polypeptide Firming Essence**

Yu, Yu [2]; Li, Shenghui [1]; Ding, Song [1]; Yang, Ke[1]; **Liu, Chenguang**[2]*;

[1] Qingdao Youdo Bioengineering Co., Ltd., Qingdao, Shandong, China;

[2] College of Marine Life Science, Ocean University of China, Qingdao, Shandong, China

*Liu, Chenguang; Room 105, Science Building, Ocean University of China, No.5 Yushan Road, Shinan District, Qingdao, Shandong, China; 13573865801; liucg@ouc.edu.cn

**Abstract**

**Background**: Currently, skin ageing is a topic of much interest, anti-aging products that claim to improve facial skin relaxation, promote firming and local lifting have a high market share and a wide range of consumer groups. Polypeptides have great development potential as anti-aging substances. The objective of this study was to assess the firming improving effect of the compound polypeptide firming Essence.

**Methods**:

In vitro evaluation: Mouse fibroblast cell (L929) viability assay by MTT assay method. Determination of Type I Collagen Content in Fibroblasts (L929).

Human test: A total of 30 Chinese women between 35~60 years old with sagging skin were enrolled, the left side is the control side, using the placebo, and the right side is the experimental side, using the compound polypeptide firming essence. After 4 weeks of continuous use, the effect of the compound polypeptide firming essence was evaluated by objective data, skin ultrasound, VISIA image and subjective evaluation.

**Results**:

In vitro evaluation: The essence had better promoting the proliferation of mouse fibroblast (L929) . Compared with the cell control group, all the essence of 1%, 0.1%, 0.01% et al.concentrations have a certain effect of promoting collagen production.

Human test: After 4 weeks, the increase in elasticity value on the experimental side is greater than the control side. The E value(Young´s elasticity modulus ) and the VE value

(Visco Elasticity) of experimental side showed significant improvement ($P<0.05$). According to the data analyzed by VISIA, the improvement in pores value on the experimental side is greater than the control side ($P<0.05$). According to skin ultrasound image, collagen intensity increased in more than 80% of volunteers. More than 90% of volunteers thought their skin was firmer and more delicate.

**Conclusion**: The Compound Polypeptide Firming Essence has an excellent effect on improving skin elasticity and firmness, it also has a good effect of fine pores, shows good application prospects in the field of anti-aging skin care products.

**Keywords**: Anti-aging; Skin elasticity; Skin firmness; Compound Polypeptide.

## Introduction.

Anti-aging, as the current mainstream skin care appeal, is getting more and more attention. Anti-aging products that claim to improve facial skin relaxation, promote firming, local lifting have a high market share and a wide range of consumer groups [1].

Compared with young skin, aging skin is dry, dull and shows some degree of laxity and local tissue sagging. According to research data, the skin elasticity value of the whole face of northern Chinese women aged 40-50 shows a downward trend with age, and the facial skin elasticity decreases rapidly after the age of 50[2]. Improving skin elasticity and firmness is important for skin anti-aging.

Polypeptides have the advantages of biocompatibility, easy absorption, safety, good water solubility and low molecular weight, etc[3], were categorized into four groups: signal peptides, enzyme-inhibitor peptides, neurotransmitter-inhibitor peptides and carrier peptides. At the same time, they have the functions of protecting, repairing damaged cells and promoting cell growth. Therefore, polypeptides have great development potential as anti-aging substances[4].

The objective of this study was to assess the firming improving effect of the compound polypeptide firming Essence.

## Materials and Methods.

1. Materials

Water, Glycerin, Hydrolyzed Sodium, Pentylene Glycol, Palmitoyl Tripeptide-5, Acetyl Hexapeptide-8, Acetyl Tetrapeptide-9, Palmitoyl Pentapeptide-4, Palmitoyl Tetrapeptide-7 . 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), PBS buffer solution, Foetal bovine serum (FBS), 0.25% of trypsin, DMEM(H), Enzyme-Linked Immunosorbent Assay(ELISA) Kit For ollagen Type I(COL1).

2. Equipments

VISIA Canfield , Dermalab Series Skin Lab Combo. Microplate reader, pipettor, blood counting chamber, carbon dioxide incubator, centrifugal machine, microscope et al.

3. Methods

3.1 L929 mouse fibroblasts viability assay by MTT assay method

L929 cells during logarithmic growth, seeded in 96-well plates, about $2\times10^3$ cells per well, Incubate overnight at 37 ° C in a 5% $CO_2$ incubator. Discard the culture medium, add samples of different concentrations, set 5 parallels for each concentration, add serum-free DMEM medium to the cell control group, and culture for 48-72h . Discard the aspirating medium, wash twice with PBS, and add 100 μL MTT (1.0 $g\cdot L^{-1}$) solutionAfter culturing for 4 h at 37 °C and 5% $CO_2$, the liquid was discarded, 150 μL of DMSO was added, and the cells were placed at 37 °C for 10 min, and the absorbance value of each well was measured at a wavelength of 490 nm.

Cell survival rate = ( absorbance of measurement group-absorbance of blank control group) / (absorbance of cell control group-absorbance of blank control group) × 100%.

3.2 Determination of Type I Collagen Content in Fibroblasts (L929) [5]

L929 cells during logarithmic growth were seeded in 96-well plates, and the number of cells per well was about $5\times10^{3,}$ and cultured overnight in a 37°C , 5% $CO_2$ incubator. Discard the culture medium, add 200 μl of samples of different concentrations, set 5 parallels for each concentration, take the complete medium without the sample as the blank control group, and cultivate for 24h±2h. The supernatant was centrifuged at 1000 rpm for 10 minutes to remove particles and polymers, and processed according to the instructions of the COL I kit, and the content of type I collagen in the supernatant was detected.

Take the absorbance OD value as the ordinate (Y), and the corresponding standard concentration of the substance to be tested as the abscissa (X), and make a corresponding curve. The content of the substance to be tested in the sample can be converted from the standard curve according to its OD value. Finally, the average of 3 replicate wells in each group was taken as the final type I collagen result.

The rate of increase (%) = (T/C-1) × 100% where:

T—the average content of type I collagen in the test substance;

C—the average content of type I collagen in the blank control.

3.3 Human test methods

A total of 30 Chinese women between 35~60 years old with sagging skin were enrolled, according to the Declaration of Helsinki, this test is voluntary by the subjects themselves, the purpose and possible risks of this test are informed before the test.

Those with one of the following conditions will not be selected: Used antihistamines in the last week or immunosuppressants in the last month; Use any anti-inflammatory drugs at the site within the last two months; Insulin-dependent diabetes; Breast-feeding or pregnant women; patients with respiratory diseases under treatment; Allergic constitution, allergic dermatitis and other people; have a history of skin disease; People who are undergoing dermatological treatment, or taking hydroxy acids, whitening products and anti-depressants within one month; Subjects with aging drugs; People in the test area who have skin damage or are judged to be unsuitable for this test; Have participated in another clinical test at the same time or have participated in facial clinical test within the last 3 months; Other iatrogenic reasons considered by the expert or professional to influence the test results[6].

The left side is the control side, using the placebo, and the right side is the experimental side, using the compound polypeptide firming essence. Both side samples were applied on he face 2 times per day( morning and night)for 4 weeks. Elasticity data measurement, skin ultrasound and skin image acquisition were obtained once a week; Subjective satisfaction of volunteers were assessed after 4 weeks [7-8]. Dermalab Series SkinLab Combo was used to easure elasticity data and get ultrasound Image. In this experiment, Skin elasticity is measured by three parameters: Young´s elasticity modulus (E), Skin retraction time (R) and ViscoElasticity (VE) combining both the elevation and retraction phase. Calculation of Youngs elasticity modulus (E) is based on measuring the distance the skin can be lifted, when applying a specific and preset vacuum to the skin inside the probe chamber. Dividing the elasticity modulus by the retraction time provides a parameter (Visco Elasticity, VE), where both the elevation phase and the retraction phase are taken into account. Retraction time (R) is the time in milliseconds it takes for the skin to retract from the peak elevation to 33% of the peak elevation. Images of all subjects were taken by VISIA .The test items and schedule are shown in Tab. -1.

Tab. -1 Test item table.

| Actions | Before | 1week | 2weeks | 3weeks | 4weeks |
|---|---|---|---|---|---|
| Informed Consent of Test | √ | – | – | – | – |

| | | | | | |
|---|---|---|---|---|---|
| Facial photography | √ | √ | √ | √ | √ |
| Ultrasound Image | √ | √ | √ | √ | √ |
| Elasticity data of E value | √ | √ | √ | √ | √ |
| Elasticity data of VE value | √ | √ | √ | √ | √ |
| Elasticity data of R value | √ | √ | √ | √ | √ |
| Subjective satisfaction | – | – | – | – | √ |

3.4 Statistical analysis

EXCEL software was used to make descriptive statistics of each measured value, including quantity, mean value and standard difference, minimum and maximum, etc.

**Results**.

1.In vitro evaluation.

1.1 L929 cell viability assay by MTT assay method.

The concentration of 0.5%, 0.1%, 0.05%, 0.01%, 0.005% and 0.001% of the polypeptide firming essence can promote the proliferation of L929 cells. The results are shown in Fig.-1.

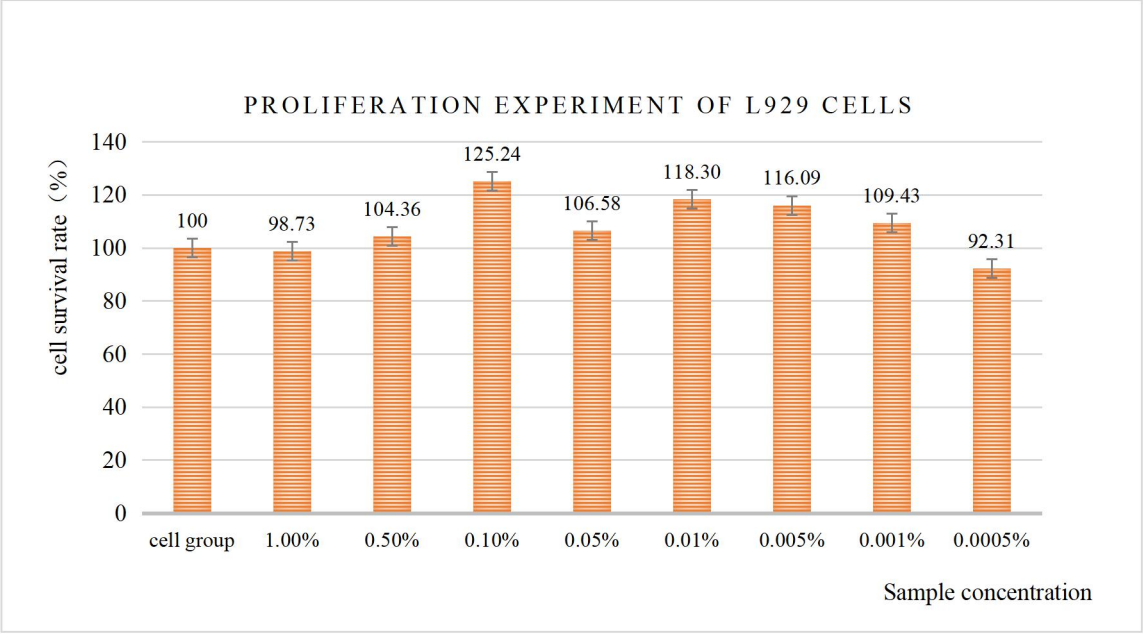


Fig.-1 Result of L929 Cell Proliferation Promoting Experiment of Compound Polypeptide Firming Essence.

1.2 Determination of Type I Collagen Content in Fibroblasts (L929)

Compared with the cell control group, all the essence of 1%, 0.1%, 0.01%, etc concentrations have a certain effect of promoting collagen production. The results are shown in Fig.-2.

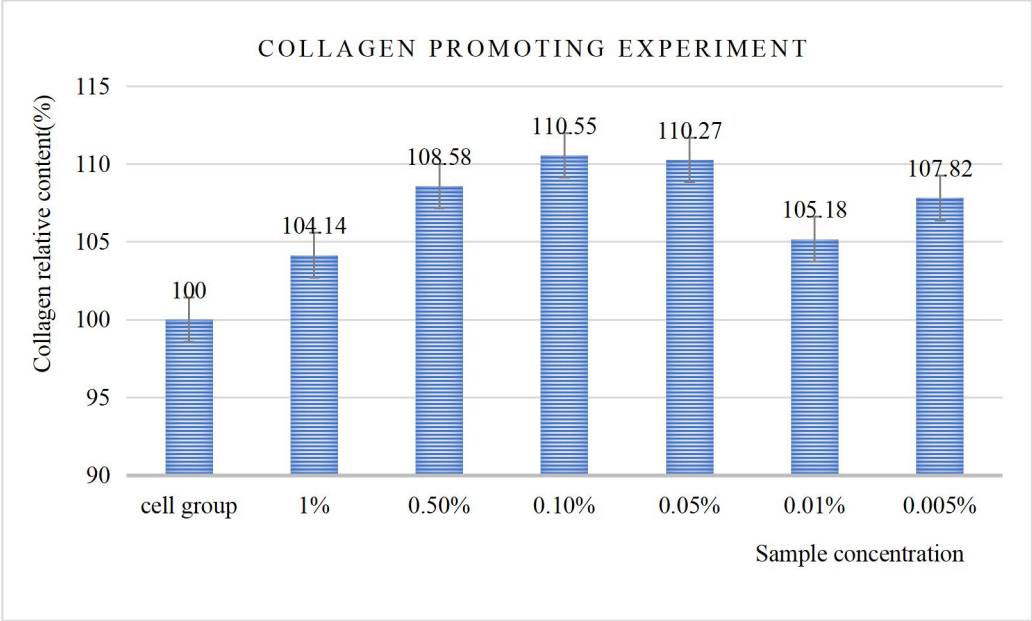


Fig.-2 Collagen-promoting test results of Compound Polypeptide Firming Essence.

2.Human test.

2.1 Skin elasticity measurement

After 4 weeks, the increase in elasticity value on the experimental side is greater than the control side. The E value(Young´s elasticity modulus ) of experimental side increased by 10.8% ($P<0.05$), the VE value(Visco Elasticity) of experimental side increased by 7.50% ($P<0.05$), the R value(Skin retraction time) of experimental side reduced by 4.97% ($P<0.05$). The results are shown in Fig.-3 and Fig.-4.

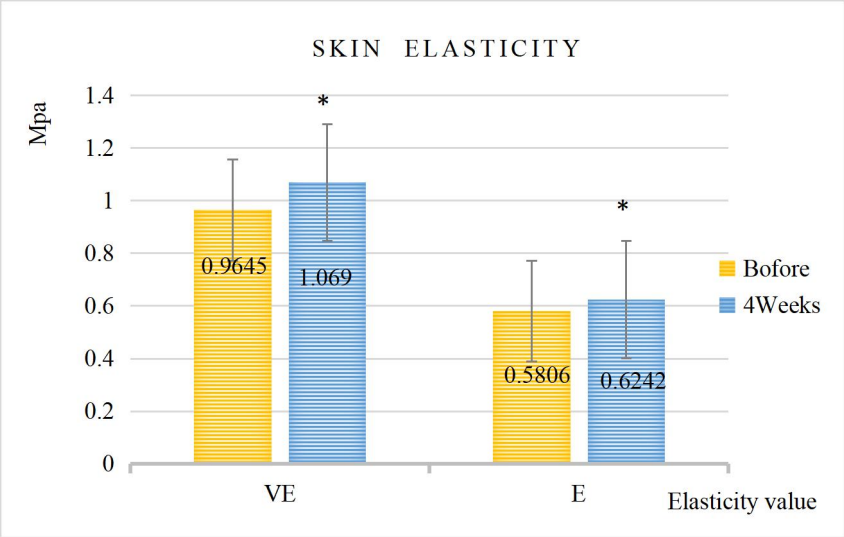


Fig.-3 Improvement of skin elasticity(E value and VE value) on experimental side.(*, adjusted p-value < 0.05).

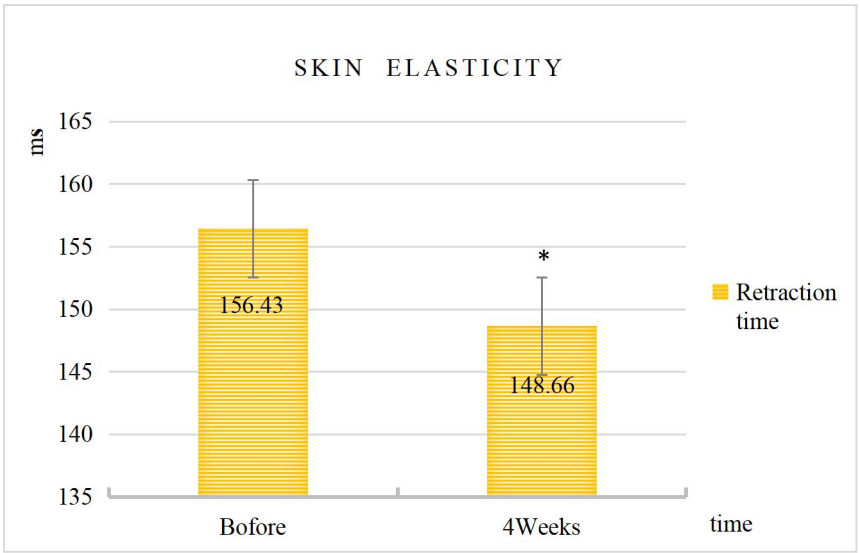


Fig.-4 Improvement of skin elasticity(R value) on experimental side.(*, adjusted p-value < 0.05).

2.2 Skin pores improvement

Decreased elasticity of the facial skin and reduction of collagen fibers in the dermis can lead to damage to the supporting structures around the skin pores, resulting in increased facial pores[6]. According to the data analyzed by VISIA, The improvement of facial pore data can be observed. The improvement in pores value on the experimental side is greater than the control side. The Feature Counts of pores of experimental side reduced by 22.15% ($P<0.05$), the Absolute Scores of pores of experimental side reduced by 27.07% ($P<0.05$).The results are shown in Fig.-5 and Fig.-6.

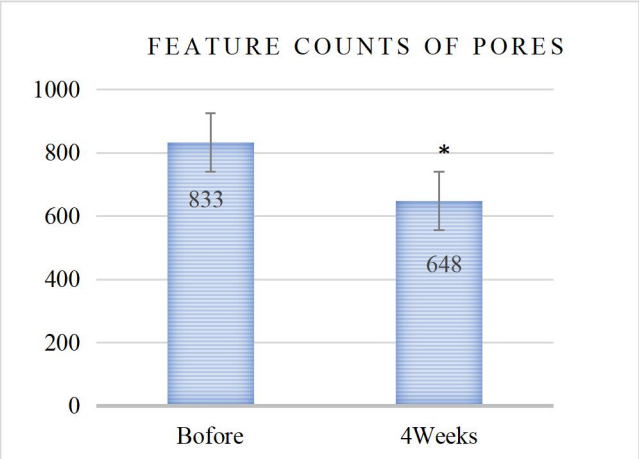


Fig.-5 Improvement of pores counts on experimental side.(*, adjusted p-value < 0.05).


Fig.-6 Improvement of pores absolute scores on experimental side.(*, adjusted p-value < 0.05).

2.3 Skin ultrasound

Ultrasound skin imaging is based on measuring the acoustic response from the skin, when an acoustic pulse is sent into the skin. In the ultrasound image the colors represent the intensity (reflection strength) of the reflected ultrasound signal. Dark color represents low intensity and white (yellowish) represents a high intensity.The firming effect of the product can be measured by the change of collagen reflection strength. According to ultrasound Image, collagen intensity increased in more than 80% of volunteers. Partial typical cases are shown in Fig.-7,Fig.-8 and Fig.-9.

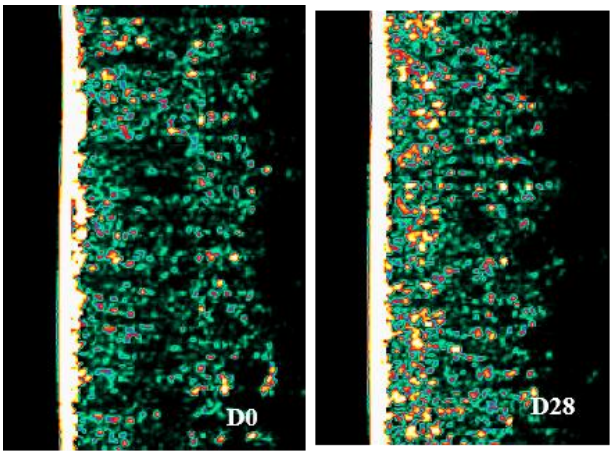


Fig.-7 skin of 48 year old woman, After using the Essence for 28 days, the collagen in the dermis of the experimental side was significantly improved.

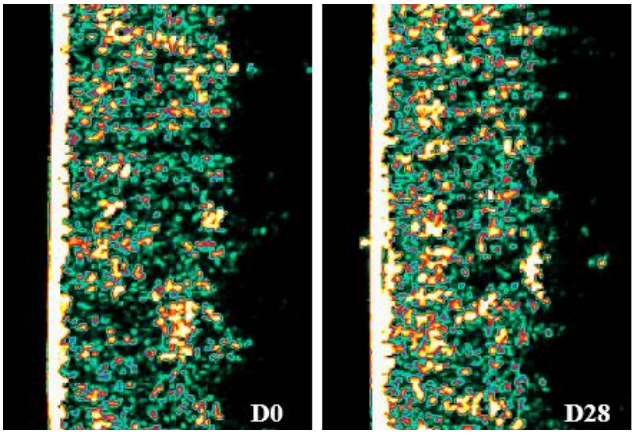


Fig.-8 skin of 38 year old woman, After using the Essence for 28 days, the collagen in the dermis of the experimental side was significantly improved, skin thickness of dermis was increased.

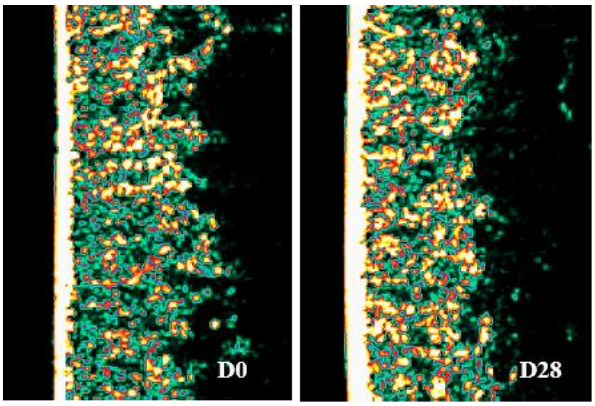


Fig.-9 skin of 36 year old woman, After using the Essence for 28 days, the collagen in the dermis of the experimental side was significantly improved, skin thickness of dermis was increased.

2.4 Subjective satisfaction of volunteers.

After the four weeks of test, the volunteers filled in the subjective satisfaction questionnaires, score from the skin moisturizing, facial sagging, slim wrinkle , overall satisfaction, etc., full of 5 points, a score above 4 represents satisfaction. More than 90% of volunteers thought their skin was firmer and more delicate. The scoring results are shown in Fig.-10.

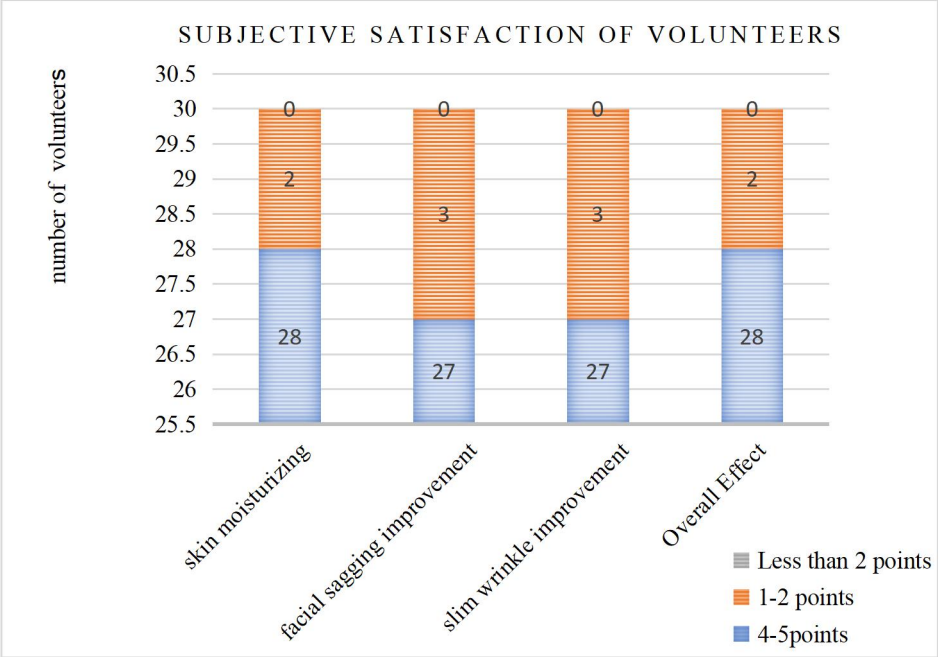


Fig.-10 Volunteer Satisfaction Statistics

**Discussion.**

Peptides have different effects on the skin especially for cosmetics. It is widely used in relaxing facial muscles and smoothing wrinklesmplished by stimulation and growth of different skin cells like human skin fibroblasts. Signal peptides can also increase elastin, proteoglycan, glycosaminoglycans and fifibronect in proliferation. By increasing matrix cell activities and consequently collagen production, the skin looks fifirmer and younger. Neurotransmitter inhibitor peptides inhibit acetyl-choline release at the neuromuscular junction and have curare-like effect [4], it is widely used in relaxing facial muscles and smoothing wrinkles. This study explored the effcet of Compound Polypeptide Firming Essence in improving skin firmness.

Young and smooth firm skin will normally be relatively easy to elevate by applying suction, and it will retract rapidly. Old and loose skin will also be easy to elevate, however, it will not retract rapidly. Therefore, what is usually considered to be skin elasticity (or smoothness, softness, firmness) is of a more complex nature and is best measured by taking both the elevation and retraction phase into account[9-10]. In this study, after 4 weeks, the E value(Young´s elasticity modulus ) of experimental side increased by 10.87% ($P<0.05$), the VE value(Visco Elasticity) of experimental side increased by 7.50% ($P<0.05$), the R

value(Skin retraction time) of experimental side reduced by 4.97% ($P<0.05$). It is proved that the essence has a good promotion effect on skin elasticity.

Collagen is a major component of the extracellular matrix and contributes to the structural stability of the skin. The different types of collagen fulfill different functions: collagen VII is a component of anchoring fibrils and stabilizes the dermal-epidermal adhesion and the extracellular matrix in the dermis contains collagen type I and III in a ratio that changes with aging[10]. Due to declining numbers of fibroblasts with increasing age, collagen and elastin synthesis is subsequently reduced, and collagen fibers are thinner with reduced density [11]. Therefore, the anti-aging effect of the product can be well observed by evaluating the promoting effect of collagen and the promoting effect of fibroblast vitality. In this study, all the essence of 1%, 0.1%, 0.001% etc. concentrations have a certain effect of promoting collagen production and promoting cell proliferation. In summary, Through the evaluation of in vitro experiments and human test, the anti-aging effect of polypeptide combination active substance on skin was verified.

**Conclusion**.

The Compound Polypeptide Firming Essence has an excellent effect on improving skin elasticity, firmness and pores. It shows good results in vitro and human evaluation and has good application prospects in the field of anti-aging skin care products.

**Acknowledgments.**

This work was supported by the R&D team of Qingdao Youdo Bioengineering Co., Ltd.

**Conflict of Interest Statement**.

NONE.

**References**.

[1] YANG Ze-ru, WEI Yue-xian, FAN Zhan-hua, et al.(2006)Evaluation of cosmetics firming efficacy based on image analysis. China Surfactant Detergent & Cosmetics, 50(12), 854-860.

[2] Yi fan, ZHANG liping. (2021)DATA ANALYSIS. China Cosmetics Review, 106-111.

[3] HE Ping.(2019)Summary of Applications of Small Molecular Peptides in

Cosmetics.Guangzhou Chemical Industry, 47(24), 14-15.

[4] F. Gorouhi, H. I. Maibach. Role of topical peptides in preventing or treating aged skin. International Journal of Cosmetic Science, 2009, 31, 327–345.

[5] George P. Majewski, Smrita Singh, Krzysztof Bojanowski. Olive leaf-derived PPAR agonist complex induces collagen IV synthesis in human skin models. International Journal of Cosmetic Science, 2021, 43, 662–676.

[6] CHENG Fang, GAO Jin-ping, YANG Sen,et al.(2017)Age-related and Regional Changes in Skin Elasticity: a Quantitative Evaluation of Han Subjects. Chinese Journal of Aesthetic Medicine, 26(2), 61-64.

[7] WANG Xiong, HUANG Xiao-bing, CHEN Xiao-ru.(2019)Effect of radio frequency combined with dot-matrix laser on skin fine lines, firmness and pores in patients with facial skin photoaging. China Medical Cosmetology 9(10), 53-57.

[8] Zhang Yali, She Wenli, Liu Hui ,et al.(2018)Evaluation and Influence of Different Wavelength Micro Laser on Skin Compactness. APPLIED LASER.38(6), 1072-1076.

[9] M.A. Ruiz Martınez, S. Peralta Galisteo, H., et al. Role of proteoglycans on skin ageing: a review. International Journal of Cosmetic Science, 2020, 42, 529–535.

[10] David Zargaran1, Florence Zoller1, et al. Facial Skin Aging: key concepts and overview of processes. International Journal of Cosmetic Science, 2022.

[11] Michael J. Flagler, Makio Tamura, Tim Laughlin, et al. Combinations of peptides synergistically activate the regenerative capacity of skin cells in vitro. International Journal of Cosmetic Science, 2021, 43, 18–529.