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Research Article

The Efficacy of An Orally Administered Supplement Inerbty® On Skin Hydration, Melanin, Elasticity, Gloss and Wrinkles: Results of A Clinical Study

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Abstract

The complex structure of skin and skin-aging need a systematic treatment for its recovery. Both the growing market of oral beauty products and their solid scientific support provide tremendous benefits to consumers who care about their facial beauty. In our research, we tested a combination of three oral beauty products – Inerbty® including key ingredients as collagen tripeptide, tremella polysaccharides, grape extract, turmeric extract, Haematococcus pluvialis and acerola cherry on the skin improvement. Thirty-five female subjects were enrolled in this clinical study. Both instrumentation analysis and clinician analysis showed positive improvement including skin hydration, melanin, elasticity, gloss and wrinkles and so on. The self-assessment of subjects showed great satisfaction rate in terms of skin improvement, willingness to reorder and so on. Not a single adverse event was reported proving the safety of the Inerbty® products.

Introduction

Driven by the "beauty economy", skin care, cosmetics, oral beauty products and other beauty related products have been increasingly purchased by consumers, driving trillions of consumption growth in related markets from the past five years. Among them, the consumption growth rate of oral beauty market is much higher than the average growth rate of other categories, from the aspects of the number of consumers and per capita consumption. The consumer growth is the main driving factor for the development of oral beauty market. In addition to the familiar collagen and grape seeds, more and more other subdivisions are gradually coming into consumers' view.

The trend of increased consumption of oral beauty products also comes from various scientific research disclosing the fact that beauty originates from the inside and how nutrition could recover aged skin [1]. It is realized by more and more consumers that administering both topical cosmetics from outside to inside the skin and orally consume nutrition from inside to the outer skin layers would exhibit significant improvement on a variety of dermal parameters [2-3].

Sometimes topical cosmetics may not be stable under UV radiation, making it necessary to use oral beauty products as a reinforcement. Resveratrol is a good example for which high activity trans-resveratrol would transform to low activity cis resveratrol once it is exposed to sunlight [4]. Skin that covers most of the body is categorized into 3 layers: the epidermis, the dermis, and the hypodermis (Figure 1) [5]. The most obvious process during aging certainly is the progressive loss of skin tissue, which amounts averagely to about 7% per decade with however large individual variations [6]. The loss of skin tissue consequently underlies most of the easily noticed morphological modifications of the skin such as wrinkles. All can be attributed to several factors such as loss of cells and loss of extracellular matrix (ECM). Cell loss, which occurs in both the epidermal and dermal layers, could lead to the loss of ECM. Also, the loss of ECM could worsen as the remaining cells

are not capable to biosynthesize materials for ECM and the matrix degrading enzymes would over express by photo-aging [6].

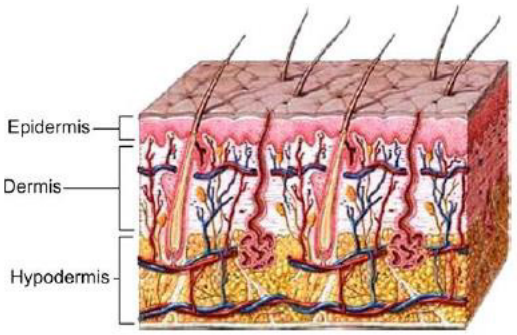

Figure 1: The 3 layers of the skin: epidermis, dermis, and hypodermis [1].

Therefore, treating skin aging is a complex issue that may need systematic combination of oral beauty products. In our study, we aim to test the efficacy of a combination of oral beauty products: collagen tripeptide beverage, grape powder pressed candy and acerola cherry pressed candy on the skin improvement from perspectives of skin hydration, skin elasticity, skin gloss and wrinkles and so on. Both equipment analysis and clinician analysis were conducted to make this investigation more scientific and repeatable.

Materials and Methods

Testing product

Inerbty® Series were provided by Guangzhou Aibaiyi Biotechnology Co., Ltd, including three products:

a) Collagen tripeptide beverage

b) Grape powder pressed candy and

c) Acerola cherry powder pressed candy

Study design/Intervention

The study was carried out as a single-blinded, randomized and self-controlled trial on the effects of 35 healthy female subjects after 56 days of oral intake. All participants signed consent agreement that manifests their benefits from this test and relevant risks. Before the inception of the study, all testing protocols and consent agreement were scrutinized and approved by Guangdong Cosmetics Ethnicity Committee, China.

Cohort

Thirty-five healthy female subjects in the age group of 25 to 45 years were screened and enrolled in the study. Thirty subjects completed the study and five withdrew due to personal reasons. The participants were supplemented with Inerbty® Series once a day.

Inclusion Criteria

Healthy female subjects from 25 to 45 years old; they are capable of reading Chinese and comprehending the consent agreement; they are willing to cooperate and complete all the tests during the study and report any adverse events.

Exclusion Criteria

Females that are pregnant or breastfeeding; any obvious facial defects including sunburn, scar, pigmented nevus, which might impair the test characterization; facial microbial infections; chronic skin diseases (such as skin tumors, rosacea, eczema, lupus erythematosus, seborrheic dermatitis, psoriasis, severe epidermal shedding); history of immunosuppressive or immunodeficiency disorders (including HIV or AIDS) or current use of immunosuppressive drugs or radiotherapy; chronic and endocrine diseases such as asthma, epilepsy, diabetes, hypertension, hyperthyroidism or hypothyroidism; participation of other clinical studies during the past 3 months; took any drugs that may affect skin status or response in the past 6 months or currently such as antihistamines, antibiotics, insulin, anti-inflammatory drugs, vitamins A, steroids, aspirin, thyroid drug; treated with facial medical treatment, such as laser treatment, chemical stripping and minimally invasive cosmetic treatment; a history of mental illness or unable to take care of themselves; participant with any of the above were excluded from the study.

Outcome measures

All tests were conducted at a third-party medical testing center called Landproof in Guangzhou, China. All measurements were performed before the first intake of the product, after 28 and 56 days.

Measurement of Hydration at Skin Surface

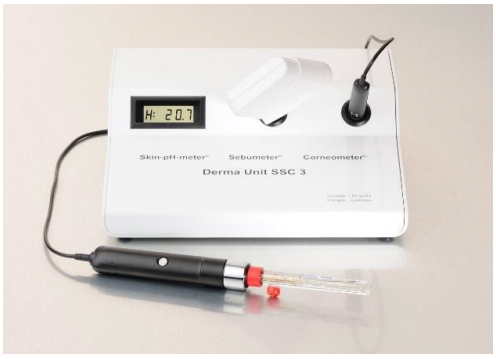

Figure 2: Corneometer®, Derma Unit SSC 3.

The hydration level of the skin surface (stratum corneum) was characterized by Corneometer®, Derma Unit SSC 3 (Figure 2). The probe allows very quick measurement (1s) that is crucial to avoid any occlusion. Substances on the skin such salts or residues

of topical applied products have only minimal impact due to capacitance measurement. Larger value reflects higher extent of hydration at skin surface.

Measurement of facial images

Multi-modality facial images of the subject's frontal, left-side, and right-side views were captured at baseline, day 28, and day 56 using the VISIA-CR. The number of spots was also analyzed by VISIA-CR with IPP software (Figure 3).

Figure 3: VISIA-CR

Measurement of Melanin

The Mexameter® MX 18 (Courage+Khazaka electronic GmbH) (Figure 4), a very easy, quick and economical tool, was applied to measure melanin index from the intensity of absorbed and reflected light at specific wavelength as recommended [10]. Larger value reflects higher melanin in the skin.

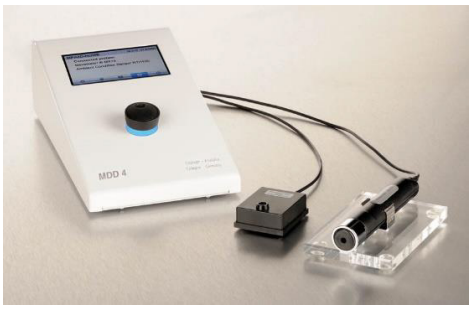

Figure 4: Mexameter® MX 18 device

Measurement of Skin gloss

Figure 5: SkinGlossMeter

The Skin Gloss Meter (Figure 5) is a portable instrument for measuring the specularly reflecting light from skin. In the SkinGlossMeter the laser light beam reflects back at the same angle as it contacts the measured surface. Scattered light that is not related to the definition of gloss is not measured. As a light source, the instrument has a built-in 635 nm red semiconductor diode laser. Larger value reflects better gloss of skin.

Measurement of Elasticity

The measuring principle of the Cutometer® (Figure 6) is based on the suction method, where negative pressure deforms the skin mechanically. Inside the probe, the penetration depth is determined by a non-contact optical measuring system. This optical measuring system consists of a light source and a light receptor, as well as two prisms facing each other, which project the light from transmitter to receptor. The light intensity varies due to the penetration depth of the skin. The resistance of the skin to the negative pressure (firmness) and its ability to return into its original position (elasticity) are displayed as curves (penetration depth in mm/time) in real time during the measurement. From these curves, parameters related to elastic and viscoelastic properties of skin surface can be calculated. The closer the value gets to 1, the more elastic the skin is.

Figure 6: Cutometer®

Measurement of skin collagen content

Using Dermalab® Combo's ultrasonic probe (Figure 7), skin thickness and dermis intensity can be measured. The main component of dermis is collagen. The thickness and strength of dermis can reflect the content of collagen. The higher the value is, the higher the content of collagen is.

Figure 7: Dermalab® Combo.

Measurement of Wrinkles

The parameters of skin wrinkles including fine lines and crow's feet are obtained by using PRIMOS Lite (Figure 8) to take 3D images of the local skin of the subjects, and then analyzed by professional analysis software. The depth, number, volume, area and length of fine lines are the parameters that can better reflect the degree of skin fine lines and crow's feet. The smaller the values of these parameters, the better the skin condition is in terms of fine lines.

Figure 8: PRIMOS Lite

Clinical Evaluation

The clinical visual grade of the subjects' facial spots, pores, crow's feet lines and nasolabial folds were scored by professional dermatologists, and the length of crow's feet lines was measured. The reference was skin aging atlas 2 - Asian type (by R Bazin & F flame, ver. 2010, Med'com Press). Lower scores reflect better improvement.

Subjective Assessments of Treatment Effects

All subjects completed the efficacy evaluation questionnaire of test products at D28 and D56. The score was given by the subjects in combination with their own feelings and with reference to the scoring standard. The efficacy score of the test products was 0-9 points (0 point indicates they are not satisfied with the efficacy of the test products; 9 points indicated that they were very satisfied with the efficacy of the test product; A score of 5 indicates a little satisfaction with the efficacy of the test product. The higher the score, the better the improvement effect of the test product on the skin problem or the better the efficacy of the test product. According to the scoring criteria, 30 subjects evaluated the efficacy of the test product after taking the test product, including skin hydration, roughness, wrinkles and pores, tightness, spots and skin color.

Adverse Events

The safety and tolerability of the administration of treatment powder were evaluated at day 28 and day 56. Professional dermatologist asked the subjects whether they had gastrointestinal discomfort, body skin tingling, itching and other symptoms or obvious signs of dry skin, desquamation, flushing and so on. During the whole study, the participants were requested to report instantly to the investigator once they experienced any uncomfortable feelings.

Statistical Analysis

Statistical analyses of this clinical study were completed using IBM Statistical Package for Social Sciences (SPSS 21.0) at an alpha level of 0.05. To evaluate primary and secondary outcome measures, analysis of variance (ANOVA) was used to compare within-group changes and group changes over time.

Results

The instrumentation analysis of skin showed great improvement including hydration in stratum corneum, melanin, skin elasticity, dermal strength and wrinkles (Tables 1-7 & Figures 9-13). The hydration in stratum corneum increased by 6.69% at day 28 and increased strikingly by 27.91% at day 56 compared to that at day 0. The skin melanin decreased by 7.97% at day 28 and maintained the similar improvement at day 56. The skin gloss improved by 9.99% and 10.50% respectively at day 28 and day 56 compared to day 0. The skin elasticity increased by 4.61% and 6.93% at day 28 and day 56 compared to day 0. The dermal strength indicating the collagen content increased by 4.67% at day 28 and the improvement almost doubled at day 56 compared to that at day 28. As collagen was recovered in the dermis, it is not difficult to see the improvement of wrinkles including crow's feet and fine lines from perspectives of length, volume, area and amount. All improvement reached statistic significances.

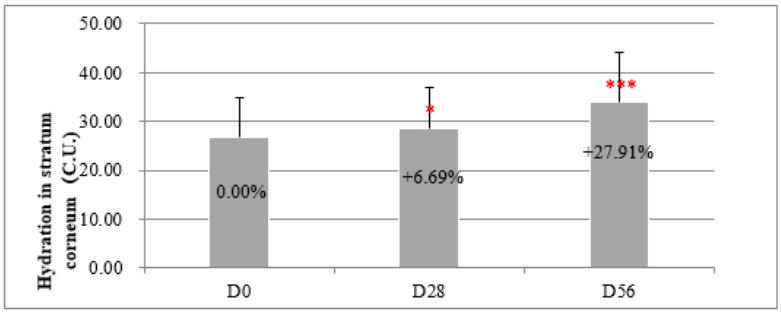

Figure 9: Improvement of hydration in stratum corneum at baseline, day 28 and day 56.
Note: * indicates changes reach a statistical significance compared to baseline ($p<0.05$)
*** indicates changes reach a statistical significance compared to baseline ($p<0.001$)

Table 1: Analysis of hydration in stratum corneum.

	D0	D28	D56
Hydration in stratum corneum (C.U.)	26.65 ± 8.16	28.44 ± 8.63	34.09 ± 9.95
p-value		0.01	< 0.001

Table 2: Analysis of skin melanin.

	D0	D28	D56
Melanin (M.U.)	169.31 ± 32.73	155.82 ± 31.95	156.63 ± 32.12
p-value		< 0.001	< 0.001

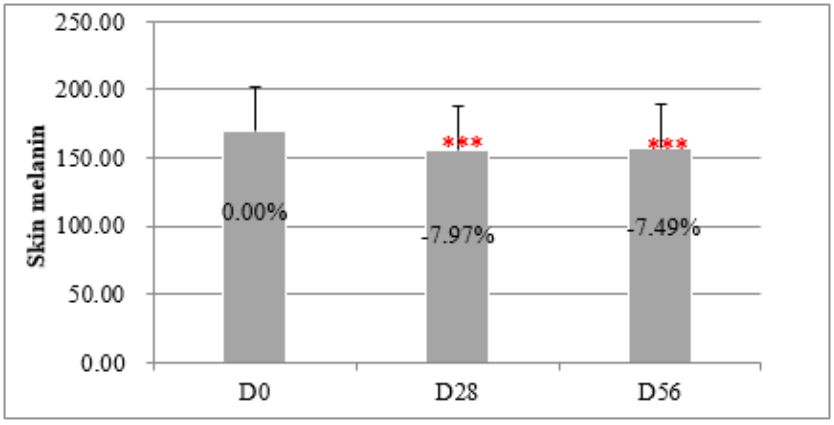

Figure 10: Improvement of skin melanin at baseline, day 28 and day 56.
Note: * indicates changes reach a statistical significance compared to baseline ($p<0.05$)
*** indicates changes reach a statistical significance compared to baseline ($p<0.001$)

Table 3: Analysis of skin gloss.

	D0	D28	D56
Skin gloss (SGU)	57.59 ± 4.58	63.34 ± 6.77	63.63 ± 5.58
p-value		< 0.001	< 0.001

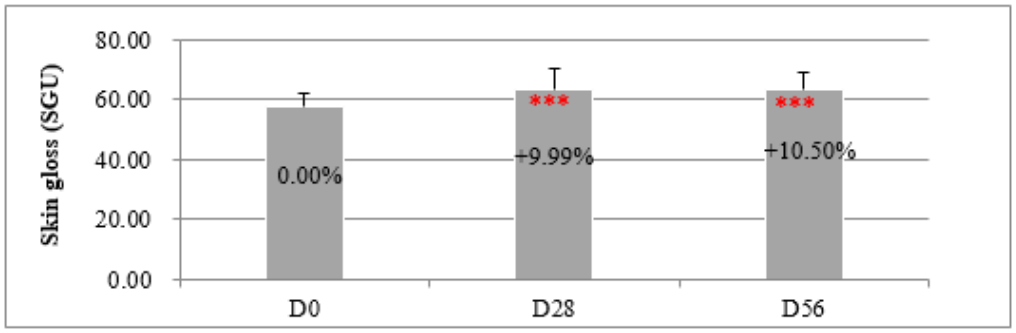

Figure 11: Improvement of skin gloss at baseline, day 28 and day 56.
Note: * indicates changes reach a statistical significance compared to baseline (p<0.05)
*** indicates changes reach a statistical significance compared to baseline (p<0.001)

Table 4: Analysis of skin elasticity.

	D0	D28	D56
Skin elasticity (R2)	0.7234 ± 0.0538	0.7568 ± 0.0530	0.7736 ± 0.0594
p-value		< 0.001	< 0.001

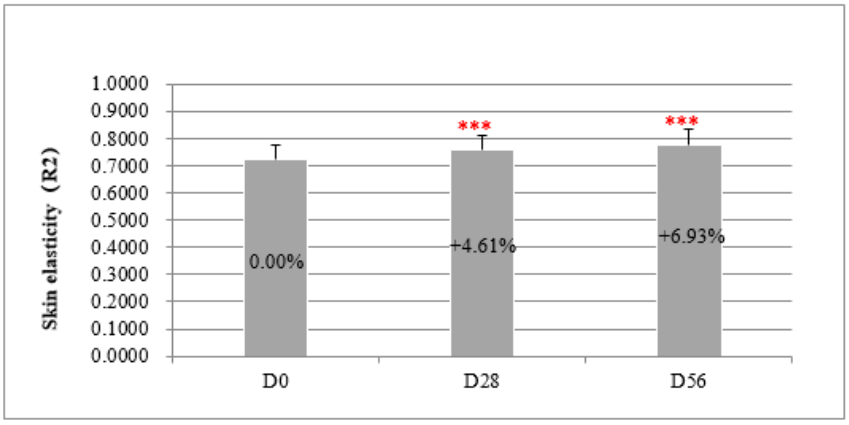

Figure 12: Improvement of skin elasticity at baseline, day 28 and day 56.
Note: * indicates changes reach a statistical significance compared to baseline (p<0.05)
*** indicates changes reach a statistical significance compared to baseline (p<0.001)

Table 5: Analysis of dermal strength.

	D0	D28	D56
Dermal strength	49.92 ± 14.02	52.25 ± 13.97	54.91 ± 14.06
p-value		0.179	0.023

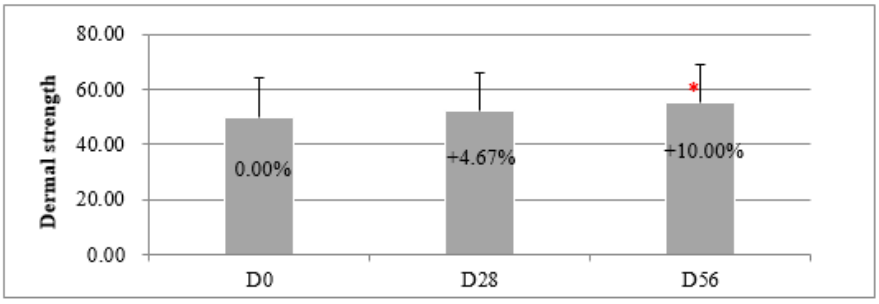

Figure 13: Improvement of dermal strength at baseline, day 28 and day 56.
Note: * indicates changes reach a statistical significance compared to baseline (p<0.05)
*** indicates changes reach a statistical significance compared to baseline (p<0.001)

Table 6: Analysis of fine lines (eye).

	D0	D28	D56
No. of fine lines	298.73 ± 89.75	216.07 ± 68.14	217.10 ± 68.21
p-value		< 0.001	< 0.001
Area of fine lines(mm2)	29.33 ± 6.65	26.03 ± 6.03	25.94 ± 6.29
p-value		< 0.001	< 0.001
Length of fine lines(μm)	102.03 ± 24.83	87.23 ± 21.44	87.30 ± 21.66
p-value		< 0.001	< 0.001

Table 7: Analysis of crow's feet.

	D0	D28	D56
Amount	317.13 ± 99.70	221.73 ± 72.21	220.23 ± 73.58
p-value		< 0.001	< 0.001
volume(mm3)	1.3330 ± 0.4016	1.2387 ± 0.3696	1.2087 ± 0.4040
p-value		0.02	0.023
area(mm2)	29.18 ± 5.20	25.45 ± 5.07	24.79 ± 5.00
p-value		< 0.001	< 0.001
length (μm)	98.93 ± 22.82	81.73 ± 17.83	82.00 ± 17.88
p-value		< 0.001	< 0.001

Beyond instrumentation analysis, an evaluation of skin improvement by dermatologists also shows the efficacy of orally administered products after 28 days and 56 days from aspects of facial spot color, facial spot intensity, facial pores, crow's feet and nasolabial fold (Table 8). All improvement reaches statistic significances.

Table 8: Clinical evaluation of skin improvement by dermatologists.

	D0	D28	D56
Facial spot color	3.63 ± 0.83	3.52 ± 0.86	3.37 ± 0.96
p-value		0.02	0.005
Facial spot intensity	3.53 ± 1.29	3.50 ± 1.29	3.28 ± 1.28
p-value		0.317	0.002
Facial pores	2.40 ± 0.42	2.12 ± 0.61	1.85 ± 0.67
p-value		< 0.001	< 0.001
Crow's feet	3.78 ± 0.73	3.53 ± 0.79	3.28 ± 0.88
p-value		0.002	< 0.001
Length of crow's feet	12.92 ± 3.52	12.64 ± 3.51	12.37 ± 3.46
p-value		< 0.001	< 0.001
Nasolabial fold	2.18 ± 0.68	1.82 ± 0.79	1.33 ± 0.91
p-value		< 0.001	< 0.001

The self-assessment including the subjective evaluation of the products from 12 aspects were summarized in Table 9. Almost over 73.33% of the 30 subjects were satisfied with the products at day 28 and continued to feel the improvement, with increased satisfaction rate at day 56. Besides, during the 56 days testing, not a single adverse event was reported.

Table 9: Self-assessment.

Self-assessment item	Satisfaction rate (%)	
	Day 28	Day 56
Hydration	80	80
Scaling	76.67	76.67
Fine lines	73.33	80
Roughness	76.67	80

Smoothness	80	86.67
Tightness	80	80.33
Pores	73.33	73.33
Gloss	83.33	83.33
Spot	70	70
Color	76.67	83.33
Satisfaction of function	80	83.33
Willingness to reorder	80	83.33

Discussion

It is undoubtful that traditional cosmetics consumers are more and more willing to embrace the oral beauty products given high market popularity and solid scientific support. As the human skin is a complex system, for which its rehabilitation needs combination of various products to recover all consequences caused by skin aging. Inerbty®, the combination of three products, mainly consists of collagen tripeptide, tremella polysaccharides, grape extract, turmeric extract, Haematococcus pluvialis and acerola cherry.

Collagen is the most significant matter in the extracellular matrix of skin. Exposed to UV radiation, reactive oxygen species (ROS) is generated, serving as a signal for several detrimental consequences. Firstly, the DNA of fibroblasts could be damaged by ROS, leading to the cell loss [7]. Besides, the overproduction of metalloproteinases (MMP) can be inappropriately activated. These protease enzymes are responsible for the decomposition of components of the extracellular matrix such as collagen and elastin fibers [8]. Therefore, except for topical application to protect skin from UV exposure, it is the most crucial step to scavenge ROS so as to prevent the cascade of detrimental reactions. In the combination of oral products, there are two key ingredients for ROS scavenge. Grape extract is rich in polyphenols such as anthocyanins, proanthocyanins and resveratrol [9].

Resveratrol is a natural antioxidant while the metabolism of resveratrol could activate a full array of antioxidant responses by Nrf2 signaling [10,11]. *Haematococcus pluvialis* is well- known as the richest source of natural carotenoid called astaxanthin, which could provide significant antioxidant activities not only via direct radical scavenging, but also by activating the cellular antioxidant defense system through modulation of the Nrf2 pathway [12,13]. As grape extract and astaxanthin are hydrophilic and hydrophobic respectively, it is believed the combination should increase the anti-oxidative effect on the skin cells regarding increased bioavailability.

To recover the collagen decomposed by UV-radiation induced MMP overactivation, orally ingested collagen tripeptide showed significant improvement in terms of wrinkle reduction including crow's feet, skin elasticity and dermal strength especially in our research. The synthesis of collagen and other extracellular matrix proteins is regulated by fibroblast in response to mechanical tension [14]. Once the collagen fibers are decomposed and fibroblasts loses mechanical strength, fibroblasts would collapse and produce less procollagen and more collagenase. This would result in further reduction of mechanical tension and thus continual loss of collagen [15]. Specific peptide's structure derived from collagen, including Pro-Hyp (PH) and Gly-Pro-Hyp (GPH), in our research recover the damage in a specific manner that outcompetes ordinary collagen peptides. It is found low molecular weight collagen peptide (PGH contained) labeled by C14 still showed high radioactivity 14 days after administered to mice [16]. Then the chemotactic effects pf PH and GPH on fibroblasts will enroll more fibroblasts to the place of collapsed collagen, where they would enhance fibroblast cell proliferation and hyaluronic acid production [17-18].

As it is known hyaluronic acid is like a sponge that locks up a great amount of water to keep skin hydrated, this still could not fully explain the improvement of hydration in stratum corneum [19]. It is found tremella polysaccharides could activate a significant channel called AQP3 for transferring water from dermis to epidermis [20]. It is found mice deficient inAQP3 have dry skin with reduced stratum corneum hydration, decreased elasticity and impaired skin fibroblast cell migration [21] (Figure 14).

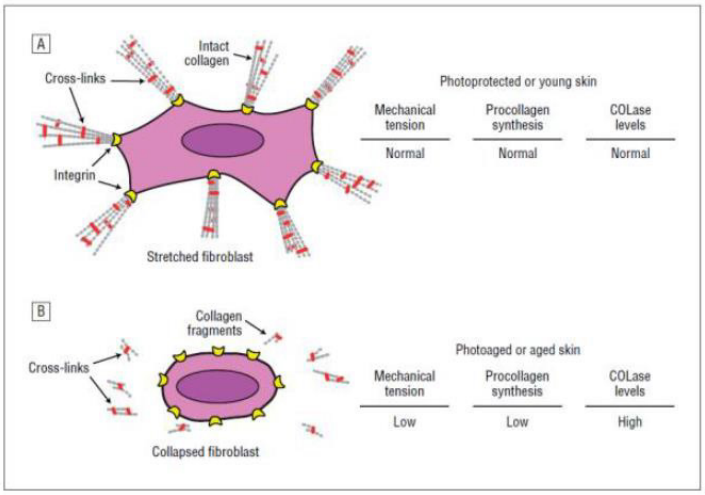

Figure 14: Fibroblast in response to mechanical tension [14].

Conflicts of Interest

The authors declared that they have no conflicts of interest to this work.

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