Study on effect of skin elasticity by polar low molecular weight(PLMW) keratin peptide

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Introduction

Obtaining biologically active peptides through microbial fermentation can be environmentally friendly as a raw material acquisition method and has economic advantages in terms of material cost burden, so various studies are being conducted recently. Biologically active peptides have very good stability and physiological activity(functionality), and are known to have no discoloration, phototoxicity, etc. because they appear based on a specific amino acid sequence. Keratin is a sulfur-containing fibrous protein that is a major component of skin, hair, nails, horns, and teeth in human. Feathers of the poultry is made of a water insoluble keratin proteins tightly bound to the alpha-keratin, which is a cystine connection. *Fervidobacterium islandicum* AW-1(*F. islandicum* AW-1) was known to produce keratinase that hydrolizes keratin substrates as the chicken feathers. Among poultry, *F. islandicum* AW-1, which produces an enzyme that decomposes chicken feathers, has been studied to reveal the metabolic process that degrades feathers through genetic analysis and the possibility of using keratin peptide as a cosmetic ingredient has been explored in various ways. In a recent previous study, hair products containing keratin peptide were manufactured and then applied to clinical study, which affects the keratin, the main component of hair, and affects the scalp and hair improvement.

In this study, *F. islandicum* AW-1 was used to produce keratin peptides by decomposing chicken feathers, and then polar low-molecular weight(PLMW) keratin peptides produced by introducing an additional process to maximize skin elasticity and use as cosmetic raw materials. We tried to confirm the efficacy related to the elasticity factor and the effect related to the elasticity of the human skin in human fibroblasts.

Materials & Methods

Keratin peptide production

Keratin peptides were produced from chicken feather incubating *F. islandicum* AW-1. After removing the cells by centrifugation. The supernatant was subjected to membrane filtration. Thereafter, freeze-drying was performed for 96h to obtain a keratin peptide powder material. After securing polar keratin peptides from this material through a hexane separation process, peptides of 10kDa or less were obtained through ultrafiltration That was PLMW keratin peptides.

In vitro study

To evaluate the elasticity-related efficacy of PLMW keratin peptides, three types of in vitro assays were performed: elastase assay, cytotoxic assay, and PIP assay. N-succinyl-(L-Ala)3-p-nitroanilide was used as a substrate for the enzyme test of the elastin-degrading enzyme, elastase, and 100mg/mL adenosine was used as a positive control. cytotoxicity test(MTT assay) and Procollagen type I C-Peptide(PIP) for confirmed to skin elasticity effect. Cytotoxicity test and PIP assay were performed using Human fibroblast HS68 cells. MTT assay was performed for the cytotoxicity test. The cytotoxicity is determined by the relative cell viability of the test group based on 100% of the survival rate of the untreated group cultured only with the cells, and it is determined that there is no cytotoxicity when the cell viability is 90% or more. In the PIP assay, The amount of collagen produced in the test group was compared with that of the control group, and the wrinkle improving effect was evaluated.

Clinical study

Clinical study was conducted on skin elasticity improvement test. In the case of clinical study, 22 adult females(Average age: 44.0±8.06) participated in the test. For the evaluation of skin improvement effect according to application of the product, it was applied to the facial area for 4 weeks. Volunteers visited the controlled, where the temperature and humidity(22±2℃, 50±10%) was controlled, and waited for at least 20 min in the controlled room. The measurement target was the cheeks, and an instrument was used to measure before use(Week 0) and after use(Week2, Week4). For skin elasticity, a Ballistometer(Dia-Stron, UK) that measures skin elasticity and a Derma torque meter (DTM310, Dia-Stron, UK) was used to measure changes in skin torsional elasticity. In addition, to measure skin color, it was measured using a spectrophotometer(CM2600D,Minolta, Japan), and skin-o-mat®(Cosmomed, Germany) was measured to measure skin water contents. The test results were analyzed before and after using the product. Before and after the test, 2 and 4 weeks after use were compared with the measured value before use(0week) and increase/decrease rate(%) of the 2 and 4week measurement value after the use.

Data Analysis and Statistics

The cell test and clinical test repeated three times and the average value was obtained. A statistical analysis was conducted at a 95% confidence interval for significance. The normality of the results was tested by the Kolmogorov-Smirnov and Shapiro-Wilk methods. After the normality test, the comparison between before and after uses was done by Paired t-test or the One-way ANOVA. The statistical analysis program used was SPSS 23.0(IBM SPSS Statistics, USA).

Results & Discussion

In vitro assay

In the elastase inhibition test, it showed a statistically significant inhibitory effect and subsequently inhibited elastase in a concentration-dependent manner(Figure 1 A). As a result of the cytotoxicity test according to the concentration of PLMW keratin peptide, the cell viability was over 90% at all concentrations used for the test, confirming that there is no cytotoxicity(no figure). In the collagen production test, it showed a concentration-dependent increase in collagen synthesis up to 400μg/ml(Figure 1 B). Therefore, the polar low molecular weight keratin peptide showed an effect on the elasticity-related factors at the cellular level.

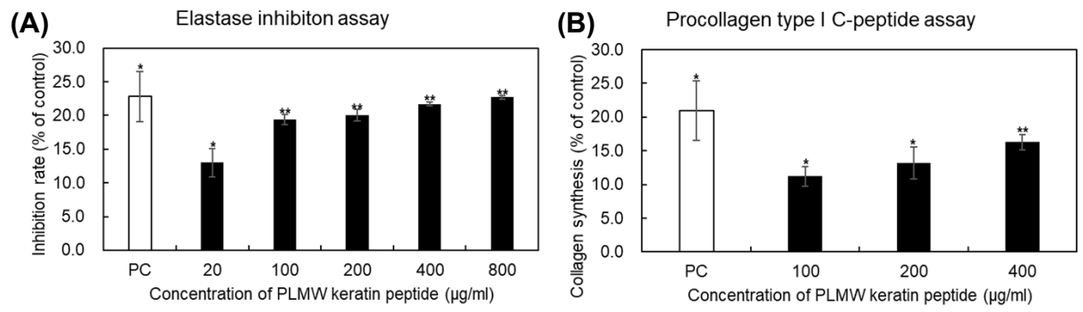

Figure 1. (A) Elastase assay results, (B) PIP assay results, Negative control vs. Positive control, Paired-t-test, **p<0.05*, ***p<0.01*.

Clinical study

After using the mask pack containing PLMW keratin peptide for 4 weeks, the instrument measurement was performed for 4 items. Skin elasticity test(Figure 2 A), Alpha value showed a statistically significant decrease after 2 and 4weeks of use and showed an improvement rate after 2 weeks and after 4weeks of use(*p<0.001*). Mean CoR values showed a statistically significant increase after 2 and 4weeks of use(p<0.05, p<0.01). In skin elasticity of skin torsion(Figure 2 B), Ur/Ue values increased statistically significantly at 2weeks and 4weeks after use(*p<0.001*). In skin water contents(Figure 2 C), there was a statistically significant increase at 2 and 4weeks after use(*p<0.001*). In skin color test(Figure 2 D), the L* value expressing skin brightness increased statistically significantly at 2 and 4weeks after use compared to before use(*p<0.001*). and the ITA* value representing the overall skin color showed that the measured value increased after 4weeks compared to before use to statistically significant(*p<0.05*).

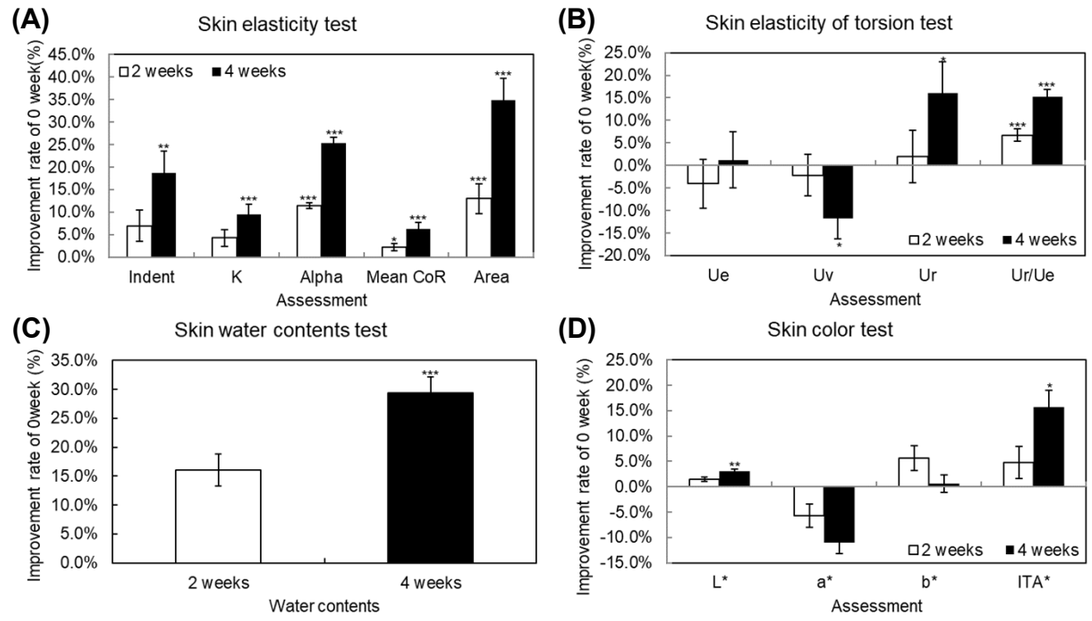

Figure 2. (A) Skin elasticity test results, (B) Skin elasticity of torsion test results, (C) Skin water contents test results, (D) Skin color test results, e control vs. One way ANOVA test, **p<0.05, **p<0.01, ***p<0.001.*

Conclusions

Focusing on keratin, which is the main component of skin, hair, and nails, PLMW keratin peptides were isolated to confirm the possibility of using keratin peptides produced by decomposing feathers by *F. islandicum* AW-1 as a cosmetic material. In order to examine the elasticity-related effect on the skin's efficacy, a mask pack, which is one of the elasticity-related in vitro study and cosmetic formulations, manufactured and clinical study for human skin efficacy. Through this study, it was confirmed that PLMW keratin peptide is effective in improving intracellular elasticity and improving human skin elasticity. This suggests that PLMW keratin peptide is a material for improving skin elasticity, and it will be used in various ways as a cosmetic ingredient in the future.

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