

The anti-aging effect of COLinus, human collagen-derived peptide, *in vitro* and *ex vivo* study

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Introduction:

As life expectancy increases, reducing visible signs of skin aging caused by various factors such as intrinsic and extrinsic factors has become a major issue [1]. Due to many of these factors, wrinkles and reduced elasticity are typical phenomena of skin aging. One of the main mechanisms of skin aging is thought to be a reduction in the amount of ECM, particularly collagen in the dermis [2]. In aged skin, the production of collagen decreases and its degradation increases, which leads to an overall reduction in collagen amount [3]. In previous studies, many products containing collagen isolated from land animals or marine animals have been used for a wide range of purposes in the cosmetics and food industries [4]. Collagen has been isolated from land animals including bovine and porcine and many marine organisms. However, associated with these sources, there is the risk of the transference of zoonotic diseases [5]. Besides, because these sources can cause immune or irritation reactions such as allergies, safety is not guaranteed for human use. In addition, collagen used in foods and cosmetics is controversial because it is not efficiently absorbed in the body due to the large molecular weight of natural collagen or partially hydrolyzed oral collagen [6]. Currently, since researches on the skin efficacy of human collagen-derived peptide had not been investigated, we started the following study for human collagen-derived peptide, which has relatively few side effects and is easily absorbed into the skin.

In this study, we developed hCOL1A2 (COLinus), small human-derived collagen peptide, with higher absorption and safer compared to natural collagen and studied for anti-aging efficacy in human skin.

Materials & Methods:

Preparing of hCOL1A2, human collagen-derived peptide (COLinus)

We generated a His-tagged human COL1A2 (hCOL1A2, COLinus) and cloned it into the pET-28a (His-tag) vector. Recombinant proteins were overexpressed in the *E. coli* strain and recombinant hCOL1A2 were purified from the supernatants using Ni-NTA resin (GE Healthcare). Purified recombinant hCOL1A2 was eluted and visualized as a single band around 20 kDa.

RNA isolation and Quantitative Real-Time PCR(qRT-PCR)

Total RNA was isolated from the cells with QiaZol (Invitrogen, USA) according to the manufacturer's instructions. RNA was reverse transcribed into cDNA using a qPCR BIO cDNA synthesis kit (PCR BIO, PCR Biosystems, USA). Using qPCR BIO SyGreen Blue Mix Lo-ROX (PCR Biosystems, USA) following the manufacturer's protocol, qRT-PCR reactions were performed in triplicates.

Immunoblotting analysis

Lysates were loaded and run on NuPAGE Tris-Acetate gels (Invitrogen). The proteins were transferred onto PVDF (Invitrogen) and blocked in skim milk. Membranes were probed with anti-Collagen Type I, anti-Collagen Type III and anti-beta actin antibody for overnight at 4°C. The secondary antibodies used were anti-mouse horseradish peroxidase-conjugated IgG antibody and anti-rabbit horseradish peroxidase-conjugated IgG antibody at room temperature.

ex vivo skin permeability and human skin tissue absorption image evaluation

ex vivo skin permeability and human skin tissue absorption image evaluation tests were conducted by requesting KSRC Korea Skin Clinical Research Center (South Korea). The amount of the test substance that was collected and penetrated the human skin tissue was analyzed with Multimode Microplate Reader (Fluorescence mode). In order to confirm the test substance absorbed into the human skin tissue, a tissue slide was prepared by fixing the human skin tissue after the reaction, and it was observed using a fluorescence microscope.

Clinical trials

Clinical trials for wrinkle improvement were implemented in eurofins CRA (South Korea). The Clinical trials were conducted to evaluate the eye wrinkle improvement effect of using the test product for 4 weeks. Eye wrinkles were photographed using ANTERA 3D[®] CS (Miravex Limited, Ireland).

References:

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4. Su Jin H, Geun-Hyung H (2020) Human collagen alpha-2 type I stimulates collagen synthesis, wound healing, and elastin production in normal human dermal fibroblasts (HDFs). *BMB Rep* 53(10):539-544
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Results & Discussion:

Effects of COLinus on the proliferation and wound healing in NHDFs

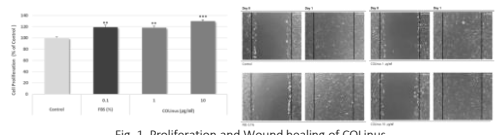


Fig. 1. Proliferation and Wound healing of COLinus.

Effect of COLinus on the production of COL-I, COL-III and Elastin expressions in NHDFs

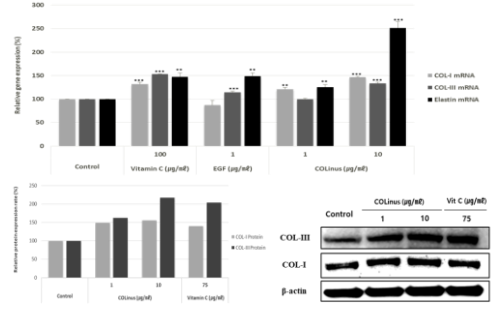


Fig. 2. The mRNA and protein expressions of skin elasticity of COLinus.

ex vivo skin permeability and human skin tissue absorption image evaluation

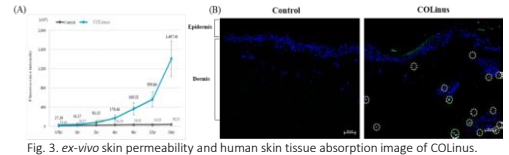


Fig. 3. *ex vivo* skin permeability and human skin tissue absorption image of COLinus.

Clinical trials

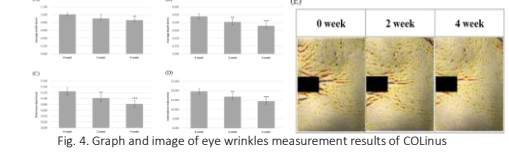


Fig. 4. Graph and image of eye wrinkles measurement results of COLinus

Conclusions:

We have developed COLinus, small human-derived collagen peptide, that is safe and permeable into the skin rather than the currently widely used animal collagen. COLinus increased growth and enhanced cell migration in NHDFs. Moreover, COLinus significantly induced COL-I, COL-III, and Elastin synthesis in NHDFs. Based on these results, COLinus is thought to be effective in preventing skin sagging and creating solid skin by not only increasing skin elasticity but also increasing tissue regeneration. Furthermore, it was confirmed that the COLinus had excellent permeability and penetrated into dermis of skin, and that it helped alleviate wrinkles around the eyes by improving the levels of various wrinkle-related factors through clinical trials.

In conclusion, COLinus, human collagen-derived peptides, can be used as a future anti-aging cosmetic ingredient for improving skin conditions.