Collagen Fiber Contraction Activity of Fibroblasts Promoted by a Human Peptide to Improve Skin Firmness

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Introduction:

1. In recent years, interest in cosmetology has increased, especially in the field of antiaging and in relation to treatments and functional cosmetics for improving skin wrinkles, sagging, and firmness.
2. When the use of the cosmetics ends, the fine wrinkles again become noticeable over time. Therefore, there remains a strong demand for a daily treatment with which to improve skin wrinkles, sagging, and firmness. Such treatments could include external medicines, cosmetics containing functional ingredients, and oral treatments.
3. The aim of the research is to develop a skin care agent that could help improve skin wrinkles, sagging, and lack of firmness caused by aging.

Materials & Methods:

1. Preparation of trypsin limited degradation product of bovine lactoferrin

A solution of bovine lactoferrin degradation product was produced, fractionated 40 times on an ODS column, and then lyophilized. After that, we examined the candidate sequences, synthesized peptides, and evaluated and confirmed the contraction-promoting effect of the collagen gels.

The peptides were synthesized by removing one amino acid each from the N- and C-termini of the peptides, and the collagen gel contraction-promoting activity was evaluated.

2. Evaluation collagen gel contraction-promoting activity of LFDP1

The human fibroblast on collagen gel was cultured with LFDP1 at 37˚C for 3 days and the area of the gel was measured with a digital caliper.

The diameters of the gels of three samples were measured and the gel contraction rate of each sample was calculated.

3. Effect of LFDP1 on the cell number of human fibroblasts

The effect of LFDP1 on the cell number of human fibroblasts was evaluated by Cell Counting Kit-8 after cultured for 1 day.

4. Analysis of mRNA expression of COL1A2 and MMP1 in cultured young and aged fibroblasts

Human fibroblasts (young and aged) were cultured for 1 day, and their RNA was extracted by RNA extraction kit. mRNA expressions of collagen type 1A2 (COL1A2) and matrix metalloproteinase-1 (MMP1) were measured using real-time polymerase chain reaction (PCR).

5. Analysis of the effect of LFDP1 on the mRNA expression of MMP1 and DDR2 in cultured fibroblasts

LFDP1 was added to human fibroblast and cultured for 3 days. RNA was then extracted using the RNA extraction kit. mRNA expressions of matrix metalloproteinase-1 (MMP1) and discoidin domain receptor 2 (DDR2) were measured using real-time polymerase chain reaction (PCR).

6. Microscopic observation of collagen fibers around cells, phospho-myosin light chain 2, and DDR2 in cultured fibroblasts on collagen gel

Collagen gel was produced in the centers of 35-mm diameter dishes with 14-mm-diameter polylysine-coated glass. It was observed with a confocal laser scanning microscope after immunostaining.

7. CD86 and CD54 expression in cultured THP-1 cells

After culturing for 2 days, expression levels of CD86 and CD54 of THP-1 cells were evaluated after immunostaining. Positive cell rates were measured with a Tali Image Cytometer (Thermo Fisher Scientific Inc.).

Results & Discussion:

Measurement of collagen gel contraction activity of LFDP1

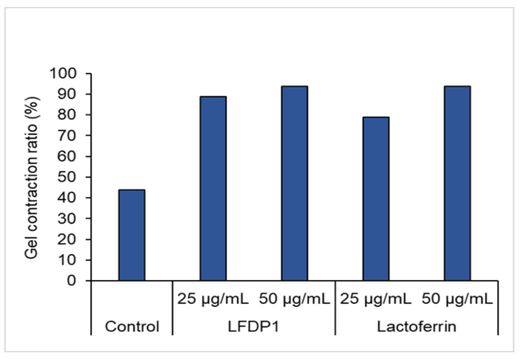

Fig.-1 Collagen gel contractile activity of LFDP1 or lactoferrin. *Left*: Collagen gel contractile activity of LFDP1 or lactoferrin when incubated with LFDP1 or lactoferrin at 25 μg/mL and 50 μg/mL for 21 days. *Right*: Images of the activity shown at 25 μg/mL exposure for 21 days.

Collagen gel contraction activity of young and aged fibroblasts and mRNA levels of COL1A2 and MMP1

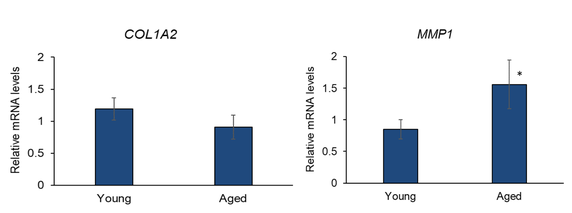

Fig.-2 mRNA levels of *COL1A2* and *MMP1* of the collagen gel containing young fibroblasts and aged fibroblasts. Mean ± standard deviation (n = 3).
*$P < 0.05$ vs. control

Effect of LFDP1 on the mRNA levels of MMP1 and DDR2 in cultured human fibroblasts

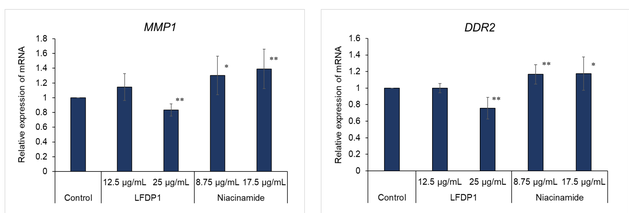

Fig.-3 Effects of LFDP1 and niacinamide on the mRNA expression levels of *MMP1* and *DDR2* in cultured human fibroblasts. Mean ± standard deviation (n = 3).
*$P < 0.05$ vs. control, **$P < 0.01$ vs. control.

Microscopic observation of collagen fibers around cells during the culture of fibroblasts on collagen gel

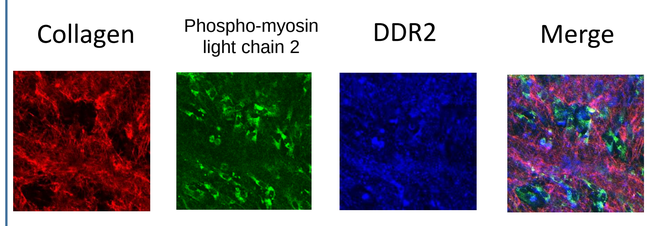

Fig.-4 Microscopic image of collagen fibers, phospho-myosin light chain 2, and DDR2 in cultured fibroblasts

Table-1 Effects of LFDP1 on the expression of CD86 and CD54 in cultured THP-1 cells

	CD86	CD54
	%	%
Control	0	0
LFDP1	0	1
4-n-butyl resorcinol	0	5

Conclusions:

LFDP1 acted on fibroblasts to increase collagen gel contractility. One of the possible mechanisms was the decrease in MMP1, which is involved in collagen degradation at the mRNA level.

Considering its stratum corneum permeability, LFDP1 may be superior to lactoferrin when used as a cosmetic skin care agent.

LFDP1 could be applied as a cosmetic ingredient to effectively increase the firmness of the skin during the aging process.

References:

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