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Treatment of human skin with an extract of *Fucus vesiculosus* changes its thickness and mechanical properties

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Synopsis

Recently the researchers found that an extract of *Fucus vesiculosus,* which is a type of seaweed, promotes the contraction of fibroblast-populated collagen gels through increased expression of integrin molecules. In this study, they investigated the effects of topical application of an aqueous extract of this alga on the thickness and the mechanical properties of human skin. A gel formulation that included 1% of the extract was applied topically to human cheek skin twice daily for five weeks. A significant decrease in skin thickness measured by B-mode ultrasound was elicited, as was a significant improvement in elasticity measured with a Cutometer as compared with controls. In cheek skin, the thickness normally increases and the elasticity usually decreases with age. These results suggest that the *Fucus vesiculosus* extract possesses anti-aging activities and should be useful for a variety of cosmetics.

INTRODUCTION

The brown seaweed *Fucus vesiculosus* is a common littoral alga found on the coasts of the Northern Atlantic Ocean, the Pacific Ocean, and the Baltic Sea. It is well known that aqueous extracts of this alga show various biological properties, such as a potent anticoagulant activity (1). Recently we found that the *Fucus* extract promotes the contraction of fibroblast-populated collagen gels and changes their mechanical properties through increased expression of integrins $\alpha 2$ and $\beta 1$ on the surface of human skin fibroblasts (2).

The fibroblast-populated collagen gel culture method is widely used as a simplified *in vitro* model of the dermis (3,4). Many researchers have studied the contraction of fibro-

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blast-populated collagen gels and have generally used that dermal equivalent model to investigate cell–matrix interactions—for example, as a model for wound healing (5,6). These results suggest that the *Fucus* extract might affect the maintenance of skin integrity, in such areas as skin thickness and mechanical properties. Therefore, in this study, we investigated the effects of topical application of a *Fucus* extract on the thickness and mechanical properties of human skin.

MATERIALS AND METHODS

FUCUS VESICULOSUS EXTRACT

Dried *Fucus vesiculosus* was purchased from Ichimaru Pharcos Co., Ltd. (Gifu, Japan). Dried *Fucus vesiculosus* was extracted with water for 24 h, with stirring at room temperature. The residue was then removed by filtration to give a slightly brown colored extract (7). The residue of this extract was about 1.5 weight/volume % (residue weight %).

TOPICAL APPLICATION

The gel formulation included 1% of the aqueous extract of *Fucus* and was applied topically on one side of the face twice daily (in the morning and evening) for five weeks. Placebo gels without the *Fucus* extract were used as controls on the other side of the face. Placebo gels included detergent (Emanon CH40; PEG-40 hydrogenated castor oil, 0.5 weight/weight [w/w] %), sodium alginate (1.0 w/w%, Mw. Ca.70000), 86% glycerol (5.0 w/w%), methylparaben (0.2 w/w%) as antiseptic agent, and purified water.

SUBJECTS AND STUDY DESIGN

Clinical testing was performed using ten healthy female volunteers (aged 23–36 years, mean 28.4 years) after giving informed consent. All measurements were performed twice daily with the volunteers in the supine position, i.e., in the morning (8:30–10:00) and in the evening (15:30–17:00). Both outer cheeks were examined before and after the application period by a specialist. Tests were carried out in a half-face, double-blind manner consisting of a five-week gel usage (twice daily applications at least) and evaluation of skin thickness and elastic properties. Panelists applied about 0.2 ml of gel per one side of cheek. All measurements were performed at exactly the same sites on the skin. Diurnal changes of skin thickness were determined by comparing the values obtained from both outer cheeks in the morning with those obtained in the evening.

MEASUREMENT OF SKIN THICKNESS

Skin thickness was measured with a 15-MHz B-mode ultrasonograph (UX-01, Rion Co. Ltd. Tokyo, Japan) as described previously (8). Briefly, the gain and dynamic range were adjusted to 24 dB and 30 dB, respectively. A typical ultrasonographic image obtained on human cheek skin is shown in Figure 1. The ultrasound image shows the epidermal echo observed close to the entry echo, then the moderately echogenic dermis, and finally

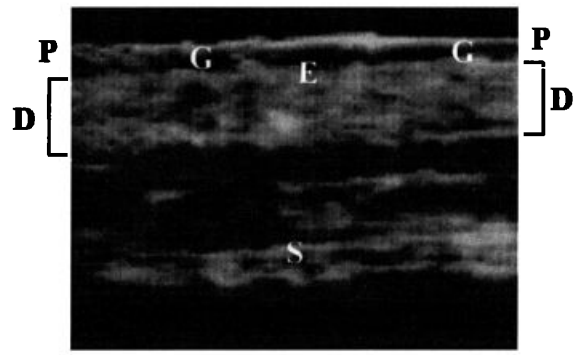

Figure 1. Typical ultrasonographic image obtained on human cheek skin. P = membrane of the ultrasound probe. G = coupling ultrasonic gel. E = epidermis. D = dermis. S = subcutaneous tissue.

the echolucent subcutaneous fatty tissue. On ultrasonographic images, the thickness from the skin surface to the plane showing discontinuity of echogenicity (representing the border between the dermis and the subcutaneous tissue) was measured at ten sites, and the mean value was calculated.

MEASUREMENT OF MECHANICAL PROPERTIES OF THE SKIN

Mechanical properties of the skin were measured with a commercially available instrument (Cutometer SEM575, Courage & Khazaka, Cologne, Germany) as described previously (9). Briefly, the time/strain mode was used with an 8-sec application of 200 hPa followed by a 2-sec relaxation period using a probe 2 mm in diameter (10). The parameters determined were immediate distention (Ue) measured at 0.15 sec, final distention (Uf) measured at 7.85 sec, delayed distention (Uv), and immediate retraction (Ur) (11). These parameters are a function of skin thickness, and thus cannot be simply compared between subjects. Each individual measurement of skin thickness was used to calculate the mechanical parameter values for the thickness of skin for a given individual. These normalized parameters were marked with an asterisk (Ue*, Uf*, Uv*, Ur*).

STATISTICAL ANALYSIS

All data were compared by the paired Student's t-test.

RESULTS

DIURNAL CHANGES OF HUMAN SKIN THICKNESS AND MECHANICAL PROPERTIES

The skin thickness of the cheek showed slight but significant changes during the day (Table I). The Ue* and Uf* values of the skin, which are indices of the distensibility of the skin and reflect its elastic properties, did not differ significantly during the day (data not shown). The Uv* value, which reflects intradermal movements of a viscous type, and the Ur* value, which is the elastic recovery, also did not change during the day (data not shown).

Table I
Diurnal Changes of Thickness of Human Cheek Skin

	Skin thickness (mm)[a]
Morning	1.298 +/- 0.119
Evening	1.258 +/- 0.147*

[a] Data expressed as mean +/- SD.
* Significantly different between morning and evening, $p < 0.05$.

EFFECT OF THE EXTRACT OF *FOCUS VESICULOSUS* ON THE THICKNESS OF HUMAN CHEEK SKIN

All ten volunteers completed the study without irritation or erythema. Before application, no significant differences were observed in skin thickness between the placebo-treated and the *Fucus* extract-treated groups measured in the morning and in the evening (Table II). However, topical application of the *Fucus* extract gel for five weeks caused a significant decrease in skin thickness measured in the morning and in the evening compared with that of the corresponding placebo-treated controls. For example, in the *Fucus* extract-treated group, nine volunteers showed a decrease in skin thickness. The difference between the *Fucus* extract-treated group and the placebo-treated group reached about 0.1 mm, which corresponded to 7–8% of the skin thickness. The application of the placebo did not elicit any change in skin thickness measured in the morning and in the evening.

Concerning a dose-dependency effect of *Fucus* extracts, gels including 1%, 2%, 3%, or 5% of extract showed similar results on skin thickness and elastic properties (data not shown).

EFFECT OF THE EXTRACT OF *FUCUS VESICULOSUS* ON THE MECHANICAL PROPERTIES OF HUMAN CHEEK SKIN

Before application, no significant differences were observed in Uf* and Ue* values between the placebo- and *Fucus*-treated groups measured in the morning and in the evening. Topical application of the *Fucus* extract or the placebo for five weeks elicited a significant difference in the Uf* values measured in the morning and in the evening (Table III). Measured in the evening, the Uf* value increased after the application of the *Fucus* extract, but did not change after the placebo treatment. The Ue* values did not change significantly, although they showed a tendency to increase after five weeks of treatment (Table IV). Neither the Ur* nor the Uv* values, which are indices of the elastic recovery or viscosity parameters, showed significant differences between the *Fucus* extract-treated and the placebo-treated groups (data not shown).

DISCUSSION

In this study, we investigated the effects of topical application of a *Fucus vesiculosus* extract on the thickness and mechanical properties of human skin. Gniadecha *et al.* (12,13) reported that diurnal changes are observed in skin echogenicity in various regions of the body by using B-mode ultrasonography, which suggests that diurnal changes in skin thickness occur. At first, we examined diurnal changes in skin thickness

Table II
Effect of *Fucus vesiculosus* Extract on Thickness of Human Cheek Skin

Measurement period	Application	Skin thickness (mm)[a]		Change with application (mm)	Difference between applications (mm)	Significance
		Before	After 5 weeks			
Morning	Placebo	1.300 +/- 0.121	1.307 +/- 0.149	0.007	-0.096	$p < 0.005$
	Fucus extract	1.295 +/- 0.120	1.206 +/- 0.160	-0.089		
Evening	Placebo	1.256 +/- 0.153	1.251 +/- 0.147	-0.005	-0.109	$p < 0.005$
	Fucus extract	1.260 +/- 0.148	1.146 +/- 0.147	-0.114		

[a] Data expressed as mean +/- SD.

Table III
Effect of *Fucus vesiculosus* Extract on the Mechanical Parameter Uf* of Human Cheek Skin

Measurement period	Application	Mechanical parameter Uf*[a]		Change with application (mm)	Difference between applications (mm)	Significance
		Before	After 5 weeks			
Morning	Placebo	0.081 +/- 0.018	0.071 +/- 0.018	-0.010	0.007	$p < 0.05$
	Fucus extract	0.084 +/- 0.016	0.081 +/- 0.017	-0.003		
Evening	Placebo	0.084 +/- 0.014	0.081 +/- 0.022	-0.003	0.010	$p < 0.05$
	Fucus extract	0.083 +/- 0.013	0.096 +/- 0.031	0.013		

[a] Data expressed as mean +/- SD.

Table IV
Effect of *Fucus vesiculosus* Extract on the Mechanical Parameter Ue* of Human Cheek Skin

Measurement period	Application	Mechanical parameter Ue*[a] Before	Mechanical parameter Ue*[a] After 5 weeks	Change with application (mm)	Difference between applications (mm)	Significance
Morning	Placebo	0.056 +/- 0.015	0.048 +/- 0.014	-0.008	0.004	N.S.
	Fucus extract	0.059 +/- 0.013	0.055 +/- 0.012	-0.004		
Evening	Placebo	0.060 +/- 0.010	0.057 +/- 0.019	-0.003	0.011	N.S.
	Fucus extract	0.059 +/- 0.011	0.067 +/- 0.025	0.008		

[a] Data expressed as mean +/- S.D.
N.S.: not significant.

by measurement both in the morning and in the evening, and slight but significant diurnal changes were observed. Therefore, all measurements were performed twice daily, i.e., in the morning (8:30–10:00) and in the evening (15:30–17:00).

The relationships among aging, skin thickness and mechanical properties of the skin have been investigated (9,11,13). In the human cheek, skin thickness increases gradually with aging (9). Topical application of the *Fucus* extract caused a significant decrease in the thickness of human cheek skin over the range of the observed diurnal period, indicating that the *Fucus* extract is a potent anti-aging ingredient. Focusing on changes in the mechanical properties of skin, topical application of the *Fucus* extract elicited a significant increase in the Uf* value and a tendency to increase the Ue* value. The Ue* and Uf* values, which are indices of skin distensibility, were significantly decreased in proportion to aging (9). These results indicate that the *Fucus* extract elicited an improvement in these mechanical properties of the skin against aging.

Concerning the effective dose of extract, gels including 1%, 2%, 3%, and 5% of extract showed similar results on skin thickness and elastic properties. These results indicate that 1% of *Fucus* extract is sufficient to elicit changes in skin thickness and elastic properties.

We recently reported that the *Fucus* extract promotes the contraction of fibroblast-populated collagen gels and changes the mechanical properties of collagen gels (2). In general, the collagen culture method is used as a dermal equivalent model, and therefore these *in vitro* effects of the *Fucus* extract on gel contraction are consistent with the *in vivo* results elicited in human skin following topical application of the *Fucus* extract.

The promotion of collagen gel contraction is caused by an increased expression of cell surface integrins (2), which mediate interactions between fibroblasts and extracellular matrix proteins (including collagen fibers) in the dermis (14–17). These results therefore suggest the possibility that a *Fucus vesiculosus* extract might alter the thickness and the mechanical properties of the skin by enhancing integrin expression of skin fibroblasts.

CONCLUSION

Topical application of a *Fucus vesiculosus* extract on human facial skin elicited a significant decrease in skin thickness and an improvement in its mechanical properties, suggesting that the *Fucus* extract possesses anti-aging activities. We propose that the extract of *Fucus vesiculosus* should be useful for a wide variety of cosmetics due to its effects on skin tightening, anti-sagging, and wrinkle smoothing.

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