Evaluation of enhancing effect of supplements on collagen release from cultured human fibroblasts using scanning acoustic microscopy

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Introduction

One of the factors that cause skin changes is the growth and proliferation of fibroblasts present in the dermis. Proteins such as collagen and elastin released by fibroblasts are important for generating skin elasticity, and fibroblast activation can be said to be directly linked to preventing skin aging.
L-ascorbic acid is a fibrillogenesis factor for collagen, and previous studies have shown that collagen release observed with Scanning acoustic microscopy (SAM) changes the acoustic impedance of the extracellular matrix[1)]. Furthermore, recent research has confirmed that Japanese herbal medicine ingredients and fruit-derived ingredients also have skin-improving effects[2)].

In this study, we aim to evaluate the collagen-promoting effect of samples A to D provided by SHISEIDO using Scanning acoustic microscopy (SAM). SAM can non-invasively measure the mechanical properties of cells and tissues that depend on their viscosity and elasticity. Using SAM, we observed collagen release's spatial distribution and three-dimensional structures[3,4)].

Material and Method

Human dermal fibroblasts were proliferated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10%fetal bovine serum (FBS) and 20µl/L Penicillin-Streptomycin with 10^4 cells/dish primarily in the CO_2 incubator, 37℃.

For observation of collagen release, we cultured fibroblasts in the PS-film dish (Honda Electronics Co., Ltd.) At 3 days in vitro (3 DIV), we applied sample supplements A, B, C, and D to cultured fibroblasts. For the control condition, we applied fibroblasts with 1,3-butanediol, a solvent of the supplements. We observed the change of fibroblast cells using SAM with a center frequency of 320 MHz to 5 DIV.

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We evaluated collagen release using measuring-focused ultrasound reflected from the cut surface obtained by SAM and converted it into an acoustic impedance image. The acoustic impedance of the target can be calculated using the following simultaneous Equation. Ultrasonic pulses are transmitted and received from the back side while scanning. On reference material with known acoustic impedance placed within the field of view for observation, we calculated acoustic impedance with the sound pressure reflectance formula. These calculations are based on the method of Hozumi et al.[5)]. The signal from the tissue is expressed by the following formula (1),

$$S_{tgt} = \frac{Z_{sub} - Z_{tgt}}{Z_{sub} + Z_{tgt}} \times S_0. \quad (1)$$

The frequency component of the signal at any frequency is represented by S. Z_{tgt} and Z_{sub} are the acoustic impedances of the target tissue and substrate respectively. The transmitted wave S_0 remains constant during observation.
The reflected signal is expressed by the following formula (2),

$$S_{ref} = \frac{Z_{sub} - Z_{ref}}{Z_{sub} + Z_{ref}} \times S_0, \quad (2)$$

Z_{ref} is the acoustic impedance of the reference material. S_{tgt} can be measured directly and *Zref* is known, but the transmitted signal S_0 incident on the reflecting surface cannot be measured. Therefore, the acoustic impedance of the target tissue is calculated as the solution of the simultaneous Equations for Z_{tgt} and S_0.

Results

All of sample supplements showed changes 48 h after the application. Above all, we focusd on sample suppliment A whose effects are unknow. Then the figures showed SAM images

immediately after and 48 h after the application of sample supplement A. We confirmed that the acoustic impedance derived from the intracellular cytoskeleton became higher in Control and Sample A after application in C mode observation. Although we could not observe a significant increase of the extracellular region in the acoustic impedance value in both Control and Sample A application in the C mode images(**Fig. 1(a, c, e, g)**), three-dimensional SAM analysis showed that the acoustic impedance on the fibroblasts changed with time in the depth direction(**Fig. 1(b, d, f, h)**). These findings hold the promise of significant contributions to our understanding of skin aging and the skin-improving effects of ingredients.

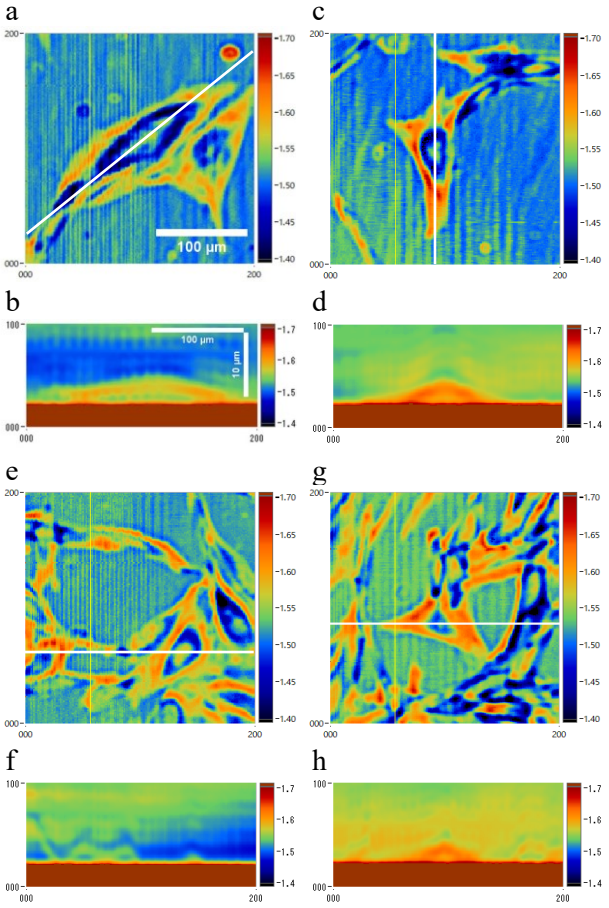

Fig.1 XY plane images (a, c, e, g) and Cross-sectional images deep-depth direction (b, d, f, h). (a, b) Immediately after application of control sample (1.3-butanediol). (c, d) 48 hours after application of control sample. (e, f) Immediately after application of sample supplement A. (g, h) 48 hours after application of sample supplement A. Scale bar = 100 µm (a, c, e, g), 100µm × 10 µm (b, d, f, h). Unit of color scale: MRaly.

Discussion

Our observation demonstrated that the acoustic impedance on the cytoskeleton area was increased after the application of the sample supplement. It might be due to an increase in procollagen production and accumulation of fibrous procollagen within the cell. The increased impedance of the cytoskeleton might reflect that G-actin proteins are polymerized and correlate with intracellular collagen. The relationship between collagen production and cytoskeletal proteins is unknown, so our observation could discover new physiological phenomena about skin dynamics. To confirm the dynamics of collagen release, we should investigate the quantification of collagen with the ELISA method.

Conclusion

The optical microscope, which requires cell fixation and/or immunochemical staining, cannot observe the extracellular matrix. On the other hand, using SAM, which doesn't require those treatments, allows noninvasive visualization of the living cell's extracellular matrix.

Acknowledgment

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