Breakthrough in improving the skin sagging with focusing on the subcutaneous tissue structure, retinacula cutis

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ABSTRACT
Skin sagging is one of the most prominent aging signs and a concerning issue for people over middle age. Although many cosmetic products are challenging the reduction of the skin sagging by improving the dermal elasticity, which decreases with age, the effects are insufficient for giving drastic changes to the facial morphology. This study focused on subcutaneous tissue for investigating a skin sagging mechanism. Subcutaneous tissue consists of predominantly adipose tissue with fibrous network structures, called retinacula cutis (RC), which is reported to have a possibility to maintain the soft tissue structure and morphology. This study investigated the effect of subcutaneous tissue physical-property alteration due to RC-deterioration on the skin sagging. For evaluating RC structure noninvasively, the tomographic images of faces were obtained by magnetic resonance (MR) imaging. Subcutaneous tissue network structures observed by MR imaging were indicated to be RC by comparing MR images and the histological specimens of human skin. The density of RC was measured by image analysis. For evaluating sagging degree and physical properties of the skin, sagging scoring and the measurement of elasticity of deeper skin layers were performed. The density of RC was correlated with the elasticity data of deeper skin layers, and the sagging scores tended to increase with decreasing the density. These results suggested that the sparse RC structure gave a decrease in the elasticity of subcutaneous tissue layer, which consequently would be the cause of facial sagging. This study would be a pathfinder for the complete elimination of skin sagging.

KEYWORDS
skin sagging, subcutaneous tissue, retinacula cutis (RC), magnetic resonance (MR) imaging, elasticity

INTRODUCTION
Decrease in the elasticity of the skin with aging is well known to cause wrinkles and sagging that are more conspicuous after the middle age. For improving the decreased skin elasticity with cosmetic products, qualitative and quantitative improvement measures for collagen in the dermis are proposed. Although the measures are found to improve the shallow wrinkles, they give only unsatisfactory effects to the deep wrinkles and sagging, resulting in an issue. On the other hand, in cosmetic surgery field, a high therapeutic achievement is obtained by subcutaneous tissue treatments. For example, lifting the superficial musculoaponeurotic system by a face-lift operation is reported to give satisfaction to patients at a higher level [1], indicating that not only the structure of the skin but also that of the subcutaneous tissue should be improved. For developing subcutaneous-tissue treatments, however, necessary knowledge regarding the relationship between the subcutaneous tissue and the sagging is still insufficient.
Subcutaneous tissue just under the dermis consists of largely subcutaneous fat and a fibrous network
called “retinacula cutis (RC)” [2, 3]. RC is thought to play a role in the structural maintenance of subcutaneous tissue for resisting tensile and gravitational force keeping its three-dimensional (3D) fibrous structure in the subcutaneous tissue [3-5]. For aiming the development of new anti-sagging treatments, this preliminary study focused on the structure of RC and investigated that changes in the network structure of subcutaneous tissue could affect the physical property of subcutaneous tissue and induced sagging. This study established a noninvasive method for analyzing the network structure of the human face with a magnetic resonance (MR) imaging and investigated the relationship between change in network structure and sagging.

MATERIALS AND METHODS

Volunteer selection
Female volunteers were selected according to the criteria; (1) age groups: 20’s and 50’s, (2) standard body-mass index (BMI) range of 18.5-25, (3) having the near-identical thickness of cheek subcutaneous tissue, which were measured with a diagnostic ultrasound imaging instrument (SSA-550A) (Toshiba Medical Systems, Otawara, Japan). The ultrasound images were collected by a linear probe operating at a frequency of 12.5 MHz was applied to the cross-point of a horizontal line from the lower edge of nasal wing and a vertical line from the corner of the eye (Figure 1) through ultrasound diagnostic gel (LOGIQLEAN) (GE Healthcare, Japan, Tokyo) applied on the skin surface. After the subcutaneous tissue was circumscribed by drawing a line along the borders between the subcutaneous tissue and dermis, and the subcutaneous tissue and muscle layers in ultrasound images, the thicknesses of subcutaneous tissue were measured at three different points and averaged. Based on the obtained data, 18 healthy Japanese female volunteers, who were 10 in their 20’s and 8 in their 50’s, were selected. Written informed consent was taken from all volunteers, and the test protocol was approved by both the ethical committees of POLA Chemical Industries and International University of Health and Welfare.

Quantitative analysis of the internal structure of subcutaneous tissue
For evaluating the internal structure of subcutaneous tissue noninvasively, the sliced sectional image of the face was obtained with MR imaging. After receiving a head coil device on the face, a volunteer lying on her back was subjected to proton density-weighted (PDW) images with an MR imager (Achieva 3.0T) (Philips Medical Systems, Best, The Netherlands). The sectional MR image of the tissue, which was cut along a horizontal line from the lower edge of nasal wing to near the ear, was used for analyzing the cheek subcutaneous tissue (Figure 1). From the sectional image, a square image which included the cheek subcutaneous tissue was isolated, subsequently analyzed a set of the following process; contrast enhancing, brightness averaging, noise removing, and binarizing, with image analysis software (ImageJ ver. 1.47) (The National Institute of Health, Bethesda, MD, USA) (Figure 2A). Large blood-vessels found in sequential sliced MR images were manually removed from the binarized images. In the binarized image, the subcutaneous tissue area and the black areas of the subcutaneous tissue were calculated.

Sagging score evaluation
After washing their faces, the volunteers were allowed to acclimate to a constant temperature and relative humidity environment at 21 ± 1 °C and 50 ± 5% for 15 min, sit on chairs, and cover their
upper bodies with gray-color cloths for reducing possible optical reflection. With keeping the situation, their faces were photographed at a 45-degree angle to a camera. According to the reported method by Ezure et al. [6], the degree of sagging at the upper cheek (Figure 1) was scored by two experienced experts referring a sagging score table, and the obtained scores were averaged.

**Measurements of the physical properties of the skin**

The physical property of the deep part of the skin was evaluated with a Cutometer MPA580 equipped with a 6 mm-diameter probe (Courage +Khazaka, Cologne, Germany), and the biological elasticity (Ur/Uf) was used as the parameter [9, 10]. The elasticity of the skin at the cross-point of a horizontal line from the lower edge of nasal wing and a vertical line from the corner of the eye was measured (Figure 1). Since Cutometer can transmit the effect of physically absorbed displacement to the subcutaneous tissue at a specific depth comparable to the diameter of the probe, the larger diameter of the probe can provide the clearer parameter of the physical property of the subcutaneous tissue [8]. Therefore, the obtained data with a probe having more than 6 mm in diameter are able to reflect the physical property of the subcutaneous tissue [11, 12]. After the skin was sucked at a reduced pressure of 30 kPa (300 mb) for 2 s and released for 2 s, the biological elasticity (Ur/Uf) was measured. The measurement was repeated 4 times on the same location, and the obtained values were averaged. The measurement was performed during the volunteer lay on her back.

**Histological analysis**

Human tissue specimens were purchased from Analytical Biological Services (Wilmington, DE). The use of human tissue specimens was approved by the ethical committees of POLA Chemical Industries. Both the skin and subcutaneous tissue were collected from the horizontal line from the lower edge of nasal wing, fixed with 10% buffered formalin solution (Wako, Osaka, Japan), embedded in paraffin, and sliced into sections with a thickness of 6 µm. The sections were stained with hematoxylin (Sigma-Aldrich, St. Louis, MO) and eosin Y (Wako), and observed with a stereo microscope (Nikon SMZ445) (Nikon, Tokyo).

**Statistical analysis**

Correlation analyses were performed by Spearman's rank correlation coefficient and Pearson's product-moment correlation coefficient. Probability less than 0.05 (p < 0.05) was considered statistically significant. All statistical analyses were performed by JMP software ver. 11.0 (SAS Institute, Cary, NC).

**Figure 1: Schematic illustration of the measuring site on the face**

The solid square on the face shows the sagging-score evaluating area. The solid line indicates the cutting line of the sectional magnetic resonance imaging. The dash line indicates a vertical line from the corner of the eye. The open circle (○) shows the site where the thickness and the elasticity of the subcutaneous tissue were measured.
RESULTS

Visualization of RC making the subcutaneous tissue of the face skin with MR imaging

Having a high soft-tissue contrast resolution, MR imaging is adequate to observe microstructures inside the subcutaneous tissue. For obtaining high-quality images in the subcutaneous-tissue regions, the face MR images were collected by allowing the head coil device to be put on the face. On the axial image at the level of the lower edge of nasal wing, this study found dark-gray (low signal-intensity) regions, which indicated harder structures, in brighter white high signal-intensity regions indicating the cheek subcutaneous tissue (Figure 2B). On MR images, differences in tissue structures are showed as differences in signal intensities expressing various shades of gray, and in PDW images, fat tissue appears brighter (higher signal-intensity), and muscle and tendon tissues having rich fibrous components appear darker (lower signal-intensity) [7, 8]. Therefore, the dark region in the white subcutaneous-tissue region was speculated to be possibly a fibrous-rich component. For confirming the histological features, hematoxylin and eosin (HE) stained specimens of the purchased human tissue was prepared from the same area of the MR images (Figure 2C). As a result, by comparing the microscopic photographs and MR images, fibrous network structures in the subcutaneous tissue on MR images were confirmed to morphologically correspond to RC structures in HE-stained tissue sections (Figure 2B, C).

Figure 2: The sectional magnetic resonance (MR) image of the face skin and the microscopic photograph of the histological specimens of the skin

(A) Sectional MR image was cut along a horizontal line from the lower edge of nasal wing to near the ear (the solid line in Figure 1). The red square shows the analyzed area including the cheek subcutaneous tissue. The white arrow head indicates the lower edge of nasal wing. (B) Expanded MR image shows the part of the cheek subcutaneous tissue and unveiled dark-gray signal-intensity regions indicating network structures in the subcutaneous tissue. (C) Hematoxylin and eosin stained skin specimens was taken from a horizontal line from the lower edge of nasal wing of the purchased human tissue. Network retinacula cutis structures are found inside the subcutaneous tissue. The yellow dash lines in both photographs B and C indicate the border between the dermis and the subcutaneous tissue, and the asterisks (*) indicate the dermis.

Decrease in the elasticity of the deep part of the skin with decreasing the network structure density in the cheek subcutaneous tissue

For finding out the relationship between sagging degree and network structures in the subcutaneous tissue, sagging in volunteers were individually compared with their observed network structures found in the tomographic MR images taken form the axial plane at the level of the lower edge of nasal wing. As a result, the slighter degree of sagging was found in the photograph, the denser network structures were observed in the subcutaneous tissue (Figure 3A-F). For confirming the
relationship between the sagging degree and network structures more precisely, the density of network structures occupying the subcutaneous tissue in the binarized MR images was calculated, and the correlation between the density of network structures and the sagging scores was analyzed. Although no significant difference was found between them, the result showed a tendency that the volunteers having higher sagging scores showed the lower density of network structures (Figure 4A and Table 1). Similarly, in spite of no significant correlation between age and the density of network structures, the result indicated a tendency that the elders had a lower density of network structures (Figure 4B and Table 1).

This study also investigated the relationship between the physical property of the deep part of the skin and network structure. The results showed the positive relationship between the Ur/Uf values of the cheek subcutaneous tissues and the densities of network structures of the volunteers, indicating that the elasticity of the deep part of the skin decreased with decreasing the density of network structures (Figure 4C and Table 1).

Table 1: Relationships between the density of fibrous network structures in the cheek subcutaneous tissue and the sagging scores, the density and the age, and the density and the elasticity of the deep part of the skin

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<th>Density of fibrous network structure (%)</th>
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<tr>
<td>Sagging score</td>
<td>-0.41</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.39</td>
<td>n.s.</td>
<td></td>
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<tr>
<td>Skin elasticity (Ur/Uf)</td>
<td>0.53</td>
<td>$&lt;0.05$</td>
<td>n.s.: non-significant</td>
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Figure 3: Comparison of the subcutaneous tissue structures in the cheek between the mild sagging and strongly conspicuous sagging

The cheek photographs were taken at an angle of 45 degree (A, B). The magnetic resonance (MR) images were taken at the cheek (C, D). The processed MR images show fibrous network structures (E, F). The left columns show the volunteers whose sagging degrees are mild (A, C, E). The right columns show the volunteers whose sagging degrees are conspicuous (B, D, F). The yellow lines in images from C to F indicate the border of the subcutaneous tissue, and the red areas show fibrous network structures. Fibrous network structures in the mild-sagging subcutaneous tissue were found to be denser than those of the strongly-conspicuous-sagging tissue.
DISCUSSION
This study investigated the effect of structures found in the subcutaneous tissue on sagging in the face. The fibrous network structure was found in the subcutaneous tissue of the face, and the precise structure of the network was clearly visualized and quantitatively analyzed by MR imaging and was morphologically identical to that of RC found in histological specimens. With decreasing the density of fibrous network in the cheek subcutaneous tissue, the elasticity of the deep part of the skin decreased, and the sagging scores tended to increase.

Anatomical and morphogenetic studies show that the subcutaneous tissue consists of mainly two components; (1) the subcutaneous fat giving volume and (2) RC, a small fibrous strand connecting the dermis to the deep fascia [2, 3]. RC is a 3D network component in the subcutaneous tissue and known to maintain the morphology of the subcutaneous tissue by providing an anti-gravity force to the tissue [3-5]. Iwanami and Tsurukiri report that the deterioration of RC in the lower eyelid of elderly subject [13], leading to a hypothesis that the cause of sagging appears on the face is due to age-related change in RC structure.

For verifying the hypothesis, this study attempted to develop a non-invasive method analyzing RC structures in the face. Herlin et al. establish a generic 3D geometric and mechanical model of the skin/subcutaneous complex by using MR images, anatomical knowledge, and histological observations, and report that RC can be visualized in MR images [14]. In their report, although RC in the face is visualized by MR imaging, high resolution images visualizing the detail structure of RC are unable to be obtained because of the thinner thickness of RC of the face than that of the hip. This study (1) established an optimized MR sequence for allowing the subcutaneous tissue to be visualized clearly on MR images and (2) confirmed fibrous network structures with dark-gray lower signal-intensity regions in the subcutaneous tissue on MR images. Since fibrous collagen-rich components express lower signal-intensity in general [7, 8], similar to RC, network structures found in the subcutaneous tissue was speculated to consist of fibrous components. Moreover, because the histological observation showed that the network structures found in MR images was

![Figure 4: The scatter plots showing the relationships between the density of fibrous network structures in the cheek subcutaneous tissue and the sagging scores, the density and the age, and the density and the elasticity of the deep part of the skin](image-url)

- With increasing the score of sagging, the density of fibrous network structures tended to decrease. (A) With increasing the age of volunteers, the density of fibrous network structures tended to decrease. (C) With increasing the elasticity of the deep part of the skin, the density of fibrous network structures tended to increase.

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morphologically identical to RC, the network structures found in the face subcutaneous tissue by MR imaging was speculated to be possibly RC. These results indicated that MR imaging was able to visualize the facial subcutaneous tissue non-invasively.

Using the method, this study analyzed the network structures, which were speculated to be RC, quantitatively after binarizing MR images. With decreasing the network structure density, the physical property of the subcutaneous tissue was found to decrease. A histotopographic study conducted by Macchi et al. indicates a possibility to reduce the elasticity of the fibroadipose connective system by qualitative and quantitative change in the properties of fibroadipose connective system including RC [15]. Although this study obtained supportive results for their conclusions, this study also found a tendency that the degree of sagging increased with decreasing the density of the network structures, in spite of no significant correlation between them. Since decrease in the elasticity of skin is reported to increase the degree of sagging [16], in this study, decrease in the density of the network structures reduced the elasticity of subcutaneous tissue, and the reduction allowed the maintenance ability of the morphology of the tissue to decrease, leading to the appearance of the more conspicuous sagging on the face (Figure 5). The reason why no significant correlation between the density of network structures and the degree of sagging was obtained in this study was speculated to be caused by the possible effect of higher variation in individual variability due to the small number of volunteers. Therefore, in the future, larger scale volunteer studies are expected to clarify the possible relationship between the network structures and the degree of sagging.

**Figure 5: Proposed mechanism of the skin sagging**
Deterioration of retinacula cutis (RC) give the reduction of RC density in the subcutaneous tissue, which decrease (1) the elasticity of the deep part of the skin and (2) the maintenance ability of the subcutaneous tissue, resulting in the appearance of the conspicuous skin sagging with gravity.
CONCLUSIONS
After establishing a non-invasive method to analyzing RC structures in the subcutaneous tissue of the face, this preliminary study found that decrease in RC reduced the elasticity of subcutaneous tissue and induced the appearance of sagging. The non-invasive method was thought to be useful for investigating possible sagging-appearance mechanisms, which never explained by previous studies, by analyzing changes in the subcutaneous tissue. The method would be expected to allow cosmetic chemists to develop sagging-improving measures including anti-sagging products.

REFERENCES