INTRODUCTION

Skin grafting is often required to treat wounds, burns, and surgical defects caused by the removal of skin tumors. Skin contracture after skin grafting is called secondary contracture. It is undesirable from functional and esthetic perspectives. It is generally accepted that full-thickness skin grafts contract less than split-thickness skin grafts. However, unexpected secondary skin-graft contracture sometimes occurs after full-thickness skin grafting. We tried to elucidate the causes of skin contracture from the viewpoint of the orientation of collagen fibers to find a way to reduce skin-graft contracture.

METHOD: First, we examined the collagen fiber orientation of the skin over the whole body in Sprague-Dawley rats. Next, two pieces of skin (width: 30 mm × 30 mm; thickness: ca. 2 mm) were stripped off a rat for grafting. The pieces were grafted to different sites so that the collagen fibers of the graft and surrounding skin ran parallel or perpendicular to each other. The collagen fiber orientation before and after the skin grafting was determined using Osaki’s microwave method, a mechanical method, and scanning electron microscopy.

RESULTS: The rat skin exhibited marked variations in collagen fiber orientation among different sites. The direction of the collagen fiber orientation corresponded to that of minimal mechanical strain. We found that the collagen fiber orientation in skin grafts remained almost unchanged after skin grafting.

Conclusions: Mismatched collagen fiber orientation between grafts and the surrounding skin is considered to be a cause of secondary contracture after skin grafting. We propose that skin grafts that minimize the difference in collagen fiber orientation between the skin graft and the surrounding skin should be selected.

(Keywords: skin contracture, collagen fiber orientation, skin grafting, mechanical method, scanning electron microscopy.)

Disclosure: The authors have no financial interest in relation to the content of this article. The study was supported in part by JSPS KAKENHI (grant no. 26670775).
Previously, one of the authors developed Osaki’s microwave method, which can be used to determine the molecular and fiber orientation of sheet materials using polarized microwaves in a cavity resonator.\textsuperscript{11–13} It was also found that the mean collagen fiber orientation was closely related to mechanical properties associated with the contraction and expansion of the skin in animals, such as calves and snakes.\textsuperscript{14–17} However, this method has not been applied to rats, which are useful for skin-graft experiments.

In this study, we assessed collagen fiber orientation in rat skin before and after skin grafting and compared the fiber orientation with mechanical properties to identify the causes of skin-graft contracture.

We considered that the contracture of grafted skin could be caused by tension derived from the differences in mechanical strain between skin grafts and the surrounding skin during movement, which may be caused by mismatches in collagen fiber orientation between the skin graft and surrounding skin.

Based on this concept, we performed skin grafts and positioned the grafts so that their collagen fibers ran parallel or perpendicular to the collagen fibers in the surrounding skin. Our findings indicated that the orientation of collagen fibers in skin grafts was not significantly altered by movement involving the skin surrounding the graft.

This study describes a skin-grafting method based on collagen fiber orientation matching between the skin graft and the surrounding skin to reduce skin-graft contracture.

**MATERIALS AND METHODS**

**Rats and Skin Grafting**

The skin from male Sprague-Dawley rats weighing ca. 600 g was used. Five rats who did not undergo skin grafting were used to prepare leather sheets. Eleven rats underwent skin grafting. The rats were anesthetized, the hairs on their backs were cut, and then two pieces of skin (size: 30 mm × 30 mm; thickness: ca. 2 mm) were stripped off from two (left and right) adjacent sites on the back near the central cranial-to-caudal direction (CCD) line. The layer containing the epidermis and dermis was used as a skin graft after the panniculus carnosus and fat layer had been removed.

The skin graft from the left side was returned to the graft bed so that its collagen fibers were oriented in the same direction as before. This graft was designated the control skin sample (C) (Fig. 1). The other graft was rotated 90 degrees relative to its initial orientation before being returned to the graft bed, and was designated the skin-graft sample (G) (Fig. 1).

In most rats, the graft take ranged from 0% to 20%, which was very low because of body movements. One rat, who exhibited a graft take of 80% for both graft sites, was used for the present experiments.

At 10 months after the skin grafting (Fig. 2), the whole skin of the rat that underwent skin grafting was converted into a leather sheet. The collagen fiber orientation of the leather sheet was examined. The rat care and experiments were performed in accordance with protocols approved by the animal care and use committee of Nara Medical University.

**Preparation of Leather Sheets**

The whole skin (thickness: ca. 2 mm) was stripped off a mature rat, and pickled in a 5% aqueous solution of hydrated lime to remove the hair. Then, the hydrated lime was removed before the skin was treated with a softening agent (to allow the fat to be skimmed off) followed by formic acid (for tanning purposes). Then, the skin sheet was stretched and pressed between boards, and dried in the air. Finally, the sheet was used as a leather sample (thickness: ca. 800–1500 μm) after the subcutaneous tissue and hairs had been removed. Thus, the leather sheet mainly

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**Takeaways**

**Question:** Is the orientation of collagen fibers a key point for reducing skin contracture after skin grafting?

**Findings:** We found that the collagen fiber orientation in skin grafts did not significantly change without effects of the skin surrounding the graft.

**Meaning:** Matching in collagen fiber orientation between skin grafts and the surrounding skin is very important to reduce skin graft contracture.

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**Fig. 1.** An image obtained just after skin grafting of the two grafts placed at different sites on the back of a rat. The left graft, which was placed back onto the graft bed in its original orientation, was designated control sample C, and its collagen fibers were, on average, oriented in the CCD. The right graft, which was rotated 90 degrees relative to its initial orientation before being returned to the graft bed, was designated skin graft G, and its collagen fibers were, on average, oriented perpendicular to the CCD.
consisted of the dermis. Because the entanglement of collagen fibers in the skin is so strong, the skin is unlikely to undergo significant changes in texture even when it is converted to leather through the treatments described above.12–15

Osaki’s Microwave Measurements

Osaki’s microwave method involves quantitatively measuring the (low) levels of anisotropy in the fine texture of collagen fiber bundles in 100-μm to 3-mm-thick sheets.14–17 In the present study, a sample (30 mm × 30 mm) cut from a leather sheet was inserted into the narrow gap between the pair of waveguides of the cavity resonator system.11–13 The sample sheet, which was rotated at a speed of 6.0 s/turn around the axis normal to the plane, was irradiated with polarized microwaves at a frequency of 12.3 GHz perpendicularly to the plane. This method is based on the concept that the mean orientation of collagen fibers for a whole sheet can be determined from the interaction between the polarized microwaves and the dipole moments of the collagen fibers. The transmitted microwave intensity (MI), which reflects this interaction, is detected at a fixed frequency by a detector at every 1 degree of rotation. The angular dependence of the MI gives the fiber orientation ratio (FOR) from the maximum-to-minimum ratio of MI and the orientation angle (β) from the direction of the minimum MI. The FOR is a measure of the degree of fiber orientation anisotropy, and β reflects the mean direction of collagen fiber orientation (ie, the degree of deviation from the CCD). Strictly, the fiber orientation value obtained from correcting the (FOR-1) value by the sample thickness should be used.

Mechanical Measurements

Strip specimens (5 mm wide × 30 mm long) for the mechanical measurements were prepared by cutting a whole rat leather in two directions—that is, parallel (line 2 in Fig. 3) and perpendicular (line 4 in Fig. 3) to the central CCD line. The thickness of each specimen was measured. Force-elongation curves for these strips were determined with an effective span of 10 mm and a stretching velocity of 20 mm/min using an RTC-1150A Universal Testing Machine (INTESCO CO., Japan), to assess the mechanical breaking strength and elongation at the breaking point.

Scanning Electron Microscopy

The fine texture of collagen fiber bundles in cross-sections was examined by SEM, with a JSM-6301F (JEOL, Japan).

![Fig. 2. An image of the back of a rat obtained at 10 months after the skin grafting. All hair has been removed. The left graft, which was returned to the graft bed in its original orientation, was designated control sample C. The right graft, which was rotated 90 degrees relative to its initial orientation before being returned to the graft bed, was designated skin graft G.](image)

![Fig. 3. Rat leather sheet samples used for the microwave measurements. Rat leather samples were obtained from three different sites on a rat leather sheet (designated d-2, d-4, and d-6; size: 30 mm × 30 mm) and used for the microwave measurements. The sampling sites were located along a fixed row that ran perpendicular to the CCD. The dotted line runs in the CCD. The leather sheet samples used for the mechanical measurements (size: 5 mm × 30 mm) were obtained from sites on line 2, which ran perpendicular to the CCD, or line 4, which ran parallel to the CCD.](image)
RESULTS

Distribution of Collagen Fiber Orientation in Rat Skin

A representative leather sheet was prepared from a rat that did not undergo skin grafting (Fig. 4). The angular dependence of transmitted MI was measured at three different sites (d-2, d-4, and d-6) along a fixed row that ran perpendicular to the CCD in a schematic rat leather sheet (Fig. 3), using Osaki’s microwave method. The orientation angle $\beta$ and the FOR were determined to be 31 degrees and 1.451 for sample d-2, 10 degrees and 1.220 for sample d-4, and -12 degrees and 1.935 for sample d-6, respectively (Fig. 5). Thus, the results showed that the collagen fibers exhibited a marked degree of orientation; that is, they were not just randomly aligned, and on average the collagen fibers were oriented parallel to the spinal column.

A distribution map of collagen fiber orientation over the entire rat leather sheet is depicted in Figure 6. Here, the length and inclination of each line represent the mean degree and direction of fiber orientation, respectively. If the fibers within a particular region were, on average, randomly aligned, the line for that region would become a point. If the length of the line were larger, the degree of fiber orientation would be larger. The inclination of each line represents the angle between the mean direction of the collagen fibers and the CCD. If the fibers were mainly oriented in the CCD, the line would run parallel to the CCD.

Figure 6 shows that the degree of fiber orientation was greater in the abdomen and proximal limbs than in the spinal column region, whereas it was less marked in the region near the spinal column than in the region in which the fibers ran from the CCD. On the other hand, the angle between the fibers and the CCD was greater in the proximal limbs than around the spinal column and abdomen. This indicates that the collagen fibers were mostly aligned parallel to the spinal column and limbs, which is supported by the SEM observations of the cross-sections of the rat leather cut in two different directions (Fig. 7).

Fig. 4. A whole leather sheet prepared from the skin of a rat that did not undergo skin grafting.

Fig. 5. Angular dependence of transmitted MI at 12.3 GHz at three different sites (d-2, d-4, and d-6) of the rat leather sheet shown in Figure 3. F is the measuring frequency, $\beta$ indicates the orientation angle determined from the direction of minimum transmitted intensity, which reflects the direction in which most collagen fibers were orientated. Fiber orientation ratio represents the maximum-to-minimum ratio of the transmitted MI, which is a measure of the degree of fiber orientation.
Rat leather strip samples were prepared in two different directions (i.e., parallel or perpendicular to the CCD). The stress-strain curves for sample d-6 showed that the mechanical stress and strain in the CCD were larger and smaller, respectively, than those in the direction perpendicular to the CCD (Fig. 8). Therefore, if the same stress is applied to two skin samples whose fiber orientation directions are very different from each other, the strain placed on the samples will differ markedly.

We also examined how mechanical stress and strain vary among different rat skin sites to evaluate the degree of expansion and contraction that can be ascribed to movement. The relationship between breaking stress and strain was evaluated in strip samples (Fig. 9). The samples were cut from positions along lines 2 and 4 of the rat leather (Fig. 3). Lines 2 and 4 were assumed to have similar mechanical properties because of the symmetry of the rat body. Samples prepared from different positions on line 2 were used to obtain mechanical measurements perpendicular to the CCD. In contrast, samples prepared from different positions on line 4 were used to obtain mechanical measurements parallel to the CCD. The breaking strain (Y) increased roughly as the breaking stress (X) decreased, although the data were variable. The relationship is expressed by the following equation:

\[ Y = -1.518X + 154.72 \]

Thus, samples with small mechanical stress values expanded more easily than samples with large mechanical stress values. This supports the idea that the rat skin at each measurement site expanded and contracted more readily perpendicular to the direction of collagen fiber orientation than in the direction of collagen fiber orientation.

**SEM Images of the Fine Structure of the Skin after Skin Grafting**

The findings of the abovementioned rat skin experiments reinforced the idea that the expansion and contraction of rat skin during body movements are related to collagen fiber orientation. SEM images of cross-sections of a skin graft (G) whose collagen fibers were oriented at 90 degrees to the CCD and the surrounding skin (S2) were
examined (Figs. 10, 11A, 11B; the cross-sections were cut along the bold arrows). Many of the collagen fiber bundles in the cross-section of skin graft G had been cut longitudinally (Fig. 11A), whereas many transverse-sectioned collagen fiber bundles were observed in skin sample S2 (Fig. 11B). Next, SEM images of cross-sections of skin graft G and the surrounding skin (S2) that were cut in the CCD were also examined. In these cross-sections, many transverse-sectioned collagen fiber bundles were observed in G (⊥) (Fig. 12A), whereas many of the collagen fiber bundles in S2 had been cut longitudinally (⊥) (Fig. 12B). It has previously been reported that the alignment of collagen fiber bundles corresponds to the orientation of collagen fibers.14,15 Significant differences in fine texture were seen between the skin graft and surrounding skin. The collagen fibers in skin graft G and the surrounding skin (S2) were, on average, orientated perpendicular and parallel to the CCD, respectively. These images also show that after the procedure, the orientation of the collagen fibers in the skin graft remained almost unchanged.

SEM showed the fine textures of the surrounding skin (S1) (Fig. 13A) were similar to the control skin graft C (Fig. 13B; the cross-sections were cut along the bold arrows), which was transplanted so that its collagen fibers were oriented in their original direction, indicating that the collagen fiber orientation of the skin graft remained unchanged after the skin grafting procedure. Many transverse-sectioned collagen fiber bundles were observed in both skin graft C and S1. The images of C and S1 were markedly different from those of G and S2 (⊥), whereas they were similar to the images of S2 and G (⊥) (Figs. 11–13).

Collagen Fiber Orientation after Skin Grafting

Previously, by examining hair pores, one of the authors found that the direction of hair growth is closely related to collagen fiber orientation.18 In the present study, the alignment of the hair pores on the surface of control rat skin graft C was similar to that seen in the surrounding skin.
In contrast, the alignment of the hair pores in skin graft G (Fig. 14A) was almost perpendicular to that seen in the surrounding skin (S2) (Fig. 14B). These examinations also indicated that collagen fiber orientation remained almost unchanged after the skin grafting. Next, we tried to determine the angular dependence of the MI at two different sites in G and S2 (Fig. 15). The orientation angle $\beta$ was determined to be 84 degrees for skin graft G and -15 degrees for the surrounding skin (S2). This also showed that the direction of collagen fiber orientation in skin graft G was almost perpendicular to that seen in the surrounding skin (S2), indicating that there was a marked difference in collagen fiber orientation between G and S2. Furthermore, the mechanical strain in skin graft G was much weaker than that seen in the surrounding skin (S2) in the direction of the waistline. This means that it was difficult for skin graft G to expand along the waistline, whereas it was easy for the surrounding skin (S2) to expand in this manner.
DISCUSSION

The findings regarding collagen fiber orientation in rat skin we obtained using Osaki’s microwave method corresponded to those we obtained using the mechanical method. The present study demonstrated that whole-body sheets of rat leather, mainly dermis, exhibited marked variations in collagen fiber orientation, depending on the sampling site. In addition, the direction of the collagen fiber orientation at each site reflected that of maximal mechanical stress/minimal mechanical strain. Our SEM observations were consistent with the collagen fiber orientation data obtained using the abovementioned two methods, even though the observations only related to the fine texture on the tissue surface. In rats, body movements accompanied by skin expansion and contraction mainly occur perpendicular to the CCD and along the waistline, whereas the magnitude of expansion and contraction in the CCD is small. This indicates that the distribution of collagen fiber orientation in rat skin is very useful for estimating the direction of skin movements.

Because the lifespan of rats is about two and a half years, the fact that the examinations were performed at 10 months is considered to represent relatively long-term follow-up. The present experimental results showed that the orientation of collagen fibers in skin grafts did not change markedly even 10 months after skin grafting.

It is important to mention movements involving skin grafts. It is known that scar contracture is likely to occur due to the force applied to wounds. If the collagen fiber orientation in a skin graft were very different from that in the surrounding skin, it would be difficult for the skin graft to expand and contract synchronously with the surrounding skin. Conversely, if the collagen fiber orientation in a skin graft were almost the same as that in the surrounding skin, the skin graft and surrounding skin would be able to move in sync with each other.

It is also necessary to discuss the application to humans of experimental results regarding skin grafts obtained in rats. Rats exhibit much better wound healing than humans. In addition, rat skin moves fairly loosely between the panniculus carnosus and muscle, which is different from humans. We focused on the collagen fiber orientation of the dermis rather than the soft subcutaneous tissue. Thus, it will be necessary to understand such differences between rats and humans.

When trying to apply this study to actual clinical practice, the collagen orientation of the entire surface in humans is currently unknown, but it is possible to predict the collagen orientation from the direction of downy hair to some extent. If so, it will be possible to select an appropriate place for preparing graft skin so that the difference in collagen fiber orientation between skin grafts and the surrounding skin should be minimized.

CONCLUSIONS

The present study suggests that matching the orientation of collagen fibers at a molecular level between skin grafts and the surrounding skin would reduce scar contracture in humans. Mismatches in collagen fiber orientation
between skin grafts and the surrounding skin are considered to cause secondary contracture of skin grafts. In the future, studies involving a greater number of cases with good graft-take are needed so that the long-term condition of skin grafts can be assessed.

Masamitsu Kuwahara, MD, PhD
Division of Plastic Surgery
Nara Medical University Hospital
Kashihara, Nara 634-8522
Japan
E-mail: makuwa@naramed-u.ac.jp

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