Collagenous Fibril Texture of the Discoid Lateral Meniscus

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Purpose: To provide the theoretic basis for treatment and to increase the understanding of the tear patterns of the discoid meniscus, we observed the collagen orientation of the discoid lateral menisci. Methods: Ten meniscus specimens were used to observe the collagen fibril orientation of the complete type of the discoid lateral menisci. The samples were observed layer by layer under a polarizing filter microscope by using Sirius red staining, and they were also observed under a scanning electron microscope. Results: The lateral discoid meniscus is classified into 7 layers based on collagen fibril orientation. The femoral surface of the discoid meniscus is covered by dense and well-arranged thick fibrils, which very much resembles a bunched streak. The fibrils show a sagittal isotropic-arranged orientation. However, the tibial surface shows an irregular and anisotropically arranged orientation. In the outer layer, a meshwork of thin fibrils has been observed. The collagen fibrils in the inner layer are radially oriented from the lateral side to the medial side. In the central layer, the peripheral collagen fibrils are displayed as dense bundles running in a circumferential pattern, whereas its medial zone shows as thin, loosely, and irregularly arranged fibrils without a bundle formation. The anterior and posterior zones of the central layer show the collagen fibrils with a straight arrangement in the radial direction. Conclusions: In the lateral middle zone of discoid meniscus, the collagen fibrils run parallel to the periphery of the meniscus. Therefore, it would be ideally suited for resisting hoop stresses. From this anatomic study, it is apparent that the peripheral portion of the meniscus is constructed to bear a load. Clinical Relevance: It is strongly recommended that the peripheral portion of the discoid meniscus should be preserved when a resection of the meniscus is mandatory. Key Words: Discoid meniscus—Collagenous fibril—Scanning electron microscope.

The meniscus functions in load bearing, shock absorption, and stability for the human knee joint. These mechanical functions are determined not only by their anatomic characteristics but also by the material properties of the meniscus. The meniscus composed of the collagen fibrils arranged with a certain orientation can provide great elasticity, and they have the ability to withstand compression forces. Some studies by Aspens et al., Bullough et al., and Kummer have depicted the normal meniscal microstructure and its collagen fibril texture by the use of different methods. Despite a few minor disagreements, their concurrent view is that the circumferential fibrils at the peripheral site can properly accommodate hoop tension from the weight-bearing function. The orientation of these collagen fibrils is believed to determine the tear pattern and treatment options so there can be no doubt that the peripheral component of the meniscus should be preserved as much as possible when a partial meniscectomy is performed.

The discoid lateral meniscus is the most common variation of the meniscus, and it is seen more often in Japan and Korea than in Western countries. It is easily torn and caught between the femur and the tibia because of its typical shape. Tear patterns of discoid menisci are more variable than the tear pat-
terns of the normal meniscus. These tear patterns are likely related to the collagen fibril arrangement of the discoid meniscus as compared with those in the normal meniscus. Treatment for the torn discoid meniscus is still a matter of debate. The same conception for treatment of normal meniscal tears can hardly be applied to the discoid meniscus because of its anomalous morphology. Presently, there are some advocates for performing a total meniscectomy despite reports warning that early degenerative changes of the articular cartilage that could result from this operation. On the contrary, partial meniscectomy is recommended to preserve meniscal function because this will prevent degenerative arthritis. However, to date, it is still unclear whether the remaining meniscus after partial meniscectomy plays a role in load bearing. This issue is closely related to collagen orientation in discoid meniscus. To our knowledge, the collagen orientation of discoid meniscus is unknown so far. In this study, we hypothesize that the collagen fibrils in discoid meniscus are also arranged in a circumferential fashion in the periphery region analogous to that of normal meniscus to resist hoop tension.

To test the hypothesis, this study observed and analyzed the collagen fibril orientation of discoid meniscus in a layer-by-layer fashion. This can provide the theoretic basis for treatment and the understanding of the tear patterns of the discoid meniscus.

METHODS

We took 10 fresh complete discoid lateral menisci from 10 patients with an average age of 37 years (range, 17 to 62); the menisci were equally assigned into 2 groups and then fixed in 4% formalin acid for 3 days. The first group was prepared for observation under a direct polarizing filter microscope (Eclipse E600; Nikon, Tokyo, Japan) by Sirius red staining. The second group was prepared for observation under a scanning electron microscope (SEM, S-2400; Hitachi, Tokyo, Japan).

Sirius Red Stain

The discoid menisci in the first group were rinsed with water for 3 to 4 hours to remove residual formalin on the tissue, and then we performed dehydration, clearing, and paraffin infiltration by using a tissue processor (Shandon, Pittsburgh, PA). The paraffin blocks were sectioned from the femoral surface to the
tibial surface of the discoid meniscus with a sliding microtome (HM400R; Microm, Walldorf, Germany) in the thickness of 5 μm. The slides were first nuclear stained by Mayer’s hematoxylin and then stained by 0.1% Sirius red solution for 1 hour. After the stained slides were differentiated with 50% acetic acid, they underwent 2 dehydration procedures with 95% and 100% alcohol, respectively. Finally, we performed clearing with xylene and mounting procedures.

**Scanning Electron Microscope**

The other 5 samples, as the second group, were subdivided into segments by uniform radial and transversal sections so as to permit a topographic classification. The segments from different layers and regions of the discoid meniscus segments were kept in 10% NaOH solution at room temperature over a period of 5 to 6 days. Subsequently, the specimens were rinsed in distilled water for 1 to 2 days, and then they were then saturated in 1% tannic acid for 4 to 5 hours. After rinsing the specimens in distilled water for 24 hours, they were counterfixed in 1% OsO4 solution. The specimens were dehydrated in a series of graded concentrations of ethanol and then freeze-cracked with a razor blade in liquid nitrogen and finally critical-point dried using liquid CO2. The dried specimens were mounted on metal stubs with a double sticky tape, and, at last, they were coated with gold. The obtained images were analyzed with an image analysis program (Nikon, Tokyo, Japan).

**RESULTS**

**Axial Section**

The polarizing filter microscope and SEM distinguished the discoid meniscus into 7 distinct layers in cross-section based on the arrangement of collagen fibril. The midportion of the meniscus is identified as the central layer, and 3 layers are symmetrically arranged in the superoinferior directions with the central layer as a symmetric axis: surface, outer, inner, and central layers (Fig 1). The femoral surface of the discoid meniscus is covered by dense and well-arranged thick fibrils, resembling a bunched streak. The fibrils show a sagittal isotropic-arranged orientation (Fig 2A). However, the fibrils of the tibial surface show an irregularly and anisotropically arranged orientation (Fig 2B). Beneath the first layer, a meshwork of thin fibrils was observed. The fibrils do not show a preferred orientation (Figs 2C and 3A). The collagen fibrils are oriented radially from the lateral side to the medial side, and perforating fibrils are also presented underlying the meshwork fibrils (Fig 2D and 3B). The peripheral collagen fibrils that display dense bundles running in a circumferential pattern are similar to

**FIGURE 3.** The Sirius red stain results (original magnification ×400). Sirius red is specific for collagen, and the collagen fibrils stained with Sirius red can appear different colors (yellow, red/orange, or green), according to their direction under polarizing filter microscopy. (A) The thin fibrils in crossing direction appear as a meshwork in the outer layer (Fig 1C). (B) Oriented radially from the lateral side to the medial side in the inner layer (Fig 1D). (C) Collagen fibrils with a circular arrangement in periphery of the central layer (Fig 1E). (D) Collagen fibrils with irregular arrangement in the medial portion of central layer (Fig 1F).
normal meniscus (Figs 2E and 3C). However, a different pattern is revealed in the medial region, which is composed of thin, loosely, and irregularly arranged fibrils without a bundle formation (Fig 2F and 3D).

Radial Section

We also observed 7 layers with differently arranged directions of the collagen fibril. The central layer shows an arrangement of bundle collagen fibrils in its external circumference and also in its thick middle perforating bundle (MPB), which is compactly connected with the joint capsule in the medium area. The central layer is the thickest, occupying approximately 75% of the full layer, and each thickness of the layer of the femoral side is approximately equivalent to corresponding layers of the tibial side (Fig 4).

Collagen Orientation of Different Zones in the Central Layer

According to the orientation of collagen fibrils in the central layer, the discoid meniscus is divided into 4 overall zones: the anterior, posterior, and middle zone, respectively, with the middle zone being subdivided into medial and lateral zones again. Both the anterior and posterior zones are coincidental with meniscal attachment portion on the bone. They show collagen fibrils with a straight arrangement in the radial direction. Collagen fibrils in the medial middle zone display an irregular arrangement, whereas collagen fibrils in the lateral middle zone represent a circular arrangement that occupies 20% of the transverse diameter of the discoid meniscus (Fig 5).

Three-Dimensional Texture

To better understand the layer arrangement of the discoid meniscus, the 3-dimensional texture of the full-layer arrangement was drawn with a synoptic method based on the previously described obtained images from the polarizing filter microscope and SEM (Fig 6).

DISCUSSION

There have been many reports, using different methods, on the normal meniscal microstructure regarding the collagen fibrillar texture.1,3,5,7 Kummer7 undertook a photoelastic analysis of the tensile trajectories, and he found that the collagen fibrils displayed an arcade-like arrangement. In contrast, some studies1,3 showed that collagen fibrils are primarily oriented in a circular direction in both the internal and external circumferences. Petersen and Tillmann5 used SEM and the split-line method to observe the orientation of collagen fibrils layer by layer. They observed the superficial network covered by a meshwork of thin fibrils, the lamellar layer containing the bundles of collagen fibrils with a radial direction arrangement, and there were intersections at various angles. They also observed that the central layer, as the main portion of the meniscus collagen fibrils, was oriented in a circular manner.
Despite a few disagreements,\textsuperscript{1,3,7} there is agreement among experts that the circular fibrils at the peripheral site can properly accommodate to the hoop tension resulting from weight bearing. The importance of the circular fibrils has also been shown by several animal experiments.\textsuperscript{17-19} Therefore, a partial rather than total or subtotal meniscectomy is always preferable if possible.

To the best of our knowledge, this is the first report that the Sirius red staining method and SEM were used to observe collagen fibril texture of the discoid meniscus. Sirius red is specific for collagen, and the stained collagen appeared in yellow, red/orange, or green, according to their direction under polarizing filter light microscopy\textsuperscript{20-22}; thus, the collagenous fibrils are easily discriminated.

The results of this study show that the individual layers of the discoid meniscus are distributed symmetrically from superior to inferior based on the central layer as an axial plane. The femoral surface of the discoid meniscus is covered by dense and well-arranged thick fibrils, and this structure resembles a bunched streak. The fibrils show a sagittal isotropic-arranged orientation. However, the fibrils of the tibial surface show an irregularly and anisotropically arranged orientation. For such an arrangement presentation on the femoral surface of the discoid meniscus, we can propose that it is caused by the global lateral condyle vigorously acting with a rolling and gliding motion in various directions. In contrast, the tibial surface of the discoid meniscus slips on the flat articular tibial surface in a limited range, on which collagen fibrils have an irregular arrangement. For maintaining the meniscal shape, the role of a meshwork of thin fibrils in the outer layer is more important than for shock absorption. The inner layer looks like a transition layer from the outer layer to the central layer; perhaps, this plays a connective role. The periphery of the central layer consists of the bundle collagen fibrils with a circular arrangement. On the contrast, the arrangement of collagen fibrils in the medial portion is irregular and without bundle fibrils. So it is believed that the periphery of the central layer is the most important portion to accommodate to the hoop tension from weight bearing. The collagen fibrils with straight arrangement in the radial direction in the anterior and posterior zone allow the meniscus to strongly attach on the tibia. The MPB of the central layer allows the discoid meniscus to tightly insert at the joint capsule, and this seems to play a role for stability.

The orientation of collagen fibrils is not only related to the principles of the treatment, but it also determines to some extent the characteristic patterns of meniscal tears.\textsuperscript{2,3,7} Because the discoid meniscus has anomalous morphology and an awkward position, it is easy to cause tears and degenerative changes.\textsuperscript{13,23} The distribution of the tear pattern has been reported in a number of studies. Hayashi et al.\textsuperscript{10} reported that the...
longitudinal tear is the most common type of discoid meniscus damage. However, Smillie\textsuperscript{12} and Bin et al.\textsuperscript{24} stated that the horizontal cleavage injury is the most frequent type, and this injury mostly occurs as a degenerative tear and might be presented as an apparently invisible substantial tear. Moreover, a study by Ferrer-Roca and Vilalta\textsuperscript{25,26} indicates that the degenerative horizontal cleavage most frequently occurs in the middle portion of the meniscus just at the portion of the discoid meniscus containing the MPB. When considering the poor vascularization in this portion, aging can easily cause a degenerative horizontal cleavage. Because the discoid meniscus is thicker than the normal meniscus because the knee joint acts in flexion and extension, rotation, and weight transmission, the discoid meniscus sustains higher shearing force between femur and tibia than normal meniscus. The MPB, in the axis, superior, and inferior layers, undergoes shearing forces in opposite directions so it is easy to cause horizontal cleavage in the middle portion of the meniscus.

Because the discoid menisci used in this study are taken from patients with symptoms, it is recognized that these menisci may have pathologic changes. However, 6 of 10 lateral discoid menisci were obtained from young patients who had only knee pain with unexplainable cause, and no grossly pathologic change was found during diagnostic arthroscopy. Five discoid menisci were occasionally found in the patients with arthritic knees during total knee arthroplasty (5 of 127 cases). One of 5 was excluded because of its degenerative horizontal cleavage; the remaining 4 obtained from the arthritic knee limited only to the medial compartment were visually intact. In clinical practice, the discoid meniscus is usually treated according to the patient’s symptoms and to the type and the degree of tear. However, the orientation of collagen fibrils, taken as an important factor, should be accurately acknowledged to determine the extent of surgical excision. Some studies\textsuperscript{14,15} showed very satisfactory results after a total meniscectomy. Nevertheless, the total meniscectomy of a lateral nondiscoid meniscus often leads to progressive osteoarthritis.\textsuperscript{27,28}

Hayashi et al.\textsuperscript{10} advocated partial meniscectomies that left 6 to 8 mm for incomplete and complete types, although they did not base their opinion on experimental data regarding the orientation of collagen fibril. Araki et al.\textsuperscript{29} found the normal transverse diameter of the lateral meniscus is between 5.0 to 13.1 mm and that of discoid menisci is between 13.1 to 30.0 mm. Our results showed that the circular arrangement of collagen fibrils occupies about 20% of the transverse diameter of the lateral discoid meniscus. So having considered the results of the previously mentioned studies, we suggest that leaving 6 mm from the periphery seems to be desirable for discoid meniscectomy.

**CONCLUSION**

The discoid meniscus can be classified into 7 layers in a symmetrical manner with the central layer as an axial plane, except for the surface layer, which shows a difference between the femoral and the tibial surfaces. All layers have their own unique fibrillar orientations. The collagen fibrils of the central layer in the anterior and posterior zone are arranged in a radial direction and, therefore, would ideally be suited for anchoring the meniscus to the tibia. Whereas, in the lateral middle zone of discoid meniscus, the collagen fibrils run parallel to periphery of the meniscus. Therefore, it would be ideally suited for resisting hoop stresses. From this anatomic study, it is apparent the peripheral portion of the meniscus is constructed to bear a load. We therefore recommend the peripheral portion be preserved for treating discoid meniscus.

**REFERENCES**