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Collagenous Fibril Texture of the Discoid Lateral Meniscus

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이 논문은 醫學碩士學位 論文으로 提出함

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2003年 12月 19日
Collagenous Fibril Texture of the Discoid Lateral Meniscus

by

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A Dissertation Submitted to The Graduate School of Ajou University in Partial Fulfillment of the Requirements for the Degree of

MASTER OF MEDICAL SCIENCES

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Collagenous Fibril Texture of the Discoid Lateral Meniscus

**Purpose:** To provide the theoretical basis for treatment and the understanding of the tear pattern of the discoid meniscus, we observed and analyzed the collagen orientation of discoid meniscus layer-by-layer.

**Materials and Methods:** Ten human complete type of discoid lateral menisci were obtained, of which collagen fibril orientation was observed in different region and layer utilizing polarizing filter microscope and scanning electron microscope.

**Results:** The lateral discoid meniscus is divided into seven layers based on collagen fibril orientation. The femoral surface of the discoid meniscus is covered by dense and well-arranged thick fibrils, resembling a bunched streak. The fibrils show a sagital isotropic arranged orientation. However, the tibial surface shows an irregular and anisotropically arranged orientation. In outer layer a meshwork of thin fibrils has been observed. The fibrils do not show a preferred orientation. The collagen fibrils in the inner layer are orientated radially from the lateral side to the medial side, and perforating fibrils are also present underlying the meshwork fibrils. In central layer, the peripheral collagen fibrils display dense bundles running in circumferential pattern. However, a different pattern is revealed in the medial portion,
composed of thin loosely and irregularly arranged fibrils without a bundle formation.

**Conclusion:** The discoid meniscus can be divided into seven layers in a symmetrical manner with the central layers as an axis, with an exception of the surface layer, which shows a difference between the femoral and the tibial surfaces. All layers have their own unique fibrillar orientations. The central layer seems to play a main role in resisting hoop tension since the peripheral portion has circumferential fashion of collagen fibers. Therefore, we can consider that preservation of the peripheral edge of the discoid meniscus would be better when resection of meniscus is mandatory.

**Key Words:** Discoid meniscus, Collagen fibril, Scanning electron microscope
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I. INTRODUCTION

The meniscus plays the roles of 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 material properties of the meniscus. Particularly the dense and tightly woven collagen fibrils in the menisci can provide great elasticity and ability to withstand compression forces.\(^\text{2,3,5,7,15,22,27}\) Some studies by Kummer,\(^\text{20}\) Bullough et al.\(^\text{7}\) and Aspden et al.\(^\text{3}\) depicted on normal meniscal microstructure regarding collagen fibrils texture with different methods. Despite a few divergences, their concordant view is that the circumferential fibrils at the peripheral site can properly accommodate hoop tension from weight bearing. These orientations of collagen fibrils are believed to determine the tear pattern and treatment options. Therefore there is no doubt that the peripheral component of meniscus should be preserved as large as possible when partial meniscectomy is performed.\(^\text{8,11,19}\)

The discoid lateral meniscus is the most common anomaly of the meniscus and more popular in Japanese and Korean than western countries.\(^\text{14,18,25}\) It is easy to be torn and occur self-degenerative changes due to its typical shape and being caught in between the femur and the tibia.\(^\text{14,15,18,23,25}\) Tear patterns of discoid meniscus are more variable than those of normal meniscus. Such as degenerative tear, horizontal tear and complex tear is common found in the discoid lateral meniscus, nevertheless
the common tear type of normal meniscus are longitudinal tear and flap tear. These tear patterns should be related to collagen fibril of discoid meniscus as in normal meniscus. Treatment for torn discoid meniscus is still on the debate. The same conception as the treatment of normal meniscal tear can hardly be applied to discoid meniscus because of its anomalous morphology. Presently, there are some advocates\textsuperscript{16,26} for performing total meniscectomy in spite of reports that warn that early degenerative change of the articular cartilage could result from this operation. On the contrary the partial meniscectomy is recommended to preserve meniscal function as a desire that will prevent degenerative arthritis.\textsuperscript{1,14} However, this option cannot be supported by either consentient clinical results or experimental data of load shearing of remained meniscus. In order to generate hoop tension, the discoid meniscus should retain collagen fibrils in circumferential fashion. To our knowledge, the collagen orientation of discoid meniscus has however never been reported so far.

This study observed and analyzed the collagen fibril orientation of discoid meniscus layer-by-layer, providing the theoretical basis for treatment and the understanding of the tear pattern of the discoid meniscus.
II. MATERIALS AND METHODS

Ten fresh obtained complete type of discoid lateral menisci from ten patients with an average age of 37 years (range 17 to 62) were equally assigned into two groups, fixed in 10% formalin acid for three days (Fig. 1). First group was prepared to being observed under direct polarizing filter microscope (Eclipse E600, Nikon, Japan) view by Sirius red staining. Second group was prepared to being observed by scanning electron microscope (SEM; S-2400, Hitachi, Japan).

A. Sirius red stain

The discoid menisci in the first group were rinsed by water for 3-4 hours in order to remove residual formalin on the tissue, and then performed dehydration, clearing, paraffin infiltration using tissue processor (Shandon, Pittsburgh, PA). To be sectioned easily, the paraffin infiltrated tissues were carried out embedding procedure to form paraffin block in certain shape. The paraffin blocks were sectioned from the femoral surface to the tibial surface of the discoid meniscus utilizing sliding microtome (HM400R, Microm, Germany) in the thickness of 5μm. The folding slices were put into water bath (Jisco, Seoul, Korea) to plane down wrinkles, the plane slice was attached on the glass slide, subsequently put into 60°C slide warmer (Jisco,
Seoul, Korea) for 1 hour to dissolve paraffin so that the slice is attached on the glass slide tightly. Before Sirius red staining, the slides were processed with xylene (Duksan, Ansan, Korea) to attain deparaffin and hydration. The hydrated slides were nuclear-stained by mayer’s hematoxylin (Merck, Darmstadt, Germany), and then stained by 0.1% Sirius red solution (0.1g siriusred (Polyscience, PA), 100ml picric acid (Sigma, MO)) for 1 hour. After the stained slides were differentiated with 50% acetic acid (Duksan, Ansan, Korea), respectively underwent twice dehydration with 95% and 100% alcohol, performed clearing with xylene and mounting procedures.

B. Scanning electron microscope

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

**Fig. 1.** Complete type of discoid lateral meniscus
III. RESULTS

1. Axial section

The polarizing filter microscope and SEM distinguish discoid meniscus into seven distinct layers in axial section by the arrangement of collagen fibril. The mid-portion of the meniscus is identified as the central layer, and three layers are symmetrically arranged in superoinferior directions with the central layer as a symmetric axis: surface, outer, inner and central layers (Fig. 2).

① Surface layer: The femoral surface of the meniscus is covered by dense and well-arranged thick fibrils, resembling a bunched streak. The fibrils show a sagital isotropic arranged orientation (Fig. 3a). However, the fibrils of the tibial surface show an irregularly and anisotropically arranged orientation (Fig. 3b).

② Outer layer: Beneath the first layer a meshwork of thin fibrils have been observed. The fibrils do not show a preferred orientation (Fig. 3c, 4a).

③ Inner layer: The collagen fibrils are orientated radially from the lateral side to the medial side, and perforating fibrils are also present underlying the meshwork fibrils (Fig. 3d, 4b).

④ Central layer: The peripheral collagen fibrils that display dense bundles running in circumferential pattern are similar to normal meniscus (Fig. 3e, 4c). However, a different pattern is revealed in the medial portion,
composed of thin loosely and irregularly arranged fibrils without a bundle
formation (Fig. 3f, 4d).

2. Radial section

Also we observed seven layers with differently arranged direction of
collagen fibril. The central layer was showed arrangement of bundle collagen
fibrils in its external circumference and thick middle perforating bundle
(MPB) that was compact connection with the joint capsule in the medium
portion. The central layer is the thickest occupying approximately 75% of full
layer, every layers thickness of the femoral side is approximately equivalent
to corresponding layers of the tibial side (Fig.5).

3. Collagen orientation of different zone in central layer

According to orientation of collagen fibrils in central layer, discoid meniscus
is divided into four zones in overall: anterior, posterior and middle zone
respectively, middle zone is subdivided into medial and lateral zone again.
Both the anterior and posterior zones are coincident with meniscal
attachment portion on bone. They show collagen fibrils with directly
arranged orientation. Collagen fibrils in the medial middle zone display
irregular arrangement, whereas collagen fibrils in the lateral middle zone
represent circular arrangement occupying 20% of transverse diameter of the
discoid meniscus (Fig. 6, 7).

4. 3-D texture

In order to better understand layer arrangement of discoid meniscus from dimension, 3-D texture of full layers arrangement was drew with synoptic method based on above obtained images from polarizing filter microscope and SEM (Fig. 8).

**Fig. 2.** Schematic drawing of the discoid meniscus
Fig. 3. The scanning electron microscopic results

a. The surface layer of femoral side (a in Fig. 2)
b. The surface layer of tibial side (b in Fig. 2)
c. The outer layer of femoral side (c in Fig. 2)
d. The inner layer of femoral side (d in Fig. 2)
e. Lateral side of central layer (e in Fig. 2)
f. Medial side of the central layer (f in Fig. 2)
**Fig. 4.** The Sirius red stain results (x 400)

a. The outer layer of femoral side (c in Fig. 2)
b. The inner layer of the femoral side (d in Fig. 2)
c. Lateral side of central layer (e in Fig. 2)
d. Medial side of the central layer (f in Fig. 2)
Fig. 5. Overall observation of the radial section

a. SEM
b. Sirius red stain x 40
Fig. 6. Collagen orientation of different zone in central layer (Sirius red stain x40)

a. Anterior zone  
b. Posterior zone  
c. Lateral middle zone  
d. Medial middle zone
Fig. 7. Synoptic drawing collagen orientation in the central layer

According to orientation of collagen fibrils of central layer, the discoid lateral meniscus is divided into four zones in overall: anterior, posterior, lateral middle and medial middle zone respectively.
Fig. 8. Synoptic drawing of collagen fibril texture
1. Surface layer
2. Outer layer
3. Inner layer
4. Lateral side of the central layer
5. Medial side of the central layer
IV. DISCUSSION

There have been many reports on normal meniscal microstructure regarding collagen fibrillar texture. A photoelastic analysis of the tensile trajectories undertaken by Kummer\textsuperscript{20} found that the collagen fibrils with supposedly running in a radial direction in the internal circumference and in a circular direction in the external circumference displayed arcade-like arrangement. On the contrary, Bullough et al.\textsuperscript{7} and Aspden et al.\textsuperscript{3} demonstrated that collagen fibrils are primarily orientated in a circular direction in both the internal and external circumferences, radial and perforating fibrils also are present. Petersen et al.\textsuperscript{22} utilized SEM and split line method to observe the orientation of collagen fibrils layer-by-layer. It reveals three distinct layers in the meniscus cross section: the superficial network covered by a meshwork of thin fibrils, lamellar layer containing the bundles of collagen fibrils with a radial direction arrangement and intersection at various angles, central layer as the main portion of the meniscus collagen fibrils orientated in a circular manner.

Despite a few divergences, a view of agreement is that the circular fibrils at the peripheral site can accommodate properly to hoop tension from weight bearing. The circular fibers act in much the same way as metal hoops placed around a pressurized wooden barrel. The tension in the hoops keeps the wooden staves in place, thus the compression of the menisci by the tibia
and the femur generates outward forces that push the menisci out from between the bones. These hoop forces are transmitted to the tibia through the strong anterior and posterior attachments of the menisci. By animal experiment, Shrive\textsuperscript{24} has shown that the hoop tension is lost when a single radial cut or tear extends to the capsular margin and that in terms of load-bearing, a single radial cut through the meniscus is equivalent to meniscectomy, thus the importance of the circular fibers is emphasized further. Either partial or complete excision of the menisci from the knees of dogs undertaken by King\textsuperscript{19} revealed that the hyaline cartilage of the articular surfaces degenerated to a degree roughly proportional to the amount of meniscus excised. On the other hand, some studies\textsuperscript{8,11,13} have demonstrated that the attempts at regeneration of menisci could be observed, however, a regenerated meniscus is frail, and they believed that the function of the regenerated meniscus in humans is probably insignificant. Once the collagen orientation is haphazard in the regenerated meniscus-like structure, the circumferential, or hoop-tension principle is not replicated. Therefore partial rather than total or subtotal meniscectomy always is preferable if possible.

The orientation of collagen fibrils is not only related to the principle of the treatment but also determines to some extent the characteristic patterns of meniscal tears.\textsuperscript{5,7,20} Beaupre et al.\textsuperscript{5} and Kummer\textsuperscript{20} found that the occurrence of radial tears in the internal circumference and of circular bucket-handle tears in the external circumference is attributed to the arcade-like arrangement of the collagen fibrils. Nevertheless Bullough et al.\textsuperscript{7}
considered that the most common longitudinal tear in clinical practice is germane to the circular collagen fibrils in deep layer, the radial fibrils rupture would lead to longitudinal tear, the circular fibrils rupture would lead to radial tear. It indicated that the meniscal tear is not only correlated to the mechanism of injury, but also depended on collagen fibril texture, so that the different segments would result in the different tear pattern.

The discoid meniscus is the most common type in meniscal anomaly that occurs more frequently laterally than medially. The incidence is higher in Korean and Japanese than in other populations.\textsuperscript{14,18,25} Watanabe et al.\textsuperscript{28} has classified the discoid lateral meniscus into complete, incomplete, and Wrisberg-Ligament types based on the degree of coverage of the lateral tibial plateau and the presence or absence of the normal posterior meniscotibial attachment. Since Wrisberg-Ligament type discoid meniscus is similar to normal shape and is unstable, it is advocated total meniscectomy. This study investigates only complete type of discoid lateral meniscus.

Best of our knowledge, this is the first time that Sirius red staining method and SEM were used to observe collagen fibrils texture of discoid meniscus. Sirius red is specific for collagen, and the stained collagen with different arranged orientation are appeared in yellow, red/orange or green, according to their direction under polarizing filter light microscopy.\textsuperscript{4,17,29}

By this study, the individual layers of discoid meniscus are distributed symmetrically from up to down based on central layer as an axial plane. 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. However, the fibrils of the tibial surface show an irregularly and anisotropically arranged orientation. For such arrangement presentation on the femoral surface of the discoid meniscus, we can suppose that it is caused by global lateral condyle vigorously acting the rolling and gliding motion with various directions. In contrast the tibial surface of the discoid meniscus slipping on the flat articular tibial surface in limited range, on which collagen fibrils is irregular arrangement. For maintaining the meniscal shape, the role of a meshwork of thin fibrils in outer layer is more major than shock absorption. The inner layer looked as transition layer from the outer layer to central layer, which perhaps plays a connective role. The periphery of the central layer consists of the bundle collagen fibrils with circular arrangement. On contrast the arrangement of collagen fibrils in the medial portion is irregular without bundle fibrils. Thus it is believed that the periphery of the central layer is the most important portion so as to accommodate to hoop tension from weight bearing. The directly arranged collagen fibrils in anterior and posterior zone allow meniscus to attach on the tibia strongly. The MPB of central layer that allow discoid meniscus to insert at joint capsule tightly seems to play a role of stability.

Because discoid meniscus has anomalous morphology and awkward position, it is easy to cause tear and degenerative change.\textsuperscript{12,23} The distribution of the tear pattern has been reported in a number of studies. Hayashi et al.\textsuperscript{14} investigated that the longitudinal tear is the most common
type of discoid meniscus damage. However, Smilie\textsuperscript{25} and Bin et al.\textsuperscript{6} stated that horizontal cleavage is the most frequent type, which mostly belongs to degenerative tear and might be apparently invisible substantial tear. Moreover, a study by Ferrer-Roca and Vilalta\textsuperscript{9,10} indicated that the degenerative horizontal cleavage most frequently occurs in the middle portion of the meniscus just as the portion of discoid meniscus containing middle perforating bundle. Regarded as poor vascularization in this portion, aging easily cause degenerative horizontal cleavage. Because discoid meniscus is thicker than the normal meniscus, furthermore, as knee joint acts flexion and extension, rotation and weight transmission, discoid meniscus is at the locking condition between the femur and tibia. MPB as an axis, superior and inferior layers undergo shear force of contrary direction, so it is easy to cause horizontal cleavage in the middle portion of the meniscus.

In clinical practice discoid meniscus is usually treated mainly according to symptom, tear degree, and type. However, the arragned orientation of collagen fibrils as a more important factor should be accurately acknowledged to determine range of excision precisely. Some studies\textsuperscript{16,26} showed satisfactory results after total meniscectomy. Nevertheless, total meniscectomy of a lateral non-discoid meniscus often leads to progressive osteoarthritis.\textsuperscript{21,30} Hayashi et al.\textsuperscript{14} advocated partial menisectomies left 6-8 mm for incomplete and complete type, which not based on the orientation of collagen fibril. Araki et al.\textsuperscript{2} found the normal transverse diameter of the lateral meniscus to be 5.0-13.1 mm, and that of discoid menisci to be 13.1-
30.0 mm. Ikeuchi\textsuperscript{16} studied 49 excised discoid lateral menisci and noted a maximum thickness of 14 mm (minimum, 4 mm). Smillie\textsuperscript{25} examined 15 discoid and 30 normal menisci and found that the discoid menisci had a thicker-than-normal central portion (especially the free margin), but the greater thickness did not extend to the periphery. Our results showed that the circular arrangement of collagen fibrils occupy 20\% of transverse diameter of the lateral discoid meniscus, and referred to above literature results, therefore we considered that leaving 6 mm from the periphery seem to be desirable for discoid meniscectomy.
V. CONCLUSION

The discoid meniscus can be divided into seven layers in a symmetrical manner with the central layer as an axis, with an exception of the surface layer, which shows a difference between the femoral and the tibial surfaces. All layers have their own unique fibrillar orientations. The central layer seems to play a main role in resisting hoop tension since the peripheral portion has circumferential fashion of collagen fibers. Therefore, we can consider that preservation of the peripheral edge of the discoid meniscus would be better when resection of meniscus is mandatory.


1995


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29. **Whittaker P, Kloner RA, Boughner DR, Pickering JG:** Quantitative

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