Three-Dimensional Architecture of the Acetabular Transverse Ligament and its Connection with the Acetabular Labrum

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SUMMARY

Background. We clarified the microstructure of the acetabular transverse ligament in terms of the construction and arrangement of the collagen fibers constituting the ligament and their connection with the acetabular labrum using light and scanning electron microscopy (SEM).

Methods. Deparaffinized blocks were treated with 2N NaOH to digest the cell matrix, allowing the collagen fibers constituting the ligament and cartilage to be observed under SEM.

Results. The acetabular transverse ligament had parallel collagen fibers with a width of 30–50 µm, which consisted of bundles with type I collagen fibrils. The collagen fibers of the transverse ligament were interwoven between the cartilage tissues of the acetabular labrum, forming a layered structure. These components seemed to be directly joined to each other histologically. However, in SEM images, the collagen fibers were not directly connected; rather, fine collagen fibers with varying diameters extending from the structures were interwoven.

Conclusions. The acetabular transverse ligament had a distinctly different microstructure than the acetabular labrum. At the connection of both tissues, we found that the microstructure was not a direct adhesion but rather a structural bond, at least with respect to collagen. The results suggest that ligaments potentially attach to tissues other than bone.

KEY WORDS

Acetabular labrum; acetabular transverse ligament; collagen fibrils; microstructure; scanning electron microscopy.

BACKGROUND

The acetabular labrum of the hip joint is not continuous, but rather horseshoe-shaped, with its base connected by the acetabular transverse ligament. This is unlike the shoulder joint with the labrum, which exists all around the glenoid fossa. The acetabular transverse ligament crosses the acetabular incision and visibly connects to the acetabular labrum at the anterior and posterior parts of the acetabular notch (1). The ligament adheres not only to the acetabular labrum but also to the bone, forming a sucker-like structure surrounding the lower part of the femoral head. It has elements that function in conjunction with the acetabular labrum which helps prevent the microinstability of the hip joint (2,3). The shape of the acetabulum varies among ethnic groups (4), and the acetabular transverse ligament and labrum are easily affected, such as in cases of inversion or damage to them, when acetabular dysplasia is present (5,6).

The acetabular diameter is smaller than the femoral head diameter when unloaded (7). The anterior and posterior corners of the lunar surface open with loading to disperse the load, but the acetabular transverse ligament is positioned anteriorly and posteriorly and is thought to spread the force between the corners and participate in load distribution (1). However, a study reported that the amount of
load on the lunar surface does not change after acetabular transverse ligament excision (8). Thus, there are many uncertainties regarding the function of the acetabular transverse ligament. However, the acetabular transverse ligament is considered to be a factor that limits femoral head reduction in the developmental dislocation of the hip, and its excision and resection release the acetabular entrance, rendering the femoral head easier to reduce to the acetabulum (9,10). The acetabular transverse ligament is easy to visualize, even in the joints with arthritic changes; thus, it is clinically useful (e.g., as an indicator of acetabular component placement in total hip arthroplasty) (11-13).

Materials and Methods
The acetabular transverse ligaments and labrums obtained from a 69-year-old woman and an 82-year-old woman during total hip replacement with femoral head necrosis and femoral neck fracture, respectively, were used for the experiments. The acetabular transverse ligaments and labrums were fixed with 10% formalin immediately after sample collection. After fixation, the specimens were immersed in a 5% ethylenediaminetetraacetic acid (EDTA; Wako Pure Chemical Industries, Osaka, Japan) solution for 2 weeks for decalcification. The specimens were then cut into 5- to 10-mm squares, fixed in 10% formalin or 4% paraformaldehyde in phosphate buffer (pH 7.4), dehydrated, and embedded in paraffin.

LM examination
Paraffin sections (5–10 μm) were stained with hematoxylin and eosin (HE; pH 2.5) or alcian blue (AB; pH 1.0 and 2.5) to assess the carbohydrate reactions histochemically and observed under LM (Nikon ECLIPSE Ci-L, Tokyo, Japan) at 2× or 4× magnification.

The slides were deparaffinized and rehydrated with decreasing ethanol passages. Masson's trichrome staining was performed as described below. After washing with running water for 3 minutes and rinsing with distilled water, the slides were treated for 10 minutes with a dedicated mordant (Muto, Tokyo, Japan) for Masson's trichrome staining. Next, the slides were washed with running water for 5 minutes and rinsed with distilled water. To differentiate the nuclei, the slides were then immersed in Carrazzi's hematoxylin for 15 minutes and rinsed in 0.5% hydrochloric acid water. In order to stain the cytoplasm and erythrocytes, the slides were deparaffinated and rehydrated with decreasing ethanol passages. Masson's trichrome staining was observed under LM (Nikon ECLIPSE Ci-L, Tokyo, Japan) at 2× or 4× magnification.

SEM examination
The remaining paraffin blocks were deparaffinized with xylene overnight and re-fixed with a 2.5% glutaraldehyde and 2.0% formaldehyde solution for SEM after observation under an optical microscope. Furthermore, after being immersed in the 2N NaOH solution at 37°C for 3 hours to digest the extracellular matrix, the samples were thoroughly washed with physiological saline and placed in 1% osmium tetroxide, 1% tannic acid, and 1% osmium tetroxide for 1 hour each, dehydrated with alcohol, and then freeze-dried with butyl alcohol. The dried specimens were coated with...
a 4-nm thick gold layer (E102 Ion Sputter, Hitachi, Tokyo, Japan) and examined using SEM (S-4800, Hitachi, Tokyo, Japan) at 5 Kv (14).

Fifty collagen fibrils from multiple microphotographs were used to determine the mean diameter of the collagen fibrils using Java ImageJ software version 1.46d (National Institute of Health, Bethesda, MD). The mean chondrocyte size was determined using 25 chondrocytes from different microphotographs at the same magnification (1000×). To investigate the acetabular labrum’s fibrous cartilage and transverse ligament, the collagen was classified as type I or II based on the diameter of the collagen fibrils (type I, approximately 150 nm; type II, 75 nm) (17).

The study procedures were conducted ethically in accordance with international standards, as required by the journal and as described by Padulo et al. (20). This study was approved by the Ethics Committee of Oita University (approval number, 1053; approval date, 22 July 2016). All experimental procedures were performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients for study participation and publication of their clinical information.

RESULTS

Macroscopic findings

A schematic drawing of the constitution of the acetabular labrum and transverse ligament is shown in figure 1a. In a normal joint, tissues of the acetabular transverse ligament and the labrum are continuous with one another (figure 1b).

LM findings

The acetabular transverse ligament is composed of bundles of collagen fibers and was, thus, eosinophilic in HE-stained specimens but negatively stained in AB-stained (pH 1.0 and 2.5) specimens. In contrast, the acetabular labrum is composed of fibrocartilage; thus, it stained bright in HE-stained specimens and strongly in AB-stained specimens (figure 2). The collagen fibers of the acetabular transverse ligament appeared to be penetrating and surrounding the cartilage tissue of the acetabular labrum in enlarged LM images of the connection of both tissues (figure 3). Masson trichrome staining revealed clear wave-depressing collagen fibers in the acetabular transverse ligament (figure 4).

SEM examination

In low-magnification SEM images of the acetabular transverse ligament, bundles of 30–50-µm wide collagen fibers were arranged in parallel (figure 5). The collagen fibrils composing the acetabular transverse ligament had a mean diameter of 161.28±16.79 (range: 133.3–189.7) nm, forming collagen fibrils classified as type I (figure 6).

In SEM images of the acetabular labrum, the cartilage tissue was composed of chondrocytes and extracellular matrix (figure 7). In high-magnification SEM images of the chon-
Figure 2. Optical microscopy images of the transition between the acetabular transverse ligament and labrum. The acetabular transverse ligament (L) consists of collagen fibers and stains negative on alcian blue (AB) staining (pH 1.0 and 2.5). The acetabular labrum (AL), on the other hand, is composed of fibrocartilage, stains slightly brighter on eosin staining, and is strongly positive for AB staining (pH 1.0 and 2.5).

Figure 3. Magnified optical microscopy images of the transitional part of the acetabular transverse ligament (L) and acetabular labrum (AL). Collagen fibers composing the L appear to penetrate the cartilage tissue.
Figure 4. Optical microscopy image of the transition between the acetabular transverse ligament (L) and acetabular labrum (AL) with Masson’s trichrome staining. The L shows clear wave-depressing fibers, while the AL shows rich cellular components around; no clear wave-depressing fibers appear to be mixed at the junction of the two tissue types.

Figure 5. Low-magnification scanning electron (SEM) microscopy image of the acetabular transverse ligament (L). The L consists of bundles of collagen fibers with a width of 30–50 nm that are arranged in parallel.

Figure 6. Horizontal ligament collagen fibers in high-magnification scanning electron microscopy (SEM) images. As treatment with 2N NaOH digests the extracellular matrix, the collagen fibrils that make up the collagen fibers are clearly observed in the inserted figure. The type I collagen fibrils are arranged in parallel and have a mean diameter of 161.28±16.79 (range: 133.3–189.7) nm.

Figure 7. Scanning electron microscopy (SEM) image of the joint lip cartilage tissue. The cartilage tissue consists of chondrocytes (CC) and extracellular matrix (M).

Chondrocytes and extracellular matrix, the extracellular matrix was densely packed with type II collagen, with a chondrocyte size of 12.52±1.71 (range: 10–15.17) µm (figure 8).

Type I collagen was also present in the acetabular labrum and appeared to cause cartilage tissue disruption, showing a parallel arrangement as seen in the transverse ligament.
Figure 8. High-magnification scanning electron microscopy (SEM) image of the chondrocytes (CC) and extracellular matrix (M). In the M, the thinner collagen fibrils are dense and have a major axis of 12.52±1.71 (range: 10-15.17) µm.

Figure 9. Ultra-low magnification scanning electron microscopy (SEM) image of the transition zone between the acetabular transverse ligament (L) and acetabular labrum (AL). The cartilage tissues are colored to differentiate between both tissues in the L and AL. The tissues of the AL seem to penetrate the tissues of the L.

Ultra-low magnification SEM images of the transition al zone between the acetabular transverse ligament and acetabular labrum revealed that the acetabular transverse ligament penetrated the cartilage tissue composing the acetabular labrum, as seen in LM images (Figure 9). Furthermore, the collagen fibers of the acetabular transverse ligament were interspersed in the acetabular labrum cartilage (Figure 10); they also surrounded and were densely surrounded by cartilage (Figure 11). At the junction of the acetabular transverse ligament and acetabular labrum, type I collagen of the transverse ligament and type II collagen of the acetabular labrum, which had a diameter of
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73.65±8.56 (range: 61.8–89.56) nm, did not bind directly. Instead, the collagen fibers of the acetabular transverse ligament and labrum were interwoven.

DISCUSSION

We assessed the microstructure of the acetabular transverse ligament with respect to the construction and arrangement of the collagen fibers constituting the ligament and their connection with the acetabular labrum. We found that the collagen fibers were interwoven between the cartilage tissues of the acetabular labrum; these structures seemed to be directly connected histologically with one another. The SEM images, however, revealed that the collagen fibers were not directly connected; instead, the fine collagen fibers with varying diameters extending from the structures were interwoven. The present study revealed the microstructure of the acetabular transverse ligament and its connection with the acetabular labrum by delineating collagen for the first time using SEM. A limitation of this study is that the effects of sex and age were not evaluated because only two samples were collected, and both subjects were older women. We also did not evaluate the attachment between the acetabular transverse ligament and bone, which may have indicated a different histological connection from that of the present result. The acetabular labrum has abundant sensory fibers, mechanoreceptors, and blood vessels in the fibrous connective tissue (16). Kapetanakis et al. also found that free nerve endings and nerve end organ presence was greater in the ventral side of the acetabular labrum (21). On the other hand, although Gerhardt et al. reported that sensory fibers and mechanoreceptors are present in the acetabular transverse ligament using LM after histological staining (22), Kilicarslan et al. concluded that it showed good vascularity with abundant free nerve fibers within the fibrous connective tissue; however, the latter group of authors did not find mechanoreceptors using neurofilament protein and S-100 protein immunohistochemistry and microscopy (23). In addition, no chondrocytes have been reported to be present in the transverse ligament, unlike in the acetabular labrum (24). However, very little is known about the microstructure of the acetabular transverse ligament. The acetabular transverse ligament is unusual in that it is a ligament that connects to tissues other than bone. In the present study, the type I collagen fibrils were found to form bundles and collagen fibers, had a width of 30–50 µm, and were arranged in parallel in the acetabular transverse ligament, as seen in other ligaments. The present study also revealed that the histological features of the acetabular transverse ligament were completely different from those of the cartilage tissue of the acetabular labrum, which is mainly composed of type II collagen fibers. The diameter of the type I fibrils of the acetabular transverse ligament was, on average, 161.28±16.79 (range: 133.3–189.7) nm in this study, which is greater than that of general type I collagen and lower than that of tendon collagen (12). In contrast, although the diameter of the type II fibrils in the acetabular labrum, which have a fibrous cartilage structure, was 73.65±8.56 (range: 61.8–89.56) nm, it was similar to that of the collagen fibrils in the hyaline cartilage (17). Further, the collagen fibers of the acetabular transverse ligament were interwoven with the acetabular labrum cartilage, forming a layered structure with collagen fibers of different diameters. These components histologically seemed to be directly joined to each other. However, in high-magnification SEM images, the diameter of the collagen fibers was rather uniform, and their distribution patterns were well segregated from the bundles composed of smaller fibers. Nogami et al. (16) reported that multiple layers of thin type I collagen are interwoven with the cartilage tissue of the acetabular labrum. Conversely, in the adhesive portion of the acetabular transverse ligament, many relatively thick bundles consisting of type I collagen were present and formed a layered structure. Therefore, type II and type I collagen can coexist and seem to give rise to various structures with distinct histological features. Regarding the attachment of the ligament to tissues other than bone, it has been found that a part of the ligamentum teres composed of type I collagen is attached to the surface and inside of the articular cartilage of the femoral head (15).

CONCLUSIONS

The present study revealed the microstructure of the transverse ligament and its attachment to the acetabular labrum. In the macrostructure of the acetabular transverse ligament, collagen fibers with a width of 30–50 µm were arranged in parallel which consisted of bundle with type I collagen fibrils. The acetabular transverse ligament had a distinctly different microstructure than the acetabular labrum with cartilage tissues. In the connection between the acetabular transverse ligament and the labrum, it was found that the microstructure was not a direct adhesion, but rather a structural bond, at least with respect to collagen. Although ligaments usually attach to bone, the results suggest that ligaments can attach to tissues other than bone. As this study only reports findings based on morphological observation, quantitative analysis, such as determining the number of cells or conducting protein analysis, should be performed in detail in future studies. New findings from future studies will contribute to the elucidation of the mechanism underlying the connection of different tissues.
UNBLINDED ETHICS STATEMENT
The study procedures were conducted ethically in accordance with international standards, as required by the journal and as described in Padulo et al. 15. This study was approved by the Ethics Committee of Oita University (approval number, 1053; approval date, 22 July 2016). All experimental procedures were performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients for study participation and publication of their clinical information.

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CONFLICT OF INTERESTS
The authors declare that they have no conflicts of interests.

REFERENCES