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# Is there crosstalk between subchondral bone, cartilage, and meniscus in the pathogenesis of osteoarthritis?

## Osteoartrit patogenezinde subkondral kemik, kıkırdak ve menisküs arasında karşılıklı etkileşim var mıdır?

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***ABSTRACT***

**Objectives:** This study aims to investigate if there is any crosstalk between subchondral bone, cartilage, and meniscus in the pathogenesis of osteoarthritis.

**Patients and methods:** Twelve female patients (mean age 64 years; range 59 to 71 years) with osteoarthritis in medial compartment were included in the study. The samples of subchondral bone, cartilage and meniscus were obtained during total knee arthroplasty. Degenerated tissue samples obtained from medial compartment were used as the experimental group (12 samples of subchondral bone and cartilage, 1x1 cm each; and 12 samples of meniscus, 1x1 cm each). Healthy tissue samples obtained from lateral compartment were used as the control group (12 samples of subchondral bone and cartilage; 1x1 cm each; and 12 samples of meniscus, 1x1 cm each). After decalcification, tissue samples were evaluated with light and transmission electron microscopy.

**Results:** In the experimental group, light microscopic evaluation of subchondral bone samples demonstrated that the cartilage-to-bone transition region had an irregular structure. Degenerated cartilage cells were observed in the transition region and bone cells were significantly corrupted. In the experimental group, light microscopic evaluation of the meniscus samples demonstrated that the intercellular tissue was partly corrupted. Separation and concentration of the collagen fibers were evident. All findings were supported with ultra structural evaluations.

**Conclusion:** Our findings indicate that degeneration of subchondral bone, cartilage, and meniscus probably plays a role in the pathogenesis of osteoarthritis with crosstalk.

***Keywords:*** Cartilage; light microscopy; meniscus; osteoarthritis; subchondral bone; transmission electron microscopy.

***ÖZ***

**Amaç:** Bu çalışmada osteoartrit patogenezinde subkondral kemik, kıkırdak ve menisküs arasında karşılıklı etkileşim olup olmadığı araştırıldı.

**Hastalar ve yöntemler:** Medial kompartmanda osteoartriti olan 12 kadın hasta (ort. yaş 64 yıl; dağılım 59-71 yıl) çalışmaya dahil edildi. Subkondral kemik, kıkırdak ve menisküs örnekleri total diz artroplasti cerrahisi sırasında alındı. Medial kompartmandan elde edilen dejenere doku örnekleri deney grubu olarak kullanıldı (12 subkondral kemik ve kıkırdak örneği, her biri 1x1 cm; 12 menisküs örneği, her biri 1x1 cm). Lateral kompartmandan elde edilen sağlıklı doku örnekleri kontrol grubu olarak kullanıldı (12 subkondral kemik ve kıkırdak örneği; her biri 1x1 cm; 12 menisküs örneği, her biri 1x1 cm). Dekalsifikasyon sonrası doku örnekleri ışık ve geçirimli elektron mikroskopisi ile değerlendirildi.

**Bulgular:** Deney grubunda, subkondral kemik örneklerinin ışık mikroskobik değerlendirmesi kıkırdak-kemik geçiş bölgesinin düzensiz bir yapıda olduğunu gösterdi. Geçiş bölgesinde dejenere kıkırdak hücreleri görüldü ve kemik hücreleri önemli oranda bozulmuştu. Deney grubunda, menisküs örneklerinin ışık mikroskobik değerlendirmesi hücreler arası dokunun yer yer bozulmuş olduğunu gösterdi. Kollajen liflerde ayrılma ve yoğunlaşma belirgindi. Tüm bulgular ultra strüktürel değerlendirme ile desteklendi.

**Sonuç:** Bulgularımıza göre, subkondral kemik, kıkırdak ve menisküsün dejenerasyonu karşılıklı etkileşim ile osteoartrit patogenezinde muhtemelen rol oynamaktadır.

***Anahtar sözcükler:*** Kıkırdak; ışık mikroskopisi; menisküs; osteoartrit; subkondral kemik; transmisyon elektron mikroskopisi.

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Osteoarthritis (OA) has been considered as a disease of cartilage degeneration. Now, it is known as a disease of the whole joint; including cartilage, synovium, bone, bone marrow, menisci, ligaments, and muscles. Osteoarthritis results from a complex system of interacting mechanical, biological, biochemical, molecular, and enzymatic factors.[1-3]

The close physical association between subchondral bone (SCB), cartilage, and meniscus suggests the possibility of biochemical and molecular crosstalk across the interface in healthy and osteoarthritic joints, and better understanding of crosstalk in bone-cartilage interface may lead to development of more effective strategies for treating OA patients.[4,5] Therefore, in this study, we aimed to investigate if there is any crosstalk between SCB, cartilage, and meniscus in the pathogenesis of OA.

## PATIENTS AND METHODS

Twelve female patients (mean age 64 years; range 59 to 71 years) with OA in medial compartment were included. The samples containing SCB, cartilage, and meniscus were obtained during total knee arthroplasty.

Degenerated tissue samples from medial compartment served as the experimental group (12 samples of SCB and cartilage, 1x1 cm each; and 12 samples of meniscus, 1x1 cm each). Healthy tissue samples from lateral compartment served as the control group (12 samples of SCB and cartilage, 1x1 cm each; and 12 samples of meniscus, 1x1 cm each).

Tissue samples were decalcified by placing into ethylenediaminetetraacetic acid solution prepared by glutaraldehyde and formaldehyde. After decalcification, tissue samples which were used for light microscopic evaluations were washed under running water for 24 hours. Increasing degrees of alcohol series (70%, 80%, 90%, 100%) were used for removing the tissue from water. Afterwards, it was passed from xylene textures and paraffin-embedded blocks were prepared. Hematoxylin and eosin staining was performed to 4-5 $\mu$m thick sections. Slides were examined with Photo-light microscope (DM4000B Image Analyze System, Leica, Microsystems, Heidelberg GmbH, Heidelberg, Germany) and Leica DFC280 plus camera.

After decalcification, tissue samples which were used for transmission electron microscopic evaluations were washed under distilled water and then placed into 4% glutaraldehyde solution to complete the fixation. Then, tissue samples were placed in 1% osmium tetraoxide for one hour, followed by fixation and staining. Afterwards, samples were dehydrated with alcohol series and tissues were placed in propylene oxide for 30 minutes, followed by a 30 minute waiting period in embedding material, enabling tissue passage into embedding material. After this, tissues taken into embedding material were placed into a rotator at room temperature for two hours, then placed into an oven at 40 $^{o}$C for another two hours. Finally, tissues were embedded into horizontal embedding blocks within the same mixture. Thin sections were cut with Leica EMUC7 ultramicrotome using a diamond knife (Leica EMUC7, Hernalser Hauptstrasse, Germany), mounted on a copper grid and stained with 2% uranyl acetate and lead citrate. The grids were examined under a Carl Zeiss EVO LS 10 TEM-SEM microscope (Germany).

## RESULTS

Hematoxylin and eosin staining of proximal tibia in the control group demonstrated cartilage cells with normal sequence in cartilage-to-bone formation region and the ossification region was seen clearly (Figure 1, 2).

Ultra structural evaluations of this group revealed that the cartilage cells changed to bone cells in sequence, the nucleus of the cartilage cells had round-shape and normal structure, the nucleus stained densely in the bone cells, and the organelles were not determined clearly because of the dense content of the cytoplasm. The matrix was homogenous surrounding the bone cells and dense collagen fibers were observed in interval space (Figure 3, 4).

Hematoxylin and eosin staining of proximal tibia in the experimental group showed that the cartilage-to-bone formation region had an irregular structure and was partly visible (Figure 5). In the transition region, degenerated cartilage cells were seen and the bone cells were significantly corrupted (Figure 6).

Ultra structural evaluations of this group demonstrated that the change of cartilage cells to bone cells was evident but these two types of cells had a more degenerated structure than the control group (Figure 7). Also, vacuolization increased in the cytoplasm of the bone cells, the surrounding matrix was more homogenized than the control group, and there was a marked dissociation in the collagen fibers (Figure 8a, b).

Hematoxylin and eosin staining of meniscus in the control group showed normal structure and common collagen fibers. Also, extensions of the

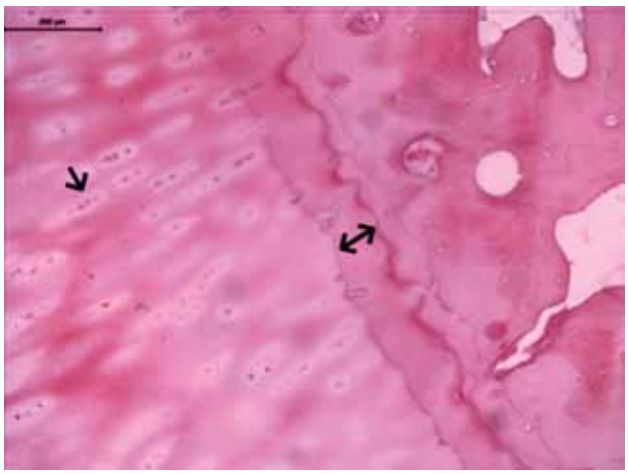

**Figure 1.** Hematoxylin and eosin staining of proximal tibia in the control group: Cartilage-to-bone formation region (↔), the cartilage cells in normal sequence (→) (H-E x 100).

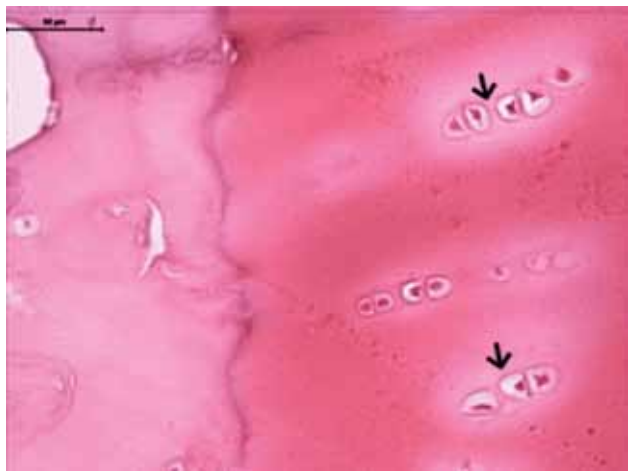

**Figure 2.** Hematoxylin and eosin staining of proximal tibia in the control group: The cartilage cells in normal sequence (→) (H-E x 400).

meniscal cells between collagen fibers were detected (Figure 9).

Ultra structural evaluations of this group revealed that the structural characteristics were the same with light microscopic findings. The nucleus had quite dense chromatin contents and also the cytoplasm was stained darkly in meniscal cells (Figure 10a, b).

Hematoxylin and eosin staining of meniscus in the experimental group demonstrated that the intercellular tissue was corrupted from place to place. Separation and concentration of the collagen fibers were evident (Figure 11).

Ultra structural evaluations of this group showed cytoplasmic density in the cells and homogenized collagen fibers surrounding the cells (Figure 12a, b).

## DISCUSSION

We determined that the intercellular tissue was corrupted from place to place in hematoxylin and eosin staining of meniscus in the experimental group. Separation and concentration of the collagen fibers were evident. The cytoplasmic density in the cells and homogenized collagen fibers surrounding the cells were seen in ultra structural evaluations of this group.

There is evidence that knee structural changes may start from meniscus. Meniscal damages may lead to pathological changes in SCB, including increased bone mineral density, bone cysts, and bone marrow lesions.[6,7] When meniscal lesions are left untreated or treated with partial meniscectomy, shock absorbing function of articular cartilage may be disturbed, the

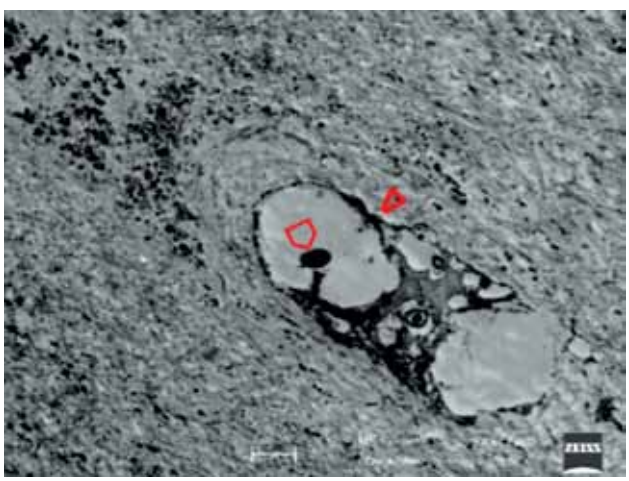

**Figure 3.** Transmission electron microscopic evaluations of proximal tibia in the control group: The cartilage cell (Δ), the cell nucleus (⌂) (Uranyl acetate-Lead citrate x10.30K).

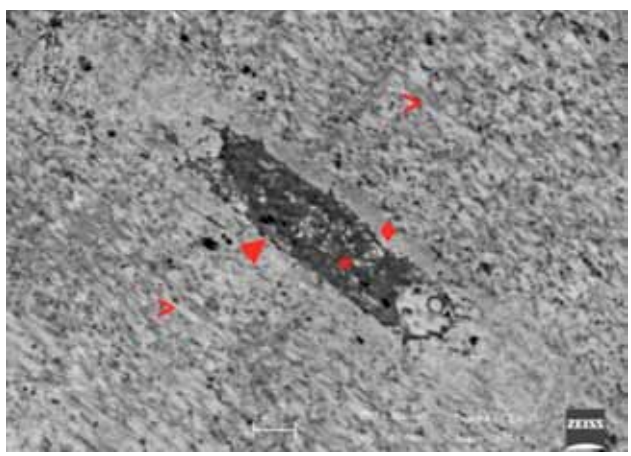

**Figure 4.** Transmission electron microscopic evaluations of proximal tibia in the control group: The bone cell (▶), the cytoplasm with dense content (*), matrix (▶), the collagen fibers (>) (Uranyl acetate-Lead citrate x10.16K).

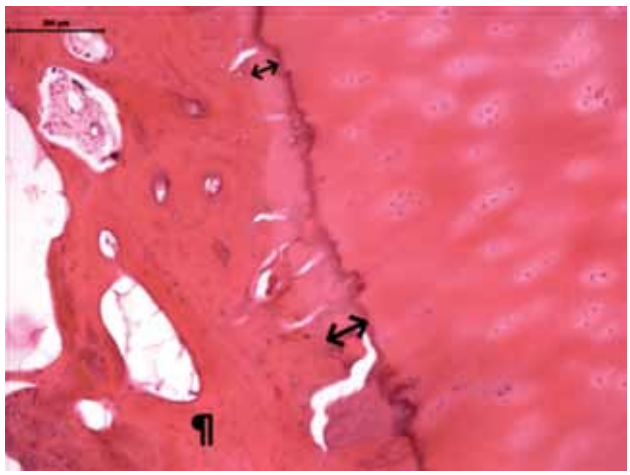

**Figure 5.** Hematoxylin and eosin staining of proximal tibia in experimental group: cartilage-to-bone formation region (↔), ossification region (¶) (H-E x 100).

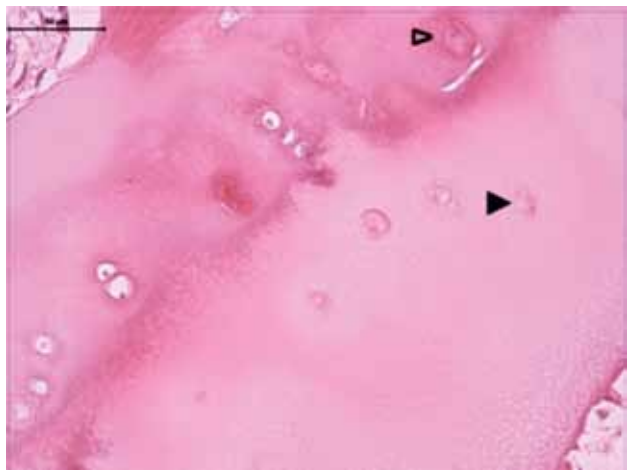

**Figure 6.** Hematoxylin and eosin staining of proximal tibia in experimental group: cartilage cell (Δ), the bone cell (▶) (H-E x 400).

adjacent articular cartilage may be exposed, and compressive and shear forces may be increased.[8]

The cartilage-to-bone formation region had an irregular structure and was partly visible in hematoxylin and eosin staining of proximal tibia in the experimental group. Ultra structural evaluations of this group demonstrated that the vacuolization increased in the cytoplasm of the bone cells, the surrounding matrix was more homogenized than the control group, and there was a marked dissociation in the collagen fibers.

The thinning of SCB may be a sign of developing OA and may cause progression in cartilage degeneration. There is strong evidence that bone marrow lesions (BML) and bone cysts have an important role in the pathogenesis of knee OA.[9] Bone marrow lesions including dense bone islands may decrease elasticity in SCB.[10] Necrotic bone fragments may be phagocytosed by osteoclasts, creating the cyst cavity. A study by Gudbergsen et al.[11] did not show any association between weight-loss and response in BML scores and failed to connect a positive BML response to clinical improvements. Eventually, these may all result in OA by playing a key role in the initiation and progression of cartilage erosion.

Hematoxylin and eosin staining of proximal tibia in the experimental group showed degenerated cartilage cells and significantly corrupted bone cells in the transition region. Ultra structural evaluations of

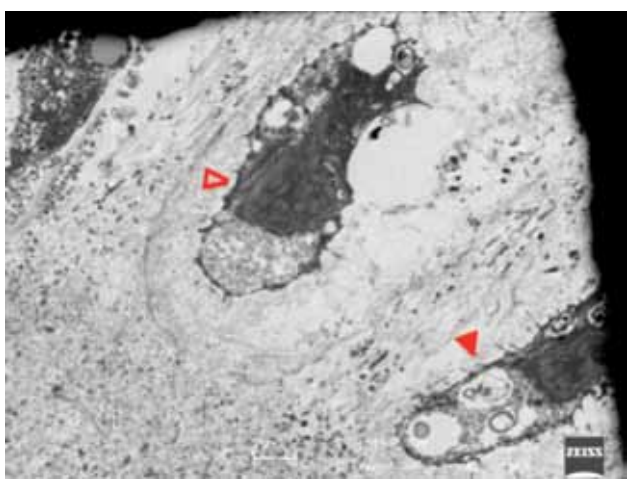

**Figure 7.** Transmission electron microscopic evaluations of proximal tibia in experimental group: cartilage cell (Δ), bone cell (▶) (uranyl acetate-lead citrate x10.16K).

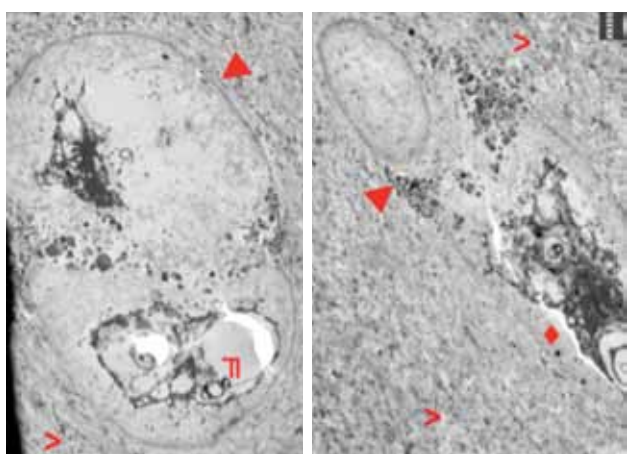

**Figure 8.** Transmission electron microscopic evaluations of proximal tibia in experimental group: bone cell (▶), vacuole (╖), matrix (◆), collagen fibers (>) (uranyl acetate-lead citrate x10.59K).

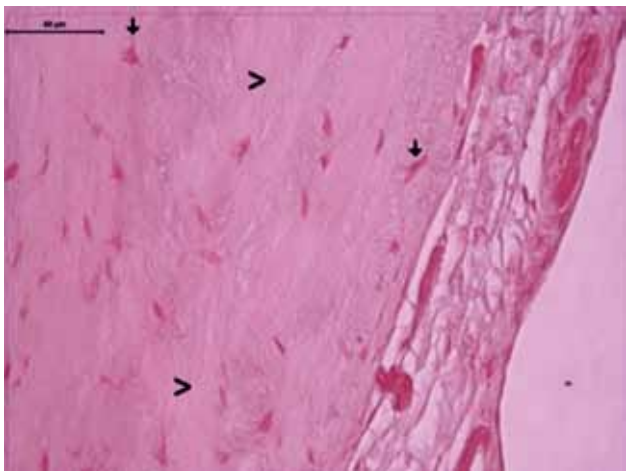

**Figure 9.** Hematoxylin and eosin staining of meniscus in control group: collagen fibers (>), meniscal cells with extensions (↓) (H-E x 400).

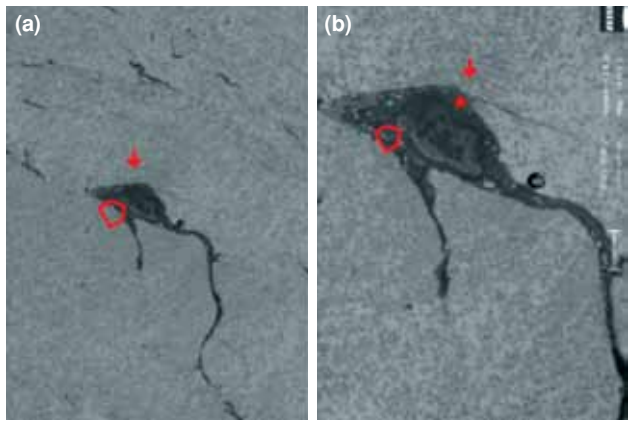


**Figure 10.** Transmission electron microscopic evaluations of meniscus in control group: meniscal cells with extensions (↓), the cell nucleus (⌂), cytoplasm with dense content (*). **(a)** Uranyl acetate-lead citrate x10.16K. **(b)** Uranyl acetate-lead citrate x24.32K).

this group demonstrated that the change of cartilage cells to bone cells was evident but these two types of cells had a more degenerated structure than the control group.

There is radiological evidence which supports that traumatic cartilage injuries have a close relationship with SCB damage like subchondral edema.[12] The primacy of alterations in cartilage versus SCB remains yet to be determined, although a close crosstalk within the osteochondral unit exists.[13] However, cartilage defects did not predict changes in bone size.[14]

High-resolution magnetic resonance imaging (MRI) may be beneficial for the discovery of the factors in the initiation and progression of the disease and differentiation of the disease subgroups. The use of quantitative MRI to assess changes in cartilage volume or thickness is promising. However, widespread use of MRI is limited by cost.

Metabolomics, the analysis of small molecules in urine, serum, synovium, and synovial fluid has led to identifying novel biomarkers for enlightening pathogenesis, diagnosis, monitoring prognosis, prevention, and treatment of several diseases including OA.[15] Knee cartilage defect severity was positively associated with urinary levels of C-terminal cross-linking telopeptide of type II collagen, a specific index for cartilage breakdown. This may lead to prevention of tibial SCB expansion and cartilage defects at an early stage, and also the development of knee OA.[16]

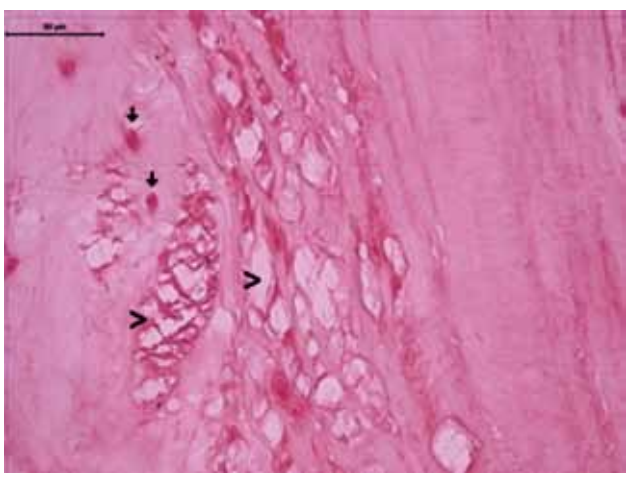

**Figure 11.** Hematoxylin and eosin staining of meniscus in the experimental group: The collagen fibers (>), the meniscal cells with extensions (↓) (H-E x 400).

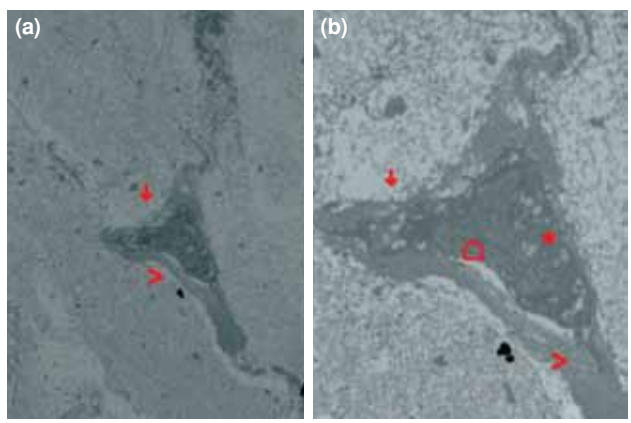


**Figure 12.** Transmission electron microscopic evaluations of meniscus in the experimental group: The meniscal cells with extensions (↓), the cell nucleus (⌂), the cytoplasm with dense content (*), the collagen fibers (>). **(a)** Uranyl acetate-Lead citrate x10.59K. **(b)** Uranyl acetate-Lead citrate x24.32K).

Increased bone turnover has been detected in the early evolution of some forms of OA. Biomarkers in OA can be categorized as burden of disease, investigative, prognostic, efficacy of intervention and diagnostic classification scheme, which were developed by the Osteoarthritis Biomarkers Network with the aim of providing a common framework for communication in the field.[17] Biochemical markers in blood, urine or synovial fluid samples may demonstrate dynamic and quantitative changes in joint remodeling. Increased rate of bone remodeling in early-stage OA may cause alteration in load transmission that predisposes to progressive cartilage loss.

It is also possible to measure cartilage volume and thickness with good precision and accuracy relative to the biological variation of the specimen with a standard laboratory micro-computed tomography (CT) system.[18] Cartilage measurements from micro-CT may improve the knowledge in the relationship between cartilage and SCB and may lead to better understanding of the OA process.

Small number of the patients is the limitation of this study.

In conclusion, degeneration of SCB, cartilage, and meniscus probably plays a role in the pathogenesis of OA with crosstalk. However, the primacy of alterations in meniscus versus cartilage versus SCB remains yet to be determined. High-resolution MRI to assess changes in cartilage volume or thickness, cartilage measurements from micro-CT, and metabolomics may be beneficial for the discovery of the factors in the initiation and progression of the disease, thus preventing the development of knee OA.

**Declaration of conflicting interests**

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