J Orthop Spine Trauma. 2019 September; 5(3): 58-61. DOI: http://dx.doi.org/10.18502/jost.v5i3.4286

Research Article

Electron Microscopy and Microanalysis of Ovis Aries Knee Joint Meniscus: Does It Have Any Similarity with Human Meniscus?

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Received: 05 April 2019; **Revised:** 20 July 2019; **Accepted:** 11 August 2019

Abstract

Background: Meniscus plays a pivotal role in normal function of knee joint and is exposed to heavy load of pressure and trauma. The aim in this study is to investigate ultrastructure of medial and lateral meniscus of Ovis aries, in addition to comparing the findings with human meniscal structure.
Methods: 14 samples of freshly-excised meniscus of ovis aries were provided. After conventional preparation, the samples were studied via electron microscopy (EM) and its elements were microanalyzed.
Results: In the macroscopic evaluation, the meniscus surface was completely smooth, but in the microscopic observation, longitudinal ridges and grooves were observed. In addition, several types of cells that were different morphologically and bundles of collagen fibers were observed. The major direction of collagen fibers was circumferentially, but there were radial fibers as well. In the microanalysis of the ovis aris meniscus, the following elements were present: sodium, carbon, and oxygen, with sodium having the highest percentage among the elements. In medial meniscus of the samples, a small amount of calcium was detected.
Conclusion: By comparing the present findings with those of other studies, many similarities were observed between ultrastructure of ovis aries and human knee meniscus. The compatibility in the ultrastructures will imply the possibility of application of this specimen for xenograft meniscal transplant procedure.

Keywords: Heterografts; Knee; Orthopedic Procedures; Ovis Aries

Citation: Nejadhosseinian M, Paragomi P, Mastery Farahani R. **Electron Microscopy and Microanalysis of Ovis Aries Knee Joint Meniscus: Does It Have Any Similarity with Human Meniscus?** *J Orthop Spine Trauma* 2019; 5(3): 58-61.

Background

Knee joint is one the major synovial joints in the body. The most common surgical operation performed on this joint is meniscectomy. This synovial joint has the largest intraarticular cartilages which play a key role in the ambulatory mechanics of lower extremity (1). One of the intra-articular components of knee joint are medial and lateral menisci. Menisci exert many actions in the joint kinetics including energy dissipation, stress absorption, and protecting cartilage against pressure (2). Hence, menisci are exposed to trauma and physical stress. Unfortunately, after meniscectomy only the peripheral annulus of meniscus is regenerated, hence the newly-built tissue lacks the original quality. Expectedly, meniscectomy increases the risk of osteoarthritis and chondrocalcinosis (3-5). Most cases with meniscal injury are of the youth and middle aged, therefore repairing meniscal damage and preventing osteoarthritis have utmost clinical significance.

Due to the specific structure of cartilage, it is a feasible candidate for various types of transplantation (6). Chondrocytes have a lower metabolic rate and lower antigenic potency as the immune system cannot easily infiltrate into this tissue. Currently, transplantation of an autologous osteochondral cylinder and implantation of autologous chondrocytes are the main options to repair articular cartilage defects (7). At the moment, no total replacement device for meniscus has met Iran's Food and Drug Administration approval (8).

Animal tissue is a candidate for xenograft transplantations. The partial replacement of non-human meniscal tissue including extracellular matrix have yielded favorable outcomes (9). Prior to decision-making, it is pivotal to examine the ultrastructure of the meniscus in other species. Previous studies have shown that the efficiency of ovis models are suitable to investigate the chondroprotective effect as sheep are at high risk of osteoarthritis following meniscectomy (10). A study by Maher et al. revealed the efficacy of implantation of scaffold tissue following partial meniscectomy in the animal model (11). Due to the prevalence of joint disorders and specifically, the financial burden of knee joint disorders, the current study is conducted with the aim to investigate the ultrastructure of medial and lateral menisci of ovis aries.

Methods

The present experimental study was conducted in Shahid Beheshti University of Medical Sciences, Tehran, Iran. This descriptive cross-sectional study began in May 2015 and was concluded in August 2016. In this study, 14 freshly-extracted menisici of Ovis aries were examined via electron microscopy (EM). These samples were prepared by classic method which included the following steps: dissection, fixation, dehydration, clearing, infiltration, embedding, sectioning, and staining.

1- Dissection: This step was performed after sedation of

the animal by chloroform. The size of the excised sections for EM had to be approximately 2 mm × 2 mm and then put into the fixative solution.

2- Fixation: The time gap between dissection and fixation had to be reduced. The major materials used for fixation included 5% solution of glutaraldehyde, 1% solution of osmium tetroxide, and Millonig fixative.
3- Dehydration: At this step, the water was extracted from the sample. Dehydration alcohol, acetone, dioxane, and dimethylformamide were the materials used for dehydration. Ethanol solutions with 10%, 20%, 30%, 50%, 75%, 90%, and 100% concentration and acetone solutions with 25%, 60%, and 95% concentration were used. It is noteworthy that after dissection, the sample tissues were washed with stilled water and buffer solution.
4- Clearing: Alcohol or acetone were extracted from the tissue and replaced with a soluble propylene oxide. This phase was launched with a mixture of 50% ethanol and 50% propylene oxide. After 10 minutes, only propylene oxide was added. This phase was set for 15 minutes and finally, the samples were ready to enter the infiltration stage.
5- Infiltration: This stage means saturating the tissue with the substance which was subsequently used for casting the sample. In the EM techniques, a mixture of propylene oxide and embedding substance (generally resin) was utilized for infiltration. Initially, a mixture of 90% propylene oxide and 10% resin was added to the samples. Afterwards, a mixture of 50% propylene oxide and 50% resin was added. Gradually, the mixture concentration was changed and the proportion of resin was increased. Ultimately, the solution contained 100% resin. Plastic substance gradually infiltrated into the tissues and prepared them for embedding.
6- Embedding: The samples were embedded in plastic casts and filled with plastic substance. After pouring the plastic into the container, the samples were embedded inside in a horizontal or longitudinal direction. The containers were exposed to 60 °C heat in oven for the plastic substance to gel.

Resin was the most useful option for embedding, because it makes the slicing to be performed in a very fine way. In this study, we used 50 cc TAAB embedding resin mixed with 25 cc Dodecenyl Succinic Anhydride (DDSA), 25 cc Methyl nadic anhydride (MNA), and 2 cc of 2, 4, 6 trimethylamino methylphenol (DMP-30). The mixture was stirred to make a homogeneous mixture. The final blend was heated to the boiling point in a capped flask. The flask was kept rotating while heating. Afterwards the flask was cooled to 2 °C by floating in ice water. The final viscosity was similar to a dense syrup. This mixture could be kept in a freezer for an unlimited time.

1- Sectioning: The tissue samples which were trimmed via a device called trimmer were hereafter called blokes. This preparatory phase was called trimming. Sectioning for EM was performed by a special kind of microtome called ultramicrotome.
2- Staining: Uranyl acetate and lead citrate were applied as two stains.

The endpoint of interest was the elemental microanalysis of medial and lateral ovine meniscal tissue.

Primarily, bivariate analysis was carried out by Pearson's chi-square test (χ^2) for categorical variables. P-value of < 0.05 was set as statistically significant. Data were entered in our data base and analyzed by SPSS software (version 20, IBM Corporation, Armonk, NY, USA).

Results

In the macroscopic evaluation, the meniscus surface was smooth with no ridge. In the EM evaluation, in fine slices with minimal magnification, the surface of meniscus was smooth with some wavy ridges with a different extent in various sections. In higher magnifications, two kinds of surface ridges were observed. One type of ripples was prominent and consisted of bulks and ridges which were detectable by scanning electron microscope (SEM). The second type of ripples which was detectable via transmission electron microscope (TEM) in 5000 and 60000 magnifications, included fine surfaces. Similar to articular surface, the meniscal surface was composed of collagen fibrils which were extensively expanded and covered by an electron-dense filamentous layer. Various types of cell morphologies were distinguished at ovine meniscus. Spindle-shaped cells with short cellular extensions were located on the superficial layers of the meniscus. In deeper layers, there were intermediate cells which demonstrated some morphologic variance: In the outer layers of cartilaginous zone, intermediate cells had multiple long fine cytoplasmic processes. Toward inner layers of the meniscus tissue, the number of processes decreased, resulting in spherical cells with no extensions. Similar to the joint cartilage, an extracellular matrix was notable inside the meniscus. The extracellular matrix was occasionally absent in the tissue. In the matrix, collagen fibrils, which are essential components of the structure, dispersed in a proteoglycan-rich matrix and filamentous mesh. On the superficial layers, fibrils were distributed. In the intermediate layers, a more complex structure was noted and the direction of fibers could not be fully inspected by TEM. Collagen fibrils with varying sizes were intermingled to form fibers and lamellas. Most collagen fibers in meniscus were arranged with circumferential orientation. However, radial arrangement of fibers was detected as well.

The microanalysis of the samples was performed, with the findings summarized in the tables and charts. Table 1 shows the mean amount of the traced elements in all medial meniscus tissues evaluated. The two medial meniscus samples had similar contents in this regard. Carbon, oxygen, sodium, and calcium were the detected elements, all of which were analyzed by standard K. The mean proportion of elements in medial meniscus is shown in the fifth column as 9.73%, 12.19%, 44.08%, and 0.3% associated with carbon, oxygen, sodium, and calcium, respectively.

Table 1. Microanalysis of Ovis aries medial meniscus

Element	Spectroscopy type	Inten corn	Std corn (%)	Element (%)	Sigma (%)	Atomic (%)
C K	ED	0.164	0.30	9.73	0.32	22.09
O K	ED	0.412	0.71	12.19	0.45	20.77
Na K	ED	0.865	0.94	44.08	1.71	52.27
Ca K	ED	0.883	1.46	0.30	0.09	0.20
Au M	ED	0.679	0.92	33.70	1.07	4.67

C: Carbon, O: Oxygen, Na: Sodium, Ca: Calcium, Au: Gold, ED: Energy-dispersive, K: constant accounting for the efficiency of the transition

Table 2. Microanalysis of Ovis aries lateral meniscus

Element	Spectroscopy type	Inten corn	Std corn (%)	Element (%)	Sigma (%)	Atomic (%)
C K	ED	0.170	0.30	13.50	0.60	25.56
O K	ED	0.424	0.71	17.64	0.84	26.07
Na K	ED	0.850	0.94	43.06	2.43	44.27
Au M	ED	0.673	0.92	25.81	1.17	3.10

C: Carbon, O: Oxygen, Na: Sodium, Au: Gold, ED: Energy-dispersive, K: constant accounting for the efficiency of the transition

Table 2 shows the elements of spectrography and the related analysis of lateral meniscus samples. The results were homogenous in all examined samples. Carbon, oxygen, and sodium were the elements detected in the lateral meniscus and all were analyzed with standard K. The mean proportion of these elements is as follows: carbon 13.5%, oxygen 17.64%, and sodium 43.06%.

The stains in EM are just means to reach better contrast. Osmium tetraoxide, due to its higher molecular weight, while keeping the texture of tissue, dyes most of their components. The application of dyes such as uranyl acetate or lead citrate will optimize the contrast even in very fine sections.

Discussion

Due to the anatomical structure and weight-bearing nature of knee joints, the menisci are predisposed to trauma and physical stress. Previous studies have revealed that meniscectomy increases the risk of osteoarthritis and chondrocalcinosis (3-5). Studies have revealed that after meniscectomy, only the peripheral annulus is regenerated, hence the new tissue has lower quality. Most cases with meniscal injury are of the youth and middle aged, therefore the prevention of osteoarthritis has clinical significance. At the moment, no total replacement device for meniscus has met Iran's Food and Drug Administration approval (8).

Due to the appropriate location of the knee joint, low metabolic rate of chondrocytes and low antigenic potency, the cartilage is the feasible recipient of transplantation. Moreover, antibodies or immunity cells do not penetrate the matrix of cartilage.

In this study, the ultrastructure of menisci in Ovis aries was assessed. A body of evidence has suggested the similar response to injury in human meniscus and the sheep models (12-14). We observed morphologically-diverse cells which are adapted to their functions. The notion of morphological adaptation in cells is well-recognized in the literature. The cells of annulus fibrosus in bovis intervertebral disc which endure high tensile forces demonstrate similarities with cells in meniscal cartilage and have multiple cellular processes (15). On the other hand, the chondrocytes which are to endure high compressive forces have circular morphology with no cytoplasmic processes (15). The similar morphology of hyaline-like cells of meniscus is due to this notion.

A number of types of superficial irregularities such as longitudinal ridges and fissures were detected via SEM. A study by Ghadially et al. has primarily postulated that in human and rabbit meniscus, varying-diameter collagen fibers are interwoven and form lamellas and fibers (16). Furthermore, the study by Bullough et al. has revealed that the meniscal collagen fibers are predominantly arranged in a circumferential direction (17), and this arrangement of collagen fibers was similar to the Ovis aries meniscus in the present study. Presumably, this structure maximizes the meniscus capability to absorb tension and energy. However, there were some differences with human knee meniscal structure as reported by Petersen et al. (18).

A microanalysis was carried out on the current ovine samples. Previous studies have examined the ultrastructure of patellar cartilage (19, 20). Nitrogen, oxygen, sodium, potassium, carbon, magnesium, fluor, phosphorus, silver, and chloride were the elements detected in these samples. The proportions of these elements in this study suggest the similar ultrastructure of human cartilage and ovine menisci samples in the present study.

A plethora of evidence already introduced the similar biomechanical properties of ovine and human menisci (21, 22). A study conducted by Sandmann et al. showed comparable level of stiffness, residual force, and compression (21). Additionally, the current results revealed comparable proportional level of elements in ovine meniscal tissue.

Our observation of meniscal tissue revealed an entwined mesh of collagen fibrils covered by electron-dense filamentous exterior surface. On the surface of the ovine meniscus, spindle-shaped cells with short cytoplasmic processes were present. Beneath the superficial layer, intermediate plane contains various cells: in the external border cells have multiple, fine, and lengthy processes. In the interior layers, the number of cell processes decrease. Therefore, in the internal surface of meniscus, circular and spherical cells were observed with no cytoplasmic processes. A study by Spindler et al. revealed the varying morphological and functional properties of cells in different layers of canine meniscus (23).

Similar to the articular cartilage tissue, a pericellular matrix is noted in the meniscal tissue. The pericellular matrix has a relatively disperse texture and it is rarely absent in the meniscus. In general, matrix collagen fibrils are the main components of the tissue extended in a matrix comprised of proteoglycan and limited filaments. In the superficial layers, these filaments are arranged parallel to the surface. Meanwhile, in the intermediate layer a more complex structure is noted and the direction of filaments could not be discerned via TEM. Collagen fibrils with varying diameters are interwoven and form fibers and lamellas. Previous studies have revealed the different orientation of collagen fibers in fibrocartilaginous texture of ovine meniscus (24, 25).

Most of peripheral collagen fibers have circumferential orientation running parallel to the peripheral border, however some fibrils with radial arrangement were visible. This structure of collagen fibers is compatible with previous studies (17, 24). The radial fibers have tensor functioning and prevent meniscal ruptures, in addition to withstanding axial stresses (2, 17). This pseudorandom architecture of collagen fibrils is similar to that reported in previous studies on humans (20). In microanalysis of medial meniscus, carbon, oxygen, sodium, and calcium were detected. Sodium was the major element of the medial meniscus as 44.08%. In the lateral meniscal tissue, carbon, oxygen, and sodium were the main elements. Like medial meniscus, sodium was the most abundant element (43.06%).

The main limitation of the current study was the low number of studied samples. On the other hand, the elaborate panel to study and analyze the ultrastructure of

ovine menisci was the main strength of this study.

Conclusion

Given the current detailed structural analysis of ovine tissue, similarities in the macroscopic inspection and ultrastructure of menisci in Ovis aries and human were observed. Given the above, these findings may suggest the possibility of meniscal xenograft transplantation from Ovis aries. This report recommends future studies further explore this prospect.

Conflict of Interest

The authors declare no conflict of interest in this study.

Acknowledgments

None.

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