*Original Article*

# ULTRASTRUCTURAL CHANGES OF THE KNEE JOINT MENISCI AFTER DAMAGE ON THEIR SITES OF INSERTION

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## Summary

The damages of the menisci are the most frequently traumas of the knee joint soft tissues. We cut the cornu anterior of the medial meniscus and then traced out the changes of the menisci. We established that 24 hours after the operation there are erythrocytes on the surface of the SSZ. On the 5th day after the operation most of the surface situated cells have erythrocytes' particles, situated in digestive vacuoles. On the 10th day after the operation there are not free erythrocytes on the surface of the SSZ. In the central part of the SSZ and the TSZ there is dismemberment of the connection between separate components of the matrix of the meniscus cartilage. We observed an increase of proteoglycans in the territorial matrix of the SSZ and in part in the territorial matrix of the TSZ. On the 30th day after the operation there are signs of an increased calcification of the cells of the SSZ and the TSZ. Restoration processes in the meniscus begin in the period 30-50 day after the operation. It can be seen an alignment of the concentrations of the proteoglycans in the matrix of the SSZ and the TSZ and calcium precipitations disappeared.

**Key words:** knee joint, meniscus, damage, insertion

## Introduction

The menisci undertake on the average 50% of the loading in the knee joint while the other 50% are transmitted directly to the articular surface of the tibia [1]. The damage of the menisci is one of the traumas of the knee joint that leads to a harsh change of the biomechanics of the knee. The damages of the menisci are the most frequently traumas of the knee joint soft tissues - on the average 60-74%, as proportion of the traumas of the medial meniscus to the lateral is 11:1 [2]. In addition the damages of the meniscus as and the ligament structures of the knee joint contributed to the instability of the knee. The degenerative changes in the meniscus were begun before the instability of the knee manifests clinically [3]. In addition the menisci have a role in the profound reception of the knee joint. Even an isolated damage of the medial menisus changes for the worse the profound reception of the knee joint [4, 5]. Likewise there is a direct connection between the damages of the menisci and the decreasing of the thickness of the cartilage in the knee joint [6]. On the other hand the biomechanic instability of the knee joint after a damage of the meniscus leads to the changes in the articular cartilage. These changes are typical of the initial stages of the osteoarthrosis [7], namely a fibrillation of the articular cartilage, only 1 month after the meniscectomy. This fibrillation was

observed in a test animal – guinea-pig [8]. These changes gradually lead to layer by layer disorganization of the cartilage and also alteration of the general morphological pattern typical for the osteoarthrosis [9, 10, 11]. It is little known about the changes in the menisci when they are damaged. We decided to investigate the changes in the menici in this condition. We cut the cornu anterior of the medial meniscus and then traced out the ultrastructural changes of the meniscus during the period of two months.

## Materials and Methods

The materials of the investigation were menisci of the knee joint of 20 Wistar rats of both sexes, aged between 8 and 12months, weighing about 250 g each. The fixation was carried out by glutaraldehyde. We used a biomechanical model of osteoarthrosis by cutting the insertion sites of the medial meniscus in the right knee. We cut the insertion site of the cornu anterior without injuring the medial collateral ligament. We repaired the integrity of the skin by a suture. The material from the left joints was used as a control for comparison. Material for investigation was taken 24 hours, 5,10,20,30 and 50 days after the intervention. The taken meniscus material was used for transmission electron microscopy (TEM) and scanning electron microscopy (SEM) and we followed the ultrastructural changes in each zone of the menisci. It was also examined ultrastructurally for proteoglycan complexes with Safranin O.

## Results

We established that 24 hours after the operation there are erythrocytes on the surface of the SSZ There were parts of the erythrocytes incorporated in the surface situated cells. The borders between the separate erythrocytes were unclear. Their cytoplasm had an electron density. The erythrocytes were agglutinated and pilled up in the lateral part of the surface. Some of them were divided by fissures. There were parts of the erythrocytes incorporated in the surface situated cells (Figure 1).

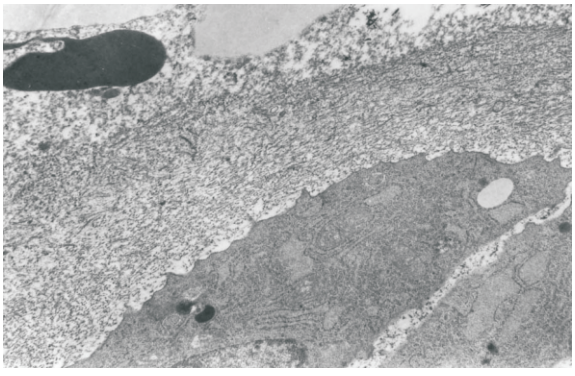


**Figure 1.** Parts of the erythrocytes incorporated in the surface situated cells of the SSZ. TEM; X-11500

On the 5th day after the operation most of the surface situated cells had erythrocytes' particles. They were smaller than the previous and were situated in digestive vacuoles (Figure 2).

There were activated chondroblasts in the Transitional Sliding Zone. We observed development of GER and enlargement of the cisterns (Figure 3). The histochemical investigation showed an increase of proteoglycans in the territorial matrix.

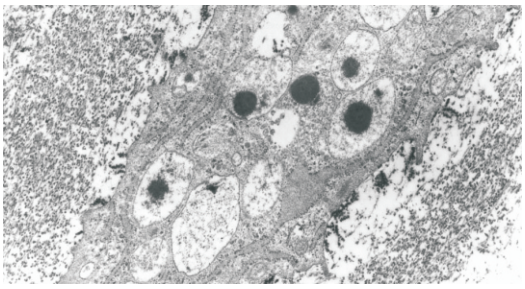


**Figure 2.** Digestive vacuoles containing erythrocytes' particles. TEM; X – 16100

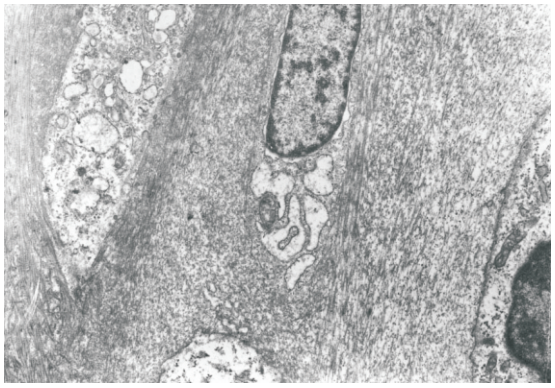


**Figure 3.** Transitional Sliding Zone. There are observed activated chondroblasts in which are seen a development of GER and enlargement of the cisterns. TEM; X-16100

On the 10th day after the operation there were not free erythrocytes on the surface of the SSZ**.** In the central part of the SSZ and the TSZ there was dismemberment of the connection between separate components of the matrix of the meniscus cartilage - oddments of the cells and teared to pieces collagen fibers. On rare occasions there were matrix vesicles and multilamellar bodies (Figure 4). Then it was observed fragmentation of the material of the erythrocytes situated in the complex lysosomes in the surface situated cells. We observed also syderosomes in the cytoplasm of these cells – granular bodies. These bodies sometimes were limited by elementary membrane and they had moderate electron density. At the same time the surface situated cells had signs of activation, namely enlargement of the granular endoplasmic reticulum. There were not changes in the profound layers in comparison with the superficial. The histochemical investigation showed an increase

of proteoglycans in the territorial matrix of the SSZ and in part in the territorial matrix of the TSZ (Figure 5).

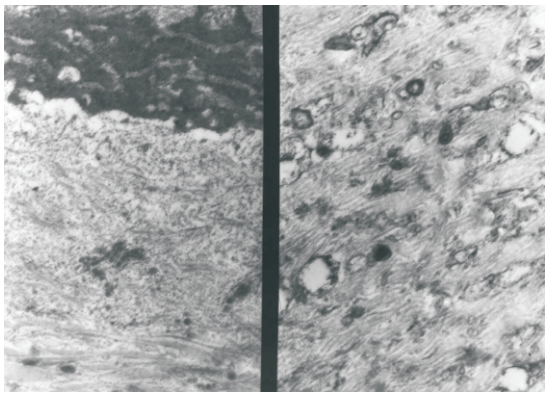
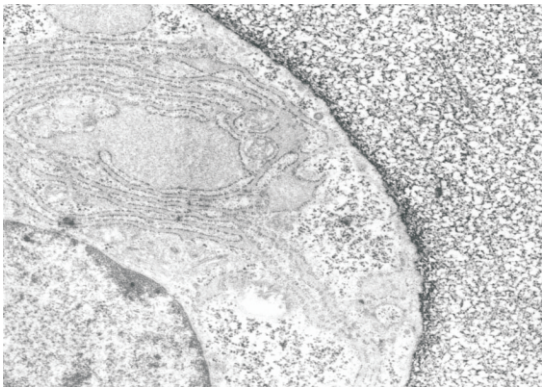


**Figure 4.** Transitional Sliding Zone. There are oddments of the cells and teared to pieces collagen fibers in the matrix – on the left. There are matrix vesicles and multilamellar bodies - on the right. TEM; X-41400



On the 20th day after the operation in the light microscopy examination was observed congestion of the superficial stripe of the meniscus and fibrillations in some places. However such fibrillations there were not in the anterior part of the medial meniscus and on the lateral one. A scanning electron microscopic investigation revealed that there were parallel situated hills and lines (furrows) with different size in the posterior part of the medial meniscus. Their edges were lightly indented whereas their endings gradually were fused with the rubbing surfaces (Figure 6).

The investigation with Safranin O showed a decrease of proteoglycans in the territorial matrix as well as the interterritorial matrix of the meniscus' cartilage close to the central zone. At the same time there was dismemberment of the collagen net.

On the 30th day after the operation there were signs of an increased calcification of the cells of the Superficial and the Transitional Zones of Sliding (Figure 7). We found more cells with signs of degeneration and oddments of the cells compared to a normal cartilage. The intercellular matrix was composed of a net of thick collagen fibers. They formed collagen bundles on the spots appointed. The quantity of specific matrix proteins was strongly decreased. The scanning electron microscopic investigation showed the presence of collagen bundles with quite rough relief, irregular slumps and elevations, and cells contacting with the articular cavity.

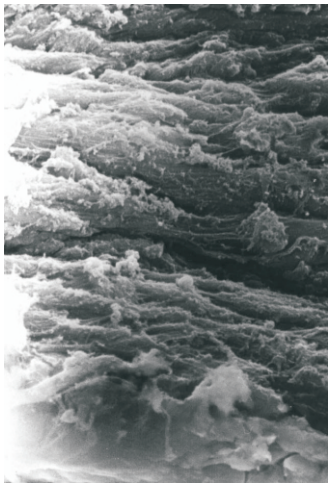


**Figure 6.** There are parallel situated hills and lines (furrows) with different size in the posterior part of the medial meniscus. Their edges were lightly indented whereas their endings gradually were fused with the rubbing surfaces. SEM; X - 6900rubbing surfaces. SEM; X - 6900

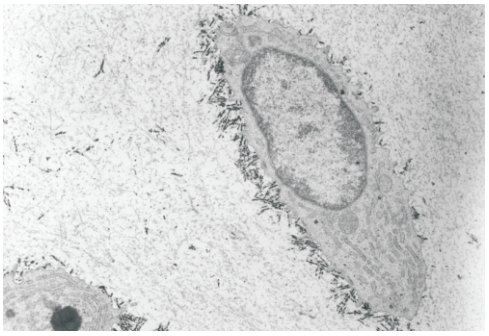


**Figure 7.** There are signs of an increased calcification of the cells of the Superficial and the Transitional Zones of sliding. TEM; X - 7130

The restoration processes in the meniscus began in the period 30-50 day after the operation. It can be seen an alignment of the concentrations of the proteoglycans in the matrix of the SSZ and the TSZ. In the next period the calcium precipitations on the surface of the cells gradually disappeared.

The collagen component of the matrix again thickened and we observed a fine net of collagen fibers type II (Figure 8). At the end of the period was seen an appearance of lamina splendens and alignment of the relief of the meniscus.

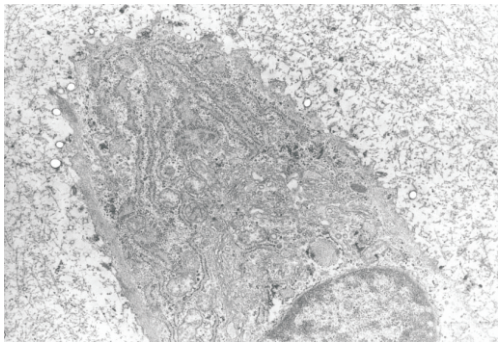


**Figure 8.** There is a fine net of collagen fibers type II in the matrix of the Superficial Zone of Sliding. TEM; X - 14030

## Discussion

The results showed that the changes in the meniscus at different traumatic injuries correspond to the changes in this structure at different models of experimental osteoarthrosis [12, 13]. This gave us a opportunity to make a conclusion that the changes in the meniscus begin in the first in his the most superficial part – a Superficial Sliding Zone and then embraced the other layers. The reason for the above mentioned is the fact that SSZ is metabolic the most active when the functional requirements are increased [14]. The structure of the Central Zone affected in the final at traumatic injuries of the knee joint. The changes in the CZ are directly related to the prior processes in the superficial layers. A similar pattern is observed both at ruptures of the cruciate ligaments of the knee joint and the lesions of the menisci. On the 50$^{th}$ day after the cutting of the meniscus was observed a pattern of developed osteoarthrosis. A fibrous and a profound layer were distinguished ultrastructurally.

We found that the process of resorption of the blood elements depends not only of the state of the synovial membrane [15], the articular cartilage [10], but and from the meniscus. It is clear that the meniscus is a functional active structure that takes participation in the process of the resorption of the hematoma in the knee joint. That is the explanation why parts of erythrocytes were found in the surface situated cells of the SSZ. These erythrocytes' particles are situated in digestive vacuoles and are surrounded by lysosomes. After the 10$^{th}$ day there are not free erythrocytes on the surface of the SSZ**.** The connection between separate components of the matrix is dismemberment in the central part of this zone. The cells have signs of degeneration, whereas in the matrix there are oddments of the cells and teared to pieces collagen fibers. On rare occasions there are matrix vesicles and multilamellar bodies. It is observed an increase of proteoglycans in the territorial matrix of the SSZ and in part in the territorial matrix of the TSZ**.**

On the 20$^{th}$ day after the operation however the concentration of the proteoglycan complexes is decreased in the territorial matrix as well as the interterritorial matrix of the SSZ. The collagen net is dismemberment.

On the 30$^{th}$ day after the operation the scanning electron microscopic investigation shows the presence of collagen bundles with quite rough relief, irregular slumps and elevations. The cells of the Superficial and the Transitional Zones of Sliding have signs of an increased calcification whereas in the intercellular matrix is observed a net of thick collagen fibers formed collagen bundles on the spots appointed. At the same time the quantity of the specific matrix proteoglycans is strongly decreased. These changes are more marked in the posterior part of the medial meniscus than its anterior part and the lateral meniscus. On the 50 $^{th}$ at the latest the restoration processes begin in the meniscus. It can be seen an alignment of the concentrations of the proteoglycans in the matrix of the SSZ and the TSZ. In the next period the calcium precipitations on the surface of the cells gradually disappeared. The collagen bundles disappeared and replaced by collagen fibers type II which formed a fine network. At the end of the period is seen an appearance of lamina splendens and alignment of the relief of the meniscus.

## Conclusions

Our results showed that the changes developed in the meniscus when there is a lesion of meniscus' insertion sites correspond to the alterations observed in this structure at different models of an experimental osteoarthrosis. This gave us a opportunity to make a conclusion that the changes in the meniscus begin in the first in his the most superficial part – a Superficial Sliding Zone and then embraced the other layers. The reason for the above mentioned is the fact that SSZ is metabolic the most active when the functional requirements are increased.

The osteoarthritis changes in the knee joint affected and the menisci. These alterations begin at the superficial zones of the meniscus and then embraced the profound zones on the analogy of the articular cartilage. At last like the articular cartilage are formed two layers – a superficial (fibrous) and a profound layer. The newly formed structure exercising the functions of a meniscus is functionally inadequate.

The menisci are functional active structures. They are affected by the osteoarthrosis together with the other intraarticular structures irrespective of the reasons causing osteoarthritis process. In addition the menisci take participation in the process of the resorption of the hematoma in the knee joint in case of there is an intraarticular hematoma. Later on the menisci take part in the recovering processes in the knee joint.

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