

Voitsenko K.,
Paltov Y.,
Kryvko Y.

Danylo Halytskyi Lviv National Medical University, Department of Normal Anatomy, 69, Pekarska str., Lviv, tel. 8 (0322) 2368443.

MORPHOLOGIC CHARACTERISTICS OF STRUCTURAL COMPONENTS OF THE KNEE JOINT CARTILAGINOUS COVERING ON THE ULTRA-STRUCTURAL LEVEL IN NORMAL STATE

Abstract. *The results of the article correspond to the plan of scientific studies conducted at Danylo Halytskyi Lviv National Medical University and are a part of the scientific research performed at the Department of Normal Anatomy «Structural Organization, Angioarchitectonics and Anthropometric Features of Organs during Intrauterine and Extrauterine Periods of Development under Conditions of Exo- and Endopathogenic Factors» (state registration number 0115U000041) during 2015 – 2019.*

Key words: *cartilaginous covering, ultra-structure, rat, norm.*

Introduction. Timeliness and topicality of the study is determined by a wide use of laboratory animals, and rats in particular, in experimental investigations [1, 2] with the purpose to simulate experimental pathologic conditions [3, 4]. In order to study pathologic changes in certain organs of animals at different stages of development of an experimental pathologic process adequately it is necessary to master fundamental knowledge of the norm on the macro-, micro- and ultra-structural levels [5-7].

The above mentioned has determined the objective of our study that includes investigation of structural organization peculiarities of the knee joint cartilaginous covering on the ultra-structural level in normal state.

Materials and methods. The material of the study was 15 mature outbred male rats with their average body weight of 80 g, and 4,5 month of age. All the animals were kept under vivarium conditions. All the work concerning the issues of their keeping, care, labeling and all other manipulations were conducted according to the regulations of «European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes» (Strasbourg, 1985), “General Ethic Principles of Experiments on Animals” approved by the First National Congress on Bioethics (Kyiv, 2001). The Bioethics Committee of Danylo Halytskyi Lviv National Medical University determined that the studies conducted correspond to ethic requirements according to the Order of the

Ministry of Health of Ukraine № 231 dated 01. 11. 2000 (minutes № 10 vdatedid 26.12. 2011).

Before taking material for biopsy examination an animal was put to sleep by means of dibutyl ether. Cartilages from the distal epiphysis of the femoral bone and proximal epiphysis of the tibia of the knee joint of rats were used as the material for ultra-structural examination. Ultra-structural specimens were prepared according to the common methods [8-10].

Results. The following results were obtained after ultra-structural examination of the layer-by-layer cellular content organization of the knee joint cartilaginous covering in the intact group of rats. Structural organization of the epiphyseal cartilage of the knee joint of intact animals demonstrated preserved architectonics of the cartilaginous tissue. The external surface of a cell-free plate is smooth, covered with a thin electron dense layer, and without any damage. A considerable amount of collagenoid microfibrils are located in the cell-free plate. They are arranged parallel to the joint surface. Single chondrocytes of the superficial area are elongated and narrow. The nucleus of the chondrocytes on the superficial area is volumetric, oval and contains an inconsiderable amount of heterochromatin in the form of a dark strip along the periphery of the nucleus. The central part of the nucleus is mainly filled with euchromatin with fine inclusions of heterochromatin. The surface of chondrocytes is uneven. Short cytoplasmic processes are visualized on it. The cytoplasm

volume of chondrocytes in the superficial area is inconsiderable. It contains a great amount of cisterns of the smooth endoplasmic reticulum, single mitochondria, short profiles of the granular endoplasmic reticulum, ribosomes, and fine electron dense inclusions. Golgi complex with single secretory vesicles is seen close to the nucleus. Fibrils of the collagen fibers in the superficial area matrix are thin, the matrix is homogeneous, and its fibrous components are accurately arranged. Chondrocytes of the transitory area are round shaped and located in the lacunas in isogenic pairs as it is shown in Figure 1.

Microvilli and emergencies are clearly visualized on the surface of chondrocytes as it is shown in Figure 2. The nucleus is mainly filled with euchromatin with inconsiderable inclusions of heterochromatin located mainly in the peripheral areas close to karyolemma. Profiles of the granular endoplasmic reticulum contain protein content, and ribosomes are visualized on its membranes. Golgi complex is well marked, vesicles are located in its cisterns. The cytoplasm contains secretory vesicles, single mitochondria, free ribosomes and single glycogen granules. In the chondrocytes of the transitory area with clearly expressed granular endoplasmic reticulum containing a considerable amount of ribosomes, the cytoplasm is mostly of a moderate electron density, which is indicative of a high activity of protein synthesis, and collagen in particular.

Chondrocytes with well developed smooth endoplasmic reticulum are visualized nearby, their cytoplasm is dark and electron dense as it is shown in Figure 3. It also contains complicated organized Golgi complex with a considerable amount of secretory vesicles with granules. Intensive synthesis of glycosaminoglycans occurs in these chondrocytes. The territorial matrix of the

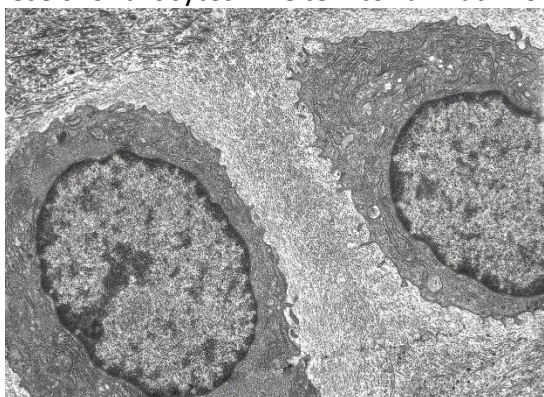
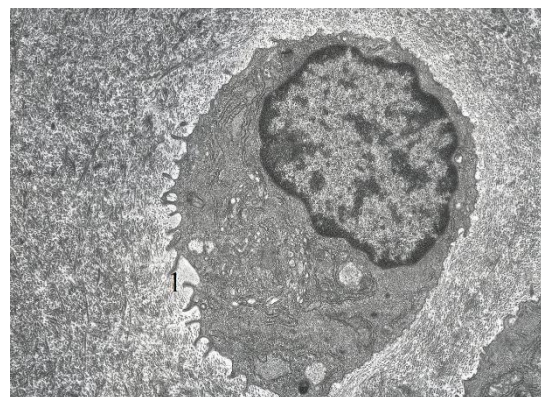


Fig. 1. Ultrastructure of chondrocytes in the transient area of the knee joint cartilaginous covering in normal state. Magnification x 6000.



Fgi. 2. Ultrastructure of chondrocytes in the transient area of the knee joint cartilaginous covering in normal state. Magnification x 6000.

1 – chondrocyte microvilli.

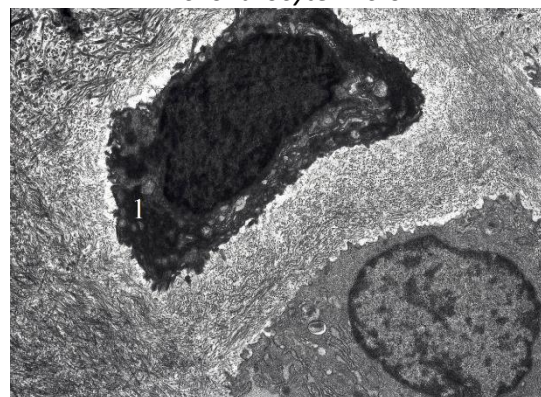


Fig. 3. Ultrastructure of chondrocytes in the transient area of the knee joint cartilaginous covering in normal state. Magnification x 6000.

1 – dark cytoplasm of the smooth endoplasmic network.

transitory area surrounding chondrocytes is preserved. It contains collagen fibrils and proteoglycans. Fibrils of collagen fibers interterritorial matrix are of various orientation. They penetrate into the perilacunar areas and form a network.

In the area of proliferating cartilage the chondrocytes are ellipsoid in shape. They are located in the content of radial columns. Their margins are irregular, they form heterogeneous processes. The nucleus is volumetric, and endoplasmic reticulum is weakly expressed. Mitosis figures of the proliferating cartilage area chondrocytes are often found. Fibrils of collagen fibers in the inter-territorial matrix are thicker compared with the collagen fibers of the superficial and transitory areas.

Volumetric mature chondrocytes with electron light cytoplasm are mainly localized in hypertrophy area. The cytoplasm contains numerous vacuoles, lipid inclusions and numerous glycogen granules, which accumulation is a signal

for ossification. Single chondrocytes are seen in different places. The volume of their cytoplasm increases due to hyperplasia of the granular endoplasmic reticulum, Golgi complex and mitochondria. Chondrocytes in the state of degeneration, necrosis, apoptosis and disintegration are found. Alternative changes are associated with reduction of organelles, increased amount of glycogen granules, lipid inclusions and cytoplasmic fibers. The nucleus of degenerating chondrocytes is polymorphic, wrinkled, with compact intensive osmiophilic homogeneous nucleoplasm. The organelles undergo reduction. Sometimes vacuolated mitochondria and single cisterns of the granular endoplasmic reticulum are found. Inter-territorial matrix of the transient area is volumetric, homogeneous, of a moderate electron density, fine-grained. It contains collagen fibrils thicker than those in the superficial area, and proteoglycan threads of different thickness. Collagen fibers are oriented in different directions. They run through the inter-territorial matrix and penetrate into the perilacunar areas.

Hypertrophic chondrocytes located far from one another and those in degeneration state are localized in the area of calcified cartilage mainly. The lacunas are wide; there are some lacunas without chondrocytes. The nuclei of chondrocytes are smaller in their volume and thick. Cytoplasmic structures are weakly organized with a considerable amount of glycogen granules and lipid inclusions.

Conclusions:

1. The external surface of a cell-free plate is smooth, covered with a thin electron dense layer. A considerable amount of collagenoid microfibrils are located in the cell-free plate. They are arranged parallel to the joint surface. Single chondrocytes of the superficial area are elongated and narrow.

2. Chondrocytes of the transitory area are round shaped and located in the lacunas in isogenic pairs. Microvilli and emergencies are clearly visualized on the surface of chondrocytes.

3. Structural organization of chondrocytes in the transitory area is indicative of a high activity of protein synthesis, and collagen in particular.

4. Volumetric mature chondrocytes with electron light cytoplasm are mainly localized in hypertrophy area. The cytoplasm contains numerous vacuoles, lipid inclusions and numerous glycogen granules. Their accumulation is a signal for ossification.

References.

1. Paltov YeV. *Morphological features of the anatomy of the arterial bed of the neck and maxillofacial area of the rat are normal. Scientific herald of the Lviv National Academy of Veterinary Medicine. SZ Gzhytsky. 2004;6-1(1):113-119. [Published in Ukrainian]*
2. Paltov YeV. *Roengenoanatomy of the arteries of the rat head and neck. Clinical anatomy and operative surgery. 2004;3(2):26-29. [Published in Ukrainian]*
3. Paltov YeV, Kryvko YuYa, Tomashova SA, Vil'hova IV, Matkivskyi RM. *The state of microstructural organization of soft tissues of periodontal disease in white rats in norm and their change at different stages of the course of experimental streptozotocin diabetes mellitus. Bulletin of the Sumy State University. 2006;2(86):36-43. [Published in Ukrainian]*
4. Paltov YeV, Kryvko YuYa. *The ultrastructural organization of soft tissues of periodontal disease in white rats is normal and the dynamics of their changes during the course of streptozotocin-induced experimental diabetes mellitus. World of Medicine and Biology. 2006;3:35-44. [Published in Ukrainian]*
5. Paltov YeV, Chelpanova IV, Fik VB, Vil'hova IV, Kyryk KhA, Kryvko YuYa. *Pathomorphological changes in layers of retina for six weeks of opioid exposure experiment. World of Medicine and Biology. 2017;2(60):146-150. [Published in Ukrainian]*
6. Paltov YeV, Kryvko YuYa. *Pathological changes in the layers of the retina after eight weeks of opioid influence at the experiment. Bulletin of problems biology and medicine. 2017;4.2(140):118-122. [Published in Ukrainian]*
7. Paltov YeV, Fik VB, Kryvko YuYa. *Pathomorphological changes in the retina layer at the end of the fourth week of opioid effect. Natural Science Readings abstracts booc; 2018 May 18; Sosnowiec-Bratislava; 2018. p. 30-32.*
8. Glauert AM. *Fixation, dehydration and embedding of biological specimens. In: Practical methods in electron microscopy. North-Holland: American Elsevier; 1975. 207 p.*
9. Stempak JG, Ward RT. *An improved staining method for electron microscopy. J Cell Biol. 1964;22(3):697-701.*
10. Reynolds ES. *The use of lead citrate at high pH as an electronopaque stain in electron microscopy. J Cell Biol. 1963;17:208-212.*