

**Voytsenko K.I.**

*Danylo Halytskyi Lviv National Medical University, Department of Normal Anatomy, 69, Pekarska str., Lviv, Ukraine*

## **MICROSTRUCTURAL CHARACTERISTICS OF THE KNEE JOINT CARTILAGINOUS SURFACE IN RATS WITHIN THE NORM**

**Abstract.** *Our study determined that the articular cartilage consists of three indistinctly separated zones of the cellular layers: tangential (superficial), transitional (intermediate) and radial (basal). Epiphyseal cartilage from both ends contacts with the osseous tissue and is divided into the zone of rest (unchanged or indifferent cartilage), zone of proliferation (columnar cartilage), zone of hypertrophy (mature or definite cartilage), zone of erosion (destruction), zone of calcification and zone of ossification (osteogenesis). The articular capsule of the knee joint is presented by two layers: fibrous and synovial.*

**Key words:** *knee joint, rats, cartilaginous.*

**Introduction.** Results of the study correspond to the plan of scientific researches performed at Danylo Halytskyi Lviv National Medical University, and are a part of the scientific-research topic of the Department of Normal Anatomy “Structural Organization, Architectonics, and Anthropometric Peculiarities of the Organs during Intrauterine and Extrauterine Periods of Development under Conditions of Exo- and Endopathogenic Factors” (state registration number 0115U000041) during 2015 – 2019.

Numerous factors of exogenous and endogenous origin promote occurrence of osteoarthritis – a chronic progressing disease of the synovial joints first of all with affliction of the hyaline cartilage and subchondrial bone portion resulting from a complicated complex of biomechanical, biochemical or genetic factors [1]. Osteoarthritis belongs to the category of heterogenic group of articular diseases of different etiology with similar biological, morphological and clinical signs [2]. Osteoarthritis is a very widely-spread disease [3]. The disease is a social and economic issue, and one of the leading grounds of a stable loss of ability to work [4].

**Objective:** the above has determined the objective of our study – to investigate the peculiarities of microstructural organization and trophic of the knee joint surface within the norm.

**Materials and methods.** 15 mature male outbred rats, 80 g of body weight, 4,5 months aged, were used as the material for the study. All the animals were kept in vivarium, and the work

concerning their keeping, care, marking, and all other manipulations were conducted according to the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes [Strasbourg, 1985], “General Ethical Principles of Experiments on Animals”, approved by the First National Congress on Bioethics [Kyiv, 2001]. The Bioethics Board of Danylo Halytskyi Lviv National Medical University determined that the conducted scientific studies correspond to the ethical requirements according to the Order issued by the Ministry of Health of Ukraine № 231 dated 01. 11. 2000 (minutes № 10 dated 26.12. 2011).

Before the material for biopsy was taken the animals had been put to sleep by means of intraperitoneal thiopental injection (in the dose of 25 mg/1kg). The material for microstructural examination was distal epiphysis of the femoral bone and proximal epiphysis of the tibia removed from rats considering topographic ratio integrity of the structural components of the knee joints. Histological specimens were made according to the common methods using hematoxylin, eosin and azan by Heidenhein method [5].

**Results.** The following results were obtained after microstructural examination of layer-specific organization of the cellular content in the knee joint cartilaginous surface among intact rats. External surface of the cartilaginous covering on histological sections of the knee joint cavity is smooth and without injuries. The articular cartilage consists of three indistinctly separated

zones of the cellular layers: tangential (superficial), transitional (intermediate) and radial (basal). A thin acellular plate stained by eosin into intensive red borders on the articular cavity. Chondrocytes of the tangential zone are small and not numerous, located in two or three layers at a certain distance from one another, oriented by their long axis parallel to the articular surface. They are elongated in shape, their nuclei are oval, and the cytoplasm volume is inconsiderable (Fig. 1). In moderately acidophilic main substance of the intercellular matrix in the superficial zone fine collagen fibers are found, located tangentially to the surface of the articular cartilage. It is evenly acidophilic stained. The content of acid glycosaminoglycans in the acellular plate and tangential zone is inconsiderable (Fig. 2). The transitional zone matrix is formed by the main substance which is acidophilic stained, moderate content of acid glycosaminoglycans and frame of collagen fibers. Collagen fibers of the transitional zone are located in different directions. Certain powerful bundles of collagen fibers arise from the transitional zone. Other collagen fibers interlace bordering perilacunar areas. Chondrocytes of the transitional zone are round, their nuclei are mostly round containing a fine nucleus located in the center of moderately acidophilic, antireflection cytoplasm sometimes containing fine vacuoles.

Chondrocytes of the cartilaginous covering of the knee joint form isogenic groups and are located within the borders of a single common lacuna. The number of chondrocyte isogenic groups is larger in the areas adjoining the tangential zone. In deeper located areas chondrocytes are in the form of columns oriented perpendicular to the articular surface (Fig. 3).

Radial (basal zone) of the articular cartilage is divided into two parts: radial proper and calcification one. Collagen fibers of the radial zone are rather thick, located radially, connected with the osseous tissue located deeper. The matrix contains a considerable number of acid glycosaminoglycans (Fig. 4). Chondrocytes in the radial zone are located solitarily or contained in the isogenic groups. These cells are volumetric, hypertrophic, with hyperchromic nucleus and antireflection, swollen cytoplasm. Closer to the site of mineralization chondrocytes with

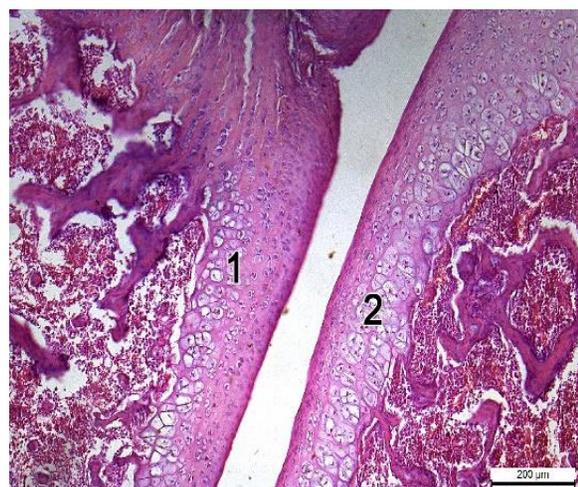


Figure 1. The knee joint cavity of a rat in the norm. Stained with hematoxylin and eosin. Microslide. Magnification x 100. 1 – articular cartilage of the proximal tibia epiphysis; 2 – articular cartilage of the patella.

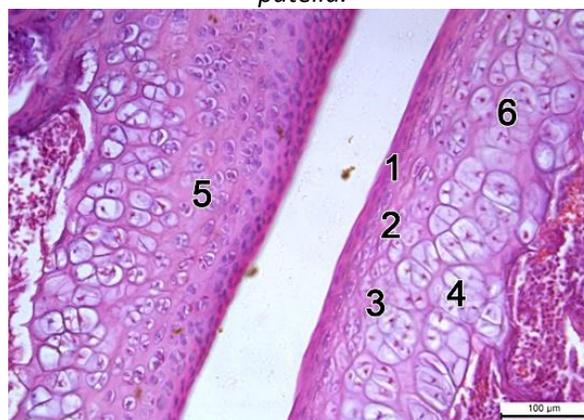


Figure 2. The knee joint cavity of a rat in the norm. Stained with hematoxylin and eosin. Microslide. Magnification x 200. 1 – acellular cartilaginous plate; 2 – chondrocytes of the tangential zone; 3 – chondrocytes of the transitional zone; 4 – chondrocytes of the radial zone; 5 – articular cartilage of the patella; 6 – articular cartilage of the distal femur epiphysis.

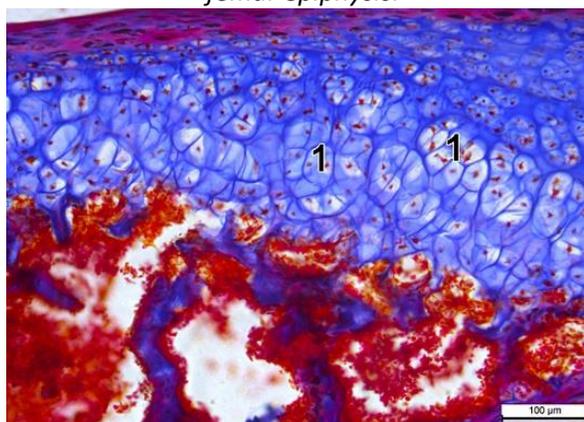


Figure 3. Chondrocytes of the cartilaginous covering of the knee joint in the proximal tibia epiphysis in the norm. Azan staining by Heidenhein. Microslide. Magnification x 200. 1 – formation of isogenic groups by chondrocytes.

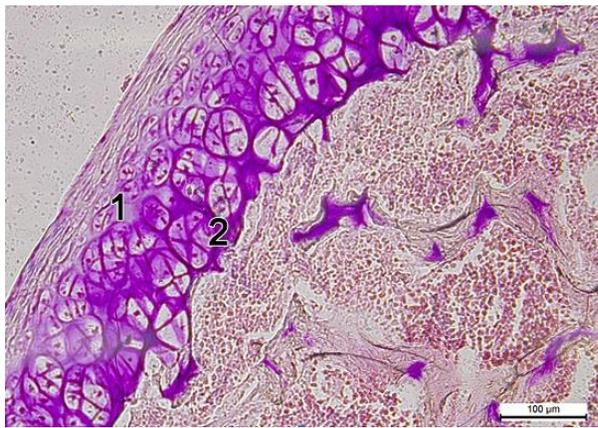


Figure 4. Chondrocytes of the cartilaginous covering of the knee joint in the area of the femur distal epiphysis of a rat in the norm. Azan staining. Microslide. Magnification x 200. 1 – moderate amount of acid glycosaminoglycans in the matrix of the transitional zone; 2 – considerable concentration of acid glycosaminoglycans in the radial zone of the articular cartilage.

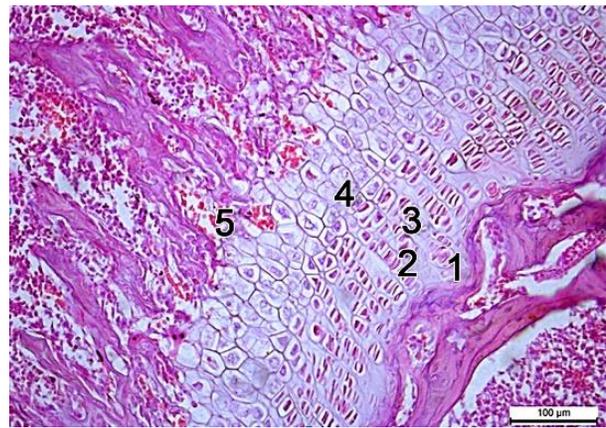


Figure 5. Epiphyseal tibia cartilage of a rat in the norm. Hematoxylin and eosin staining. Microslide. Magnification x 200. 1 – zone of rest; 2 – zone of proliferation; 3 – zone of hypertrophy; 4 – zone of destruction; 5 – zone of osteogenesis.

destructive changes often occur. They contain pyknotic nucleus and weakly outlined antireflection cytoplasm. In the calcification zone along the whole articular surface there is moderately winding basophilic line. Single, moderately filled with erythrocytes capillaries are visualized in the area of calcified cartilage penetrating from the subchondral osseous tissue. The subchondral osseous plate is closely associated with the zone of calcified cartilage, formed by osseous trabecular, and contains a number of blood vessels and nerve elements.

Epiphyseal cartilage from both ends contacts with the osseous tissue and is divided into the zone of rest (unchanged or indifferent cartilage), zone of proliferation (columnar cartilage), zone of hypertrophy (mature or definite cartilage), zone of erosion (destruction), zone of calcification and zone of ossification (osteogenesis) (Fig. 5).

The zone of rest (unchanged cartilage) contacts with the osseous tissue of the epiphysis and contains two layers – external and internal. The contact of the cartilage and bone is firm, and the main substance of the bone trabecula joins partially calcified main cartilage substance. The areas of osseous-chondral connection are visualized, where the main cartilage substance continues into the matrix of the osseous tissue (Fig. 6). Cellular pool of the external layer is presented by fine chondrocytes of an oval or fusiform located solitarily. Calcification of the

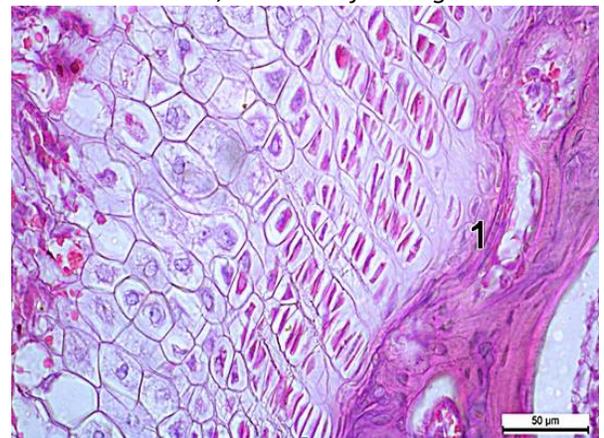


Figure 6. Epiphyseal tibia cartilage of a rat in the norm. Hematoxylin and eosin staining. Microslide. Magnification x 400. 1 – area of osseous-chondral connection.

main cartilage substance is more expressed in the peripheral areas of the epiphyseal cartilage than in the center. In the point of a direct contact of the bone and cartilage vessels sometimes are seen contacting with non-mineralized cartilage. In the internal layer of the superficial zone there is typical hyaline cartilage which main substance is stained basophyically. Chondrocytes of this layer are oval or round in shape, with well-marked lacuna wall, and they rarely form isogenic groups. The proliferation signs of internal layer chondrocytes of the superficial zone are lacking. Collagen fibers of the superficial zone are mostly located parallel to the trabecula of the subchondral osseous tissue. The majority of chondrocytes of the proliferation zone is located in the plane of the longitudinal axis of the metaepiphyseal plate forming elongated columns. Chondrocytes are fine, thickened or wedged-cubic shape. The amount of pale basophilic matrix is

moderate, collagen fibers are located longitudinally, bordering and organizing proliferating chondrocytes into columns. Proximal ends of the columns mostly contain chondrocytes at the stage of differentiation. The zone of hypertrophy contains volumetric mature chondrocytes mostly of cubic shape, located in the columns forming isogenic groups surrounded by a moderate amount of basophilic matrix and collagen fibers. The amount of irregular stained main cartilage substance between the columns increases. Chondrocytes with dystrophic and necrotic changes are visualized. Alternative processes in chondrocytes increase in a distal direction. Swelling, antireflexion and vacuolization of the cytoplasm are determined in these cells. Certain chondrocytes suffer from necrotic changes. In addition to destructive changes in the zones of destruction and calcification there is focal mineralization of the cartilaginous matrix and resolution of the cartilaginous tissue. The remains of chondrocytes with the signs of autolysis are visualized. Structural elements of the bone marrow, blood cells penetrate into deep areas of the ossification zone, vessels filled with erythrocytes grow into them. Rather often osteoblasts producing osteoid are found. In some places the remains of partially calcified intensive basophilic cartilage and newly formed immature enchondral osseous tissue are visualized. In the above areas on the basis of the cartilaginous matrix osseous trabeculae begin to form (the areas of immature osseous tissue) containing a considerable amount of acid glycosaminoglycans (Fig. 7). Non-mineralized

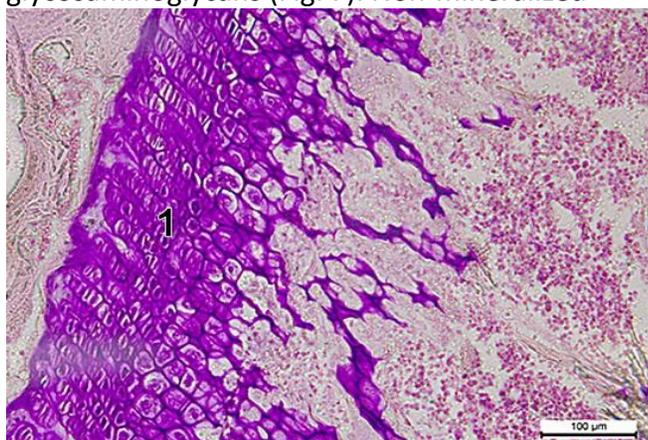


Figure 7. Covering of the knee joint in the area of the epiphyseal tibia cartilage of a rat in the norm. Azure staining. Microslide. Magnification x 200  
1 – considerable amount of glycosaminoglycans in the epiphyseal cartilage and newly formed osseous plates.

young osseous tissue is presented by thin oxyphilic trabecula (primary spongiosa). Intra-trabecular spaces of this zone in addition to reticular-fibrous tissue contain less differentiated cellular elements. In the areas of the secondary spongiosa trabecula made of laminar osseous tissue are visualized.

In the zone of resorption the fragments of partially calcified intensive basophilic cartilage are found, as well as dirty oxyphil newly formed immature osseous tissue containing a considerable amount of acid glycosaminoglycans.

The articular capsule of the knee joint consists of two layers: fibrous and synovial membrane. The fibrous layer consists of the layers of collagen fibers located between the periosteum. Synovial membrane of the articular capsule contains three layers. The internal layer, covering, faces the articular space and consists of 3-4 lines of closely located thickened synovial cells. Synovial cells of the covering layer are located irregular, on different layers, parallel extended to the longitudinal axis of the joint. The basal membrane separating covering cells from the matrix is lacking. The middle layer – superficial collagen-elastic – is built in the form of a thin plate and scattered fibroblasts. In addition to the fibrous structures located longitudinally it contains single lymphocytes, tissue basophils, localized mainly round capillaries. The majority of capillaries are located under the synovial cells of the covering layer. It should be noted that the vessels are located differently both in the deep and superficial collagen-elastic layer. Deep collagen-elastic layer contains the bundles of collagen and elastic fibers and fibroblasts. Synovial membrane of two kinds was determined in the experimental animals: fibrous or adipose (a number of adipose cells were localized in the synovial layer of the adipose type).

**Conclusions:** 1. Our study determined that the articular cartilage consists of three indistinctly separated zones of the cellular layers: tangential (superficial), transitional (intermediate) and radial (basal). 2. Epiphyseal cartilage from both ends contacts with the osseous tissue and is divided into the zone of rest (unchanged or indifferent cartilage), zone of proliferation (columnar cartilage), zone of hypertrophy (mature or definite cartilage), zone of erosion (destruction),

zone of calcification and zone of ossification (osteogenesis).

3. The articular capsule of the knee joint is presented by two layers: fibrous and synovial.

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