FOLIA MEDICA CRACOVIENSIA
Vol. LIX, 3, 2019: 81–93
PL ISSN 0015-5616
DOI: 10.24425/fmc.2019.131138

Morphofunctional features of articular cartilage structure

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Abstract: Background: Articular cartilage is highly-organized nonvascularized tissue which is responsible in humans for pressure absorption under load, as well as for the smoothness of the opposite tangential bone surfaces.
The purpose of our research is to study structural and functional features of articular cartilage at light-optical level by using state-of-the-art research methods of bone-cartilage tissue.
Material and Methods: The study was conducted on samples of femoral heads. Hyperfine sections were subject to hematoxylin and eosin, Van Gieson's and PAS staining. In order to identify the receptor profile of chondrocytes and the features of protein arrangement in extracellular matrix we undertook an immunohistochemical study.
Results: An articular cartilage is quite organized tissue. As any other organ, it has parenchyma and stroma. Parenchyma is represented by one type of cells — chondrocytes, which, depending on how deep they are located in cartilage, have a different shape, size and functional features. The chondrocytes and extracellular matrix have different degrees of receptors expression.
Conclusions: The cartilage is being constantly self-renewed, what is manifested by means of a rather slow division of the surface-located chondrocytes and programmed death of dystrophic-modified cells. The features of extracellular matrix structure determine the originality of cell location in different areas of cartilage tissue. Due to synthesis of specific proteins, chondrocytes self-regulate properties of cartilage tissue.

Key words: articular cartilage, chondrocytes, extracellular matrix, receptors expression, femoral head.

Introduction

Articular cartilage is highly-organized nonvascularized tissue which is responsible in humans for pressure absorption under load, as well as for the smoothness of the opposite tangential bone surfaces [1, 2]. Its uniqueness consists in aparent dominance of extracellular (territorial) matrix (ECM) over cellular composition represented by one type of cells (chondrocytes) and complete absence of blood vessels that makes the nutrition of cartilage possible only for account of extracartilaginous sources [2, 3]. Moreover, structural and functional features of its organization are exclusively subject to its own regulatory mechanisms due to the absence of any innervation [4, 5].

Hyaline cartilage of bone articular surfaces is of quite structured layerwise texture with different content of chondrocytes and ECM which can change with age [6]. Its structure is divided into two clearly-defined parts — noncalcified and calcified cartilage the boundary between which there is a "wavy" line (in foreign literature better known as "Tidemark"). According to various sources, noncalcified cartilage includes from 3 to 6 zones, which are characterized by specific cellular composition and ECM features [3].

Although some researchers consider cartilage as inert tissue, in fact, chondrocytes are metabolically-active cells that need constant flow of nutrients whose shortage results in the development of tissue degeneration [2, 7]. Throughout its existence, there is a process of its continuous renewal affected by chemical and mechanical environment [1, 8].

Therefore, the aim of our research is to study structural and functional features of articular cartilage at light-optical level by using state-of-the-art research methods of bone-cartilage tissue.

Material and Methods

The study was conducted on 10 samples of femoral heads (whirlbones) from male patients, who died of a disease not associated with pathology of the musculoskeletal system, obtained during autopsy (the average age of patients was 42 ± 4.5 years). Using a saw with a replaceable blade, 4 pieces (from the top, bottom, front and rear surfaces) were cut out from each sample 0.5 × 1.0 cm in size and 1.0 cm deep. The tissue was subject to fixation in Carnoy's fluid for 24 hours. Then it was placed into decalcifying solution (70% ethanol, and formic acid 1:1). The specimen removal was carried out when there was an evidence of finally completed decalcification (assessed by a needle test). Dehydration and paraffin-filling were performed in a rotary-type unit like "TM-4M".

Hyperfine sections (3–5 mkm) made on a rotary microtome were subject to hematoxylin and eosin staining using the standard technique. Features of

collagen fibers and glycosaminoglycans arrangement were studied with the help of histochemical Van Gieson's and PAS stain. In order to identify the receptor profile of chondrocytes and the features of protein arrangement in ECM we undertook an immunohistochemical study. The decamouflage of atigens was carried out in a water bath (balneum) "VB-4" at the temperature of 97–98°C. The antigen-antibody reaction was visualized by using a detection system "Ultra Vision Quanto Detection System HRP DAB Chromogen" ("Thermoscientific", USA). It included blocking of peroxidase endogenous activity by hydrogen peroxide, blocking of nonspecific background stain using "Ultra V block", intensification of the reaction "Primary Antibody Amplifier Quanto" and final visualization by means of diaminobenzidin (DUB) with nuclear restaining by means of Mayer's hematoxylin. In the course of the studies, we used antibodies to proteins Ki-67, p53, bcl-2, MMP1, OPN, S100 ("Thermoscientific", USA) (Table 1). This study was approved by the Bioethics Commission of the Medical Institute of Sumy State University (protocol No. 24 from 07.02.2018). and was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization/Good Clinical Practice guidelines.

Table 1. List of antibodies for immunohistochemical reaction.

Antibody	Host	Clone	Dilution	Cellular localization
Bcl-2	*Mouse*	100/D5	1:100	*Cytoplasmic*
S100	*Mouse*	4C4.9	1:150	*Cytoplasmic*
P53	*Mouse*	SP5	1:100	*Nuclear*
Ki-67	*Rabbit*	SP6	1:100	*Nuclear*
MMP1	*Rabbit*	polyclonal	1:50	*Cytoplasmic*
OPN	*Rabbit*	polyclonal	1:100	*Cytoplasmic*

Measuring the size of cartilaginous and subchondral bone tissue was conducted within the morphometric program "Digimizer". Mathematical calculations were performed with the help of Microsoft Excel 2010 with the annex Attestat 12.0.5.

Results

Histological investigation revealed that the articular cartilage thickness varies depending on its location on the femoral head reaching its peak (up to 4 mm) on the bottom and lateral surfaces. Minimum indices are found on the upper part (up to 1 mm). Articular cartilage is divided into 2 parts — noncalcified and calcified cartilage, the boundary between which is a "wavy" line that passes in a zigzag manner at a minor distance from the subchondral bone tissue (Fig. 1). The share of noncalcified cartilage, regardless of its position, covers about 5–8%. The structure of

noncalcified cartilage can include four areas: acellular, surface (tangential), transition and deep (radial). In most cases, the boundaries between those zones are vague.

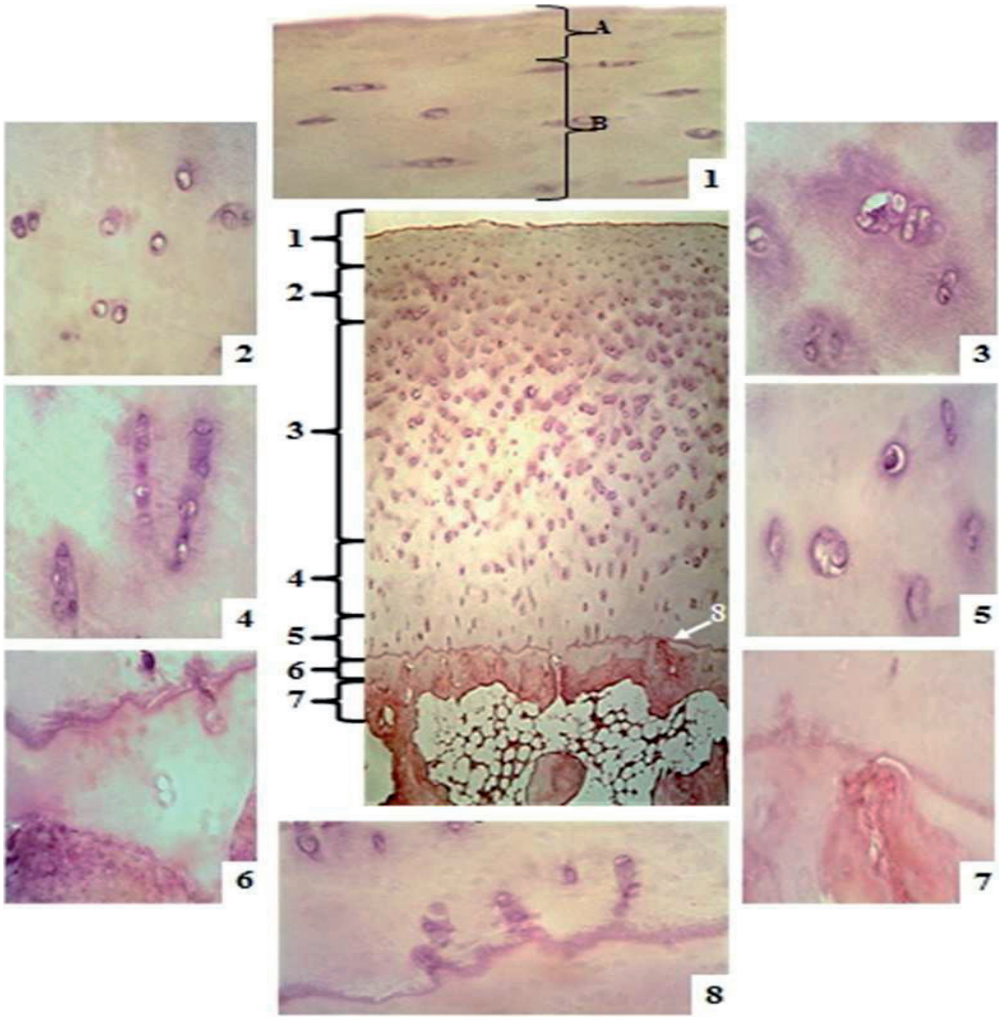

Fig. 1. Cross section (transverse section) of articular cartilage of femoral head. **1.** A — an acellular zone, B — a tangential (surface) zone. **2.** An intermediate layer of the transition zone. **3.** A layer of isogenic cell groups in the transition zone. **4.** A layer of columns in the radial (deep) zone. **5.** A layer of hypertrophic cells in the radial zone. **6.** A calcified cartilage. **7.** A subchondral bone. **8.** A wavy line. Stained with hematoxylin and eosin. Magnification: central image — × 40, 1–8 — × 400.

The acellular zone of articular cartilage, which is directly in contact with synovial fluid, is presented as ECM in the complete absence of chondrocytes (Fig. 1.1A). It accounts for between 1% and 3% of the total thickness of cartilage tissue.

Tangential zone covers about 10% of the total thickness of cartilage tissue. This layer is one of the richest in chondrocytes where the cells are of elongated shape and arranged parallel or at the right angle to the articular surface (Fig. 1.1B). Chondrocytes in this zone are almost completely filled with the nucleus, cytoplasm is represented as a thin rim around the nucleus.

Transition zone is divided into two layers — an intermediate layer and a layer of isogenic cell groups. Cell density becomes lower in the intermediate layer. Chondrocytes are placed separately, at a certain angle to the surface; they are of polygonal and round shape, contain one, sometimes two basophilic nuclei and relatively greater amount (compared to the surface area) of weakly eosinophilic cytoplasm (Fig. 1.2). The layer of isogenic cell groups is represented by groups (up to 1–4 chondrocytes) and clusters (5 or more chondrocytes) of cells which have one nucleus each, more pronounced quantity relative to eosinophilic cytoplasm (Fig. 1.3). The grouped chondrocytes are presented as rounded formations that with deepening first become of triangle, and then of elongated form. The transition zone takes 40–65% of articular cartilage's total thickness.

Deep zone is also divided into two layers — a layer of columns and a layer of hypertrophic cells. The availability of elongated 2–6 cells in the form of a chain that are perpendicular to the joint surface and subchondral bone is the main characteristic of the layer of columns (Fig. 1.4). The cells which are located closer to the "wavy" line undergo degenerative changes and in some cases, take the apoptotic death pathway. In the layer of hypertrophied cells, chondrocytes are mostly arranged individually although their clusters can be found in the form of chains (Fig. 1.5). Chondrocytes in this area are of relatively larger dimensions, they are marked with the degenerative changes and gradually progressive signs of apoptosis, sometimes empty gaps are available. The deep zone covers about 15–30% of the thickness of joint cartilaginous tissue.

The deepest is the area of articular cartilage (calcified cartilage) which mainly consists of ECM where chondrocytes are located separately and individually (Fig. 1.6). This site is in direct contact with the subchondral bone (Fig. 1.7) which has different thickness and sometimes reaches a noncalcified cartilage. The boundary between the two types of cartilage is a double circuit line (a "wavy" line) of intense basophilic, eosinophilic tinge (Fig. 1.8). The wavy line in the areas free of calcified cartilage reaches the bone tissue. In some places it is interrupted due to penetration of closely placed chondrocytes (Fig. 1.6,8).

While studying the autopsy material stained with hematoxylin and eosin, it was found that chondrocytes which are in transition and deep zones of articular cartilage have a certain positional zonality relative to their ECM. They are closer to its intense distal edge with a triangular "tail" towards the articular surface shaping "comets" (Fig. 2) which is connected with the peculiarities of nutrients' inflow to the chondrocytes.

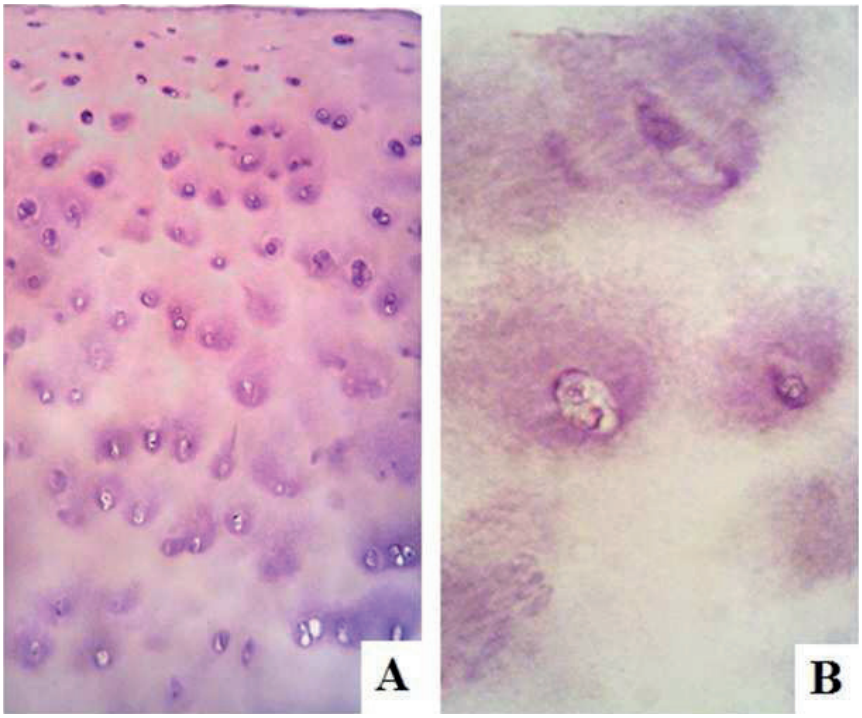

Fig. 2. Cross section (transverse section) of articular cartilage of femoral head. Stained with hematoxylin and eosin. Magnification: A — × 100, B — × 400.

We have divided the entire ECM into 3 parts: pericellular (lacunar), territorial (capsular) and inter-territorial (Fig. 3). Pericellular matrix directly surrounds all the chondrocytes. It is barely visible in the intact cartilage tissue (PAS stain) in the form of a thin, intensively colored rim around cells (Fig. 3A). Its thickness is no more than 3 mkm. Outside of it, there is a much more spacious territorial matrix which has the lowest color intensity on hydrocarbon (Fig. 3B), but intensely accepts basic colorants at staining the agents with hematoxylin and eosin (Fig. 1). The surface area and intermediate layer of the transition zone are almost completely filled with the territorial matrix (Fig. 3.1) with a tendency to gradual decrease in its content in the composition of ECM in deeper located areas of articular cartilage (Fig. 3.2,3). It is followed by inter-territorial matrix which is at PAS stain perceives colorants to a greater extent than the territorial matrix, while less than pericellular matrix (Fig. 3C). It begins to appear in the intermediate layer of the transition zone with a subsequent increase when approaching the wavy line (Fig. 3.2,3). In the calcified cartilage, chondrocytes are deprived of pericellular and territorial areas and surrounded only by the calcified

ECM. The wavy line is moderately evident at PAS stain and clearly demarcates two parts of cartilage tissue (Fig. 3.4).

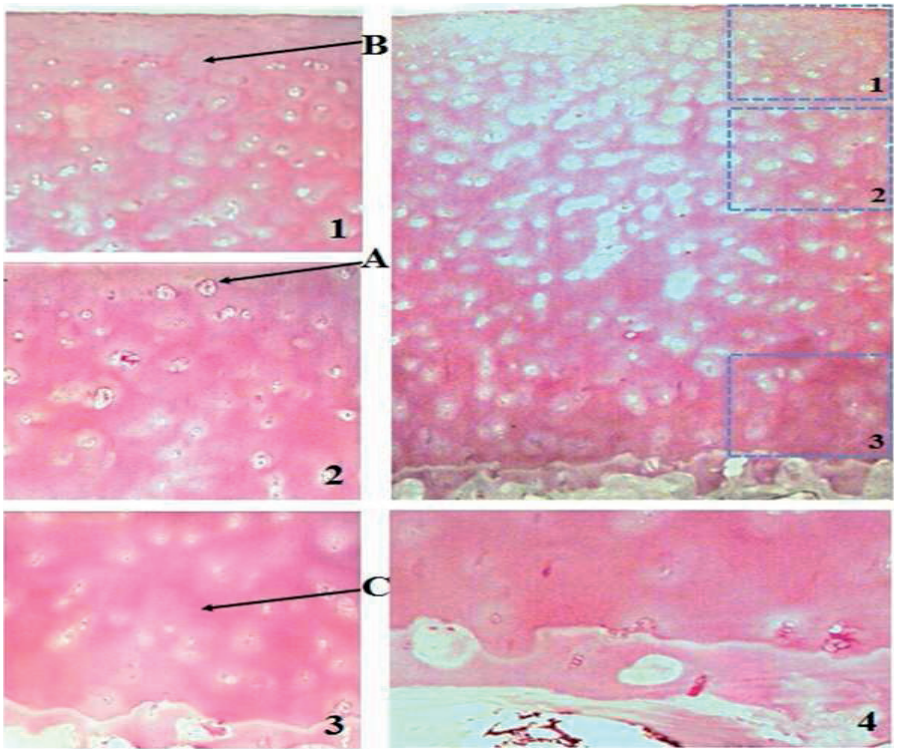

Fig. 3. Cross section (transverse section) of articular cartilage of femoral head. **1.** The acellular and surface zone. **2.** The transition zone. **3.** The radial zone. **4.** A wavy line, a calcified cartilage and a subchondral bone. **A** — pericellular matrix, **B** — territorial matrix, **C** — interterritorial matrix. Stain — PAS. Magnification: large image — × 40, 1–4 — × 100.

In order to identify the features of collagen fibers arrangement in the articular cartilage we carried out Van Gieson's stain. The fibers are painted in bright red (Fig. 4). It was found that the highest collagen density was in the upper (acellular and surface areas) and lower parts (deep zone and calcified cartilage) of articular cartilage (Fig. 4.1,3). The lowest indicators of its content were found in the transition zone (Fig. 4.2). The "wavy" line is also intensely colored in red. In addition, in the lower deep zone sectors there can be traced dash-like focuses located parallel to the "wavy" line that are also magenta positive (Fig. 4.4). Collagen fibers are available only in the interterritorial matrix. Having originated from the subchondral bone tissue, they pass through the entire thickness of cartilage tissue reaching its surface area.

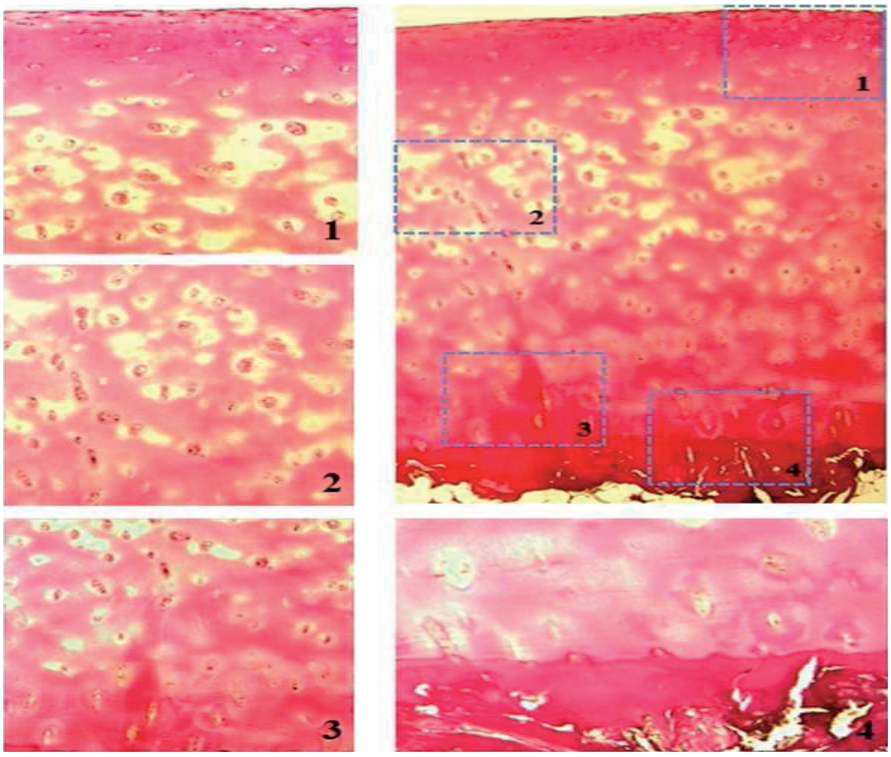

Fig. 4. Cross section (transverse section) of articular cartilage of femoral head. **1.** The acellular and surface zone. **2.** The transition zone. **3.** The radial zone. **4.** A wavy line, a calcified cartilage and a subchondral bone. Stain — Van Gieson's. Magnification: large image — × 40, 1–4 — × 100.

In the course of immunohistochemical study, it was found out that articular cartilage includes solitary cells (in the intermediate layer of the transition zone) with positive nuclear reaction concerning the expression of receptors Ki-67 (Fig. 5.1). Their number does not exceed 1% of the total number of chondrocytes. Within the layer of hypertrophic cells in the deep zone of noncalcified cartilage, as well as in calcified cartilage there are chondrocytes (up to 10% of the cells in these areas) which have cytoplasmic expression of antiapoptotic protein bcl-2 (Fig. 5.2,3). All the 100% of hyaline cartilage cells express receptors to protein S100, which is manifested by the availability of intense cytoplasmic reaction (Fig. 5.4–6). Moreover, even empty gaps (chondrocytes deprived of the core) have intensive stain demonstrating the presence in them of S100 protein receptors (Fig. 5.6). Intranuclear receptors to proapoptotic protein p53 become apparent only in the middle of the articular cartilage (the transition zone) with a tendency to its strengthening in deeper areas (Fig. 5.7,8). P53-positive granules were also available in the territorial ECM. While studying the receptors to OPN, its cytoplasmic expression was found in deeper-seated

chondrocytes and their territorial ECM (Fig. 5.9,12). Instead, proteinase proteins MMR1 were detected in most chondrocytes' cytoplasm of the transition and deep zones and interterritorial ECM (Fig. 5.10,11). Receptors p53 and OPN were intensely expressed in the "wavy" line area (Fig. 5.7–9).

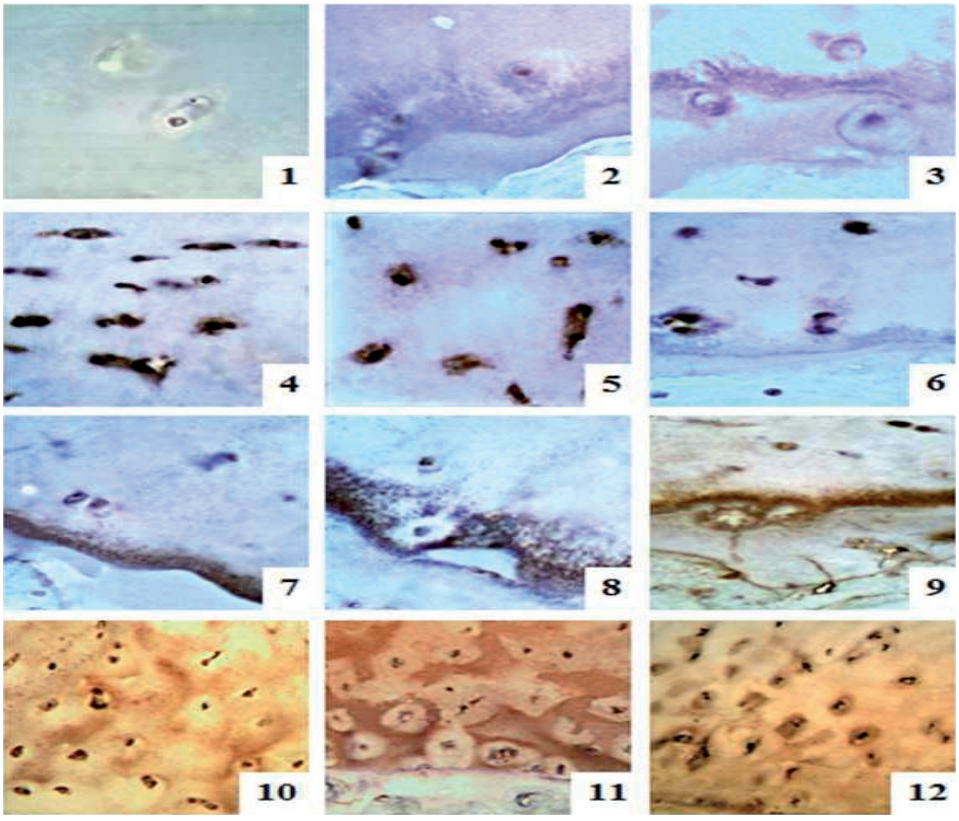

Fig. 5. Cross section (transverse section) of articular cartilage of femoral head. Immunohistochemical study of receptors. **1.** Ki-67. **2–3.** bcl-2. **4–6.** S100. **7–8.** p53. **9, 12.** OPN. **10–11.** MMP1. Magnification: 1–10 — × 400, 11–12 — × 100.

Discussion

As a result, the study ascertained that articular cartilage is quite organized tissue. As any other organ, it has parenchyma and stroma. Parenchyma is represented by one type of cells — chondrocytes, which, depending on how deep they are located in cartilage, have a different shape, size and functional features [1, 3].

Division into zones is carried out in view of the cellular composition and quality characteristics of ECM. Thus, acellular zone is characterized by complete absence of

chondrocytes and significant saturation of tissue with collagen fibers. The latter have parallel (relative to the articular surface) arrangement and high density, providing the resistance of cartilage to compression and stretching, and acting as the first barrier to the penetration of various pathological components of synovial fluid [1]. The same tendency is typical of the surface area which is characterized by considerable saturation with chondrocytes. Taking into account the functional inertness of cells in the tangential zone and considerable saturation with collagen fibers, it is possible to state that this cartilage section is a cambial regenerative layer and it is responsible for tensile counteraction of cartilage tissue.

In the transition zone of articular cartilage, chondrocytes show slight signs of proliferative activity (low Ki-67expression) and take part in the synthesis of glycosaminoglycans (availability of PAS-positive substance in ECM). Slow chondrocytes division (less than 1% of the cells) results in the formation of isogenic cell groups which in the process of embedding, first take a rounded, then elongated shape. This occurs due to their movement along parallel collagen fibers which are arranged perpendicular to the subchondral bone tissue. In the deep zone, reinforced functional activity of chondrocytes can be observed, due to which tissue acquires elastic capacity (binding of glycosaminoglycans with water), counteracting the constant pressure of cartilage tissue surface layers [9].

As chondrocytes approach the "wavy" line, they undergo degenerative changes trigging the programmed mechanisms of their death (availability of expression of proapoptotic p53 proteins), in contrast to which cells try to survive by activating their antiapoptotic mechanisms (positive expression of protein bcl-2 receptors) [10–12]. So, despite the available information about reparative inertness of hyaline cartilage [13], we found out that this tissue is able to renovate at the expense of continuous, though slow, division of chondrocytes and their preprogrammed disposal.

The articular cartilage area, which is closest to the subchondral bone tissue, is characterized by hypocellularity, hypofunctionality, pronounced dystrophic-apoptotic changes of chondrocytes, its saturation with collagen fibers. Thous points to its role in internal resistance to compression due to the gradual change of qualitative characteristics of the supporting tissue (transition from rather flexible cartilaginous tissue to denser bone tissue) that prevents from the development of cartilage atrophy because of pressure when in contact with mineralized bone tissue.

In our study, much attention is also devoted to studying the genesis and function of the so-called "wavy" line. We agree with the idea that this morphological formation is the result of ingress of chondrocytes' metabolic products and ECM degeneration remains when passing through the cartilage tissue of synovial fluid components [14, 15]. While reducing pressure on a joint, it reaches the deepest areas, repelling the degradation remains of collagen, chondrocytes, glycosaminoglycans to the periphery, as indicated by its positive stain at histochemical methods of

investigation and identifying the receptors p53 and OPN. The availability of dash-like staining near the "wavy" line only confirms this fact by pointing to different pressure from the synovial cavity. By the way, the movement of nutrients is bilateral (from the synovial cavity and the bone marrow), that gives them double-circuit (two-contour) look. "Wavy" line is not an accidental formation, it serves as a bilateral barrier on the way of propulsion of various components, both from the synovial cavity and the bone marrow. Its affection can be a key point in the development of joint diseases. However, it is important to remember that the pathological changes of normal structure in different organ promotes the development of combined and complicated diseases which leads to unpredictable consequences [10, 15, 16].

The availability of MMR1 expression in the interterritorial matrix (due to their activation in a neutral environment) and OPN in the territorial matrix indicates the progressive disorganization of cartilaginous tissue in the deeper areas and its affinity with bone tissue which is reached at its constant renewal. Exactly those indices can be the first indicators of cartilage disorganization at rheumatic diseases [17, 18]. The expression of almost all receptor cells to protein S100 is the feature of hyaline cartilage chondrocytes. The presence of S100 protein can indicate the ability of tissue to synthesize the collagen of type II, their participation in reparation and regeneration of cartilage, cell acquisition of the ossifying properties, etc. [19]. It is the degree of expression of proteins MMR1, OPN and S100 that can be a marker of adaptive property of cartilage tissue to changing different load on a joint.

Summarizing the above, the operational diagram of hyaline cartilage of bone articular surfaces can be represented as follows. The cells located in the surface zone are in refractory period (adiaphoria). If there is need for regeneration, they start to multiply, forming isogenic groups, and gradually descend into the transition area. There, they show their pronounced functional activity. As deep zone is reached, degenerative changes start to arise (that can be connected with oxygen and food deficiency) and they come into the apoptotic way of death. The cells located close to the subchondral bone tissue acquire the ability to influence the quality of ECM they have synthesized (synthesis of MMR1, OPN, S100 proteins). Throughout the cartilage, chondrocytes are located exclusively along the collagen fibers that explains both the shape of cells and their isogenic groups. Fibrous tissue originates from the subchondral bone being directed to the surface area continuously and perpendicularly. Then, it changes its direction to parallel relative to the outer surface of the cartilage and reaches the synovial cavity. The nutrition of cells occurs due to the delivery of nutrients from the synovial cavity and the bone marrow, which is comparable to a tidal wave. This explains the emergence of the "wavy" line, its double-circuitness, dash-like formations in the deep area and specific location of the territorial matrix around chondrocytes (in the form of "comets").

Conclusions

What conclusions can we say that articular hyaline cartilage is highly-organized tissue, which even in the absence of innervation and vascularization maintains a clear structure. In the course of human life, cartilage is being constantly self-renewed, what is manifested by means of a rather slow division of the surface-located chondrocytes and programmed death of dystrophic-modified cells. The features of extracellular matrix structure determine the originality of cell location in different areas of cartilage tissue. The qualitative composition of each of the cartilage layers due to location of collagen fibers and the features of glycosaminoglycans content is responsible for its inherent function.

Due to synthesis of specific proteins, chondrocytes self-regulate shock-absorbing and absorbent properties of cartilage tissue quickly and efficiently responding to changes in static and dynamic load on a joint.

List of abbreviations

ECM — extracellular matrix
MMP1 — matrix metalloproteinase 1
OPN — osteopontin

Consent for publication

The patients gave written consent to the use of her personal data and the publication of this article.

Availability of data and materials

The main results of histological and immunohistochemical studies presented in figures and text of this article.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors agreed to be accountable for all aspects of the work and ensuring accuracy and integrity and approved the final version of this manuscript.

Funding

The work was carried out with the support of the research theme № 62.12.01-01.17/20 ЗП

Ethics approval and consent to participate

This study was approved by the Institutional Review Board at the Sumy State University and was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization/Good Clinical Practice guidelines.

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