Morphohistological study of regeneration of knee joint cartilage defects in an experimental model under the influence of nuclear magnetic resonance therapy

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Despite the variety of modern methods of treatment, the problem of hyaline cartilage regeneration is still relevant. Purpose of the study: to determine the effect of nuclear magnetic resonance therapy on the regenerative ability of simulated defects in the cartilage tissue of the knee joint in rats, to assess the dynamics of microscopic changes in articular cartilage in the main and control groups. The study was performed on 60 sex-mature rats. The defects were created in the area of the articular surfaces of the left knee joint - 30 defects in the main group and 30 defects in the control group. The right knee joint of both study groups was used as the norm. Medical (antibiotics, anti-inflammatory, analgesic) therapy was carried out for 3 days after the surgery. On the 4th day after surgery, rats of the main group were given nuclear magnetic resonance therapy for 60 minutes, for 7 days. After 7, 14, 21, 28 days after therapy, histological analysis of cartilage regenerate was performed. 28 days after the use of nuclear magnetic resonance therapy, the height of cartilage regenerate in rats was 82.12±8.89 μm in the intervention group and 56.34±7.82 μm in the control group. Cartilage regenerate in rats after nuclear magnetic resonance therapy was close to the structure of intact hyaline cartilage. However, complete regeneration did not occur, as evidenced by the smaller thickness of the articular cartilage compared to that in the right knee joint. In the control group, the formation of the regeneration had pronounced signs of dysregeneration. The cartilage tissue in the area of the defect, was predominantly fibrous in the nature with areas of necrosis. Nuclear magnetic resonance therapy contributes to the formation of articular cartilage in the defect - cartilage regenerate, which in its histological structure approaches hyaline cartilage.

Keywords: nuclear magnetic resonance therapy, rats, cartilage regenerate, chondrocyte proliferation, experiment.

Introduction
Degenerative joint disease, such as osteoarthritis (OA), is one of the most common pathologies among people, especially after the age of 65, and is a leading cause of disability [4, 10, 15]. Knee joint injuries account for up to 14% of the total number of lower extremity injuries that lead to articular cartilage damage.

Articular cartilage is a type of connective tissue that evenly distributes pressure on the bone, acting as its shock absorber and protection. Given the fact that cartilage tissue is avascular, it is quite difficult for damaged cartilage to recover on its own. Therefore, the restoration of damaged cartilage is achieved due to the proliferation of chondrocytes and the synthesis of the extracellular matrix, which consists of collagen fibers and proteoglycans [5].

The occurrence and progression of degenerative changes in cartilage tissue are associated with various factors, including genetic features, aging, trauma, and environmental exposure [1]. The main pathogenetic factor in the formation of cartilage defects of the knee joint is its destruction, which develops as a result of a mismatch in the distribution of mechanical load and the cartilage's ability to resist it. In case of traumatic damage to articular cartilage tissue, metaplasia or dysregeneration usually occurs, as a result of which hyaline cartilage is replaced by fibrous cartilage [2]. Traumatic damage to articular cartilage leads to degenerative-dystrophic damage to the joint and the occurrence of irreversible secondary arthrotic changes [3, 11]. Despite significant achievements in the study of the
mechanisms of articular cartilage degeneration and the development of new treatment methods, the problem of hyaline cartilage regeneration remains extremely relevant [14, 17, 20].

The purpose of the study: to determine the effect of nuclear magnetic resonance therapy on the regenerative capacity of simulated cartilage tissue defects of the knee joint in rats, to evaluate the dynamics of microscopic changes in articular cartilage in the main and control groups.

Materials and methods

All manipulations with animals were carried out in accordance with the law of Ukraine "On the protection of animals from cruel treatment" dated December 9, 2015 [18]. The experimental animals were cared for in accordance with generally accepted recommendations, requirements and provisions for the care of laboratory animals: the Helsinki Declaration of the General Assembly of the World Medical Association (2000); Provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes" (Stpaczbyg, 1985) [7]. The research protocol was approved at a meeting of the Bioethics Commission of National Pirogov Memorial Medical University, Vinnytsia, Ukraine (protocol No. 7 dated September 16, 2021).

Surgical interventions. The study was performed on 60 sexually mature rats weighing 250.0±50.0 g. In all animals, in aseptic conditions, under intravenous anesthesia with Ketamine solution (50.0 mg/kg), after treating the operative field with Betadine 10 % three times, a skin incision was made and the underlying capsule of the left knee joint through a medial parapatellar approach, measuring ±2-3 mm. Using a scalpel (size 15), a full-layer cartilage defect was created. The open wound was washed with an antiseptic solution of Dekasan, followed by layer-by-layer suturing of soft tissues. The postoperative wound was treated with Sterilium and an aseptic bandage with Betadine 10 % was applied. The first 3 days after damage simulation, for comparative analysis, the right knee joint in both study groups without injury were examined. In all experimental groups, general and local complications were not observed in the postoperative period.

After medical treatment for the animals of the main group, starting from the 4th day after surgery, a cartilaginous program of nuclear magnetic resonance therapy was carried out daily, for 60 minutes, for 7 days. Nuclear magnetic resonance therapy was not used for the control group of animals.

For comparative analysis, the right knee joint in both groups of animals was not damaged.

Rats from the main group were removed from the experiment after 7 (group O1), 14 (group O2), 21 (group O3) and 28 (group O4) days (6 animals for each term) after a one-week course of nuclear magnetic resonance therapy by administering lethal dose of anesthetic (sodium thiopental was administered intramuscularly at the rate of 90 mg/kg). In the control group of animals, nuclear magnetic resonance therapy was not performed, but the rats were removed from the experiment at the same time of the study as from the main group, which corresponded to 17 (group K1), 24 (group K2), 31 (group K3) and 38 (group K4) days (6 animals for each period) from the beginning of the operation.

Histological analysis. To evaluate the morphological changes, the knee joints of the left hind limb of rats of the main and control groups, as well as the knee joints of the right hind limb involving the distal part of the femur and the proximal part of the tibia, were isolated, fixed in a 10 % solution of neutral formalin and decalcified. Preparations were prepared according to standard methods, axial histological sections of joints (5 from each animal) with a thickness of 5-7 μm were stained with Weigert's hematoxylin and eosin, as well as Van Gieson's picrofuchsin [22].

Microscopy and photography were carried out using a light microscope EUROMEX microscopes Holland IScope 1153-PLI. Images were acquired and processed using the "ImageFocusAlpha" program.

Animals were withdrawn from the experiment at intermediate stages of articular cartilage regeneration (7, 14, 21, 28 days) to study the dynamics of the recovery process.

Morphometric studies of articular cartilage at the level of the distal part of the femur and the proximal part of the tibia were performed using the "ImageFocusAlpha" software for the "EUROMEX microscopes Holland IScope 1153-PLI" microscope. The total height of the articular cartilage, the height of the surface, intermediate and zone of calcified cartilage were measured. The intermediate (or mid) zone was considered the layer of articular cartilage from the surface zone to the basophilic line (tidemark) according to the recommendations of Gerwin N. and co-authors [8].

In all experimental groups, general and local complications were not observed in the postoperative period.

Statistical studies. The results of the morphometric study were processed using the Microsoft Excel computer program and presented in the form of M±m. Comparison of mean values was performed using Student's t-test.

Results

To compare the structural changes in the articular cartilage after injury, we examined the cartilage structure of the right knee joint of both study groups without injury.

Morphologically, the following zones could be distinguished in the cartilage: the surface layer, separated from the joint cavity by a thin acellular eosinophilic plate, which contained single flat chondrocytes: the middle layer, with rounded and oval chondrocytes, which were evenly
located in isogenic groups; deep layer where chondrocytes formed columns. The subchondral bone plate was directly adjacent to the deep layer. In the surface layer, elongated chondrocytes, arranged in 2-3 layers along the articular surface, contained large oval hyperchromic nuclei with a thin rim of cytoplasm. The intercellular matrix had a uniform, weakly eosinophilic coloration. In the middle layer, near the surface, isogenic groups of chondrocytes contained 2 to 4 cells. In the deeper layers, chondrocytes formed columns. The basophilic line was visualized along the entire length of the articular surface. The zone of calcified cartilage contained chondrocytes in expanded capsules located at a distance from each other. Such cells had hyperchromic nuclei surrounded by vesicular cytoplasm. Closer to the subchondral bone, chondrocytes had pyknotic nuclei with weakly contoured cytoplasm. Some lacunae did not contain cells. In some places, blood vessels penetrated from the bone tissue to the calcified cartilage (Fig. 1, 2). The described structural features of articular cartilage were characteristic of all articular surfaces of rats that were not traumatized. However, the thickness of the articular cartilage was different, which is related to the long period of the study.

Group O1. In the case of application of nuclear magnetic resonance therapy, a high density of osteoblasts was determined in the newly formed coarse-fiber bone tissue. The central part of the defect was occupied by granulation tissue that completely covered the bottom of the defect and contained a significant number of blood capillaries of various diameters (Fig. 3). The thickness of the regeneration tissue was 33.45±5.17 μm (Table 1).

Group K1. In this research group, the regeneration process was less pronounced. In the regenerate of the bottom of the defect, the formation of bone tissue in the form of coarse-fibered bone trabeculae with a small number of osteocytes was noted. On the outer surface of the bone trabeculae of the subchondral bone, there are numerous osteoblasts in the form of a palisade. The granulation tissue is represented by scattered islands of fibroblastic different cells (Fig. 4). The thickness of the regeneration tissue was 20.53±3.07 μm (see Table 1).

In rats that did not undergo MBST, the articular cartilage

![Fig. 1. The structure of the knee joint of an intact rat. 1 - femur cartilage. 2 - articular cavity. 3 - anterior horn of the meniscus. 4 - tibial cartilage. Hematoxylin and eosin staining. Magnification x100.](image1)

![Fig. 2. The structure of the knee joint of an intact rat. 1 - surface zone. 2 - middle zone. 3 - zone of calcification. 4 - subchondral bone tissue. Hematoxylin and eosin staining. Magnification x400.](image2)

![Fig. 3. Regenerative tissue O1. 1 - coarse fibrous bone tissue. 2 - blood vessels. 3 - granulation tissue. Hematoxylin and eosin staining. Magnification x400.](image3)

**Table 1.** The thickness (μm) of the regeneration cartilage of the left knee joint in different experimental groups of animals.

<table>
<thead>
<tr>
<th>Term, days</th>
<th>Group of animals</th>
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<td>7</td>
<td>33.45±5.17</td>
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<td>14</td>
<td>48.54±7.03</td>
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<tr>
<td>21</td>
<td>64.57±7.86</td>
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<tr>
<td>28</td>
<td>82.12±8.89</td>
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**Note:** p - the reliability of the differences in the average values of the thickness of the regeneration cartilage of the knee joint of the main group compared to the control group in all terms of the experiment.
near the injury zone underwent structural changes. In particular, areas of cartilage without chondrocytes were detected in the surface zone, and the matrix was stained eosinophilic. In the middle zone, some isogenic groups did not contain chondrocytes. The basophilic line throughout the area of the articular cartilage covering the femur was uneven and discontinuous. Significant areas without chondrocytes and uneven staining of the matrix were also observed in the zone of calcified cartilage. At a distance from the site of transchondral damage, structural changes in the articular cartilage were less pronounced.

Fig. 4. Regeneration tissue K1. 1 - coarse fibrous bone tissue. 2 - granulation tissue. Hematoxylin and eosin staining. Magnification x400.

Fig. 5. The structure of articular cartilage K1. 1 - subchondral bone tissue. 2 - intermediate zone of articular cartilage. 3 - absence of chondrocytes in the surface zone of articular cartilage. Hematoxylin and eosin staining. Magnification x100.

Fig. 6. The structure of articular cartilage K1. 1 - subchondral bone tissue. 2 - zone of calcification of articular cartilage. 3 - basophilic line of articular cartilage. 4 - intermediate zone of articular cartilage. 5 - absence of chondrocytes in the surface zone of the cartilage. Hematoxylin and eosin staining. Magnification x400.

Fig. 7. The structure of regeneration tissue O2. 1 - subchondral bone tissue. 2 - regeneration tissue. Hematoxylin and eosin staining. Magnification x100.

Fig. 8. The structure of regeneration tissue O2. 1 - subchondral bone tissue. 2 - newly formed cartilage tissue. 3 - regeneration tissue. Hematoxylin and eosin staining. Magnification x400.

Fig. 9. The structure of articular cartilage K2. 1 - subchondral bone tissue. 2 - dense designed connective tissue. 3 - blood vessels. Hematoxylin and eosin staining. Magnification x400.
and were manifested by a decrease in the density of chondrocytes in the surface zone, inhomogeneity of the color of the matrix, and unevenness of the basophilic line (Fig. 5, 6).

**Group O2.** In rats, which were subjected to MBST after the articular cartilage defect was applied, the histospecies showed a traumatic injury area filled with lamellar bone tissue, which usually did not differ in structure from the adjacent maternal subchondral bone (Fig. 7). On the surface of the defect, which bordered the cavity of the joint, a formation of uneven thickness of the cartilage layer was found. Chondrocytes, which were densely arranged and formed 2-3 rows of isogenic groups, had 2 cells each. They contained basophilic cytoplasm and large rounded nuclei (Fig. 8). The thickness of the regeneration tissue was 48.54±7.03 μm (see Table 1).

**Group K2.** In rats that did not undergo MBST after the articular cartilage defect, the regeneration tissue did not completely fill the defect area. The formation of dense connective tissue, which contained numerous fibroblasts, was noted on the surface bordering the joint cavity. Their long axis was directed parallel to the surface. The bone tissue was located along the perimeter of the defect, from the sides of the parent bone to the center, and contained coarse-fibered bone trabeculae with a significant density of osteocytes and osteoblasts on the outer surface (Fig. 9). The number of fibroblasts in the fibrocartilaginous tissue was higher, compared to the same one, in the previous period of the study. The thickness of the regeneration tissue was 35.27±6.64 μm (see Table 1).

**Group O3.** In the rats treated after the articular cartilage defect was applied, 21 days after nuclear magnetic resonance therapy, in the area of damage, lamellar bone tissue, as in the previous period of the study, did not differ in structure from subchondral bone. On the surface of the defect, which bordered the cavity of the joint, the formation of fibrous cartilage, uneven in thickness, was found. In the middle zone, isogenic groups of cartilage contained 2-3 to 4 chondrocytes, in contrast to the previous period of observation, when isogenic groups contained 2 chondrocytes each. The territorial cartilage matrix is more pronounced in contrast to the previous term of the study. The thickness of the regeneration tissue was 64.57±7.86 μm (see Table 1).

**Group K3.** In the control group of animals that did not undergo MRI, bone trabeculae were located directly under the fibrocartilage layer, had irregular edges and resorption lacunae with osteoclasts and osteoid layering in other areas. The damaged articular surface was covered with fragments of fibrous cartilage. Most of the chondrocytes were destructively changed. Small foci of chondroblast hyperplasia were detected. Fibrous cartilage also formed on the articular surface adjacent to the defect site, replacing the articular cartilage and, without a clear boundary, transitioning into the connective tissue that was located on part of the surface of the preserved articular cartilage. The regenerate was filled with fibrous tissue, its average thickness was 45.42±4.38 μm. The fibers were located mainly parallel to the articular surface. The free surface of the fibrous tissue looked smooth, but the tissue itself formed intra-articular growths. In places, foci of destruction (necrosis) were noted in the fibrous tissue. Fibrous tissue...
contained well-developed blood vessels of the hemomicrocirculatory bed. In addition, on the periphery of the defect area, cells of both damaged and preserved hyaline cartilage were found under the fibrous tissue. That is, excessive (beyond damage to the articular surface) growth of fibrous tissue under which the subchondral bone plate with the applied defect was located was determined (Fig. 10). At the edges of the defect, the plate was unevenly thickened. Because of the bone tissue defect, hemocapillaries and reticular tissue penetrated the area of articular cartilage damage (see Table 1).

Thus, in treated animals, cartilaginous tissue was found in the defect zone, which in terms of histological structure approached the structure of articular hyaline cartilage of intact joints. According to the macroscopic (surface, color, density) characteristics, the regenerate had all the properties of the newly formed hyaline articular cartilage (Fig. 11), which was indicated by the increased number of chondroblasts in the deep zone of the cartilage and eosinophilia of the intercellular matrix. However, the superficial zone was represented by one layer of flattened cells oriented parallel to the surface of the joint (Fig. 12). The thickness of the newly formed cartilage tissue was less than the similar tissue in intact joints and was 82.12±8.89 μm (see Table 1).

Group K4. The articular cartilage, near the injury zone, underwent structural changes. In particular, chondrocytes were absent in the surface zone, and the matrix was stained eosinophilic. In the middle zone, the formation of isogenic groups containing 3-4 cells was noted, some isogenic groups did not contain chondrocytes at all. The basophilic line throughout the area of the articular cartilage covering the femur was uneven and discontinuous. Zones without chondrocytes and uneven staining of the matrix were observed in the zone of calcified cartilage. At a distance from the defect site, structural transformations in the articular cartilage were less pronounced and were manifested by a decrease in the density of chondrocytes in the surface zone, heterogeneity of the color of the matrix, and unevenness of the basophilic line. A high density of fibroblasts was determined in it (Fig. 13).

The articular cartilage, located on the edge of the wound, was degeneratively changed, there was a reduced number of chondrocytes and a thickened zone of calcified cartilage. The cells of the surface layer of articular cartilage were destroyed. In the middle layer, chondrocytes were unevenly located in isogenic groups. The deep layer, where the chondrocytes formed columns, the subchondral bone plate was directly adjacent to the deep zone (the zone of calcification was not clearly visualized) (Fig. 14). The thickness of the cartilage was 56.34±7.82 μm (see Table 1).

Discussion

In the course of the histological analysis of defects of the articular cartilage of the knee joint in an experimental model under the influence of nuclear magnetic resonance therapy (MBST), significant morphological changes were found, which testify to the positive role of MBST in cartilage regeneration. At the first stage of the study, it was established that the control group, which did not receive MBST, showed pronounced signs of cartilage...
degeneration. In particular, wide zones of necrosis were detected, as well as loss of the cartilage matrix and a decrease in the number of chondrocytes.

In the group of animals subjected to MBST, the articular surface at the site of injury was lined with a newly formed covering of cartilage tissue, and the regenerate had all the properties of hyaline cartilage, which was indicated by an increased number of chondroblasts in the deep zone of the cartilage and eosinophilia of the intercellular matrix. Histological analysis also showed an increased number of living chondrocytes, as well as signs of their greater differentiation compared to the control group, which also indicates stimulation of cartilage regeneration under the influence of MBST.

Our study was also accompanied by a comparative analysis with the work of Gerwin N. and co-authors conducted in 2010 [8]. The authors of this paper recommended using criteria for evaluating histopathological changes in cartilage in osteoarthritis. In our study, we similarly divided the cartilage into three zones: superficial, deep, and calcification zone, and the use of MBST contributed to the improvement of morphological indicators.

In the context of the histological changes found in our study, the molecular aspects mentioned in the study by Chen H. and co-authors [5] should also be taken into account. The maintenance of a higher level of chondrocyte differentiation may be related to the molecular mechanisms that support the proliferation and differentiation of these cells.

Our study in a control group of rats is also consistent with the study by Bets I. G. and co-authors, conducted in 2018 [2], in which the authors showed the stages of articular cartilage regeneration after a full-layer defect of cartilage tissue, resulting in the formation of fibrous cartilage tissue. Restoration of hyaline cartilage at the site of damage did not occur at all stages of the study. However, in our study, in the group of animals that underwent nuclear magnetic resonance therapy, regeneration in the defect zone of subchondral bone tissue and articular cartilage was more pronounced, as indicated by the thickness of regenerated articular cartilage. Also, in the group of animals that underwent nuclear magnetic resonance therapy, the cartilage tissue in the area of regeneration had signs of hyaline cartilage.

In the perspective of further developments, we plan to conduct a study on the restoration of articular cartilage within three months.

**Conclusion**

1. It has been proven that the use of nuclear magnetic resonance therapy for transchondral damage of articular cartilage contributes to the formation of cartilage regenerate within 28 days in the cartilage defect, which in its structure is close to the structure of intact hyaline cartilage.

2. After transchondral damage to the articular cartilage, it does not fully recover within 28 days, as evidenced by the smaller thickness of the regenerate compared to that of the right knee joint.

3. In the control group of animals without nuclear magnetic resonance therapy, after traumatization of regenerate formation, there were few signs of dysregeneration. The tissue formed in the damaged area had signs of fibrous cartilage with areas of necrosis.

4. The experiment needs a longer period to study the full recovery of articular cartilage.

**References**


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МОРФОГІСТОЛОГІЧНЕ ДОСЛІДЖЕННЯ РЕГЕНЕРАЦІЇ ДЕФЕКТІВ ХРЯЩА КОЛІННОГО СУГЛОБА В ЕКСПЕРИМЕНТАЛЬНОЙ МОДЕЛІ ПІД ВПЛИВОМ ЯДРЕННОЇ МАГНІТО-РЕЗОНАНСНОЇ ТЕРАПІЇ

Фіщенко В. О., Король А. П., Юсупова Д. В.

Незважаючи на різноманіття сучасних методів лікування, проблема регенерації гіалінового хряща залишається надзвичайно актуальною. Мета дослідження - визначити ефект ядерної магніто-резонансної терапії на регенераційну здатність модельних дефектів хрящової тканини колінного суглоба у щурів, оцінити динаміку мікроколінічних змін суглобового хряща в основній та контрольній групах. Дослідження виконано на 60 статево-вікових щурах. Повноцінні дефекти створені в ділянці суглобових поверхонь лього колінного суглоба - 30 дефектів в основній групі та 30 дефектів в контрольній групі. Правий колінний суглоб двох досліджуваних груп використовували в якості контрольної. Через 3 дні після хірургічного втручання проводили необхідну медикаментозну терапію (антимікробні, протизапальні, аналгезуючі). На четверту добу після операції шурям основної групи зastosовували ядерну магніто-резонансну терапію по 60 хвилин впродовж 7 доб. Через 7, 14, 21, 28 діб після операції проводили мікроскопічний аналіз хрящевого регенерату. Через 28 діб після застосування ядерної магніто-резонансної терапії виявлено різницю хрящевого регенерату у щурів становила 82,12±8,9 мм в основній групі та 56,34±7,8 мм в контрольній. Хрящевий регенерат у щурів після ядерної магніто-резонансної терапії за будовою наближався до структури неушкодженого гіалінового хряща. Однак, повної регенерації відбувалося, про що свідчить менша товщина суглобового хряща порівняно з правим колінним суглобом. У контрольній групі формування регенерату мало виражені ознаки дисрегенерації. Хрящова тканина в ділянці дефекту мала переважно фіброзний характер із зонами некрозу. Ядерна магніто-резонансна терапія сприяла формуванню в дефекті суглобового хряща хрящового регенерату, котрий за своєю гістологічною будовою наближається до гіалінового хряща.

Ключові слова: ядерна магніто-резонансна терапія, щур, хрящовий регенерат, підкорення хондроцитів, експеримент.

Author’s contribution
Fischchenko V. O.: conceptualization, resources, providing equipment for the experiment, supervision.
Koral A. P.: software for morphometric research, histological analysis.
Yusupova D. V.: data visualization, formal analysis and verification, conducting an experiment, project administration, methodology and writing an original draft, review writing and editing, processing of statistical data.