Features of the reparative chondrogenesis course in induced joint cartilage defect and application of cartilage protector

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Abstract. The article reflects information on morphological features of joint cartilage in normal and at induced damage. In animals of the experimental group, the effect of "Stop Arthritis" preparation on the structural and functional cartilage state in the regenerative process dynamics was evaluated. The research was performed based on the Department of Anatomy and Histology of Animals named after Professor A.F. Klimov MSAVMIB — MVA named after K.I. Skryabin. Gonarthrosis modeling was performed on 13 clinically healthy rabbits of Chinchilla breed at the age of 5 months, on which surgical intervention was carried out to create a bilateral articular cartilage defect in the kneecap unit region by excising a fragment of articular cartilage to the subchondral bone. As a result of the studies it was revealed that spontaneous articular cartilage healing after a surgical trauma occurs with the participation of connective tissue pannus, which is obviously formed both on the side of the articular capsule and the subchondral bone. In this case, healing is accompanied by subchondral osteoporosis, as well as the development of deforming arthrosis in the kneecap block area. It was shown that the use of "Stop Arthritis" cartilage protector optimizes reparative chondro- and osteogenesis in damaged tissues of the knee joint, which is confirmed by the formation of a holistic articular coating with organ-specific zonal differentiation.

1 Introduction

Currently, pathologies of the musculoskeletal system occupy the second place after infectious diseases among pet diseases, with about half of them associated with joint lesion. The most difficult problem is treating animals with degenerative-dystrophic pathological processes in large joints - osteoarthritis [1-5].

The osteoarthritis development is known to result from the interaction of mechanical and biological factors leading to an imbalance between degradation and synthesis of articular cartilage matrix and subchondral bone. This is accompanied by cartilage softening, its thinning and sclerosis. Osteoarthritis risk factors are hereditary predisposition, age, intra-articular injuries, past inflammatory joint diseases (arthritis), excess body weight. In the early

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stages, the picture of osteoarthritis is characterized by latency of clinical manifestation, which complicates X-ray diagnostics of this arthropathy. In this regard, osteoarthritis is detected on the basis of the pain syndrome appearance in the animal and motor activity restriction, corresponding to the progression of structural changes in the tissues of the joint. Improvement and development of new methods of treating animals with this pathology remains one of the topical veterinary medicine problems [6-9].

One of the effective ways of arthrosis therapeutic correction is the use of cartilage protectors [10-15]. In this regard, we have undertaken a study aimed at testing a modern cartilage protector containing glucosamine in induced arthrosis.

Objective: to evaluate the course of reparative chondrogenesis on the arthrosis model under the influence of cartilage protector preparation Stop-Arthritis.

2 Material and methods of research

Research was conducted based on the Department of Anatomy and Histology of Animals named after Professor A.F. Klimov MSAVMIB — MVA named after K.I. Skryabin.

The study was performed on rabbits of Chinchilla breed at the age of 5 months, on which surgical intervention was carried out to create a bilateral articular cartilage defect in the kneecap unit region by excising a fragment of articular cartilage to the subchondral bone.

The keeping conditions, surgical intervention and removal from the experiment met the requirements governing the humanist attitude of laboratory animals. All experimental studies: keeping, care and euthanasia were carried out in accordance with the requirements of the orders of the Ministry of Health of the USSR No. 755 of 12.08.1977, No. 701 of 27.07.1978, “Sanitary rules for the arrangement, equipment and maintenance of experimental and biological clinics (vivarium)”, the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific purposes” (1986) and the “Helsinki Declaration of the World Medical Association” (1964).

Table 1. Experiment design.

Group No.	Number of animals	Research tasks in the group
1 — intact animals	5	To study the structure of articular cartilage in norm
2 - control (surgery with cartilage damage, spontaneous regeneration within 3 months)	10	To study the structure of articular cartilage regenerate in spontaneous healing of experimentally reproduced arthrosis
3 — experiment (surgery+Stop-arthritis for 3 months) 3 months - the term of preparation use stated in the instructions	10	To study the articular cartilage regenerate structure during healing of experimentally reproduced arthrosis under the influence of cartilage protector

Before the surgery, animals were anesthetized according to the following scheme:

- anocithesia: atropine solution 0.1% - 0.2 ml subcutaneously, rometarum 2% -0.3.
- anesthesia: zoletil 0.5% - 0.5 ml i.m.
- anesthesia of the skin and subcutaneous tissue by introducing a solution of novocaine 0.5% - 4ml into the kneecap area.

3 Results of the study

3.1 Structure of intact cartilage

On the basis of anatomical preparation of the rabbit's knee joint, it was established that the articular cartilage of the kneecap block on the femur has a plane, smooth, shiny surface peculiar to animals of other taxonomic groups (Fig. 1).

Fig. 1. Macromorphology of the kneecap block intact cartilage of the rabbit femur distal epiphysis.

In microscopic examination, it was differentiated into typical structural zones: surface, medium and deep (Figure 2). Subchondral bone lies close to the cartilage forming a powerful layer with numerous vascular channels and intertrabecular spaces.

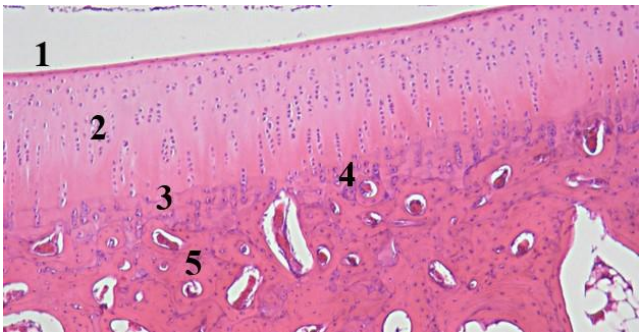

Fig. 2. Micromorphology of the intact articular cartilage of the rabbit's kneecap block. General view. 1 - surface zone, 2 - middle zone, 3 - deep cartilage zone, 4 - tidemark, 5 - subchondral bone contains cavities with blood vessels. Hematoxylin and eosin, lens 4, oc. 10.

The thin surface zone is characterized by singly or pairwise arranged chondroblasts oriented tangentially. In the middle zone, the most significant in thickness, chondrocytes form isogenic groups, which are arranged in the form of vertical columns.

The deep zone of articular cartilage is characterized by a mineralized matrix distinguished by weak basophily. At the boundary between it and the non-mineralized mid-zone cartilage, there is a tidemark, which is locally duplicated. The deep zone directly contacts the subchondral bone, from which bone marrow cavities are introduced into the cartilage with numerous blood vessels, often located near tidemark and not invasing it.

Characteristics of intact articular cartilage according to morphometric indicators is presented in Table 2.

Table 2. Characteristics of intact articular cartilage of the rabbit's kneecap block

Indicators	Values
Total thickness of articular cartilage, μm	308,0±19,9
Thickness of individual zones of articular cartilage, μm	
surface	32,9±6,7
middle	221±19
deep	60,8±11,8
Thickness of subchondral bone, μm	From 173,0±38,7 to 304,0±76

3.2 Results of spontaneous healing of induced damage to articular cartilage in animals of control group 3 months after surgery

Macroscopically, the studied animals showed an extensive defect filled with regenerate (Figure 3). The peripheral regenerate part is thickened and characterized by a ring shape. The central part forms a cavity and is thinner than the peripheral. Outside the area of surgical intervention, signs of deforming arthrosis are revealed on the crests of the kneecap block.

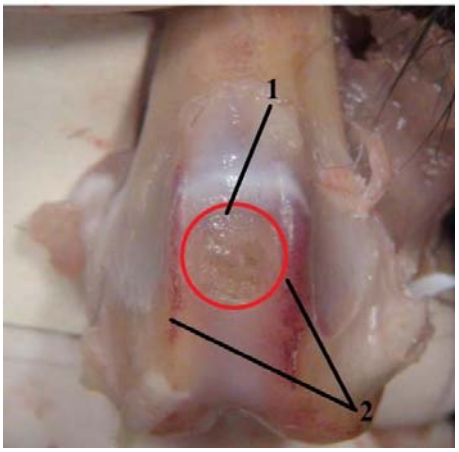

Fig. 3. Macromorphology of distal femur epiphysis in animals of control group (3 months after surgery). 1 - the area of surgical trauma is filled with a regenerate of a heterogeneous structure, 2 - crests of the kneecap block with signs of deforming arthrosis.

In microscopic examination, the structure heterogeneity of the regenerate filling defect area has been established (Figure 4).

Macroscopically thickened and having a ring shape, its peripheral part is formed by fibrous cartilage with signs of reparation. At the same time, necrosis centers remain in the area of healing, undoubtedly supporting the inflammatory process in surrounding tissues. At the border of the destruction area there is compensatory thickening of the preserved articular cartilage with an increased number of isogenic groups located in the form of rosettes and nests, which may indicate process of reparative regeneration. The defect area is made by fibrous cartilage with identified fibers, as well as numerous cartilaginous cells of varying degrees of morphological maturity forming isogenic groups. On the surface of the cartilage there are numerous defects in the form of attritions and cracks of different localization and extent, which are also found on the boundary of the regenerate and preexisting articular cartilage.

The sharp thinning of the subchondral bone and the presence of numerous extensive intertrabecular cavities on it that determine the rarefaction of its structure and indicate the presence of osteoporosis signs is attracting attention.

Fig. 4. Micromorphology of the articular cartilage destruction area in animals of control group 3 months after surgery. Cut through the peripheral regenerate part. The area of surgical trauma is filled with fibrous cartilage (1), articular cartilage with signs of reparation is visible along the defect edges (2), as well as with the necrosis area (3). The subchondral bone (4) is thinned. Cartilaginous regenerate contains cracks, attritions (arrows). Hematoxylin and eosin, lens 4, oc. 10.

In the central part of the healing region, the regenerate is thin and retracted, formed by a thin layer of dense connective tissue, which is located on the rarefied subchondral bone (Figure 5).

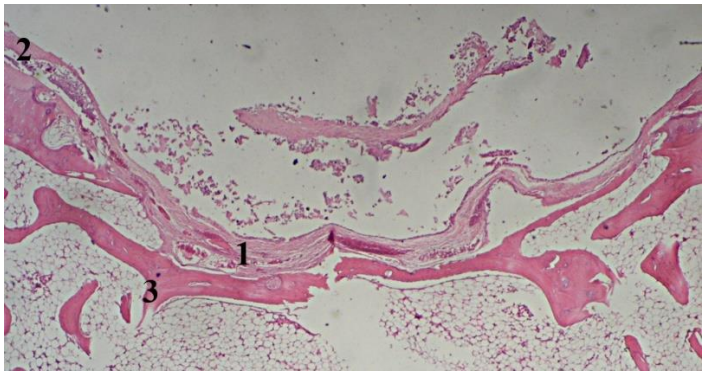

Fig. 5. Micromorphology of the articular cartilage destruction area in animals of control group 3 months after surgery. The area of the surgical trauma is filled with connective tissue (1), articular cartilage with signs of reparation is visible along the defect edges (2). Subchondral bone (3) is rarefied. Hematoxylin and eosin, lens 4, oc. 10.

At the defect boundary, the articular cartilage contains isogenic cells groups arranged in the form of nests. The connective tissue pannus contacts intertrabecular spaces of the subchondral bone, connective tissue is also identified in some of them. Most intertrabecular spaces contain fatty bone marrow.

Connective tissue regenerate is well vascularized, represented by structured tightly packed bundles of collagen fibers, contains numerous fibrocytes.

The characteristics of the regenerate by morphometric indicators are presented in table 3.

Table 3. Characteristics of articular cartilage regenerate of the rabbit's kneecap block in the control group (3 months after surgery)

Indicators	Values
Thickness of connective tissue pannus in the center of the focal damage, μm	155,0±42,6
Thickness of cartilaginous coating on the periphery of the damage center, μm	225,0±58,2
Thickness of subchondral bone, μm	From 64,3±18,5 to 225,0±56,5 (focal)
in the damage focal center	to 87,4±16,8
on the periphery of the focal damage	74,0±26,5, single areas of osteosclerosis — 238,0±15,5

3.3 Results of spontaneous healing of induced damage to articular cartilage in animals of experimental group 3 months after surgery and application of Stop-Arthritis cartilage protector

After surgery, the defect area in animals of the experimental group was composed of a cartilaginous coating of whitish color with an uneven, locally thinned surface (Figure 6). There were no phenomena of deforming arthrosis on the crests of the kneecap block.

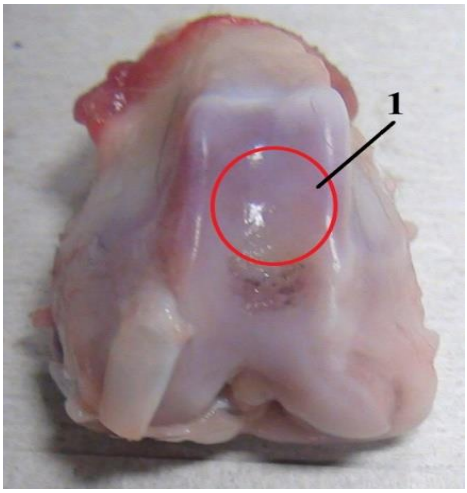

Fig. 6. Macromorphology of distal femur epiphysis in animals of the experimental group "Surgery+Stop Arthritis" 3 months after surgery. The surgical trauma area is filled with a regenerate (1) with thinning foci (dark inclusions on the regenerate surface).

In microscopic studies it was found that the cartilaginous defect is filled with a regenerate, which structure bears a resemblance to such of articular cartilage: it is differentiated into surface, medium and deep zones (Figure 7). Notably, many chondroblasts and chondrocytes are located in amplified lacunas. Cystic cavities have been identified in the middle and deep zones, sometimes communicating with cavities of the subchondral bone.

The regenerate matrix often has fibrillation signs, it is represented by a thin fibrous structure with focal micro cracks. The subchondral bone is quite massive, with signs of active structural rearrangements. Chondroid proliferates are still identified in its intertrabecular spaces.

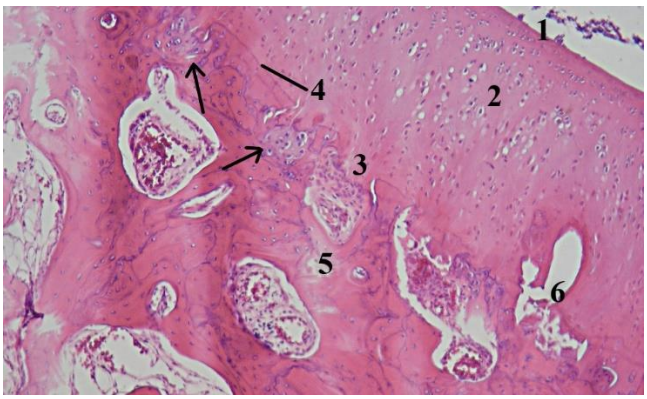

Fig. 7. Micromorphology of cartilaginous regenerate in animals of experimental group 3 months after surgery. In the cartilaginous regenerate structure, surface (1), middle (2) and deep (3) zones, the front of mineralization (4) are visible. 5 - subchondral bone, chondroid proliferates (arrows) are visible in its cavities. 6 - cystic cavity. Hematoxylin and eosin, lens 10, oc. 10.

The characteristics of the regenerate by morphometric indicators are presented in table 4.

Table 4. Characteristics of articular cartilage regenerate of the rabbit's kneecap block in the experimental group "Surgery+Stop-Arthritis" (experimental period of 3 months)

Indicators	Values
Total thickness of cartilaginous coating, μm	345,0±25,1
Thickness of subchondral bone, μm	Heterogeneous changes: from 138,0±28,6 (small osteolysis in some animals) up to 369.0±10.3 (osteosclerosis in other animals)

4 Conclusion

Therefore, as a result of the studies it was revealed that spontaneous articular cartilage healing after a surgical trauma occurs with the participation of connective tissue pannus, which is obviously formed both on the side of the articular capsule and the subchondral bone. At the same time, fibrous cartilaginous regenerate is formed during reparation at the defect area periphery, and connective tissue in its central part. Healing was accompanied by subchondral osteoporosis, as well as the development of deforming arthrosis in the kneecap block area. All this indicates the structural and functional inferiority of regenerate and subchondral bone and compensatory involvement of para-articular tissues in the process 3 months after injury.

Under the influence of the "Stop Arthritis" cartilage protector, a cartilaginous regenerate is formed in the area of damage. According to our data, this is due to inducing proliferative activity of preserved cells of the articular cartilage's middle and deep zone, as well as through the development of chondroid proliferates in intratrabecular spaces of the subchondral bone. From here, proliferates penetrate zones with necrotized, damaged cartilaginous coating, forming many foci for its restoration. Reparative processes are also identified perifocally from the defect area, which leads to its filling with cartilaginous regenerate, the organization of which is similar to such of articular cartilage. Unlike the control group, thinning of the subchondral bone and signs of deforming arthrosis were not observed.

Thus, under the influence of the Stop-Arthritis preparation, reparative chondrogenesis is stimulated both in the preserved articular cartilage and in the subchondral bone. The obtained data should be considered in the development of strategy and tactics of therapeutic correction of arthropathies of different genesis.

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