Experimental Morphological Study of the Effects of Subchondral Tunnelization and Bone Marrow Stimulation on Articular Cartilage Regeneration

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The articular cartilage was studied under conditions of experimental osteoarthrosis with tunnelization of the subchondral zone and injection of autologous bone marrow into the channels. Histomorphological studies showed that tunnelization of the subchondral zone with injection of autologous bone marrow into the channels stimulated reparative regeneration of the chondral tissue by inhibiting destruction of the joints.

Key Words: *articular cartilage; osteoarthrosis; tunnelization; regeneration*

The development of degenerative processes in gonarthrosis is now explained by dysfunction of the joint and microcirculatory disorders in the articular terminals [1,4,6]. The biochemical environment (bioactive substances forming during necrotization of bone chips, bone marrow protein bioregulators) and biophysical factors (blood oxygen level, pressure) are essential for the cell proliferation, osteo- and chondrogenic differentiation under conditions of subchondral tunnelization [5,8,11]. Bone marrow stromal stem cells are the least differentiated precursors of chondro- and osteoblasts in an adult organism. Studies on experimental models showed that injection of autologous bone marrow stromal cells into the articular cartilage at the site of injury stimulated its regeneration [7,9,10].

We studied the articular cartilage under conditions of experimental osteoarthrosis creation with subsequent tunnelization of the subchondral zone and injection of autologous bone marrow into the channels.

MATERIALS AND METHODS

Osteoarthrosis (OA) was induced in 12 mongrel dogs by immobilization and compression of both knee joints with crossing of the femoral artery. The adequacy of the model was tested 28 days after immobilization by morphological studies on 5 sacrificed animals. In the rest animals tunnelization of the subchondral zone with injection of autologous bone marrow was carried out on the right knee joint 7 days after OA simulation. The bone marrow was collected from the proximal humeral metaphysis with Kassirsky's needle. Four tunnels were made in each femoral and tibial condyle and 0.2 ml bone marrow substrate was injected into each tunnel. The animals were scarified 14, 28, and 90 days after tunnelization. Experimental material was divided into 3 series. Series I were results of the articular cartilage studies in experimental OA (n=10), series II were the left joints with OA without tunnelization (n=7), and series III were right tunnelized joints with OA (n=7). All manipulations on animals were carried out in accordance with the requirements of the Ministry of Health of the Russian Federation to the work of experimental biological clinics and of the "European Convention for Protection of Vertebrates Used for Experimental and Other Research Purposes" [3]. Paraffin sections of the femoral condyles, stained with hematoxylin and eosin and with alcyan blue at pH 1.0 (histochemical reaction to sulfated glycosaminoglycans, sGAG) were analyzed. The concentration of sulfur (ωS, weight%), sGAG marker, was evaluated in non-decalcinated specimens

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of articular cartilage, embedded in epoxy blocks, by x-ray electron probe microanalyzer INCA Energy 200 (Oxford Instruments Analytical) mounted on a JSM-840 (Jeol) scanning electron microscope. In addition, large (up to 8 mm^2) semithin epoxy sections of the articular cartilage with the sublying subchondral bone stained with methylene blue and basic fuchsin, were prepared for comparative morphometric studies. The micropreparation images were digitally processed on a DiaMorf complex and analyzed using Video-Test-Master-Morphology software. The thickness of the articular cartilage (h, μ), chondrocyte volume density (VV_{cc}, %) in tissue, chondrocyte numerical density (NA_{cc}, μ^{-2}), and percentage of empty lacunas (NA_{el}) and isogenic groups (NA_{ig}) in the total sample were evaluated. The articular cartilage of intact animals (n=5) served as the morphometry control. The digital material was analyzed by methods of variation and nonparametric statistics using AtteStat 1.0 software [2] in Microsoft Excel 97 electron tables.

RESULTS

Studies of the articular cartilage in series I (OA) showed that the interstitium was no longer homogenous, there were foci of fibers separation, part of chondrocytes were destroyed, and the content of cells with caryopiknosis increased (Fig. 1, *a*). The counts of chondrocytes in the intermediate and deep zones dropped, solitary cells were scattered or formed isogenic groups; the cells in the isogenic groups were at different stages of degeneration (Fig. 1, *b*). The degenerative changes were the maximum in chondrocytes of the intermediate zone; they were equally far from the vascular and synovial trophic factors (about 50% cells destroyed). The staining of the basophilic line was uneven, the line was fragmented, and there were ruptures, the bone marrow pannus penetrating into the cartilage (Fig. 1, *c*). The calcified cartilage zone was thickened in some places. Histochemical reaction to sGAG was significantly reduced and the surface zone interstitium was not stained, while in the intermediate and deep zones intensely stained sites alternated with pale blue or colorless sites. Electron probe microanalysis showed a significant reduction of sulfur content (Table 1). Morphometry showed a significant (p<0.001) thinning of the cartilage, reduction of numerical density of chondrocytes, their volume density was almost 2-fold lower than in the control, and the percentage of empty lacunas increased (Table 1). Loosening of bone trabeculae, loss of their thickness, and enlargement of spaces between them were found in the subchondral zone, in parallel with erythrocyte aggregation and "prethrombosis" of blood vessels in the microcirculatory network.

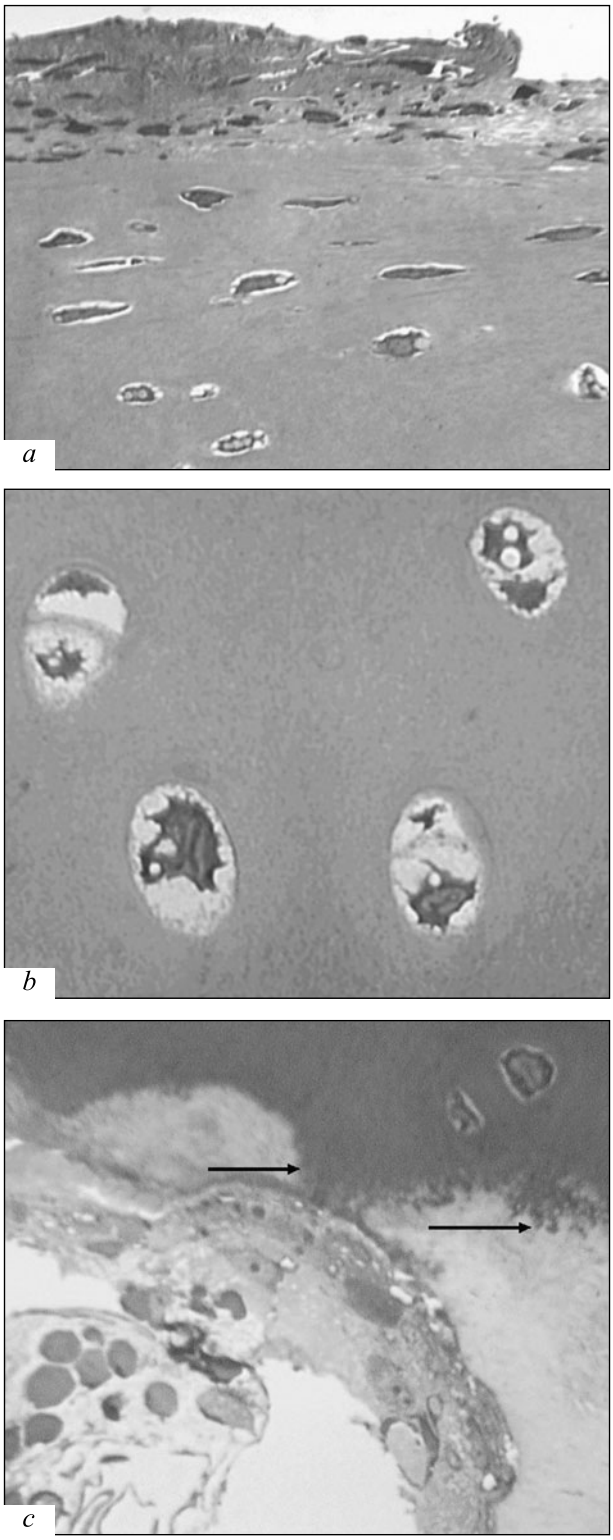

Fig. 1. Experimental series I. *a*) surface zone of cartilage; *b*) intermediate zone, ob. 40, ×12.5; *c*) deep zone, broken basophilic line (arrows), ob. 16, ×12.5. Semithin section, methylene blue and basic fuchsin staining.

In series II of after 14 days separation of fibers in the interstitium with disclosure of collagen fibers

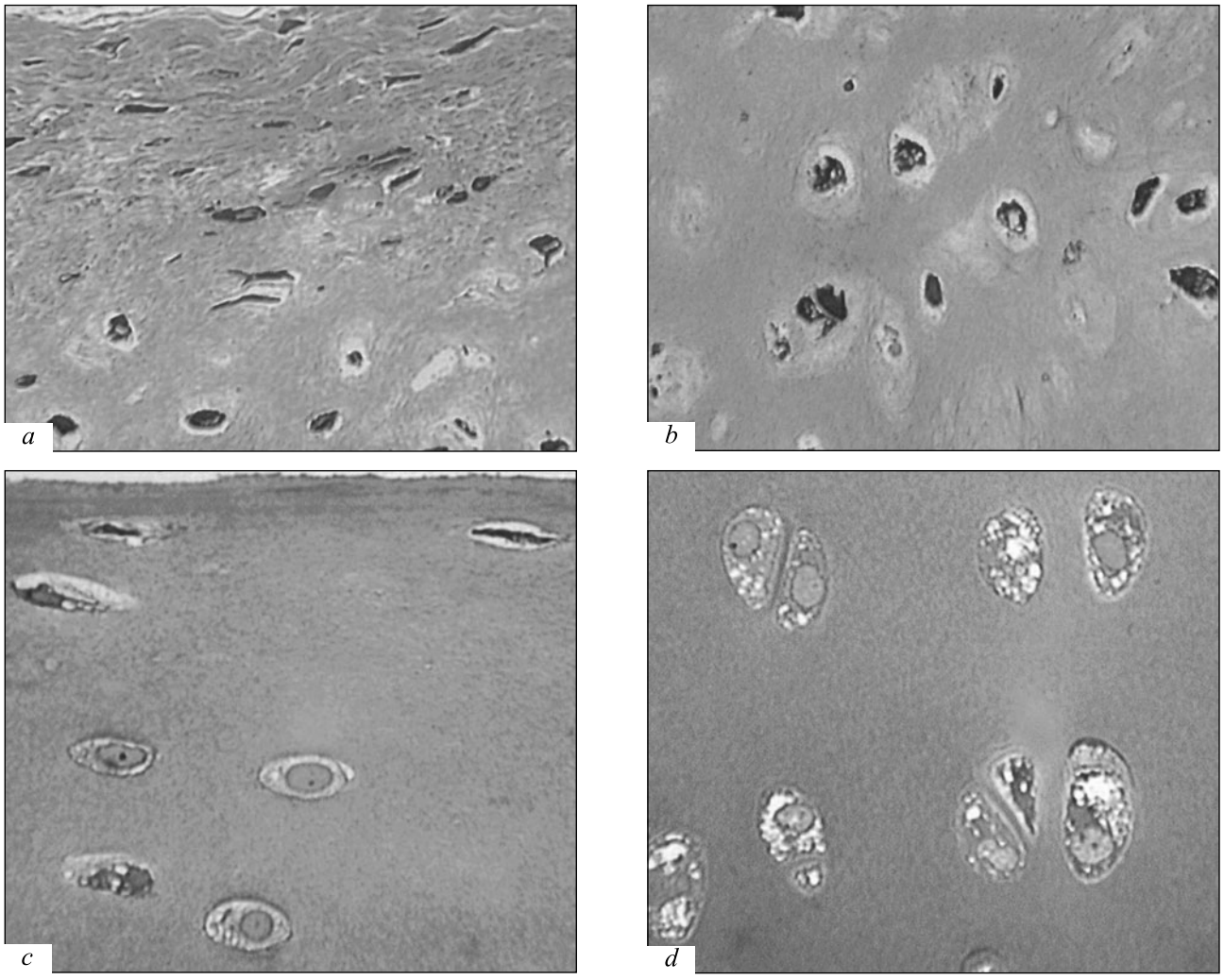

Fig. 2. Articular cartilage 28 days after OA simulation: surface zone – experimental series II (*a*) and III (*b*); intermediate zone: series II (*c*) and III (*d*). Semithin sections, methylene blue and basic fuchsin staining, ob. 40, ×12.5.

in the articular cartilage persisted, the greater part of chondrocytes were in a state of destruction, and empty lacunas were found. Reparative regeneration of the cartilage consisted in increase of cell count, volume density of chondrocytes, and number of isogenic groups with functionally active cells, and also a slight increase of sulfur content in comparison with OA simulation (Table 1). After 28 days the incidence and volume of sites with separation of fibers increased (Fig. 2, *a*), as did the number of empty lacunas in the intermediate zone (Fig. 2, *b*). The numerical and volume densities of chondrocytes virtually did not differ from the values during the previous period. The thickness of the cartilage was significantly greater than in series I and control because of more pronounced disorganization of the surface zone interstitium, swelling of the main substance and collagen fibers. Destructive changes progressed after 90 days, foci of fiber separation with disclosure of collagen fibers were still found in the articular cartilage. Numerous empty lacunas were found in all zones, particularly in the intermediate, in which cell-free fields were detected (Fig. 3, *a*); the interstitium stained with alcyan blue looked colorless. The cytoarchitecture of the deep zone changed: the columnar disposition of cells was lost. Sites with disrupted basophilic line were still found in some cases. The numerical density of chondrocytes increased in comparison with the previous periods of experiment at the expense of cell increase in the surface zone. Alcyan blue staining was focal, its intensity was reduced significantly, the content of sulfur being significantly lower than during the previous periods and the values in series I. Increase in the percentage of empty lacunas and reduction of the percentage of isogenic groups in parallel with progressive reduction of sGAG content indicated failure of the cartilage compensatory potential. A significant ($p<0.001$) thinning of the cartilage looked justified (Table 1).

TABLE 1. Quantitative Characteristics of the Femoral Condylar Articular Cartilage (*M*±*m*)

Parameter		h, μ	NA_{cc}	NA_{el}, %	NA_{ig}, %	VV_{cc}, %	ωS, weight%
Control		475.5±1.3	6.2±0.56	13.6	14.5	9.03±0.46	1.26±0.02
OA simulation	series I	318.6±2.7*	5.37±0.30*	43.39	23.80	4.60±0.36*	0.70±0.02*
14 days	series II	563.2±13.1*+	5.70±0.34*	31.3	16.2	5.5±0.3+*	0.810±0.025+*
	series III	457.8±1.9*o+	9.9±0.4*o+	15.6	16.4	7.4±0.3*o+	1.02±0.03*o+
28 days	series II	510.9±1.8*+	6.4±0.6*+	35.7	16.1	5.4±0.3*+	0.720±0.015*
	series III	480.9±1.3*o	8.4±0.4*o+	16.1	21.03	6.80±0.34*o+	0.850±0.025*o+
90 days	series II	326.6±6.3*	8.6±0.6*+	40.3	8.9	6.09±0.3*+	0.56±0.03*+
	series III	465.8±4.4o+	9.05±0.60*o+	14.6	14.6	7.02±0.30*o+	0.99±0.02*o+

Note. The significance of differences in parameter h was evaluated by Student's test ($p<0.001$), in parameters VV_{cc}, NA_{cc}, and ωS by Wilcoxon's test ($p<0.05$). Significant difference *vs*. *control, +series I, °series II.

Activation of osteoblasts was found in some sites of the subchondral zone at the interface with calcified cartilage, while in other sites osteoclastic resorption predominated.

In series III sites of fiber separation in the articular surface were still seen after 14 days, but they were smaller and less incident than in series II. Formation of a dense small-looped network of young trabeculae was seen in the subchondral zone in foci of active remodeling; spaces between the bars in sites of bone formation were filled with the red bone marrow in which lymphoid and blast cells predominated. Clear-cut signs of inhibition of the articular cartilage destruction were detected after 28 and 90 days. No foci of fiber separation were found in the greater part of observations (Fig. 2, *c*; Fig. 3, *b*). The homogeneity of the surface zone interstitium was restored, the basophilic line was intact. Isogenic groups of 2, 3, and 4 members appeared in the intermediate zone, and the territorial matrix was forming in them (Fig. 2, *d*). The number of actively secreting cells increased; they were markedly hypertrophic. An increase of sulfur content and intensity of alcyan blue staining of the interstitium increased in comparison with series I and II.

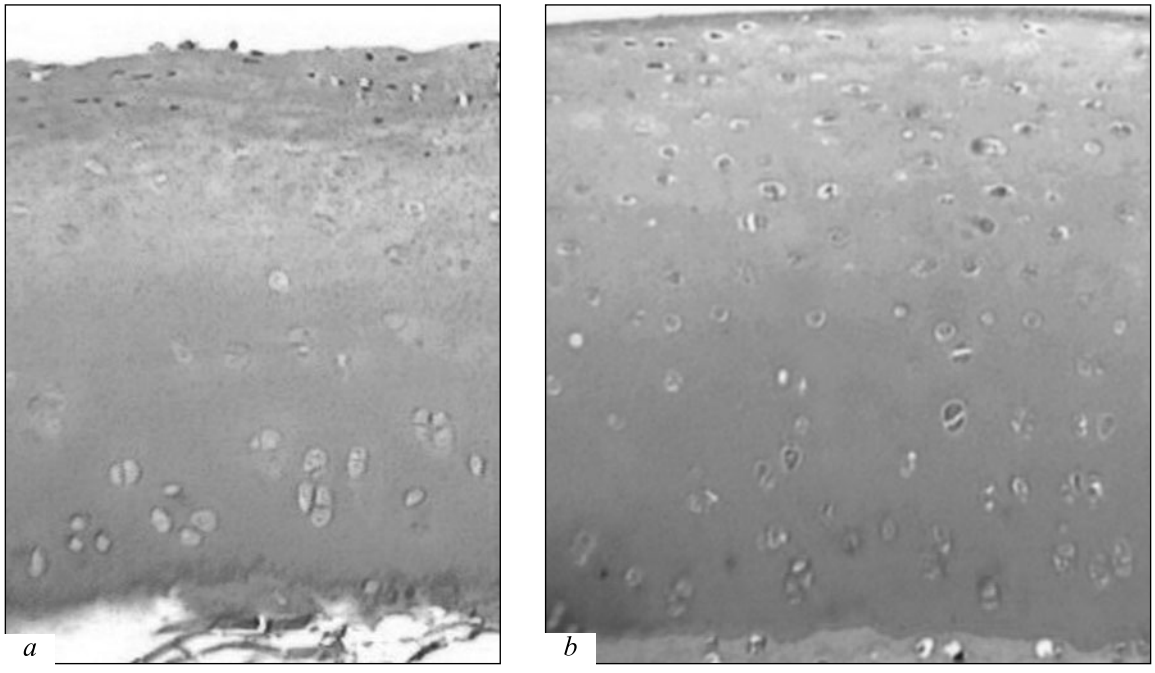

Fig. 3. Articular cartilage 90 days after OA induction: experimental series II (*a*) and III (*b*). Semithin sections, methylene blue and basic fuchsin staining, ob. 6.3, ×12.5.

The thickness of the cartilage, numerical and volume densities of chondrocytes were higher than in series II. The percentage of empty lacunas and isogenic groups were comparable with the values in the control. Functioning capillaries feeding the hyaline matrix were found in the subchondral zone. Proliferation together with enlargement of chondrocytes, increase of sGAG content resultant from higher synthetic activity of the cells could be regarded as reactive changes of adaptation genesis, at the expense of which the reparative regeneration of the cartilage was realized.

Hence, tunnelization of the subchondral zone with injection of autologous bone marrow into the channels stimulated the reparative regeneration of the cartilage tissue, inhibiting the joint destruction. Signs of the articular joint regeneration were also found in the left joints without tunnelization, but these signs were slight, and the destructive changes progressed with prolongation of experiment.

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