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Role of Subchondral Bone in the Restoration of Articular Cartilage

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The role of subchondral bone in the restoration of the articular cartilage was determined in experiments on mongrel dogs subjected to gonarthrosis modeling followed by subchondral zone tunneling and introduction of bone marrow suspension into the canal. Light microscopy, computer histomorphometry, and electron probe microanalysis showed that stimulation of functional activity of chondrocytes was achieved via correction of homeostasis of the cartilage and subchondral bone and improvement of tissue trophic. Restoration of the microarchitecture of the subchondral bone and improving its vascularization after tunneling with the introduction of bone marrow suspension into the drill holes enhances chondrocyte metabolism and recovers their functionality.

Key Words: *osteoarthrosis; subchondral bone; tunneling; articular cartilage; regeneration*

The interest to the study of subchondral bone (SCB) was spurred by new information on the pathogenesis of osteoarthrosis. For instance, it became clear that this disease manifested not only by the loss of the articular cartilage, but also by changes in the bone tissue, which are probably the primary cause and trigger of cartilage degradation [1]. It is known that osteoarthrosis develops in zones of dynamic overload of SCB that cause redistributive microcirculatory disturbances contributing to the development of subchondral osteosclerosis [7]. The key role of SCB in triggering articular cartilage degradation consists in the synthesis of a number of cytokines and growth factors and their transport to the overlying cartilage [14]. Substantiation of the role of SCB in the pathogenesis of osteoarthrosis prompted the search for new treatment technologies directed to not only cartilage, but also bone tissue [1].

Modified techniques of subchondral tunneling are widely used in clinical practice [4,5,12]. Leading world scientists support the view that studies of the efficiency of osteoperforation techniques in the treatment of cartilage damage, indications for their use, development of new ways to stimulate chondrogenesis and normalization of physiological parameters of articular environment including genetic engineering technologies are promising and should be given high priority [11]. Our previous experimental and morphological studies have demonstrated that cell technologies in subchondral tunneling are highly effective in stimulating articular cartilage regeneration [8].

The purpose of this study was to evaluate the role of SCB in the restoration of the articular cartilage during the treatment of osteoarthrosis.

MATERIALS AND METHODS

Experiment was carried out on 3-5-year-old mongrel dogs (n=16) of both sexes weighing 12-15 kg. Maintenance of animals, surgery, and post-operative care was performed in accordance with the requirements of the Ministry of Health of the Russian Federation to Experimental and Biological Clinics. All manipulations on animals were examined and approved by the Ethics Committee of Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics.

In all animals, experimental osteoarthrosis (EOA) was previously modeled by intersection of the femoral artery followed by immobilization of both knee joints

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for 28 days. The adequacy of the model was confirmed by morphological examination of the joint [10]. On day 8 after EOA, extra-articular subchondral tunneling was performed on the right joint with introduction of bone marrow suspension obtained from the proximal metaphysis of the humerus into the drill holes. Four drill holes were made in each femoral and tibial condyle. The left joint was not drilled. The animals were sacrificed 28 days after EOA and on days 14, 28, and 90 after subchondral tunneling.

Experimental data were divided into three series. The first one included the results of joints exanimation during EOA; series II, left joints after EOA; series III, right joints after EOA. The joints from 5 intact animals served as the controls.

We assessed the status of SCB, bone tissue and bone marrow structures in the epiphyses of the femur. To study the vascular bed, we used a method of injecting blood vessels with 10% gelatin-India ink solution followed by dissection and clarification of the preparations. Paraffin and celloidin sections of femoral condyles stained with hematoxylin and eosin and by Masson's trichrome stain were used for light microscopy. Calcium concentration in the subchondral bone (ωCa, wt.%) was evaluated using X-ray electron probe microanalyzer INCA Energy 200 (Oxford Instruments Analytical) mounted on a scanning electron microscope the Jeol JSM-840.

Histomorphometrical studies were performed on serial paraffin sections and semithin epoxy slices of the articular cartilage harvested from enlarged (up to 8 mm^2) area below SCB [9] and stained with methylene blue and basic fuchsin. Images of the slides were digitized using DiaMorph hardware-software system and examined using VideoTesT-Master-Morfologiya software.

Digital data were analyzed by the methods of descriptive statistics. Significance of differences was evaluated by Wilcoxon's test using AtteStat 1.0 [2] and Microsoft Excel 1997 software.

RESULTS

In series I, vascularization of the subchondral zone was deteriorated after EOA (Fig. 1, *a*). Light-optic microscopy of semithin slices showed signs of impaired microcirculation, *i.e.* stasis of microvessels. In areas of loading adjoining the calcified cartilage (CC), foci of enhanced bone formation were observed as well as thickening of the trabecular bone, on the surface of which active osteoblasts were found (Fig. 2, *a*). In least loaded zones, signs of inhibited osteoblast activity and prevalence of osteoclastic resorption were seen (Fig. 2, *b*). Calcium content was significantly ($p<0.05$) reduced to 10.09±0.01% (normally, 21.85±1.86%). In the epiphysis, signs of osteoporosis were seen as rarefaction of the spongy bone. Significant ($p<0.05$) reduction of the volume density of the trabeculae to 23.4% was noted in comparison with the control (48.9%).

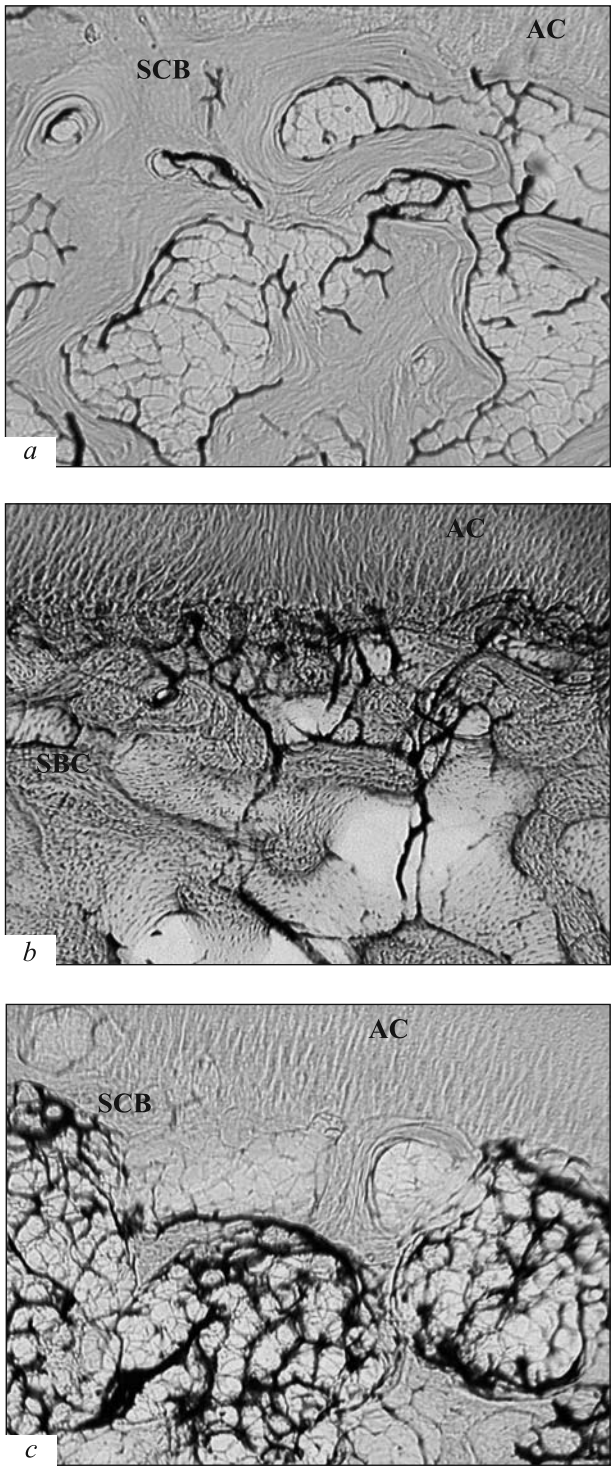

Fig. 1. Vasculature of subchondral part. Injection with Indian ink solution. Cleared specimen, ×30. *a*) Series I, EOA; *b*) series II, 90 days after EOA; *c*) series III, 90 days after EOA. AC: articular cartilage.

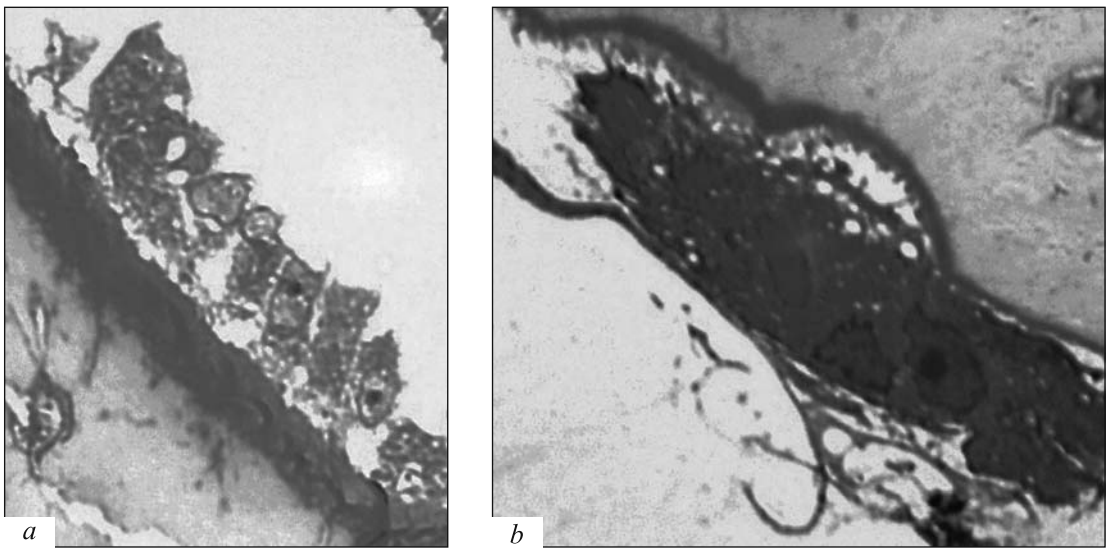

Fig. 2. Bone trabecula, series I. Semithin section stained with methylene blue and basic fuchsin, ×50. *a*) Active osteoblasts on the surface; *b*) osteoclast resorbing the basic substance of the trabecula, ×1250.

In series II, reduced vascularization of the subchondral zone (Fig. 1, *b*), impaired microarchitecture of bone trabeculae with uneven dye absorption, splitting along the glue line with the unmasking of bone plates persisted in joints that were not subjected to tunneling with increasing duration of the experiment. Calcium level in SCB was significantly reduced after EOA ($p<0.05$), it was 12.77±2.64% after 14 days, 11.37±1.83% after 28 days, and 15.18±1.96% after 90 days. In the epiphysis, the volume density of the trabeculae continued to decline 14 days after EOA ($p<0.05$) to 11.6%; it was increased 28 and 90 days after EOA up to 20.5 and 28.4%, respectively, but did not reach the control level ($p<0.05$).

In series III, in the drilled joints after introduction of the bone marrow suspension into the drill holes, vascularization was improved in the subchondral zone in close proximity to the cartilage (Fig. 1, *c*). A dense trabecular network was formed in foci of "active remodeling", intertrabecular spaces were filled with red bone marrow, which occupied larger area in the subchondral departments than in series II. Numerical density of the bone marrow cells in the subchondral parts of the epiphysis was 16.1±1.4 per μ^2 14 days after tunneling in series III, which was significantly ($p<0.05$) higher than the similar value in series II (8.9±0.7 per μ^2). Calcium levels in SCB were significantly ($p<0.05$) increased in comparison with experimental series I and II up to 15.57±2.16% on day 14, 15.27±2.54% on day 28, and 18.34±3.36% on day 90 after tunneling; the values were still reduced in comparison to intact normal. In the epiphysis, the volume density of the trabeculae increased ($p<0.05$) in comparison with series I to 30.4% on day 14 and to 30.9 and 39.3% on days 28 and 90 after tunneling, respectively.

Assessment of the status of the articular cartilage conducted by us earlier [8] suggested the development of degenerative changes in series I, corresponding to osteoarthrosis stages 1 to 2. In series II, enlargement of empty lacunae in the articular cartilage along with progressive reduction of sulfur concentration attested to exhoustion of the compensatory capacity of the cartilage by day 90 after EOA. Sites of fiber separation on the joint capsule were still seen, opened lacunae with chondrocytes were revealed. In series III, there were no sites of fiber separation in most of the observations by the end the experiment, the number of functionally active cells were characterized by pronounced hypertrophy increased, sulfur level and cartilage thickness were elevated.

In the knee and hip joints, cartilage nutrition is realized primarily through SCB capillaries. Subchondral bone plate in the zone of direct contact with the cartilage has microscopic protrusions containing blood capillaries. Some of them penetrate into the zone of CC (under pathological conditions, also through the basophilic line of not calcified cartilage) [1,3,15]. Cells in CC remained viable due to its monolithic fusion with well-vascularized bone.

The experimental model of osteoarthrosis developed by us [10] confirms the important role of microvessels of subchondral zone in the nutrition of the articular cartilage. Due to circulatory disorders and limitation of the function in the articular cartilage, trophic insufficiency develops because of pathological changes in subchondral zones nourishing the deeper layers of the cartilage. In SCB, bone resorption was

enhanced, focal osteosclerosis was detected, and significant reduction in calcium level was found.

Several clinical studies confirmed that osteoarthrosis increased the rate of both components of bone remodeling, namely, resorption and bone formation. The predominance of one or another process depends on the stage of the disease [1,6]. Trabecular thickening in SCB is not always accompanied by the increase in bone mineralization or osteoid enlargement [13].

Restoration of the microarchitecture of the SCB and improving its vascularization after tunneling with introduction of the bone marrow suspension into the drill holes were accompanied by the improvement of the histological structure of the cartilage. Vascular invasion into the zone of CC together with rich vascularity of SCB ensured diffusion of nutrients from the SCB into the cartilage. In the articular cartilage, the number of active hypertrophied secretory cells was increased [8]. Hypertrophied chondrocytes in the deeper layers of cartilage are known to perform a specific function. The cytoplasm in these cells is in a state of gel and retains 85-90% water. Due to this hydrophilic content, these cells mediate effective diffusion of oxygen and nutrients into the cartilage [3].

Thus, stimulation of functional activity of chondrocytes is performed by correction of cartilage and SCB homeostasis and improvement of tissue trophic. Improvement of chondrocyte metabolism and their functionality are achieved due to restoration of the microarchitecture of the SCB and enhancement of its vascularization after subchondral tunneling with introduction of bone marrow suspension into the drill holes.

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