METHODS

Stimulation of Regeneration of Hyaline Cartilage in Experimental Osteochondral Injury

M. Sh. Khubutiya, I. Yu. Kliukvin, L. P. Istranov, V. B. Khvatov, A. B. Shekhter, A. Yu. Vaza, I. V. Kanakov, and V. S. Bocharova

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A model of osteochondral intra-articular defect in rats is presented. During spontaneous healing, the stage of formation of granulation tissue is followed by its replacement with bone and fibrous tissue. Chondroinductive properties of collagen 1 sponge used for defect filling manifested in the formation of fibrous cartilage with fields of hyaline cartilage. Filling of the defect with collagen 1 sponge containing bone powder stimulated regeneration of the bone tissue and hyaline cartilage.

Key Words: *collagen; hyaline cartilage*

Intra-articular fractures constitute about $^1/_3$ of all skeletal injuries in traumatology. Restoration of the anatomical integrity and congruence of articular surfaces in intra-articular fractures is an important aspect of the treatment of these injuries essential for prevention of posttraumatic arthrosis. Repair of extensive traumatic defects of the articular cartilaginous tissue is carried out by the surgical method. Good results are attained by using cartilage autotransplantation, but this procedure involves damage to intact sites of the joint [5]. The most prospective methods are repair of the articular cartilage by transplantation of cultured auto- and allogenic mesenchymal cells and chondrocytes into the site of defect [2,13, 14]. In case of large articular cartilage defects, these technologies are effective only with autologous cells [14]. Large-scale introduction of transplantation of autologous chondrocytes in medical practice is impeded by the absence of specialized laboratories, trained staff, and norm-setting documents. In addition, more and more reports indicate that the procedure of cell production under artificial conditions is not safe. There is high probability of damaging the cell genome during forced cell proliferation [1]. Moreover, the environmental signals intrinsic of the wounds during the acute period and actively stimulating reparation in the zone of the osteochondral defect [7,9] are attenuated during the period needed for the production of the sufficient volume of cell material.

It can be hypothesized that creation of adequate conditions for targeted migration of bone marrow cells into the osteochondral wound and for chondrogenic differentiation of mesenchymal cells can lead to recovery of the damaged hyaline cartilage.

We developed a method for stimulation of reparative histogenesis of the hyaline cartilage in osteochondral injuries by using a collagen matrix.

MATERIALS AND METHODS

The study was carried out on outbred albino rats (200-230 g) in accordance with the Order of the

N. V. Sklifosovsky Institute of Urgent Care, Moscow, Russia. ***Address for correspondence:*** khvatov@yandex.ru. V. B. Khvatov

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Two transplant types were used to modulate the reparative process: sponge with collagen 1 and sponge with collagen 1 and bone powder (crumbs). Collagen 1 was obtained as described previously [3].

Transplants (collagen matrix) for filling of the osteochondral defect were prepared by lyophilization of 2% collagen 1 solution. Bone powder was added to one variant of filling material. The powder was prepared by grinding acetone-degreased rat bones in 1:1 weight proportion (50 ml 2% collagen 1 solution+1 g bone powder).

Osteochondral defect was simulated on the patellar surface of the femoral condyles in the knee joint. The knee joint was opened with a longitudinal anterointernal parapatellar incision under general anesthesia (ketamine), the patella was dislocated outwards, and a defect 2.5-3 mm deep was drilled with a 2.3 mm drill on the patellar surface of the condyles. The defect was filled with collagen matrix, the patella was set, the articular sac and skin were sutured. The operation was carried out on both lower limbs simultaneously. No immobilization was used. The animals were kept in individual cages until complete healing of the skin wound.

The experiment was carried out in 3 groups of rats, 16 per group. In group 1 (control), the osteochondral defect healed spontaneously. In group 2, the defect was filled with collagen 1 sponge and in group 3 with sponge from collagen 1 with bone powder.

Repair of the osteochondral defects was evaluated morphologically. Morphological studies were carried out 1 and 2 weeks and 1 and 3 months after surgery. Four animals were used for each period (a total of 48 rats). The animals were sacrificed by lethal dose of hexenal (intraperitoneally), the knee joints were removed and fixed in 10% neutral formalin. The specimens were decalcinated with Biodek and embedded in paraffin. The sections (4-5 μ) were sliced with a microtome and stained with hematoxylin and eosin, picrofuchsine after van Gieson, and with toluidine blue for detection of acid glycosaminoglycans (the main component of cartilaginous matrix proteoglycans).

RESULTS

Morphological analysis of the defect in the patellar surface of the femoral condyles in control rats 1 week after surgery showed that in the majority of animals the bone defect was filled with organizing fibrin and erythrocytes with signs of formation of immature loose connective tissue in deeper areas.

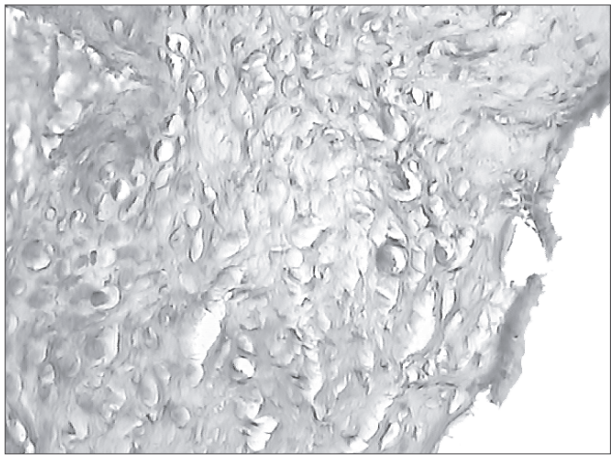

Fig. 1. Spontaneous healing of osteochondral defect (control) 3 months postoperation: the defect is filled with compact fibrous connective tissue without signs of cartilaginous tissue regeneration. Here and in Figs. 2, 3: hematoxylin and eosin staining (×400).

After 2 weeks, the intensity of inflammatory changes decreased and maturation of the granulation tissue was noted. Bone tissue regeneration in the depth of the defect was just starting; osteoid bars were just forming. One month after surgery, inflammatory infiltration disappeared, granulation tissue was transformed into mature connective tissue, and osteogenesis in the depth of the defect was intensified. Three months after surgery, the defect shrank in size and its upper layers closer to the surface were filled with compact fibrous connective tissue without signs of cartilage tissue regeneration, while the deeper zone was filled with mature bone regenerate occupying about 4.5 of the initial defect volume (Fig. 1).

In group 2, the transplant was completely absorbed and replaced with granulation tissue as soon as 1 week after transplantation. The greater part of

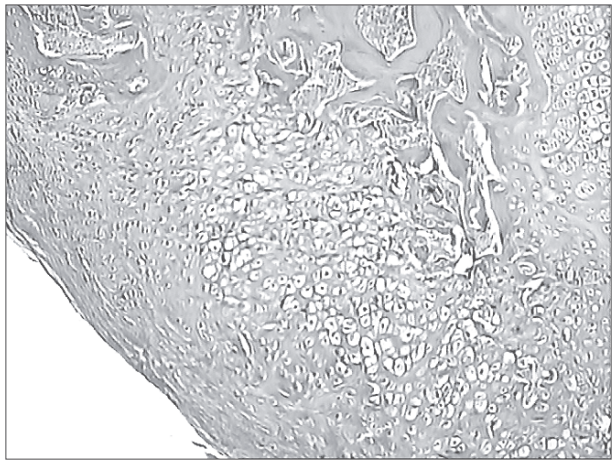

Fig. 2. Healing of osteochondral defect filled by a sponge from collagen 1: 3 months postoperation.

the defect volume was filled with new bone tissue, with small foci of regenerating cartilage in the surface layer. After 1 month, no collagen transplant was detected; in addition to bone regenerate, there were separate foci of hyaline and fibrous cartilaginous tissue regeneration. After 3 months, fields of hyaline and fibrous cartilage were seen (Fig. 2). The defect surface was covered by the connective tissue.

In group 3, new maturing bone tissue occupied $^4/_5$ of the defect volume just 1 week postoperation; the outer layer was formed by the mature connective tissue without signs of cartilage regeneration. After 2 weeks no implanted collagen was detected; bone tissue was mature, forming numerous foci of chondral regeneration. The remaining fragments of bone crumbs were surrounded by columns of differentiated chondrocytes (Fig. 3, *a*). One month after surgery, large fields of fibrous and hyaline cartilage formed, which were separated from the joint surface with a connective tissue layer. After 3 months, the defect zone was filled with bone tissue and regenerated cartilage (Fig. 3, *b*) covered by a narrow connective tissue layer. The site of the defect was undetectable at visual inspection of the sliding surface of the femoral condyle (Fig. 3, *c*).

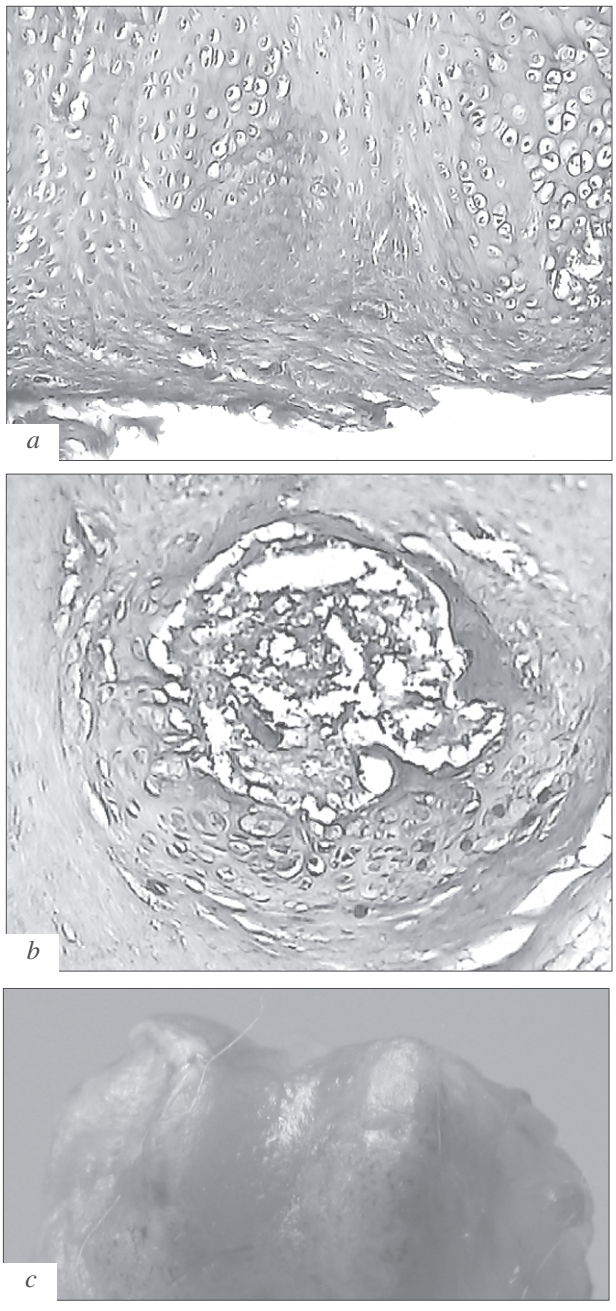

Fig. 3. Healing of ostochondral defect filled with collagen 1 sponge with bone powder. *a*) 2 weeks postoperation: signs of bone tissue maturing; *b*) 3 months postoperation; *c*) 3 months postoperation: sliding surface of femoral condyle.

Hence, the defect was sooner replaced with new bone tissue in experimental groups. This fact attests to osteogenesis induction by collagen material filling the defect cavity. In group 2, regeneration of fibrous and hyaline cartilage of different degree of maturity was observed. In group 3, the process of chondral tissue regeneration and maturation was even more intense during delayed periods. Despite the formation of mainly hyaline cartilage, no full-value recovery of the external chondral plate was attained. The chondral regenerate was covered by a narrow layer of the connective tissue (Fig. 3, *b*).

Bearing in mind that chondrocytes and mesenchymal cells actively release collagen proteins (a most important component of chondral tissue matrix), presumably, under conditions of articular osteochondral defect and direct contact with bone marrow mesenchymal cells, these cells repopulate the chondral tissue by chondrocyte precursors in the presence of a collagen matrix. In addition, unique microenvironment appears at the site of the wound in acute injury. This microenvironment activates bone marrow cells located in the adjacent intact tissues. However, in order to realize their functions during regeneration of the destroyed tissue, precursor cells have to migrate into the zone of injury using the interstitial matrix proteins (mainly collagens), which are absent from the osteochondral wound during the early periods after the injury.

The type of collagen for osteochondral defect filling is essential. The interstitial hyaline cartilage matrix consists from mainly collagen 2, providing the work of the joint during excessive exercise, but the periosteal and perichondral cells contain collagen 1 in their cytoplasm. In addition, cells involved in tissue restoration, carry receptors to collagen 1 on their surface [8,15]. Introduction of collagen 1 into the osteochondral wound defect leads to creation of the necessary conditions for fixation and directed migration of cells, participating in the wound

process, to the zone of injury, in which the microenvironment stimulates chondrogenic differentiation of mesenchymal precursors.

New data on the effects of growth factors on articular cartilage development and regeneration were published [4,10]. The injury modifies the homeostasis and cell composition of tissues and hence, the signal microenvironment of mesenchymal cells [7,9]. Of the microenvironmental factors, the osteal morphogenetic proteins with their inductive effects attract special attention [4]. Bone powder added to the collagen matrix, initiated the development of hyaline cartilage, presumably due to the stimulatory effects of osteal morphogenetic protein.

The results of experiments indicate that transplants from collagen 1 with bone powder are characterized by osteo- and chondroinductive effects creating conditions for the realization of bone marrow mesenchymal cell potential and providing healing of the osteochondral defect with restoration of the hyaline cartilage. Importantly, collagen 1-based implantation material with bone powder is cheap and can be stored for a long time and used when needed.

Hence, we developed an experimental model for studies of healing of osteochondral intra-articular traumatic defects in rats. During spontaneous healing of osteochondral intra-articular defect, the articular surface is restored through the stage of granulation tissue formation and its subsequent replacement with the bone and fibrous tissue without cartilage formation. The collagen sponge filling the osteochondral intra-articular defect had a favorable impact on the course of the early postoperative period. The chondroinductive characteristics of the matrix manifest by the formation of a fibrous cartilage with fields of hyaline cartilage. The implant consisting of collagen 1 with bone powder is characterized by pronounced osteo- and chondroinductive effects. Filling of the osteochondral defect with collagen matrix with bone powder accelerated bone tissue regeneration and led to the formation of hyaline and fibrous cartilage.

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