Use of Primary Human Fetal Chondroblast Culture for Xenotransplantation into Rat Articular Cartilage Defect

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The possibility of xenotransplantation of human fetal chondroblasts was studied. Filling of the rat articular cartilage defect with a tissue-engineering construction based on primary culture of human fetal chondroblasts and chitosan gel caused no immune rejection over 60 days and provided the formation of organotypical regenerate due to proliferation and differentiation of donor fetal chondroblasts and their integration in the recipient cartilage tissue.

Key Words: *articular cartilage; chondroblasts; xenotransplantation*

Cell technology is a modern approach to disease treatment [2], including treatment of traumas of the cartilage tissue of different etiology [7,12]. Tissue-engineering constructions based on primary culture of autologous chondrocytes are usually employed for the correction of these injuries [4-6]. However, the phenotype of chondrocytes can be changed during *in vitro* culturing, collagen-2 and proteoglycan synthesis decreases, the cells acquire a fibroblast-like shape and synthesize collagen-1 [11]. In addition, low proliferative activity of chondrocytes and difficulties in regulation of their differentiation *in vitro* rule out the production of the needed volume of cell material within short time. The main factor impeding the use of autologous tissue-engineering constructions is shortage of donor material, which necessitates the search for its alternative sources [3] and experimental verification of the possibility of donor cell integration into recipient tissues [9,10]. Fetal cells are regarded as a universal modulus for cell reconstruction and permit creation of stable stems of new normal tissue in zones of injury [2].

We studied the possibility of xenotransplantation of human fetal chondroblasts into the rat articular cartilage defect.

MATERIALS AND METHODS

Fetal chondroblasts were isolated from growth plates of vertebral bodies of human embryos (gestation week 12, aborted material was received from licensed institutions of Ministry of Health of the Russian Federation in accordance with ethical standards, with written informed consent). Primary chondroblasts were cultured through 4 passages *in vitro* [1]. The proportion of low, medium, and highly differented chondroblasts was evaluated at each passage. The tissue engineering construction was created with passage 2 cells and chitosan gel. All manipulations needed for xenotransplantation of human fetal chondroblasts into the rat articular defect were

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carried out in accordance with requirements of the Geneva Convention for Animal Protection.

The defect (reaching the subchondral bone) was created in the intercondylar fossa of the rat femoral bone with a dental bore (2.5 mm in diameter). The defect was left free in group 1 (n=10), filled with chitosan gel in group 2 (n=10), and in group 3 (n=5) with a mixture of passage 2 primary culture of fetal chondroblasts (3×10^5 cells) and 50 μl chitosan gel. All animals were intramuscularly injected with 0.3 ml lincomycin once daily for 2 weeks after surgery.

The animals were observed for 60 days, after which they were sacrificed under ether narcosis, and fragments of the distal epiphysis of the femoral bone were isolated for morphohistological analysis. The material was fixed in 10% neutral formalin, decalcified in buffered Trilon B, dehydrated in ascending alcohols, clarified in xylene, embedded in celloidine paraffin, and serial sections were sliced. The preparations were stained with hematoxylin and eosin, acid glycosaminoglycans (GAG) were detected by alcyan blue staining after Stidman. Morphometrical characteristics were evaluated using AxioVision software.

The data were statistically processed using Student's t test, the differences were significant at $p\leq0.05$.

RESULTS

On day 60 after the injury to the articular cartilage, a dark-brown oval regenerate was clearly seen in the intercondylar fossa in group 1 rats. The interface between sawed articular cartilage and the regenerate was identified at the photo-optic level (Fig. 1, *a*). The edges of sawed articular cartilage were uneven, covered with compact connective tissue (Fig. 1, *b*); the size of the cartilage injury increased to 3.89±0.39 mm *vs.* the initial wound (2.5 mm), which can be explained by destruction of the articular cartilage caused by inflammatory reaction. The resultant regenerate did not completely fill the defect, was cone-shaped with an apex beyond the articular surface, and was covered with compact unformed connective tissue. The central parts of the regenerate were formed by fibroreticular tissue with developing primitive osseous bars. Numerous blood vessels were seen in the deeper layers of the regenerate (towards the depth of the defect bed), with signs of active osteogenesis around these vessels (Fig. 1, *c*).

Oval regenerate located in the intercondylar fossa was clearly seen macroscopically in group 2 rats on day 60; in contrast to the regenerate in group 1, it was white. Histological study showed that the contour of the sawed articular cartilage was blurred (Fig. 1, *d*). The size of the cartilage defect increased to 3.39±0.37 mm in comparison with the initial wound. The regenerate was formed from compact unformed fibrous connective tissue completely filling the defect and protruding beyond the articular cartilage surface (Fig. 1, *e*). Invasion of blood vessels into the regenerate and ejection of poorly differentiated cells from the epiphyseal spongy bone were seen on the side of the subchondral bone plate towards the articular surface (Fig. 1, *f*).

Macroscopically non-uniform regenerate with dark and light areas was seen in group 3 rats during the same period. Histological study showed that the resultant regenerate completely filled the detect (Fig. 2, *a*). The diastasis between the wound edges was 2.61±0.23 mm, this virtually not differing from the size of the initial wound. Numerous groups of 4-8 cells were located at the terminals of the sawed cartilage area; the main substance around these cells exhibited intense alcyan-positive reaction (Fig. 2, *b*). The space between articular cartilage section and regenerate was filled by moderately alcyan-positive substance (Fig. 2, *c*) in which solitary cells with signs of destruction were seen. Due to heterogeneity of the cellular composition, the peripheral and central zone of the regenerate could be distinguished. The upper third of the regenerate in the peripheral zone (at the interface with intact articular cartilage) was presented by large oval cells (Fig. 2, *d*). Histochemical reaction detected summary acid GAG in the cell cytoplasm, territorial and interterritorial matrix. The intensity of the reaction was higher at the periphery of cell lacunas.

The regenerate was covered with homogenous alcyan-positive substance on the side of the articular surface. Signs of formation of layers typical of native articular cartilage were noted in the central zone of the regenerate (Fig. 2, *e*). The cells of the forming tangential layer were oval, with clearly seen cytoplasm moderately stained with hematoxylin. The plane of cell location was parallel to the articular plane. The cells in the deeper layers of the regenerate were oval or round. The planar location of cells arranged in columns perpendicular to the articular surface was changed. The cells and interstitium in the base of regenerate were separated from the subchondral bone by lamellar bone tissue (Fig. 2, *f*). The regenerate cells at the interface with bone tissue had no signs of destruction. High levels of acid GAG was histochemically detected in their cytoplasm, territorial and interterritorial matrix. The absence of significant differences between the size of articular cartilage defect and size of initial wound

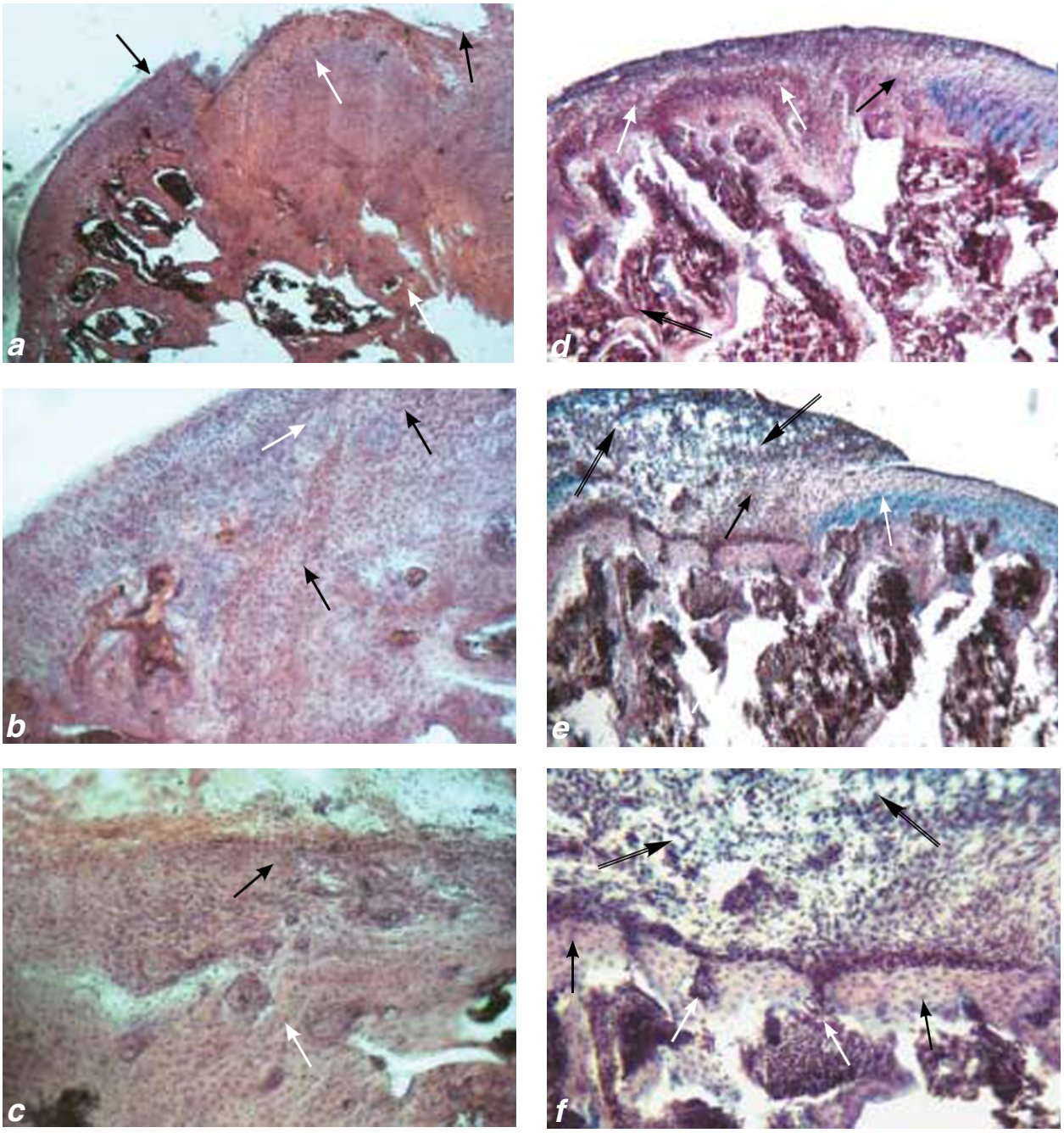

Fig. 1. Rat articular cartilage regenerate. *a-c*) no treatment (hematoxylin and eosin staining); *d-f*) rat cartilage defect filled by chitosan gel (Alcyan Blue staining). *a*) femoral bone articular cartilage section. Dark arrow: border of sawed off articular cartilage; light arrow: regenerate (×40); *b*) interface between sawed off articular cartilage and regenerate. Dark arrow: border of articular cartilage section; light arrow: regenerate (hematoxylin and eosin staining; ×100); *c*) articular cartilage regenerate. Dark arrow: surface zone; light arrow: deep zone of regenerate (hematoxylin and eosin staining; ×100); *d*) surface of articular cartilage of the rat femoral bone. Dark arrow: articular cartilage section line; light arrow: defect (×40); *e*) integration of fibrous tissue of the regenerate and femoral bone articular cartilage. Dark arrow: compact unformed fibrous tissue of regenerate; light arrow: cartilaginous tissue of articular surface; double arrow: chitosan (×100); *f*) central zone of articular cartilage regenerate. Dark arrow: subchondral plate; light arrow: invasion of vessels in the regenerate zone; double dark arrow: rejection of poorly differentiated cells (×200).

and clear-cut contour of the regenerate on the side of cartilage section suggested the absence of pronounced inflammation in the wound during the posttransplantation period in this group of rats in contrast to groups 1 and 2, in which signs of productive inflammation were seen. It seems to be due to the absence of expression of HLA proteins by fetal chondroblasts, reduced reactogenicity of the

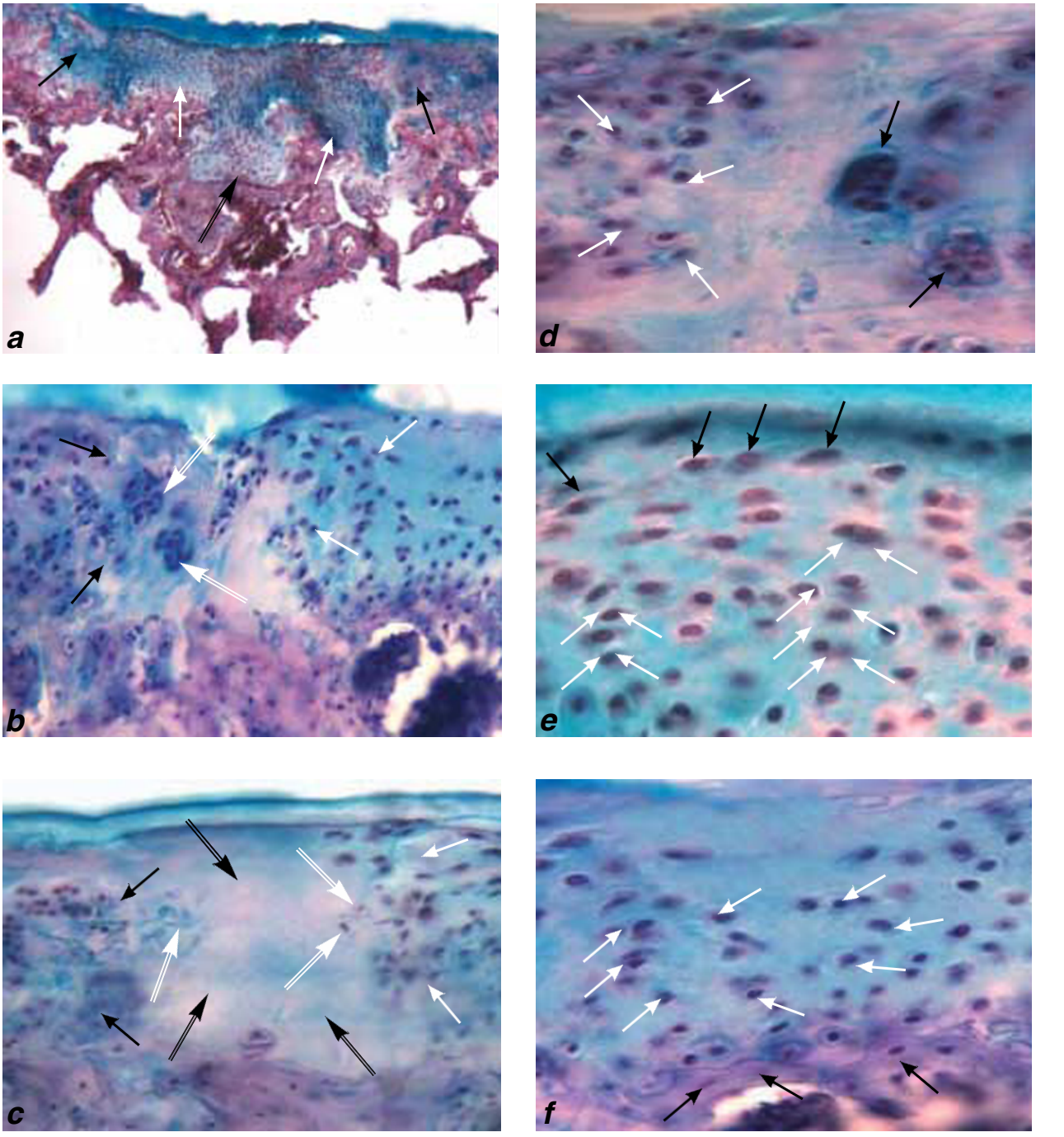

Fig. 2. Regenerate of rat articular cartilage after filling by chitosan gel and primary culture of human fetal chondroblasts (Alcyan Blue staining). *a*) surface of femoral bone articular cartilage. Dark arrow: contour of articular cartilage section; light arrow: regenerate; double arrow: regenerate base (×100); *b*) interface between sawed off articular cartilage and regenerate. Dark arrow: intact site of articular cartilage; light arrow: regenerate; double arrow: chondrocyte clusters (×200); *c*) a site of matrix between articular section and regenerate. Dark arrow: articular cartilage; light arrow: regenerate; double dark arrow: interstitium; double light arrow: cells with signs of destruction (×200); *d*) peripheral zone of articular cartilage regenerate. Dark arrow: chondrocytes in intact portions of articular cartilage; light arrow: regenerate cells (×400); *e*) central zone of articular cartilage regenerate. Dark arrow: cells of the forming tangential layer; light arrow: forming column structures (×400); *f*) base of formed articular cartilage regenerate. Dark arrow: subchondral plate; light arrow: regenerate cells (×200).

xenotransplant due to purification of chondroblast culture from leukocytes (immune response co-stimulators) during culturing, and the use of chitosan gel as the immunomechanical barrier. The gel separates the recipient from the transplant, provides viability of transplanted cells, does not prevent dif-

fusion of biopolymers synthesized in the cells [8]. In addition, chitosan gel fills and supports the defect volume, is characterized by high biocompatibility, blocks the synthesis of inflammation mediators, suppresses the destructive effects of free-radical compounds on intercellular matrix, and reduces activity of proteolytic enzymes [13].

We conclude from these data that use of tissue engineering constructions based on primary culture of human fetal chondroblasts (passage 2) and chitosan gel causes no acute immune rejection over 60 days and provides the formation of organotypical regenerate due to proliferation and differentiation of donor fetal chondroblasts and their integration in the recipient cartilaginous tissue.

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