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Chondroplasty Efficacy of Bone Matrix

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Abstract

Aim: To study the chondroplasty efficacy of the bone matrix obtained using an original technology in restoring cartilage defect of the knee joint.

Material and Methods: Marginal defects were modeled on the surface of the distal end of the femur in 40 adult male Wistar rats. The bone matrix obtained using an original technology was implanted in the damaged area in animals of the experimental group. Material was investigated by means of light microscopy, transmission and scanning electron microscopy, and electron probe X-ray microanalysis.

Results: It was found that the bone matrix implanted did not cause an immune rejection reaction, activated reparative chondrogenesis for a prolonged period. In the area of articular cartilage lesion, the regenerate acquiring cellular and histochemical characteristics of the hyaline cartilage tissue was formed. The chondroinductive properties for the bone matrix were ensured by localized growth factors and morphogenetic proteins released during osteoclastic resorption.

Conclusion: The application of the bone matrix as a stimulator of chondrogenesis is theoretically reasonable and has a good perspective in treatment of damages and diseases of the articular cartilage.

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Introduction

Articular cartilage damages or loss due to congenital abnormalities, degenerative joint diseases or traumas affect quality of life of injured people of all age groups. This health problem is expensive [1, 2]. The main techniques of chondroplasty include reparative chondrogenesis stimulation by perforation of the subchondral bone at the base of the defect, restoration of the damaged area by anatomic auto- and allografts, and cell-based technologies [3]. However, the fibrous cartilage developing in the defect area during osteoperforation is inferior to the hyaline cartilage in terms of their biomechanical characteristics. The application of autografts is associated with additional injurious exposure and posttraumatic inflammatory process, enhances blood loss and length of surgery and is limited by impossibility of taking a required volume of autogenous material particularly in children, as their donor regions are small and taking tissues may result in a localized growth disorder [4]. When using allografts, the avital (cadaveric) material which lacks vital chondrogenic cells is applied. Ethical and moral issues occur when there is a potential risk for transmission of various diseases and recipient's infection by prion agents, hepatitis C and B viruses, HIV infection. Histoincompatibility and a number of severe immune complications are often followed by abscess formation and graft rejection in the postoperative period develop [5]. The application of cell technologies and tissue bioengineering is difficult because of low proliferative chondroblast activity, aseptic inflammation progression when a substantial number of the implanted cells die or lose their phenotype, trophism of the regenerate gets broken [6]. The use of pluripotent bone marrow stromal cells, which are able to differentiate towards chondrocytes under the influence of the microenvironment of the cartilage defect area [7], is followed by spreading the cells throughout the joint cavity, formation of chaotic disordered cytoarchitectonics of the regenerate, formation of thick cicatrices, synechiae and joint stiffness [4]. Despite numerous developed techniques, none of them lead to the full organo-specific restoration of the articular hyaline cartilage [1]. This indicates the relevance of the issue and immediacy of the present research.

The aim of the work was to study the chondroplasty efficacy of the bone matrix obtained using an original technology in restoring cartilage defect of the knee joint.

Material and Methods

Forty adult male Wistar rats weighing 340-390 g. were used in this study. The animals were divided into two groups: experimental and control of 20 rats each. All procedures were performed in accordance with the Order of the Ministry of Health and Social Development of the Russian Federation №708n of August 23, 2010 "On approval of laboratory practice regulations" and approved by the Ethics Committee of FSBI "Russian Ilizarov Scientific Center "Restorative Traumatology and Orthopaedics" of Ministry of Healthcare of the Russian Federation (Record №3 (16) of September 2, 2010). Experiments were carried out at the laboratory and the vivarium of the Russian Research Centre "Restorative Traumatology and Orthopaedics".

Intervention Description

Blind-ended window defects 2.5-3 mm in diameter prior to penetration into the subchondral bone were modeled on the patellar surface of the distal end of the right femur by a dental bur in rats under general anaesthesia (Rometar 8 mg/kg and Zoletil 4 mg/kg of body weight intramuscular) in the operating room. Sterile granular allogenic bone matrix (2-3 mg) obtained from long bones of rats on the original technology was placed in the defect area in animals of the experimental group [2]. Animals in the control group did not undergo any additional procedures, and the defect area was left to heal under a blood clot. In 7, 15, 30 and 60 days after surgery the animals were taken out of the experiment (5 animals were used at each time point).

Research and Outcomes Registry Methods

Femoral bones operated on were fixed in the paraformaldehyde and glutaraldehyde solution and embedded in paraffin (after decalcification) and araldite (without decalcification). Paraffin sections were stained with hematoxylin-eosin, Van Gieson's picro-fuchsin and Alcian blue at pH 2.5 and 1.0 to determine histochemical markers of the hyaline cartilage: non-sulfated (nsGAG) and sulfated glycosaminoglycan (sGAG) respectively. The content of glycosaminoglycans was determined in conventional units (c.u.) by defining Alcian blue

concentration in paraffin sections due to the presence of copper in it (Alcian blue 8GS, $C_{44}H_{42}Cl_2CuN_{12}S_2$), using energy-dispersive X-ray spectrometer (X-ray electron probe microanalyzer) INCA-200 Energy (Oxford instruments, England) [8]. The structure of the regenerate was studied on paraffin sections with a light microscope AxioScope.A1 and a digital camera AxioCam (Carl Zeiss MicroImaging GmbH, Germany), in Araldite blocks with a scanning electron microscope JSM-840 (Jeol, Japan) (after dosed removal of the embedding medium by 4% sodium etiolate solution) and on ultra thin sections (after slice contrast with uranyl acetate and lead citrate) with a transmission electron microscope JEM-2010 (Jeol, Japan). Ultra thin sections were achieved by means of an ultramicrotome LKB-8800 (LKB, Sweden).

Statistical analysis of quantitative data was performed by using the software StatSoft STATISTICA 6.1.478 Russian, Enterprise Single User Version: 6.1.478. The findings were presented as an average (*M*) and a non-sampling error (*m*). The statistical significance level of intergroup differences was evaluated using nonparametric Mann-Whitney U test for independent samples. Differences were considered statistically significant at $p < 0.05$.

Results

The implanted granules of the bone matrix were of 50-200 μm in diameter and had a well-ordered highly porous structure. The pores corresponded to the regions of the localization of osteocitarian lacunae and bone canaliculi from which cells and other non-mineralized organic components of the bone matrix had been removed. Granule calcification was homogeneous. The content of osteotropic macroelements such as calcium, phosphorus, sulfur and magnesium was not different from their concentration in the mineralized bone matrix.

Seven days after surgery, the joint capsule was hyperemic and edematous in animals from the control and experimental groups. The cartilage covering became dull. The signs of inflammatory response and alterative-destructive changes affecting all components of the meta-epiphysis were identified in the defect area. Foci of the organized hematoma infiltrated by fibrin clots, little differentiated cellular elements, neutrophil granulocytes, macrophages, mast cells, extravascular erythrocytes and lymphocytes were disclosed. Leukocyte necrotic masses containing lysed cells and fibrin layers were revealed. The articular cartilage was scarified. The defect area was partially replenished with granular and little differentiated loose connective tissue with the signs of edema and few vessels. Statistically significant changes in nsGAG and sGAG content in the articular cartilage defect area at this stage of the experiment were not observed (Table 1).

Fifteen days after surgery, numerous leukocytes, fibroblasts, bundles of collagen fibers, granulation tissue and a considerable number of dilated and filled with blood vessels were disclosed in the defect central area in the control group. Cellular fibrous elements of inflammation in animals of the experimental group were not revealed. The implanted granules of the bone matrix resembled cystic cavities formed during decalcification of the specimen in histological processing and due to biodestruction. Macrophages and functionally active osteoclasts with multiple nuclei and ruffled borders were localized at the surfaces of these cavities and within them. Dilated sinusoids were surrounded by proliferating perivasculocytes. There were no hemorrhages or foci of perifocal inflammation in the defect. Active fibroblastic proliferation and intense neoangiogenesis were noted. The layers of chondrogenic cells at the different stages of differentiation were located on the granule surface. The articular cartilage defect area was partially filled with a newly formed hyaline cartilage, the intercellular substance of which contained mainly nsGAG (Table 1).

The defect margins merged with the margins of the "maternal" articular cartilage where cell nests with isogenous groups of chondrocytes were identified. The presence of the latter gave evidence of a proliferative activity of chondroblasts which were one of the cellular sources of the cartilaginous tissue filling the defect. The cells arranging around the implanted granules were the second source of chondrocytes. Glycosaminoglycan concentration in the defect area in experiment exceeded the control values more than twice (Table 1).

Thirty and sixty days after surgery, a considerable volume of the defect area was filled with loose and dense connective tissue in the control group (Figure 1 a, b, c).

Table 1. Glycosaminoglycan content in the area of the knee joint cartilage defect in the control group of animals (Control) and in the bone matrix granule implantation (Experiment) (*M* ± *m*, c.u.)

The period of the experiment, days	Control		Experiment	
	nsGAG	sGAG	nsGAG	sGAG
	(n=5)	(n=5)	(n=5)	(n=5)
7	0.18 ± 0.01	0.11 ± 0.01	0.20 ± 0.01	0.12 ± 0.01
15	0.19 ± 0.01	0.12 ± 0.01	0.49 ± 0.01[1]	0.26 ± 0.01[1]
30	0.21 ± 0.01	0.16 ± 0.01	0.56 ± 0.02[1]	0.54 ± 0.01[1]
60	0.22 ± 0.01	0.24 ± 0.01	0.59 ± 0.03[1]	0.70 ± 0.01[1]

Note. [1] Statistical significance of intergroup differences: p<0.01.

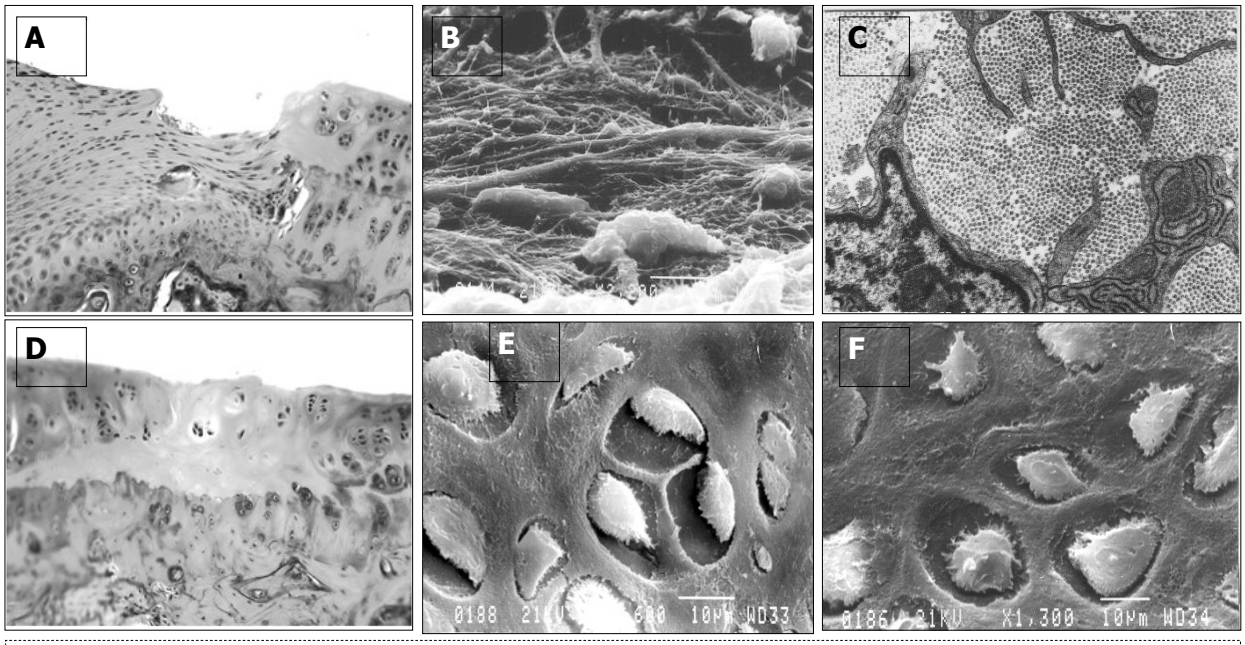

Figure 1. The defect area of the knee joint cartilage in the control (a, b, c) and experimental (d, e, f) groups of animals sixty days after surgery: a, b, c – the defect is replaced by fibrous connective tissue; d, e, f – the defect is filled with a newly formed hyaline cartilage; a, d – paraffin sections, stained by hematoxylin-eosin. Objective lens 10, ocular lens 10; b, e, f – scanning electron microscopy, magnification 1300; c – transmission electron microscopy, magnification 5000.

The newly formed cartilaginous tissue in the form of isogenous groups of chondrocytes was apparent only at the edges of the defect. In the experimental group of animals, the articular cartilage defect was almost completely filled with the newly formed hyaline cartilage with a smooth surface (Figure 1 d, e, f). This hyaline cartilage was growing from the defect margins where the developed isogenous groups of cells were located.

The surface of the newly formed hyaline cartilage acquired the intrinsic luster of the articular cartilage of intact animals. The cell population of the cartilage regenerate was represented mainly by proliferating chondroblasts. Its structure had not had a distinctive specificity of the articular cartilage yet. Thirty days after surgery, the content of non-sulfated and sulfated forms of glycosaminoglycans in the defect in experiment exceeded the control values more than twice, but did not differ significantly against each other. Whereas sixty days after surgery, the sulfated forms of glycosaminoglycans prevailed. It gave evidence of a higher maturity degree of the newly formed cartilage tissue (Table 1) reaching the maturity degree intrinsic to the articular cartilage of intact animals.

Discussion

A mature hyaline cartilage is known to have insignificant potential for regeneration due to a low cell density and little mitotic activity of chondrocytes [6, 9]. Large articular cartilage defects are substituted by a biomechanically inadequate fibrous cartilage followed by osteoarthritis development [1]. Tissue engineering methods have provided alternative opportunities for treatment by applying a cell-based therapy when combined with synthetic substitutes of the extracellular matrix and biologically active factors for a functional replacement of the articular hyaline cartilage [8]. The present study showed that the bone matrix granules implanted into the defect area produced an apparent chondromodulating effect activating reparative chondrogenesis. The regenerate acquiring cellular characteristics of the hyaline cartilage tissue was developing in the articular cartilage defect area at an early stage. An integral cartilage covering was formed.

The impaired edges of the cartilage surface were gradually smoothing out resulting in the whole or partial functional recovery of the joint activity. Localized growth factors and bone morphogenetic proteins discharged during osteoclastic resorption supplied the implantation granules with the chondroinductive properties [10].

Conclusion

The research carried out showed that the application of the granular bone matrix obtained using the original technique as a stimulator of chondrogenesis and a corrector of destructive disorders in the cartilaginous tissue in injuries of the articular cartilage was theoretically reasonable and promising in a clinical practice in treatment of the articular cartilage injuries and diseases.

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