

Estimation of the chondrocyte number in the articular cartilage by combined distraction osteosynthesis of the tibia (Ilizarov apparatus and plating)**T.A. Stupina, M.A. Stepanov**

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Introduction The number of chondrocytes in the articular cartilage of the femoral condyles and talus junction surface in the conditions of distraction osteosynthesis of the tibia with the Ilizarov apparatus combined with plating was estimated. **Material and methods** Seven dogs underwent tibial lengthening at a daily rate of 1 mm for 4 steps and to 14–16 % of the initial length of the segment with the use of the Ilizarov apparatus and plating. Stages of the study were days 30 and 90 in the fixation phase when only plating was left. Histomorphometry of semi-thin sections of the articular cartilage was carried out with the help of an *Opton* photomicroscope (Germany), *DiaMorf* software complex (Russia) and *VideoTest-Master* morphology program. The proportion of chondrocytes (N_{ch} , %), the proportion of empty lacunae ($NN_{em.lac}$, %), and isogenic groups ($NN_{is.gr}$, %) were determined in the total sample volume of 200 lacunae. A control group was articular cartilage of five intact dogs. **Results** The articular cartilage of both articular surfaces retained the zonal structure. Disorder in the homogeneity of the superficial part of the surface zone and destruction of a part of chondrocytes were noted. In the articular cartilage of the femoral condyles, the N_{ch} value was lower than in the control animals by 5.74 %. $NN_{em.lac}$ was increased 2.3 times while $NN_{is.gr}$ values decreased to 2.06 % in the femoral condyle articular cartilage after 30 days of fixation period with the plate. After 90 days of fixation, the N_{ch} values were lower with respect to the controls by 3.73 %. Low values of $NN_{is.gr}$ (4.3 %) and high values of $NN_{em.lac}$ (27.54 %) were maintained. In the articular cartilage of the talus, N_{ch} was lower than in the control group by 1.98 %; $NN_{em.lac}$ increased 1.5 times and $NN_{is.gr}$ decreased to 4.7 % relative to controls after 30 days of fixation. After 90 days, the value of $NN_{em.lac}$ remained at the same level as in the previous experiment stage. The values of $NN_{is.gr}$ were comparable with the control group. **Conclusion** The technique of distraction osteosynthesis of the tibia in combination with plating is of low traumatic effect on the adjacent joints. A comparative analysis of quantitative parameters of the articular cartilage revealed that the most vulnerable site was the articular cartilage of the femoral condyles.

Keywords: combined osteosynthesis, Ilizarov apparatus, plate, articular cartilage, chondrocytes, morphometry

INTRODUCTION

One of the most important issues in limb lengthening is functional disorders in the joints adjacent to the operated segment. Pathological changes in the articular cartilage, that may also happen when the method of controlled transosseous osteosynthesis is used, often complicate the treatment of orthopedic patients [1]. It was established in our earlier studies that changes of the destructive and reparative nature occur in the articular cartilage of adjacent joints by tibial lengthening and their degree of expression depends on the elongation conditions [2, 3].

The amount of chondrocytes in the articular cartilage hardly reaches 2–3 % from the total mass. Accordingly, the recovery is significantly reduced and is proportional to the number of cells. Chondrocytes play an exceptional role in maintaining the state of the extracellular matrix in the processes of normal vital activity and in the development of

degenerative diseases. An increase in the number of actively proliferating and producing cells is the main source in the articular cartilage recovery process [4].

Present requirements to the quality of medical care and treatment comfort dictate their priorities. The most important of them are the quality of life of a patient and reduction in the duration of inpatient treatment.

In order to shorten the period of the patient's wearing the Ilizarov apparatus and to reduce the complication rate, the technique of distraction osteosynthesis of the tibia in combination with a plate was tested in the experiment [5].

Purpose Estimate the number of chondrocytes in the articular cartilage of the femoral condyles and talus trochlea during distraction osteosynthesis of the tibia in combination with plating.

MATERIAL AND METHODS

The studies were conducted on 7 mongrel dogs of both sexes at the age from one to 5 years, weighing 12–

15 kg. The maintenance of animals, surgical interventions and euthanasia was carried out in accordance with

the requirements of the Ministry of Health of the Russian Federation to the work of experimental and biological clinics (Order of the Ministry of Health of the USSR No. 755 from 1977). All manipulations on animals were reviewed and approved by the ethical committee of RISC for RTO.

All the dogs underwent a surgery of transosseous osteosynthesis in the right tibia with the Ilizarov apparatus in the operating room under general anesthesia. Osteotomy of the tibia was performed at the border of the middle and upper third of the tibia. Through a small additional incision in the soft tissues on the medial surface of the segment at the level of its proximal metaphysis, a plate of the original construction [5] was placed on the diaphysis of the tibia when the soft tissues had been separated from the bone. The plate extended from the upper to the lower third of the tibia and did not contact with the wires of the Ilizarov apparatus. The plate was fastened with 3 screws to the proximal fragment of the tibia and the distal screw was inserted only in the upper part of the plate groove. The screw was not attracted tightly to the plate, so that it was able to slide in the groove during the distraction. Elongation was carried out in the manual mode at a rate of 1 mm per day for 4 steps to lengthen 14–16 % of the initial length of the segment. Distraction continued for 2–28 days, depending on the length of the tibia. On the day of the end of

the distraction, the apparatus was removed. The regenerate was located at the level of the solid part of the plate which prevented deformities at the level of the regenerate after removal of the apparatus. The dogs were withdrawn from the experiment by an overdose of sodium thiopental after 30 and 90 days of plate fixation.

After the knee joint and tarsus articulation exposure, the samples of the articular cartilage of the femoral condyle and talus trochlea joints measuring $2-3 \times 4-5$ mm were cut out perpendicular to the articular surface using a scalpel. Histomorphometric studies were performed using serial semi-thin sections of the articular cartilage along with subchondral bone of a large area (up to 8 mm^2) [6], which were stained with methylene blue-basic fuchsin.

Images of micropreparations were digitized with a photo-microscope *Opton* (Germany) at the *DiaMorf* complex (Moscow) and analyzed with the *VideoTest-Master Morphology* program. The proportion of chondrocytes (N_{ch} , %), the proportion of empty lacunae ($NN_{em.lac}$, %), and isogenic groups ($NN_{is.gr}$, %) were estimated in the total sample volume of 200 lacunae. The articular cartilage of five intact dogs was studied morphometrically.

The digital material was analyzed according to the variation and nonparametric statistics with the *AtteStat* program, version 1.0 in Microsoft Excel 97 spreadsheets.

RESULTS

The light optical study of half-thin sections revealed similar changes in the articular cartilage of the femoral condyles and the talus joints at all the time-points of the experiment. The articular cartilage of both joint surfaces retained the zonal structure (**Fig. 1A, Fig. 2A**). The destructive changes were manifested by disorders in the homogeneity of the superficial part of the surface zone and disorders in the structure of a part of the chondrocytes (**Fig. 1 B, C**). The isogenic groups of cells were rare and were noted in the intermediate and superficial zones. An increase in empty lacunae encountered in the articular cartilage of the femoral condyles was noted mainly in the intermediate and deep zones while in the articular cartilage of the talus articulations - in the superficial zone. In all the samples, the integrity of the basophilic line was not broken.

The histomorphometric analysis showed that the portion of chondrocytes was 71.89 % in the articular cartilage of the femoral condyle of the intact animals while isogenic groups made 14.5 % and empty lacunae occupied 13.6 % (**Fig. 3**). After 30 days of plate fixation, the value of chondrocyte portion in the total sample size was significantly ($p < 0.05$) lower than in the control by 5.74 %. The portion of empty lacunae in-

creased 2.3 times. At the same time, the proliferative activity decreased, the portion of isogenic groups was 2.06 %. After 90 days of fixation with the plate, the portion of chondrocytes increased insignificantly as compared with the previous period. When compared with the control, this parameter was reduced by 3.73 %. Low values of the isogenic groups portion (4.3 %), high values ($p < 0.05$) of the empty lacunae (27.54 %) were maintained.

In the articular cartilage of the talus articulations in intact animals, the portion of chondrocytes was 79.3 %. The isogenic groups made 9.9 % and empty lacunae made 10.8 %. After 30 days of plate fixation, the portion of chondrocytes in the total sample volume was lower ($p > 0.05$) than in the controls by 1.98 %, and the portion of empty lacunae was increased 1.5 times. The portion of isogenic groups decreased to 4.7 % relative to the control dogs. After 90 days of fixation with the plate, the values of the fraction of empty lacunae remained at the same level as at the previous experiment time-point. An increase in the proliferative activity of chondrocytes was noted. The values of the isogenic groups portion were comparable with the values in the control group.

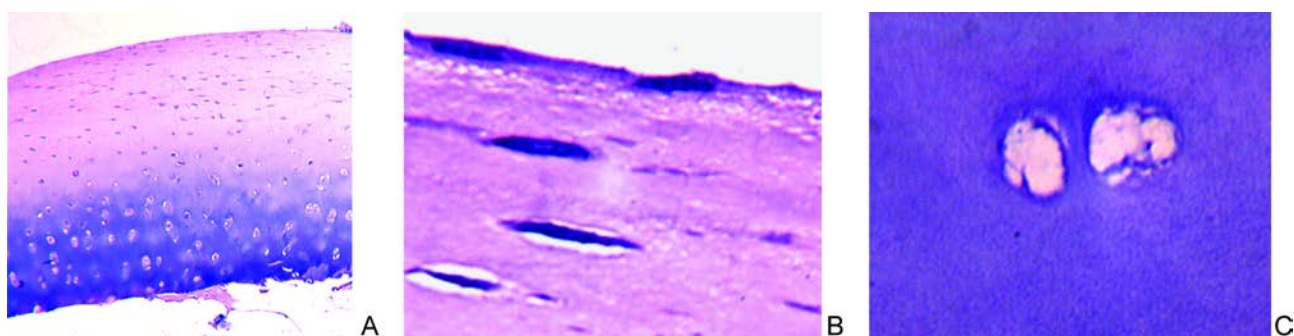


Fig. 1 Articular cartilage of the femoral condyle at 90 days of fixation with the plate only: semi-thin sections, methylene blue-basic fuchsin stain; lens – 6.3; eyepiece – 12.5× (A); lens – 100 mm; eyepiece – 12.5× (B, C). A – general view; B – superficial zone of the cartilage; C – empty lacunae in the intermediate zone of the cartilage

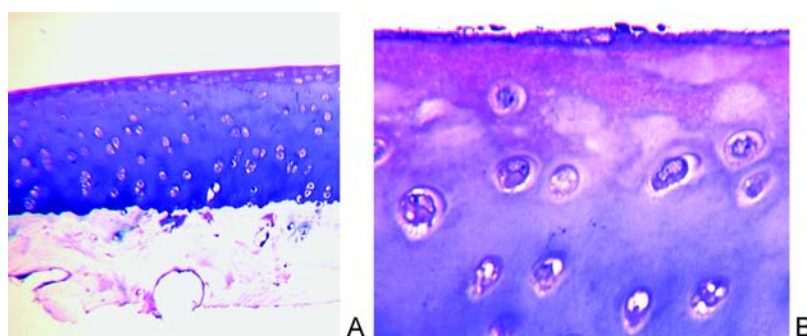


Fig. 2 Articular cartilage of the talus articulation at 90 days of fixation with the plate; semi-thin sections, methylene blue-basic fuchsin stain; A – general view (lens – 6.3, eyepiece – 12.5×); B – empty lacunae in the superficial zone of the cartilage (lens – 40, eyepiece – 12.5×)

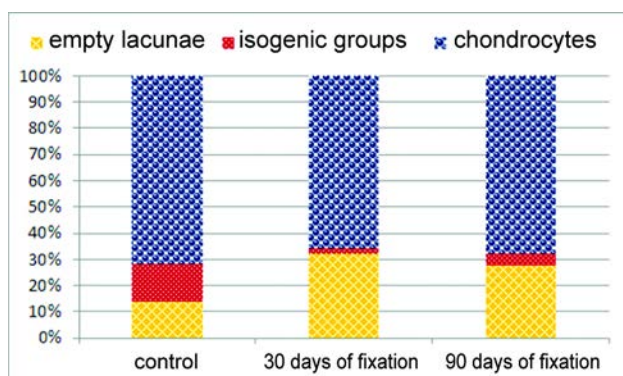


Fig. 3 Morphometric parameters of the articular cartilage of the femoral condyle

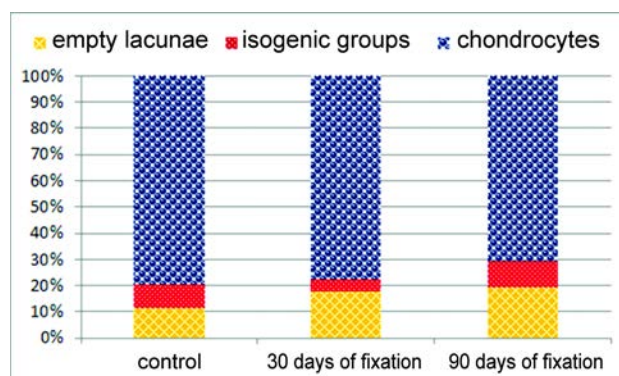


Fig. 4 Morphometric parameters of the articular cartilage of the talus articulations

DISCUSSION

Thus, the light optical study of semi-thin sections of the articular cartilage of the femoral condyles and the talus trochlea articulations under the conditions of tibial elongation at a rate of 1 mm per day made with 4 increments and in combination with plating revealed similar changes: homogeneity disorders in the intercellular substance of the upper superficial part of the surface zone and destruction of a part of chondrocytes. The articular cartilage of both articular surfaces preserved the zonal structure. The integrity of the basophilic line was not disturbed.

The prevalence of osteoarthritis varies in these

joints. Its incidence in the ankle joint is much lower as compared with the knee joint [7, 8].

The histomorphometric method enabled to reveal the differences in the state of the articular cartilage of the femoral condyles and the talus articulations. Thus, the quantitative analysis showed that in intact animals the processes of chondrocyte death were more pronounced in the articular cartilage of the condyles of the femur than in the articular cartilage of the talus trochlea ($NN_{em.lac.}$). The same pattern was also observed in the experiment. The most vulnerable in these experimental conditions was the articular cartilage of the femoral

condyles. The degree of damage to the articular cartilage of the condyles of the femur is characterized by an increase in the $NN_{em.lac}$ to a greater degree than in the articular cartilage of the talus articulations at all terms of the experiment. Thereby, the values of $NN_{is.gr}$ reduced sharply, and the number of chondrocytes was also reduced to a greater extent.

Our previous studies of the Ilizarov distraction osteosynthesis of the tibia in the mode of 1 mm with 4 daily steps during 30 days of the experiment revealed that the chondrocyte death in the articular cartilage of the femo-

ral condyles was less pronounced [9].

Chondrocytes are the only type of cells that form cartilage and maintain a dynamic equilibrium between synthesis and degradation of the extracellular matrix [4]. Destruction of the cartilage is primarily associated with the death of chondrocytes, and then with the loss of the matrix. Accordingly, the recovery processes are determined by the degree of the biosynthetic activity activation of the surviving chondrocytes, their proliferation, post-proliferative restoration of biosynthetic activity, and participation in matrix reorganization.

CONCLUSION

The results of the study show that the technique of the Ilizarov distraction osteosynthesis of the tibia combined with plating is low traumatic for the adjacent joints. The zonal structure of the cartilage, the integrity of the baso-

philic line is preserved. A comparative analysis of the quantitative parameters of the articular cartilage revealed that the most vulnerable was the articular cartilage of the femoral condyles in these experimental conditions.

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