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## Osteochondral graft from the pre-Achilles area for repair of ankle joint articular surface defects and lesions

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**Introduction** Management of osteochondral lesions of the talus trochlea involves surgical debridement of the necrotic cartilage and underlying bone, and then repair of the defect with an osteochondral autologous graft. The use of MSCT, MRI allows us to detect changes in the ankle joint at the initial stage, when the salvage operation is effective. **Purpose** Histological substantiation of the possibility to use osteochondral autologous grafts from the pre-Achilles area of the calcaneus for management of osteochondral defects in the damaged talus trochlea. **Material and methods** We substantiate a new technique for obtaining an osteochondral autologous graft to repair osteochondral defects of the talar trochlea resulting from various lesions by means of a morphological study of the following microslides: cartilage and underlying bone tissue of the talar trochlea (cadaver material), necrotic area of the talus, and the cartilage portion of the pre-Achilles area of the calcaneus. For comparison, preparations of the cartilage tissue from the lateral condyle of the femur were investigated. **Results** Investigations of the specimens of the pre-Achilles cartilage of the calcaneus revealed a large number of chondrocytes in the extracellular matrix, presence of its all cartilage zones (superficial, middle, and deep ones). This was a proof that this cartilage can be considered as an autograft material.

Keywords: pre-Achilles area, osteochondral defect, talus, talar trochlea, osteochondral lesion, chondrocyte, "tide mark", extracellular matrix

#### INTRODUCTION

The problem of managing osteochondral defects of the damaged talar trochlea (ODTT) remains challenging at present [1, 2]. To date, there are several surgical treatments for ODTT, each of them having its merits and shortcomings such as the problem of the "painful donor site", difficulty of positioning the graft into the recipient bed on the talus, presence of several invasive stages of harvesting, cultivation and implantation of the donor material. The use of mosaic osteochondroplasty with the use of osteochondral autograft (OA) for the ODTT repair meets the goals of treatment as it provides a radical cleaning of the necrotic focus, elimination of the chronic inflammation focus in the joint, and restoration of the ankle joint biomechanics. The measure results in reduction of patient's disability and rehabilitation periods. Currently, the Tsivyan NNIITO of the Ministry of Health of Russia applies a patented method of surgical ODTT treatment with the use of OA from the pre-Achilles area of the calcaneus [3].

The purpose of this work is the histological substantiation of the possibility of using OA from the pre-Achilles calcaneus area for the treatment of osteochondral defects in the damaged talus trochlea. For this purpose, morphological studies of micropreparations of cartilaginous and bone tissue of the pre-Achilles zone of the calcaneus were conducted. For comparison, the preparations from joint cartilage and the adjacent bone tissue of the lateral condyle of the femur were examined.

### MATERIAL AND METHODS

With the permission of the ethics committee of the Tsivyan NNIITO (No. 006/14 of September 12,

2014), the micro-preparations of the articular cartilage and the underlying bone of the talus trochlea

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(cadaveric material), the cartilaginous area of the pre-Achilles zone of the calcaneus, the cartilaginous tissue of the lateral condyle of the femur and the necrotic area of the talus were examined. The age of the patients (n=12) was from 30 to 60 years. The preparations were fixed in a 10 % solution of neutral

# Cartilage and underlying bone tissue of the talar trochlea

There is no clear division into zones in the articular cartilage of the talus trochlea. There are superficial chondrocytes of flattened shapes (Fig. 1); chondrocytes of the upper part of the middle zone, one or two in the lacuna, and chondrocytes located in columns in the deeper part of the middle zone, and hypertrophied deep-zone chondrocytes. Dystrophically altered cells, mostly non-nuclear, are located in the homogeneously eosinophilic dense matrix of the deep cartilaginous zone. There are cells with a preserved nucleus, but with a calcified cytoplasm. The cartilaginous and bone tissues are separated by a distinctly intensive basophilic line which is the border zone of the articular cartilage that ensures the preservation of the cartilaginous tissue (Fig. 1 b). Down from the basophilic line, there is a zone of calcified cartilage, adjacent to the bone tissue. The bone tissue is of the lamellar type with uneven lines of adhesion and a large number of osteoblasts, which indicates the process of restructuring.

## Necrosis area of the talar trochlea

The preparation shows bone fragments and necrotic bone marrow. Thin bone tissue is almost devoid of cells; there are denuclearized cells in the preserved part of the bone architecture. Necrotic masses are visible in some areas. Macrophages are identified. In the regeneration zone, tissue fibrosis is observed, characterized by fibrin fibers and rare spindle-shaped cells. The process of necrosis occurs with a practically preserved cartilaginous tissue (in some places, necrotic changes penetrate the cartilaginous tissue). All the zones characteristic of articular cartilaginous tissue can be identified: superficial, middle and deep. The extracellular matrix is defibrillated and unevenly stained. The basophilic line is destroyed in some places, and the process of necrosis has penetrated into the cartilagformalin. After decalcification in a solution of EDTA and paraffin dehydration, the preparations were stained with hematoxylin and eosin. The resulting specimen were analyzed and digitalized with the Axio Observer Z1 microscope (Carl Zeiss, Germany) and the ZEN 2012 software (Blue edition).

### RESULTS

inous tissue (**Fig. 2**). Detachment of the cartilage tissue from the bone is seen. The changes revealed in the cartilaginous tissue confirm the need for an operation to repair affected cartilaginous tissue with a graft of a healthy cartilaginous tissue.

# **Pre-Achilles area of the calcaneous (cartilage and underlying bone tissue)**

Cartilaginous tissue is presented by an extracellular matrix with chondrocytes immersed into it. The cartilaginous zones can be seen: superficial, middle and deep zones. It is possible to identify the basophilic line and the calcified zone adjacent to the basophilic line from the side of the bone tissue. The matrix of the superficial zone is intensely stained. The chondrocytes of this zone have a flattened cytoplasma and a rounded basophilic nucleus. The cells are arranged in parallel rows (**Fig. 3a**).

Further, chondrocytes are located by 1-2 in the lacuna, the matrix is stained unevenly. Closer to the basophilic line, there are a few chains of chondrocytes that form columns. The matrix of this zone is most intensely stained relative to the other zones. The basophilic line is clearly delineated (**Fig. 3b**). Chondrocytes are immersed into it in several places. The area of calcified cartilage adjacent to the basophilic line is thin or absent in some places. The bone is immature: a lot of osteoblasts and fuzzy lines of adhesion. The study of the preparations, depending on the age, found that the total number of chondrocytes decreases and the dystrophic changes affecting the intercellular matrix are enhanced with the age.

There is a large number of chondrocytes in the intercellular matrix and all the zones are present (superficial, middle, deep). Also, a well-marked basophilic line and the adjacent calcified zone indicate that this cartilage is structurally and functionally preserved and can be used for grafting.



**Fig. 1** Paraffin sections, hematoxylin-eosin stain; magnification  $\times 100$ : **a** – surface area of the talus articular cartilage; **b** – basophilic line and chondrocytes of the middle and deep zones of the articular cartilage of the talus



Fig. 2 Paraffin sections, hematoxylin-eosin stain, magnification  $\times 200$ . Zone of necrosis of the talus trochlea. Destruction of the basophilic line, necrosis process extended into the cartilaginous tissue



Fig. 3 Paraffin sections, hematoxylin-eosin stain, magnification  $\times 200$ : **a** – chondrocytes of the superficial, middle and deep zones of the pre-Achilles area of the calcaneous; **b** – basophilic line and column-arranged chondrocytes of the pre-Achilles area

# Articular cartilage and underlying bone tissue of the lateral femoral condyle

All the zones, characteristic of this type of tissue, are presented in the articular cartilage of the lateral condyle of the femur: a thin superficial layer (intensely stained barrier), an intermediate zone with isogenically located groups of cells (**Fig. 4a**) and chaotically located columns of chondrocytes, a deep zone with hypertrophied chondrocytes. Groups of cells are located in lacunae, the boundaries of which merge. The basophilic line is narrow; there are areas where it borders on the bone. The ossified zone is almost not detected. A thin layer of the subchondral bone is visualized (**Fig. 4 b**).



**Fig. 4** Paraffin sections, hematoxylin-eosin stain, magnification  $\times 100$ : **a** – chondrocytes of the superficial and middle zones of the cartilage tissue of the lateral femoral condyle; **b** – basophilic line chondrocytes of the middle and deep zones of the cartilage tissue of the lateral femoral condyle

### DISCUSSION

It was found in the course of the work, that the majority of young and working age patients with severe pain of an unclear genesis suffered osteochondral lesions [4]. Various positions for taking X-rays suggested by different authors do not allow to reliably diagnosing ODTT. It seems that the administration of multispiral computed tomography (MSCT) to patients with "causeless" pain in the ankle should become the rule. Indeed, MSCT is the method of choice for the diagnosis of ODTT [5].

This cannot be accepted completely as far as MSCT does not allow estimation of the prevalence of concomitant edema of the spongy bone substance of the affected talus trochlea. Additional information may be obtained with MRI. The need for this information is debatable. There are works emphasizing the need for preoperative conservative treatment for a preoperative prevention of graft rejection, progression of aseptic necrosis in the ODTT zone [6].

Also, surgical approaches to the treatment of ODTT remain controversial. Despite the questionable effectiveness due to irradicality, a number of authors reported on the continued use of multifocal osteoperforation of the talus trochlea as an independent method [7]. Undoubtedly, multifocal osteoperforation takes its deserved place in the complex of surgical treatment as an additional method [8]. Time has confirmed the need for radical intervention on the cystic formation, in connection with which such non-radical operations as subchondral microfracture, debridement, exocclusion of the necrosis zone, etc. have ceased to be used by orthopedists as independent methods. The reason for this is the need to replace an emerging defect. Since the end of the twentieth century, there has been a considerable progress in this direction [9, 10, 11, 12]. To repair the emerging defect, implantation of autologous chondrocytes, bone autografts, allografts, allohemiarthroplasty [13, 14] were used.

To date, the methods listed above seem exotic since they show their positive properties, apparently, only in the hands of their authors. The method of choice which still remains is obviously the replacement of the defect with an osteochondral autograft by the Hangoody method. Nevertheless, the undoubted positive features of the Hangoody technique are levelled by its unacceptable shortcoming, and namely, the "painful" donor site in the knee area [15]. Various authors suggested techniques that reduce the traumatic nature of the Hangoody technique but the problem has not been solved. The technique suggested by our clinic may provide the solution to this problem. However, it is necessary to continue the collection of clinical material to prove its viability. When introducing it, we needed proofs of the eligibility of using an osteochondral autograft from the pre-Achilles area for clinical purposes. The data presented in a number of studies allowed us to state that this graft is osteochondral and is suitable for achieving the goals of ODTT treatment due to its morphological properties.

Undoubtedly, the methods of ODTT management will develop further. It would be very valuable to reduce the invasiveness of the surgical intervention due to the introduction of the mosaic osteochondroplasty with minimally invasive methods under the control of arthroscopy. It will permit to abandon the need for osteotomy of the malleoli. But to date, mosaic osteochondroplasty, according to many experts, remains a method of choice.

There are no data on the description and characterization of the cartilaginous tissue from the pre-Achilles area of the calcaneus in the available domestic and foreign literature. Therefore, to identify the structural and functional features of this cartilaginous tissue, a morphological study of cartilaginous tissue from the pre-Ahilles area of the calcaneus was conducted. A comparative analysis of the preparations of the articular cartilage and the bone of the talus trochlea, the cartilage of the pre-Achilles area of the calcaneus, the cartilaginous tissue of the lateral femoral condyle and the zone of necrosis of the talus was carried out. The articular cartilage of the talus trochlea is presented by a uniformly homogeneous matrix, in which the cells are located diffusely. The articular cartilage features three zones: superficial, intermediate and deep (basal) ones [16]. Chondrocytes of the zones

are different in volume, shap, ability to differentiate, density of distribution in the matrix, and ultrastructural organization. The least differentiated cells in the articular cartilage are located in the upper part of the intermediate zone, and the most differentiated are in the deep one [17]. Each of the cartilage tissues studied by us featured superficial flattened types of chondrocytes; the intermediate ones, located isogenously by 1-2 in the lacuna, and separate groups of cells located in columns; and deep hypertrophied chondrocytes. Also, the presence of a separating basophilic zone, ensuring the preservation of cartilaginous tissue, bone tissue with signs of restructuring, was revealed. The obtained data confirmed the possibility of using osseo-cartilaginous OA from the pre-Achilles area of the calcaneus. The investigation of the necrosis zone of the talus trochlea detected all the signs of destruction: fragments of bone tissue, necrotic bone marrow, and necrotic changes penetrating into the cartilaginous tissue in some places. All the changes that were revealed indicate the need to replace the affected tissue with an autologous graft.

### CONCLUSION

The study of the cartilage preparations of the pre-Achilles calcaneus area showed a large number of chondrocytes in a homogeneously stained intercellular matrix, the presence of all cartilage zones (superficial, middle, deep), and also the preserved basophilic line. Thus, this cartilage is structurally and functionally preserved and can be considered as a material for autologous grafting.

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