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AUTOLOGOUS REGENERATIVE STIMULANTS FOR BONE ALLOGRAFT IMPLANTATION

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There are many different surgical techniques for bone reconstruction. However, biological reconstruction methods are being increasingly developed. The main purpose is not only to fill up defects, but to stimulate the processes of reconstruction and regeneration of bone as a complete organ. In this report, we describe the basic principles of orthobiology and the essential orthobiological materials. A clinical case is presented where a combination of allogeneic osteoplastic materials with autologous platelet-rich plasma is used to reconstruct a cavity defect in the tibia.

Keywords: orthobiology, bone regeneration, bone defect, bone graft, allogeneic bone graft material, bone marrow aspirate, platelet-rich plasma.

INTRODUCTION

Orthobiology is a conceptual concept, which includes a group of biological materials and substrates promoting bone regeneration. Such include bone grafts, osteoplastic materials, growth factors, regulatory protein, and cellular biomedical products [1, 2]. The role of orthobiology in bone healing lies in osteoconduction, osteoinduction and osteogenesis, which are part of the "diamond concept" proposed by Giannoudis et al. [3], where the authors identified four basic conditions for successful bone healing: potent osteogenic cell populations, osteoconductive matrix scaffolds, osteoinductive stimulus, and mechanical stability.

Bone transplantation

Bone transplantation in the classical concept is possible only if the bone tissue is preserved in its native form and is applicable only for two types of materials – autologous bone and allograft. Autografts have three functional properties (osteoconductivity, osteoinduction and osteogenicity) and demonstrate the highest ability for osseointegration and remodeling; therefore, they are rightfully considered the 'gold standard' for bone grafting. However, their use is limited because they can be used in a small volume and there is a need to form an additional access for collection of donor fragments [4]. Spongy bone autografts are the most commonly used type of materials because they contain a small number of osteoblasts and osteocytes, with a high content of living multipotent mesenchymal stromal cells (MSCs) and they create an osteogenic potential for neoosteogenesis from the graft. Moreover, proteins contained in the autograft allow maintaining the natural osteoinductive potential [2]. In the early post-autograft period, at the stage of hematoma and inflammation, the contained MSCs allow quick formation of granulation tissue; necrotic tissues are removed by macrophages, and graft neovascularization occurs [3].

Allogeneic bone materials

Unlike autografts, allografts are immunogenic and exhibit rejection reactions, which are caused by antigens of the major histocompatibility complex (MHC) [5]. The initial osseointegration phase is accompanied by severe inflammation due to immune response, causing necrosis of osteoprogenitor cells [6]. The necessary conditions for the use of allografts should be considered a decrease in immunogenicity and the conduct of donor/recipient compatibility studies, by analogy with organ transplantation [7]. Another problem is the risk of infection transmission, which has been resolved in most countries in the world thanks to the widespread development of a network of tissue banks and advanced processing technologies [4]. Due to immune response, purified decellularized and delipidized bone grafting materials (DDBGM) are very popular in clinical practice [8]. Purification of bone tissue of bone marrow cells and lipids and then of mineral-collagen matrix significantly reduces the degree of inflammatory response during DDBGM implantation, but does not prevent it. According to various literary sources, the probability of an immune response after implantation of such materials is about 10% [9]. Demineralized

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bone matrix (DBM) is a highly purified, allogeneic bone derivative, a material devoid (by more than 40%) of the mineral component, while preserving collagenous and non-collagenous inducer proteins [10] that determine osteoinductivity. Having plasticity and high degree of biodegradation, the process of osseointegration and remodeling of the implanted DBM is more intensive than with non-demineralized bone materials [4].

The clinical outcome of a reconstructive-restorative surgery depends on the patient's health status, the tissues surrounding the recipient bed, and the quality and functional characteristics of the implanted materials. To improve the functional properties of bone grafting materials, they are used in combination with autologous bone marrow aspirate and/or autologous platelet-rich plasma [2, 11, 12]. The use of such combinations is a simple, affordable and effective way to reduce the risk of immune reactions after implantation, increase the osteoinductive potential and impart osteogenic properties to materials.

Autologous bone marrow aspirate

Autologous bone marrow aspirate (ABMA) contains 2 types of adult stem cells: hematopoietic stem cells (HSCs) and MSCs. The main mechanism of ABMA as a stimulator of bone regeneration, is realized due to MSCs content, which differentiate into osteoblasts in the presence of specific growth factors and cytokines. The mediated mechanism of action of ABMA is the effect of cytokines derived from MSCs on endothelial cells, which promote angiogenesis.

Autologous platelet-rich plasma

The use of autologous platelet-rich plasma (aPRP) as a biogenic stimulator of regeneration is a fairly popular and widespread method in orthopedics. Regenerative potential is achieved through a cascade of reactions and release of growth factors contained in platelet-rich plasma [13]. Besides, plasma platelets are able to release over 300 molecules that are responsible for complex intercellular and extracellular interactions [14]. Unlike soft tissue, bone regeneration is a long process. In this regard, many researchers suggest the use of thrombin-activated aPRP in the form of a dense fibrin clot to create conditions for slow release of the factors contained in it [16].

The main orthobiological materials and their comparative characteristics are presented in Table.

CASE

Patient M., born in 1979, medical record card No. H2019-10342, was treated at the 12th department of the Priorova National Medical Research Center of Traumatology and Orthopedics from December 18, 2019 to December 24, 2019 for post-traumatic deformity of the proximal third of the right tibia. Posttraumatic type II medial right-sided gonarthrosis. Cyst of the upper third of the central-medial right tibia. Old damage to the body and anterior horn of the medial meniscus, partial damage to the anterior cruciate ligament of the right knee joint. Moderate right knee synovitis. The patient complained of swelling and pain in his right knee during physical exercise, impaired lower limb function. Examination revealed a deformity of the proximal metaphysis of the right tibia along the anterior surface and a varus deformity of the tibia. The right knee contours were unchanged, and edema was moderate. The right thigh muscles were satisfactorily developed. On palpation, the internal articular cavity was painful. Positive Baykov's symptoms. Capsular ligament apparatus: anterior drawer test (–/+), Lachman test (–), posterior drawer test (–), Varus stress test (–), Valgus stress test (–). Joint movement was full, painful when flexing mainly along the inner surface. Patella movements were painless. No vascular or neurological disorders in the limb were revealed at the time of examination. The patient underwent X-ray and multispiral computed tomography (MRI), which diagnosed a cyst of the proximal central-medial metaphysis of the right tibia (Fig. 1).

Punch trephine biopsy of the cyst was performed; cytological examination revealed no atypical cells. Given the clinical and diagnostic data, a decision was taken to surgically reconstruct the abnormal focus – combined

Table

Comparative biological properties of bone grafts and autologous regenerative stimulants

Autologous and allogenic orthobiological materials				
Material type/functional properties	Osteoconduction	Osteoinduction	Osteogenicity	Osseointegration
Cortical autograft	+	+	+	+
Spongy autograft	+++	+++	+++	+++
Cortical allo-implant	+	+/–	–	+
Spongy allo-implant	+	+/–	–	++
Demineralized bone matrix	+	++	–	++
Autologous bone marrow aspirate	–	++	+++	+++1
Autologous platelet-rich plasma	–	+++	+	++2

Note. [1] – effect on the osseointegration process is achieved due to the content bone marrow-derived multipotent mesenchymal stromal cells; [2] – effect on the osseointegration process is achieved due to the content of growth factors.

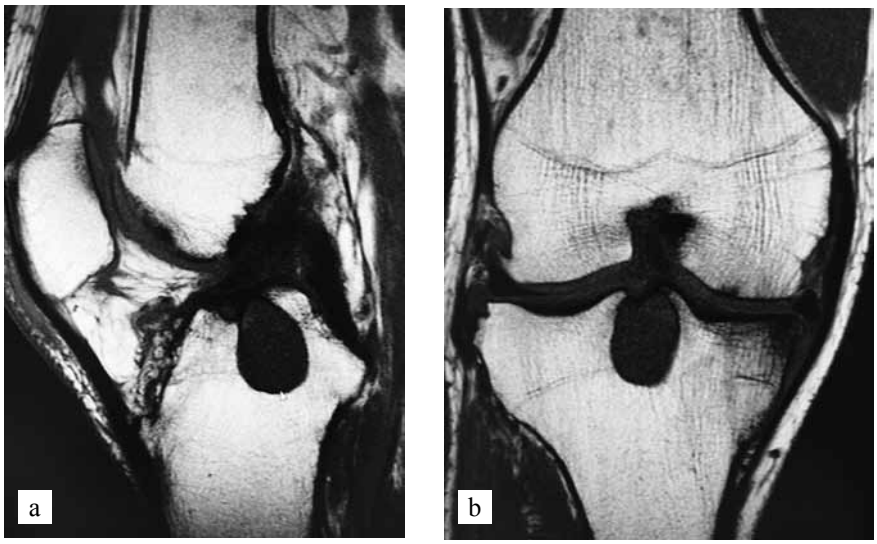

Fig. 1. Magnetic resonance imaging (MRI) of the knee. Tibial cyst. a) sagittal plane. b) frontal plane

bone grafting with allogeneic cancellous implants impregnated with aPRP. Allogeneic cancellous chips and Perfoost blocks from the Tkanevoy Bank of the Priorova National Medical Research Center of Traumatology and Orthopedics (RU # FSR 2009/05682 dated December 17, 2018) and a sets for preparation of aPRP and activation platelet-rich plasma Regen BCT-SP (R-BCT-SP) and Regen ATS-SP (R-ATS-SP) produced by Regen Lab SA, Switzerland were used to perform the surgical procedure.

The permission of the ethics committee was not required for the surgical intervention since the materials used have registration certificates. Blood sampling and preparation of autologous plasma were done prior to surgery, according to the manufacturer's instructions.

***Surgery process.** The operation was performed under spinal anesthesia, with the patient in the supine position, with reoperative antibiotic prophylaxis. The right lower limb was fixed in a knee brace in the middle third of the thigh. After treating the operating site three times with antiseptic solutions, a blood-squeezing tourniquet was applied from the lower third of the leg to the lower third of the thigh, then a pneumatic tourniquet was inflated in the upper third of the thigh. Under the control of an electronic optical transducer, below and lateral to the superficial goose foot, a 5 cm longitudinal skin incision was made, skin-subcutaneous flaps were mobilized, and access to the bone was made. An osteotomy was performed along the needle inserted into the cyst cavity of the central-medial metaphysis of the right tibia, then a canal was formed using a drill. The defect cavity was repeatedly washed with aseptic solutions, and the walls were treated. Bone-grafting materials were fragmented to the required size, then autologous plasma was added to them and plasma activation using thrombin was performed.*

The resulting combined bone-grafting material was placed in the defect cavity, and it was impacted for uniform distribution in the cavity. For the purpose of intraoperative control, a picture was taken with an electronic optical converter. Wound suturing was performed in layers.

CT multiscan was performed the next day after surgery. According to visual data obtained in three planes – axial, frontal, sagittal (Fig. 2) – there was a uniform filling of the defect with bone-grafting material.

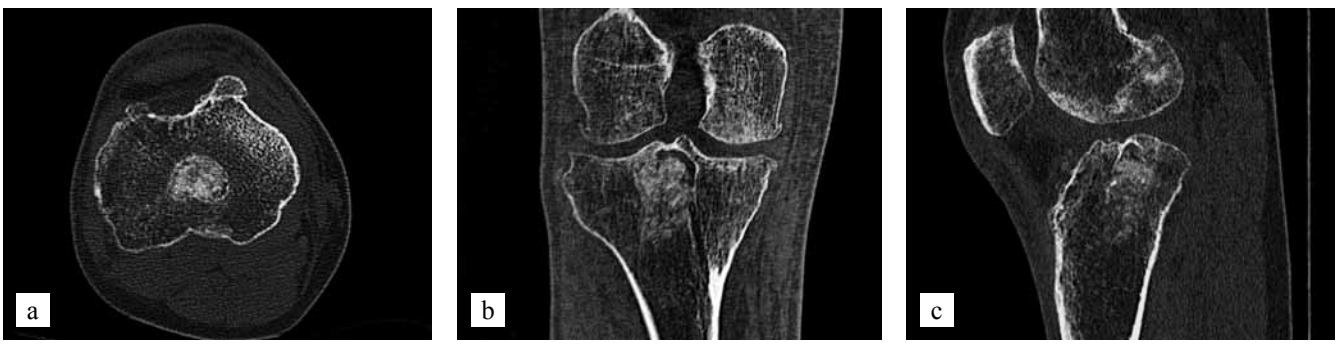

Fig. 2. Multispiral computed tomography (MSCT) of the knee joint. Tibial cyst after bone grafting. a) axial projection; b) frontal projection; c) sagittal projection

DISCUSSION

Analyzing the literature on this topic [1–3, 12, 15, 17], we should note the tendency that reconstructive and grafting interventions on bone tissues are aimed not only at addressing pain, restoring function, filling a defect or eliminating deformity, but also at stimulating regenerative processes. This explains the development of such a conceptual approach in orthopedics as orthobiology, since orthobiological products and their combinations can create the necessary conditions for achieving this goal.

Transplantation of frozen massive bone-cartilaginous allografts for bone tumors was performed by M.V. Volkov in 1960–70s [18]. The author described 145 cases, of which half of the results were unsatisfactory, which was due to insufficient understanding, at that time, of the mechanisms of transplant immunology. One of the first studies on the histocompatibility of cartilage tissue was carried out by Langer and Gross [19], where it was shown that intact articular cartilage does not cause humoral immune responses. This is due to the fact that antibodies are unable to penetrate through the dense cartilage matrix and reach the chondrocytes. This peculiarity of cartilage tissue allows transplantation without the necessary HLA compatibility studies [20], making the cartilage an "immune-privileged" tissue. Bone tissue, on the contrary, has enough immunogen; its transplantation in its native form requires the necessary compatibility studies to reduce the rejection risks and degree of immune response [20, 21]. Modern possibilities of laboratory screening and understanding of the mechanisms of transplant immunology and immunosuppression create the necessary conditions for the development of tissue transplantation, which is confirmed by literature data. For example, C. Krettek et al. [17] describes positive clinical results after transplantation of allogeneic osteochondral blocks and massive grafts. The use of allogeneic grafts from femoral heads from living donors is very popular among many orthopedic surgeons in the USA and European countries [22, 23]. In Russia, due to gaps and conflicts in legal regulations on tissue donation and transplantation, the lack of a network of regional tissue banks and the complexity of interaction of specialists in the sequence from donor to recipient patient, bone tissue transplantation remains a difficult surgical procedure to access [24].

Allogeneic bone grafting materials are the most popular and often used method in reconstructive surgery. The ability to process bone tissue to a mineral-collagen or demineralized matrix reduces immunogenicity and minimizes the likelihood of complications associated with it. In Russia, the production of materials is not subject to uniform standards on which the tissue processing and sterilization technology is based [8, 25]. As a result, bone grafting materials differ in their properties. This complicates the repeatability and predictability of clinical outcomes, and sometimes leads to post-implantation complications [9].

The efficiency of autologous bone marrow aspirate use to improve bone regeneration processes, both alone and in combination with osteoplastic materials, has been confirmed by many studies. Gianakos et al. [26] described the results of 35 animal studies in which BMA was used in long bone defects. Healing occurred in 100% of cases, and 90% reported significant improvement in earlier bone healing on histologic/histomorphometric assessment. Hernigou et al. [27] described the use of concentrated BMA concentrate after centrifugation in the pseudarthrosis of the tibia in 60 patients, of whom fusion was achieved in 53. Desai et al. [28] described the positive results of the use of BMA in combination with osteoplastic materials in false tibial joints. Schotter and Warner [29] published data indicating a positive effect when using BMA in combination with allogeneic bone grafting materials.

Sanchez [30] and Gallasso [31] published clinical cases of the use of aPRP to accelerate fracture healing. A positive effect was achieved in all cases. Kesyan G.A. et al. [11] described a number of clinical cases, which also noted the positive effect of the use of aPRP in combination with osteoplastic materials for the treatment of pseudoarthrosis. Despite the widespread use of aPRP to stimulate bone regeneration, the data on the effectiveness of its use remain controversial. Peerbooms et al. [32] reported that there is no beneficial effect of using aPRP to stimulate bone regeneration. Chahla et al. [15] published an analytical review of the literature, which reflected the analysis of 105 studies, of which 16% fully describe the characteristics of the cellular composition and the content of growth factors, and only 10% describe in detail the protocol for preparing aPRP. The need to standardize aPRP preparation protocols and guidelines for its use is described by many authors [15, 16]. Relying on recommendations from the same publications, it should be noted that to stimulate bone regeneration, thrombin-activated plasma in the form of a dense fibrin clot, which is able to form and maintain the required shape and slowly release the growth factors contained in it, is necessary. For this reason, we used the Regen BCT-SP (R-BCT-SP) and Regen ATS-SP (R-ATS-SP) kits, described in our clinical case.

CONCLUSION

The use of bone grafts and bone grafting materials in combination with bone marrow aspirate or platelet-rich plasma reduces the degree of immune response, promotes osseointegration and remodeling processes, which expands the possibilities of using surgical methods for biological reconstruction of bone tissues. To stimulate bone regeneration, it is recommended to use thrombin-activated platelet-rich plasma in the form of a dense fibrin clot. Only in this case can one create and

maintain the necessary shape of the material and ensure slow release of growth factors.

The authors declare no conflict of interest.

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