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Available methods to enhance regenerative potential of plastic materials for bone defects replacement in orthopedics. Part 1. Autologous platelet rich plasma

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Abstract

The main method of surgical treatment of patients with post-traumatic disorders of bone regeneration is the use of bone grafting. Until now, the optimal plastic material has been a bone autograft, which use involves additional trauma to the patient. Alternative materials that are used for grafting have only an osteoconductive effect, of varying effectiveness. To optimize the properties of plastic materials, giving them an osteostimulating effect, they can be used in combination with biologically active substances. A source of biologically active substances can be platelet-rich plasma, platelet lysate and red bone marrow. This literature review includes a description of three main methods to stimulate osteogenesis. The first part examines the mechanism of action of platelet-rich plasma, indications and contraindications for its use, describes the results of treatment when platelet-rich plasma is used to stimulate

osteogenesis. Platelet-rich plasma is a product of a human native blood obtained by centrifugation. The output is a high concentration of platelets in a small volume of plasma, which contain growth factors and cytokines that have a direct effect on the regeneration process. Local platelet-rich plasma therapy is performed to stimulate osteogenesis. Autologous platelet-rich plasma with growth factors contained in α-granules of platelets is injected into an allogeneic graft or into a fracture zone. The aim of this article is to summarize the results of treatment using platelet-rich plasma to improve bone regenerative potential in orthopaedics.

Keywords: bone regeneration, pseudoarthrosis, platelet-rich plasma, growth factors, bone grafting

Conflict of interests Authors declare no conflict of interest

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AAOS, American Association of Orthopedic Surgeons

ASC, adipose-derived stem cells

BMP, bone morphogenetic protein

IGF, insulin-like growth factor

IL, interleukin

MSC, mesenchymal stem cells

PDGF, platelet-derived growth factor

PRP, platelet rich plasma

TGF, transforming growth factor

TNF-α, tumor necrosis factor alpha

VEGF, vascular endothelial growth factor

Introduction

An impaired reparative regeneration of bone tissue in the treatment of patients with fractures, especially in the context of the growing share of high-energy trauma and injuries in general, is an urgent problem in orthopedics and traumatology [1]. In traumatic injuries, an impairment of reparative osteogenesis is observed in a high percentage of cases and, according to various authors, ranges from 2.5% to 25.0%. Delayed consolidation and false joints, as a complication of the treatment of limb fractures, occur in 0.5-2.7% of cases [2]. Fractures of skeletal bones that develop as a result of high-energy trauma, even with adequate treatment, often do not heal, and false joints are formed. Impaired processes of fracture consolidation increase the treatment duration and can further lead to disability, which makes 7.8%-33.1% of cases in the proportion of patients with this nosological form [3]. Of the long bones, the tibia and fibula (14%) and the femur (13.9%) most often fail to unite. [4]. It is necessary to study the stimulation of osteogenesis both in cases of impaired reparative processes and repeated operations, and also in primary osteosynthesis in order to possibly prevent complications in the form of impaired fracture union.

An effective solution to the problems of fracture consolidation can be achieved by searching for and implementing new methods for optimizing repair osteogenesis. Recent studies show that the improvement of methods of fracture reposition and fixation alone is not sufficient for the treatment of trauma patients, which makes the studies using local factors for stimulating reparative osteogenesis relevant [5].

An important problem is the replacement of bone tissue defects that can be formed as a result of injuries, cancer, or infection, degenerative and inflammatory diseases. Approximately 2 million bone defect replacement surgeries are performed annually in the United States alone.

The number of surgical interventions for intra-articular and multi-comminuted fractures with bone defects is growing every year. Various types of bone grafting are traditionally used to replace defects. A modern traumatologist has a large number of plastic materials in his arsenal – from autologous bone to materials obtained using genetic engineering. The best option for bone grafting is the use of autologous bone, since it does not cause an immune response, contains osteostimulating proteins and living cells, including osteogenic ones. The main disadvantage of autologous bone is that its using is associated with the necessity to traumatize the donor site, which leads to an extension of the operation time, the appearance of additional gates of infection and the development of bone deficiency in the area of the donor site, and pain in the postoperative period. In this regard, other plastic materials are widely used: xenobone, allobone, artificial implants [6, 7]. Traditional materials used for bone grafting have only osteoconductive properties of varying capacity [8]. The results of their use after graft reconstruction are different, often unsatisfactory, which is associated with many factors: poor quality of native bone tissue, graft resorption, and lack of osseointegration. There may be non-fusion or slow consolidation in the site where the material did not get. In order to improve the properties of osteoplastic materials and bring their efficacy closer to autologous bone, various methods have been used to increase their regenerative potential by saturating them with biologically active substances, living cells.

In recent years, the methods that use cellular technologies in patients after severe injuries and in the postoperative period have become increasingly popular. The next decade is expected to face the greatest development of cellular technologies in the field of "regenerative surgery", along with therapeutic medicine and endocrinology. Cellular technologies are a set of methods that include various options for cell

transplantation, engineering, gene therapy, and cytokine therapy. However, the use of each technique has its own advantages and disadvantages.

In the literature, there are various definitions of the mechanisms of influence on osteoreparative processes. In this literature review, we will use the definitions of R. von Versen (1993) [9]:

- Osteoblastic osteogenesis is the stimulation of fusion by transplantation of deterministic osteogenic prodromal cells that have their own potential for bone formation (autotransplantation of spongy bone).

- Osteoconduction is performed by using transplanted biological or synthetic material, which acts as a conductor (conductor) for the germination of blood vessels. Then there occurs the penetration of various cellular elements from the bone bed, with which the graft is connected by granulation tissue, resorbed and gradually replaced by a new bone.

- Osteoinduction occurs through the phenotypic transformation of non-specific mildly differentiated cells into bone cells under the effect of specific substances. With this option, it is possible to achieve the formation of bone tissue in soft-tissue structures of experimental animals (ectopic osteogenesis).

- Osteostimulation is the exposure to certain factors that contribute to the strengthening of already ongoing osteogenesis processes, i.e. stimulate them.

In this review, modern methods of stimulating reparative processes are discussed, which are the most accessible for implementation in a multidisciplinary hospital. These include the use of autologous platelet-rich plasma, autologous human platelet lysate, and autologous human bone marrow aspirate. In contrast to the use of bone morphogenetic protein, stromal-vascular fraction of adipose tissue, autologous bone

marrow concentrate, the use of these methods does not require special expensive equipment and is technically easy to implement.

Purpose of the analysis of special literature

The analysis of special literature was aimed at summarizing the available data on current approaches to improving the regenerative potential in emergency traumatology.

Search strategy for literary sources

The search for sources was carried out using the electronic databases of scientific literature PubMed and eLibrary. We used the following keywords: bone healing stimulation, autologous bone marrow, bone graft, PRP, lysate and their corresponding terms in Russian. The depth of information search was 20 years. To analyze and evaluate the literature data, criteria for including sources in the analytical study were determined.

The criterion for including sources in the study was the presence of the full text of the article or the structured one, with specific quantitative data of the abstract.

Exclusion criteria: clinical examples, abstracts, unpublished papers, studies that show signs of "duplication" (similar study protocol, groups, number of patients, etc.). In case of detection of "duplicate" articles, we selected a source that was later in the publication date.

Platelet-rich plasma. Definition. Indications and contraindications for use

Human bone tissue has a fairly good natural ability to regenerate. However, this ability has its limitations, and defects exceeding the critical size that impede the ability to heal spontaneously. Thus, the development

and clinical implementation of effective methods of bone regeneration is of paramount importance.

In recent years, the use of autologous platelet-rich plasma for stimulating and improving regeneration at the site of injury has been rapidly gaining momentum in the world practice. Platelet-rich plasma (PRP) is a product of a human's own blood. For its manufacturing, a high concentration of platelets is obtained in a small volume of plasma. Both plasma and its preparation contain growth factors that play an important role in the initial stage of bone healing and regeneration [10, 11].

Currently, PRP therapy is included in the consensus of the American Association of Orthopedic Surgeons (AAOS). In the publications of 2014-2015, indications and contraindications to PRP therapy were defined and comments on the use and safety were given. In 2016, the level of evidence for the use of PRP in the treatment of osteoarthritis was shown [12].

According to the AAOS Guidelines, the indications for administering the platelet-rich plasma are as follows [12]:

- Acute conditions: tendinitis, acute soft tissue injuries;
- Chronic conditions: tendinopathy-achillobursitis, epicondylitis, plantar fasciitis, rotator cuff tendinopathy, patellar tendinitis or ligamentitis, Adductor-Rectus-Symphysis (ARS)-syndrome, myopathies, osteoarthritis.
- Combination with surgical techniques: treatment of chondropathies, support of tendon suture, spinal fusion; treatment of meniscus injuries, anterior cruciate ligament injuries; in combination with a bone graft, the treatment of osteomyelitis, as a component of stem cell transplantation.

Contraindications to platelet-rich plasma administration [12]:

- Hematological pathologies with platelet dysfunction, critical platelet and fibrinogen levels, unstable hemodynamics, blood clotting disorders;
- Acute infectious diseases, septicopyemia, inflammatory processes of various localizations, skin infections in the injection area;
- Low level of compliance (dementia and other mental illnesses).
- Anemia;
- Malignant tumors, especially hematological or bone ones.
- Chronic liver diseases;
- Taking aspirin.

Pre-injection recommendations [12]

To ensure the efficacy and safety of PRP therapy, the patient should know and follow the following recommendations before the procedure:

- - cancel corticosteroids 2-3 weeks before the procedure
- - cancel nonsteroidal anti-inflammatory drugs 1 week before
- - at least 5 days before the procedure, it is recommended to stop taking anticoagulants
- - increase the fluid intake 24 hours before the procedure
- - in some cases, on the eve of the procedure, it is recommended to take sedatives.

Platelets contain more than 300 biologically active molecules that are released upon activation and subsequently influence the process of tissue regeneration [13].

Production of platelet-rich plasma

PRP is usually obtained from whole blood collected in a test tube containing an anticoagulant. The available registered protocols for preparing the PRP drug are based on centrifugation, but the methods of cell separation differ. Both single and double centrifugation of sampled blood is used [14, 15]. The result of this method of manufacturing the drug is obtaining a large concentration of platelets in a small volume of plasma.

There are many methods for manufacturing PRP; each of them has certain properties in terms of the ability to concentrate platelets and the processes to release certain growth factors. For more effective human bone repair, the ideal platelet concentration of the PRP used should be at least $1000*10^9$/L of cells per liter, and the total volume be at least 3-5 ml, especially in the treatment of extensive defects [16]. It has been proven that both lower and higher platelet concentrations do not improve wound healing. An important parameter of PRP is the preservation of the maximum number of functionally complete platelets, since these cells are the source of growth factors and other biologically active substances. When thrombin is added to PRP, platelets are activated and growth factors are released from their alpha granules. This composition is called tPRP [17]. Some authors use a 10% solution of calcium chloride ($CaCl_2$) or thrombin to activate PRP. Thrombin as a clotting agent for platelet gel formation can lead to rapid platelet activation and mass release of growth factors. Intensively released growth factors are removed from the wound before they can affect the cells (70% is released within 10 minutes and almost 100% is released within 1 hour). When a platelet gel is formed using $CaCl_2$, the release of growth factors can be slowed down. $CaCl_2$ activates and coagulates PRP, forming autogenic thrombin from

prothrombin, which ultimately leads to the formation of a loose fibrin matrix that releases growth factors within 7 days [18].

Human platelets are multifunctional cells, but they have only one physiological way of implementing their reparative functions, which is directly related to granules and degranulation. This creates a certain difficulty in choosing an adequate tactic for treating tissue defects with platelets. At the same time, there is no doubt that platelets are a very valuable and promising object for solving many medical and biological problems [19].

The importance of selecting platelet doses with a high level of functionally active cells has been repeatedly mentioned. According to some data, the fraction of the platelets containing granules (D pl. gr.) both in the blood of donors and in platelet concentrate usually does not exceed 74-75%; D pl. gr. over 80% is observed only in some patients, primarily at the peak of thrombotic complications [20]. Experience shows that platelet doses with a normal value of D pl. gr. (35-75 %) are effective both in transfusion, and also in solving other clinical problems [21].

History of platelet-rich plasma use

The positive effect of autohemotherapy has been known to doctors since the beginning of the XX century. In 1905, a German surgeon August Bihr first applied this method to accelerate the healing of fractures. He injected the patient's native blood into the hip and found that it was a kind of irritant to the body and allowed for faster healing of fractures. It is important to note that, according to his observations, the healing rate increased in various pathological conditions by up to 30%, compared with conventional methods of treatment [22]. Since then, specialists who used autohemotherapy in their practice have noted the faster healing of wounds, ulcers, purulent skin diseases, and the activation

of reparative processes in fractures, sprains, and soft tissue injuries [23]. In the mid-twentieth century, a scientist from the University of California M. Urist proposed the term "osteoinduction". He defined osteoinduction as the ability to cause ectopic osteogenesis, i.e., to form bone in soft-tissue structures. He proved that this effect was posed by a complex of non-collagenic proteins present in a partially demineralized allogeneic graft, which were called "bone morphogenetic proteins" (BMP). In 1965, he published the results of a study in which he summarized his experience of using PRP as a factor contributing to an increase in the amount of osteoinductive morphogenetic protein (BMP) in the blood to stimulate osteogenesis in defects of the facial skeleton bones. His work was based on the data on the pronounced osteogenic and chondrogenic activity of peptides contained in platelet alpha granules [24]. It should be noted that currently osteoinductive morphogenetic protein is not produced in Russia) and its cost is extremely high, so its use in Russia is rather limited.

In 1969, J. Folkman et al. used PRP to nourish endothelial cells of the microvascular bed and preserve the integrity of the vessels in organs for transplantation [25].

In the late 80's of the twentieth century, Robert Marx described a technique for obtaining PRP and applying it as a gel in dentistry [26]. In the 1990s, Eduardo Anitua wrote that the use of PRP in the form of a gel is associated with accelerated bone regeneration, and also proved the presence of specific receptors for platelet growth factors in bone tissue [19].

In 2001, Russian scientists R.R. Akhmerov and R.F. Zarudiy developed the method of "Plasmolifting^{TM}", namely, obtaining and using an injectable form of PRP in dentistry and dermato-cosmetology [27]. To date, the use of platelet-rich autoplasma to accelerate bone and soft tissue

growth has become a real breakthrough in dentistry, sports medicine, traumatology, orthopedics, and surgery.

The mechanism of action of the growth factors from platelet-rich plasma on bone regeneration

Platelets play an important role in primary wound healing. During bleeding, they are activated and contribute to the release of a large number of growth factors and cytokines involved in the repair process. Platelet-rich plasma with a platelet concentration of at least $1000*10^9$/L in 5 ml of plasma contains 3-5 times more growth factors than in conventional plasma, which stimulates healing processes [18]. The cellular responses that follow tissue damage are controlled by platelets and released growth factors. Platelets release a large number of growth factors and cytokines after adhesion and aggregation to form a fibrin clot. To stimulate reparative osteogenesis, local PRP therapy is performed, i.e. the infusion of autologous platelet-rich blood plasma with growth factors contained in platelet α-granules into the fracture zone. These substances have an oligopeptide structure and are characterized by a high degree of affinity and a relatively long time of primary exposure to the receptors of induced osteogenic progenitor cells [28, 29]. The PRP injection allows in some cases avoidance of additional surgical trauma, shortening the consolidation time and patient's rehabilitation period. The advantage of PRP as a matrix for cells is that PRP is an autologous and non-toxic agent. It is safe by nature, there is no risk of such infectious disease transmissions, as HIV, hepatitis, Creutzfeldt-Jakob disease, etc. There is no risk of developing immunogenic reactions that are observed when using allo- or xenografts, either. Moreover, artificial recombinant growth factors often require the transfer by synthetic or animal proteins, while PRP, on the contrary, can act as their carrier itself [19, 30]. Since the first

use of PRP with autobone, a large amount of scientific data has been accumulated, including the use of PRP in clinical practice [31].

Autologous platelet preparations have demonstrated the ability to alter the natural pathway of bone healing in several ways. Their action is associated with an increase in the concentration of growth factors and bioactive proteins that are released by activated platelets and can help regenerate the tissues with a low healing potential, restoring biomechanical properties to the properties of normal bone. The use of PRP increases the release of chemical mediators into the microenvironment of the damaged area. Each of these factors has its own role.

Platelet-derived growth factor (PDGF). PDGF is found in platelets, especially in alpha granules, as well as in cells such as macrophages, endothelial cells, monocytes and fibroblasts, and in the bone matrix [32]. It has a heterodimeric structure consisting of two different chains A and B. Homodimers AA and BB are also found in platelets and show the similar activity. The reason for having three different forms remains unclear. There is an assumption that differentiated binding to the receptors of various cells, such as endothelium, fibroblasts, macrophages and bone marrow stem cells, is necessary. As a result of the presence of platelets in a blood clot, PDGF is the first growth factor in a wound that stimulates revascularization, collagen synthesis, and bone regeneration [33]. The role of PDGF in the wound healing process is to stimulate mitogenesis to increase the number of regenerative cells, to stimulate angiogenesis, and activate the macrophages responsible for wound cleansing and being a secondary source of growth factors [34].

Transforming growth factor (TGF). Among the TGFs found in PRP, TGF-1 and TGF-2 are the main growth and differentiation factors that are involved in bone and connective tissue regeneration. TGF

promotes extracellular matrix production, stimulates the biosynthesis of type I collagen and fibronectin, and induces the bone matrix formation. Accordingly, TGF can both initiate, and also support bone tissue repair processes, as well as affect the remodeling of a maturing bone graft [35]. However, the most important functions of TGF-1 and -2 are chemotaxis and mitogenesis of preosteoblasts, as well as the ability to stimulate the collagen accumulation during the connective tissue healing and bone formation. In addition, this factor suppresses the formation of osteoclasts and bone resorption, which contributes to the predominance of bone generation over resorption. TGF can also trigger the signaling pathway of osteoprogenitor cells (osteoprogenitor is a mesenchymal cell-precursor of osteogenesis) synthesizing BMP, regulating the expression of growth factors in bone and cartilage tissue [36].

Insulin-like growth factor I (IGF-1). IGF-1 is the third important protein found in blood platelet granules. IGF-1 stores in the bone matrix, endotheliocytes, and chondrocytes are released during bone regeneration and are responsible for bone formation and resorption [37]. The presence of IGF-1 in platelets can have an effect on osteoblasts and preosteoblasts, initiate osteogenesis, and inhibit bone cell apoptosis and expression of the mesenchymal collagen enzyme, reducing its destruction. In addition, IGF-1 can bind to a specific receptor on the cell membrane and stimulate the cells that are involved in osteogenesis. Studies have shown that applying IGF-1 to the surface of rat molars can promote cementogenesis, and, in combination with PDGF, bone generation on the implant surface. The biological effect of IGF-1 can be regulated by IGF-binding proteins that can transport IGF-1 and increase its half-life [38].

Effect of platelet-rich plasma angiogenesis factors on bone repair

Osteogenesis requires a sufficient blood supply, and at the final stage of remodeling of enchondral ossification, the specific matrix metalloproteinase can cause a degradation of cartilage and bone, promoting vascular growth. There are two independent pathways of angiogenesis: one depends on VEGF and the other on angiogenin. VEGF mainly influences the neonatal vascular growth and specific endothelial cell mitogen, while angiogenin mainly has an effect on the generation of large vessels and the formation of collateral circulation. This is necessary for angiogenesis in the bone graft at early stages and for further ossification. Local application of vascular endothelial growth factor (VEGF) has been shown to accelerate the germination of small vessels, promote aggregation and ossification of skeletogenic cells. Adipose-derived stem cells (ASCs) may also be involved in this process [39]. So, J. Holstein et al. revealed the activation of angiogenesis during bone regeneration in a mouse model of cranial defects [40].

A sufficient amount of VEGF in PRP and a rapid mobilization of growth factors can promote a local vascular growth. Several factors are thought to be associated with an increase in the PRP potential for vascularization, including plasma concentration, Ca^{2+} activation, VEGF release, platelet generation, and histomonocyte count in white blood cells [41]. S. Kim et al. infused PRP (containing a sufficient amount of VEGF and PMP and peripheral blood mononuclear cells (PBMCs include lymphocytes (T, B and NK cells), monocytes and dendritic cells) without using heterophilic peripheral blood granulocytes (neutrophils, eosinophils, basophils) in the bone defect of the rat skull. They found that PRP enriched with angiogenic factor can lead to faster and more intensive generation of new bone in a critical-size bone defect. The researchers

suggested that using PRP can prolong the short-term effect of rapid initial angiogenesis. Also, B. Annabi et al. studied a bioactive platelet-derived lysophospholipid called S1P and pointed out the crucial role of S1P-/EDG-1-mediated angiogenic and survival events in the regulation of microvascular network remodeling by mesenchymal stem cells (MSCs), which may provide a new molecular link between hemostasis and angiogenesis [42]. Bone marrow mesenchymal stem cells play an important role in vascular growth, especially in ischemic tissues and tumors. It is known that VEGF can aggregate MSCs into new vessels and regulate the differentiation of MSCs into vascular cells. Ball et al. found that VEGF-A can stimulate PDGF receptors and regulate the formation and transformation of MSCs, implying that VEGF-A/PDGF receptors can influence the aggregation of MSCs in the ischemic region, promoting vascular generation [43]**.**

Platelet-rich plasma as a source of inflammatory cytokines for bone repair

There is growing evidence that inflammation plays a vital role in the early stage of fracture healing. Inflammatory responses involve a number of biochemical and cellular alterations, which extent correlates with the extent of the initial injury. Dense granules of platelets contain histamine, serotonin, dopamine, calcium, and adenosine. During aggregation, platelets are activated and secrete growth factors, cytokines, and hemostatic factors that play a special role in the early stages of the internal and external pathways of the blood coagulation cascade [44]. Histamine and serotonin released from platelets increase capillary permeability, which allows inflammatory cells to have greater access to the wound area and activate macrophages [45]. Activation of adenosine

receptors modulates inflammation during wound healing. The main proinflammatory cytokines responsible for the early response are interleukins IL-1, IL-6, and tumour necrosis factor alpha (TNF-alpha) [46]. The expression of both IL-1 and TNF-β in fractures is biphasic, with a peak at the beginning of fracture repair, followed by a second peak in the transition from chondrogenesis to osteogenesis during enchondral maturation [47]. In a study on mice with a hip fracture and a knocked out gene responsible for interleukin-6 synthesis, delayed remodeling and mineralization of bone callus were noted, and the ability of TNF-alpha and IL-1beta to attract osteoblasts was revealed [48]. Moreover, another study in mice using human bone fragments showed the leading role of TNF-alpha in stimulating bone fusion and found that PRP can inhibit the release of IL-1 from activated macrophages [49].

Studies using platelet-rich plasma to improve bone fusion

Experiments have shown that the use of PRP with a bone graft can significantly improve the quality of bone healing in a rabbit model. M. Hakimi et al. demonstrated that PRP in combination with an autologous sponge graft leads to a significantly better bone regeneration compared to the isolated use of an autologous sponge graft in critical size defects on load-bearing long bones of mini-pigs in vivo [50]. Meanwhile, Y. Yamada et al. in an experiment on a dog model found that the combination of MSCs with PRP leads to faster bone maturation [51]. In the study by B.Han et al. PRP was used as an autologous source of growth factors, improving the quality and intensity of osteogenesis [52]. The use of isolated cells with a biocompatible matrix in combination with PRP increases the effect of growth factors on these cells.

A.F. Giovanini et al. evaluated the effect of PRP and autograft on the synthesis of type III and type I collagens, as well as the effect of bone tissue progenitor cells on CD34+ in a model of bone defect on the skull of 23 rabbits. They found that the use of PRP can inhibit bone deposition, as well as increase the ratio of type III to type I collagen and chemotaxis of CD34+ progenitor cells [53]. The reparative role of human platelets has been actively studied for the past 20 years, but many aspects of using platelets are still unclear [16, 54]. Good results of using PRP in the conservative treatment of degenerative changes in joints and ligaments (knee pain, tennis player's elbow, etc.) have been obtained [55]. However, when using PRP to stimulate bone regeneration, the clinical results obtained are ambiguous, and the evidence base is insufficient [56]. In 2008, G.M. Calori et al. conducted a prospective randomized trial comparing the results of treatment using rhBMP-7 and PRP in 120 patients with non-union of limb long bones [57]. Bone fusion was achieved in 68.3% of cases (41 of 60 patients) in the group of patients treated with PRP, and in 86.7% (52 of 60 patients) in the group where rhBMP-7 was used. The mean time period of clinical fusion was four months in the PRP group, compared to 3.5 months in the rhBMP-7 group. The results obtained indicated a worse regeneration in the treatment with PRP. Another study examined the PRP efficacy in 132 patients with delayed consolidation after surgical treatment at the Military Medical Research Institute in Warsaw between 2009 and 2012 [58]. Bone fusion was achieved in 108 patients (81.8%) after PRP administration, while no improvement was observed in 24 patients (18.2%). The PRP efficacy was also found to depend on the administration site. Thus, the complete fusion (mean at 3.5 months) was observed in the proximal tibia, while fusion in the proximal humerus was achieved only in 63.64% (mean at 3.2 months). The PRP efficacy in the treatment of long bone non-union can

also be found in a more recent study in 94 patients [59]. Autologous PRP (>2,000,000 platelets/mL) in a volume of 15-20 ml was injected directly into the defect site. Fusion was evaluated monthly for the formation of bone "bridges" according to X-ray data up to a 4-month period. In 82 patients (87.23%), a complete union had been achieved by 4 months without complications. However, a PRP negative effect on bone union also has found [60, 61].

The N. V. Sklifosovsky Research Institute for Emergency Medicine conducted an experimental study in which the effect of PRP in combination with collagen on the regeneration process in the rat distal femur was studied. Allogeneic collagen was obtained from rat tails, converting it to gel. Platelet-rich rat plasma was prepared using the Messora method. The platelet concentration in the obtained PRP was 1100-1300 × 10^3 /μL. A defect with a diameter of 2 mm was formed in the femoral condyle. In group 1 (the comparison group), after inducing a bone defect, the wound was sutured without filling it with plastic material. In group 2, the bone defect was filled in with rat collagen (in amount of 150-200 μl). In group 3, a mixture of rat collagen/allogeneic PRP in a ratio of 1:1 was used to fill the bone defect, and this ratio was noted to be the most optimal. The dynamics of bone repair in the defect area was studied on histological preparations stained with hematoxylin and eosin and according to Van Gieson. Analyzing the results obtained, the authors came to the conclusion that the use of PRP in combination with collagen releases growth factors from platelet granules, which makes it possible to reduce the period of bone defect repair in rats by 2-fold. In addition, the use of PRP reduces the intensity of the inflammatory response in the bone defect area [21].

The same Institute developed a method for morphofunctional analysis of human platelets using fluorescence microscopy of vitally stained platelets

while maintaining their functional activity. The study involved 300 donors. Unseparated blood platelets were studied in 200 patients, 50 samples of platelet concentrate obtained by apheresis, 40 hematological patients and 10 with thromboembolic complications were examined. Platelet concentration and aggregation were measured. In the course of the experiment, a direct correlation was established between the aggregation activity and morphofunctional parameters of donor platelets [62].

The Department of the Medical University (Istanbul) studied the PRP effect on fracture fusion. An experiment was conducted on rats, in which a monocortical defect was inflicted in the femur bones. We formed three groups. In the first group, no treatment was performed. The second group received PRP. Animals from the third group did not undergo osteotomy to determine comparative biomechanical tests. PRP was prepared by taking venous blood, adding sodium citrate as an anticoagulant, and then it was centrifuged once for 8 minutes at 1800 rpm. Platelets were activated with a sodium chloride solution and administered into the defect. After 4 weeks, a histological evaluation was performed, significantly more complete healing of the bone defect was revealed in the PRP group compared to the group without treatment. After 9 weeks, the biomechanical characteristics of the newly formed bone were compared, which were objectively better compared to the group where plasma injection was not performed [63].

Kuban Medical University analyzed the treatment results in 16 women with fractures of the radius distal metaepiphysis. It was noted that A-PRP therapy in patients of the study group (n=6) contributed to better coping with clinical and radiological manifestations of the fracture compared to the control group (n=10), where patients did not receive A-PRP therapy. According to the study results, the time period of fracture consolidation was shortened by 3-4 days, which made 9.5±1.1 % of the

mean time of fracture consolidation in patients of the control group. Significant detectable changes in bone tissue in the form of X-ray signs of fusion were seen by the 20th day; soft osseous callus while stimulating reparative osteogenesis with platelet-rich plasma became X-ray contrasted by the 20th day. [64].

In the German clinic named after Heinrich Heine, the study was conducted to investigate properties of PRP in combination with an autograft for the replacement of a radial diaphysis defect in a rabbit model was conducted. In the first group, an autograft and PRP were used, while in the second group, only an autograft was used. Radiographic and histological evaluation confirmed the predominance of platelet-rich plasma compared to the control group [65].

In Israel, Dr. Gabriel reviewed the most common uses of PRP and the ambiguity of its use in the treatment of lateral epicondylitis (tennis player's elbow), low back pain, alopecia, osteoarthritis, bone regeneration, and in dental practice [66]. According to Ranly et al., PRP can even slow down bone formation [67]. Thus, the studies using PRP in spinal fusion have shown that the addition of the drug reduces the rate of regeneration. The presence of excessively high concentrations of growth factors was suggested to slow down directed osteogenesis.

Often, the actual content of biologically active substances in PRP is not taken into account, although it is known that human cells, including osteoblasts, lose their viability in conditions of an excess of growth and differentiation factors. Thus, TGF-β1 normally promotes the transformation of MSCs into osteoblasts, activating osteogenesis in the area of bone damage, but high doses of this factor lead to the opposite effect [54]. A similar effect is exerted by TNF-alpha that prevents pathological transformation of cells: at low concentrations (up to 50 ng/mL) of TNF-alpha, bone growth is observed, and at high

concentrations (over 100 ng/mL), a mass death of osteoblasts and chondroblasts occurs [68].

A Cochrane systematic review with evidence level 1A was published, which included 4 studies in 114 patients in whom PRP was used for maxillofacial surgery. These studies evaluated the efficacy of PRP used in combination with bone grafts in maxillary sinus plastic surgery. The authors reported that there was no statistical difference between the group of patients who had PRP added to the bone graft and the group in which this was not done. There was no difference reached in clinical outcomes, failure rates, or complication rates [69]. L. Griffin et al. received insufficient evidence and a limited number of studies in a systematic review evaluating the use of PRP for limb bone regeneration [70].

Conclusion

To increase the regenerative abilities of bones and soft tissues, platelet rich plasma has recently been widely used, and indications and contraindications for its use have been developed and formed. However, along with many publications about the positive effect of using platelet rich plasma, there are a sufficient number of registered cases indicating the inefficacy of this method.

Conflicting results of the PRP efficacy of may be associated with different methods of its preparation and application. The efficacy of platelet rich plasma depends on the quality of a particular patient's platelets, health status, the impact of external factors, and the method of obtaining plasma.

The N.V. Sklifosovsky Research Institute for Emergency Medicine uses morphofunctional analysis to assess the qualitative composition of platelet rich plasma, which makes it possible to determine the

concentration of growth factors in platelets. This is necessary for the most efficient use of platelet rich plasma. Meanwhile, the quality of the platelets per se is not taken into account. When determining the morphofunctional status of platelets using this method, it is possible to optimize the selection of the platelet rich plasma dose. Platelet factors are located in secretory vesicles and are released only when platelets are activated. After the release of factors from the granules, which occurs within a very short period of time, the platelet does not synthesize them again and is, in fact, a "one-time" cell. Also, the platelets can remain inactive for a long time, break down gradually and do not release biologically active substances at all. Platelets tend to have poor adhesion to the bone surface, even after the bone demineralization. Therefore, there is a risk that the effect of platelet rich plasma moving to the defect area will not occur. This has been shown in clinical examples, when after the platelet rich plasma administration, there was no stimulation of bone fusion. In a few studies, where platelet rich plasma was used to treat bone defects, an accelerated bone fusion was found, but the efficacy of platelet rich plasma was still lower than that of bone morphogenetic protein.

Growth factors contained in platelet granules can stimulate the growth and differentiation of the present living cells only, so it is impossible to achieve the effect of a bone defect healing simply by injecting platelet rich plasma there. It is necessary to create conditions for the appearance of mildly differentiated cells in the defect zone and ensure the presence of osteoconductive material on which fibroblasts can be fixed and begin their differentiation under the impact of the microenvironment, including the growth factors. Also, when in contact with osteoconductive material, in particular, type 1 collagen, platelets are actively degranulated.

Platelet rich plasma can be used for adjuvant therapy in many surgical procedures in maxillofacial surgery and orthopedics, potentially improving bone regeneration and preventing the development of false joints.

The use of platelet rich plasma has a number of advantages: low cost, efficacy and safety of application with strict compliance with the protocol. Current clinical studies generally show good results, especially in the treatment of soft tissues, but there is currently no high level of clinical confirmation.

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