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Available methods to enhance regenerative potential of plastic materials for bone defects replacement in orthopedics. Part 3. Use of autologous human red bone marrow

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Abstract

The previous two articles described the use of platelet-rich plasma and platelet lysate. This part of the literature review examines the mechanism of red bone marrow action, indications and contraindications for its use. The results of treatment for delayed consolidation of bone fractures are also described. Hematopoietic stem cells give rise to all cellular components of the circulating blood, such as red blood cells, lymphocytes, neutrophils, and platelets. The most rational way to stimulate bone regeneration is to use the patient's own biological material. The aim of this article is to summarize the results of treatment using autologus bone marrow to improve bone regenerative potential in orthopaedics.

Keywords: osteogenesis, bone marrow, bone grafting, angiogenesis, stem cells

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MSC - mesenchymal stem cells
RBM - red bone marrow

Introduction

In two previous articles: "Available methods to enhance regenerative potential of plastic materials for bone defects replacement in orthopedics. Part 1. Autologous platelet rich plasma" and "Available methods to enhance regenerative potential of plastic materials for bone defects replacement in orthopedics. Part 2. Use of autologous human platelet lysate", we reviewed the general issues, mechanism of action, and methods of application of platelet-rich plasma and platelet lysate. In this article we shall discuss the action of red bone marrow, the principles of its use, and the results of treatment.

The maintenance of bone tissue in a normal physiological state occurs due to a strict balance between the bone formation and resorption. After damage, any tissue is replaced by connective tissue, forming a scar. Bone and liver are exceptions. After damage, bone tissue and liver tissue are completely restored.

Bone wound healing is a complex multi-step process that involves different types of cells, the extracellular matrix, and many signaling

molecules. In the process of repair after bone injury, several types of cells and tissues are involved to achieve union. Unfortunately, in 10-15% of patients with fractures the regeneration process may be impaired, which leads to a delayed fracture union or non- union [1].

The process of bone union can be divided into 5 separate, but overlapping phases: 1 - hematoma formation, 2 - inflammation phase, 3 - soft callus formation, 4 - hard callus formation, 5 -remodeling [2–6]. The hematoma formation occurs as a result of damage to the vessel. Platelets, erythrocytes, and innate immunity cells (neutrophils, mast cells, eosinophils, basophils, macrophages, dendritic cells, NK cells) are the first to enter the fracture area [7–9]. Based on their secretion profile, innate immune cells create a pro-inflammatory state, as a result of which the cells of adaptive immunity (the body's ability to neutralize foreign and potentially dangerous microorganisms that have already entered the body earlier) and mesenchymal stromal cells are attracted to the damaged area. For the normal course of the reparative process, a transition from the pro-inflammatory to the anti-inflammatory phase is necessary. The transition to an anti-inflammatory state initiates a revascularization in the area of the fracture, which is another condition for successful union [10]. At the next stage, the fibrocartilaginous tissue fills the fracture area and forms a soft callus for a primary stabilization of the fracture site. Cartilage tissue matures and hypertrophies, its mineralization begins. Hard bone callus is formed by newly formed bone tissue, which replaces hypertrophied chondrocytes. The last step is to remodel the fracture site [11]. In humans, this process can take up to several years, depending on the general condition of the patient, the type and location of the fracture [12].

Aim. To summarize the available information about modern approaches to ways to improve the regenerative potential in emergency traumatology.

Literature search strategy

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

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

Exclusion criteria: clinical cases, abstracts, unpublished works, studies with signs of duplication (similar study protocol, groups, number of patients, etc.). In case of detecting duplicate articles, a later source was chosen, according to the date of publication.

Major part

The adult human skeleton has two types of bone marrow: yellow and red. The yellow bone marrow undergoes fatty involution and is inactive. Red bone marrow contains two known populations of adult stem cells, hematopoietic and mesenchymal (stromal) ones. Hematopoietic stem cells give rise to all cellular components of the circulating blood, such as red blood cells, lymphocytes, neutrophils, and platelets. Another population of stem cells consists of mesenchymal stem cells (MSCs), which are also known as bone marrow stromal cells. MSCs can differentiate into connective tissue cells such as osteoblasts, osteocytes, adipocytes, and chondrocytes and can proliferate in vitro. The ability of MSCs to differentiate into bone-producing cells determines the possibility of their clinical use in orthopedic injuries to improve fracture union and treat bone defects. The best approach is to use the patient's own biological material to stimulate bone regeneration. The results of experimental and

clinical studies indicate a positive effect of MSC transplantation on the course of the reparative process [13]. It was revealed that due to their osteogenic differentiation, the pool of osteoblasts and the volume of newly formed bone tissue increase, and the time for restoring the bone integrity is reduced [14].

The most important component of a bone marrow aspirate for use in orthopedic trauma is the population of osteoprogenitor cells. The term "connective tissue precursors" was proposed more than 2 decades ago after the assay of MSC colony formation became available in vitro, i.e. the determination of the number of stem and progenitor cells of a given tissue by culturing together with their activators and cells that accelerate proliferation [15]. This definition includes not only the evaluation of native stem cells derived from expanded cell populations, but also describes a general approach that encompasses the full diversity of native cell populations. Thus, connective tissue progenitor cells include all heterogeneous native populations of progenitor and stem cells that operate in one or more connective tissues: bone, fat, cartilage, fibrous, muscle. Moreover, the term "connective tissue progenitors" includes colony-forming cells, which are native stem or progenitor cells present in tissues. MSCs are found in the bone marrow and are multipotent cells with the ability to differentiate into osteoprogenitor or chondroprogenitor cells based on molecular signals from their local environment. Then osteoprogenitor cells can differentiate into osteoblasts under the influence of cytokines and growth factors [16]. Differentiation of osteoprogenitor cells and their ability to stimulate the formation of bone tissue are enhanced by osteoinductive factors also contained in bone marrow aspirate [17]. Even when administered intravenously, MSCs reach the injury site and recruit bone-forming progenitor cells to the site between fragments. Dreger et al. evaluated the effect of MSCs in fractures of the femoral bones in mice [18]. It has been found that the timing of MSC

application is a key factor for successful treatment outcome. Another study analyzed the results of using MSCs in nonunion fractures in mice. Cultured mouse MSCs were injected intravenously 24 hours after the fracture and an improved bone formation was observed.

When using bone marrow in humans for local stimulation of osteogenesis, the question arises of where it is more expedient to obtain MSCs: from bones or from adipose tissue, and what concentration is needed to restore the impaired healing process. P. Hernigou et al in their study determined the necessary concentration of progenitor cells in autologous human bone marrow to successfully treat nonunions. It amounted to more than 1500 progenitor cells/cm^3. In this study, the aspirate contained not only osteoblast progenitor cells, but also other mononuclear cells that also stimulate bone regeneration [19]. Currently, in orthopedics, autologous bone marrow taken as an aspirate is most often used as the basis of cell therapy [20]. T. S. Lindholm and M. R. Urist were ones of the first to add raw bone marrow aspirate to the bone matrix to improve bone union [21]. Later J. F. Connolly et al. published studies on the successful clinical use of raw bone marrow aspirate by percutaneous administration for the treatment of tibial nonunions [22]. There are many studies on the use of bone marrow aspirate in osteonecrosis, nonunions, bone defects, surgical arthrodesis, distraction osteogenesis, chondral defects, arthritis, tendinopathies to improve healing after and during tendon repair. Despite the obtained results, there is no mass application of cell populations of bone marrow aspirate.

A full-fledged course of a regeneration processes at the tissue level can only be ensured at the cellular level. Moreover, all tissues (bone, cartilage, muscle, fat) contain rare and often heterogeneous populations of progenitor cells, which proliferation can be activated for subsequent differentiation and formation of new connective tissue. When cultivating in vitro, the cells obtained from a population of colony-forming

connective tissue progenitor cells, if they meet certain criteria, can be classified as mesenchymal-stromal or stem cells. The International Society for Cell Therapy has adopted a definition of standard terminology, as well as minimum criteria for when cultured cells can be considered mesenchymal stromal stem cells - based on the ability of adhesion under standard cultivation conditions. Other criteria for classifying these cells as mesenchymal stromal-stem cells include the presence of surface markers CD70, CD90, CD105, the absence of hematopoietic markers CD44, CD45, CD14, CD19 and HLA-DR, as well as their ability to differentiate into osteoblasts, adipocytes and chondroblasts in vitro [19].

From a clinical point of view, one of the factors slowing down the regeneration of bone and cartilage tissue is the relative loss or lack of local stem and progenitor cells [23]. Therefore, osteogenesis is often stimulated by transplantation of osteogenic stem and progenitor cells to the site where a new tissue formation is required [24]. To do this, a cancellous bone autograft is used. It is usually taken from the iliac crest. This intervention has been performed for decades and is the prototype of cell transplantation and remains optimal for stimulating bone regeneration [25, 26]. The cancellous bone autograft includes a bone matrix that has an osteoconductive effect, providing a matrix over which cells can migrate. It also contains soluble stimulants that act on the cells inside the cancellous autograft and adjacent tissues, accelerating the proliferation, migration and differentiation of local progenitor cells, which contributes to osteogenesis. However, the most important biological component of a cancellous graft is the presence of connective tissue progenitor cells with the ability to differentiate into new osteoblasts (also known as connective tissue osteogenic progenitor cells) [26]. Due to the significant surgical invasion during autograft harvesting, as well as the risk of pain at the donor site, bleeding, and the possibility of developing infectious

complications, many surgeons have chosen an alternative method for obtaining osteogenic connective tissue progenitor cells [27–30]. Stem cell sampling is carried out using a special needle, which is inserted directly into the pelvic bone, where the main reservoir of bone marrow is located. This method is devoid of the disadvantages described above, and in combination with an allograft or other suitable scaffold material, it shows similar properties to a cancellous autograft in almost all respects. In addition, other systems have been developed for harvesting cancellous bone marrow and fat from the medullary canal of long bones, such as the Reamer Irrigator Aspirator (RIA) System - DePuy-Synthes [31].

Attempts to improve cartilage regeneration are based on the transplantation of connective tissue progenitor cells from which cartilage can form (known as chondrogenic connective tissue progenitor cells). When microfracturing stimulates the bone marrow, physical conditions are created for the migration of connective tissue progenitor cells from the subchondral layer and bone marrow into the joint to replace a cartilage defect by forming cartilage or fibrocartilaginous tissue.

Transplantation of osteochondral columns, usually cylindrical in shape, into the defect area involves both mechanisms: the action of connective tissue progenitor cells from the bone marrow and from cartilage tissue [32]. Both methods involve the implantation of cells with the ability to proliferate and differentiate into the area of the defect to replace it.

Red bone marrow (RBM) concentrate is also used, for which preparation industrial installations are used. The concentration of bone marrow cells after treatment is 4.5 ± 0.5 times higher than the concentration of those in the collected aspirate. Most of the studies that describe the results of using RBM, the latter was used in the treatment of cartilage defects with good results.

Numerous fundamental animal studies have investigated the ability of MSCs to stimulate bone formation. In a systematic review, A. Gianakos et al. summarized the results of 35 animal studies in which bone marrow aspirate concentrate (BMAC) was used to treat bone defects of critical size [33]. The studies reviewed used a variety of animal models, including rabbits, rats, mice, goats, dogs, sheep, and pigs. They found that in studies where statistical processing was performed, in 100% (14/14) of cases, clear radiological signs of increased osteogenesis, in 81% (13/16), a larger average bone volume was seen when using microcomputed tomography; and in 90% (19/21) of cases, more intense bone formation was determined by histological analysis compared with control groups. The limitations of those studies were the wide variety of animal and bone defect models used, as well as an unknown applicability of the results to clinical practice in humans. However, numerous clinical outcome studies have been published and their results are comparable to those of these animal models [33].

Autologous bone marrow harvesting technique from the iliac crest

The largest number of publications on bone marrow harvesting from the iliac crest belongs to P. Hernigou and his research group. Through rigorous approach and analysis, they have developed several methods of bone marrow harvesting technique to obtain the highest possible concentration of MSCs [24]. Typically, bone marrow harvesting is performed as follows:

− Depending on the site of sampling and the intended site of injection, the patient is placed on the operating table either on his back, on his side or on his stomach.

− Both areas of the iliac crests and the limb (where the bone marrow will be injected) are covered and treated in a standard manner.

− A disposable or reusable cut-tip trocar needle (e.g., Jamshidi or Lee-Lok needles) is used, which is inserted through a small skin incision along the iliac crest.

− The needle is inserted manually through the cortical plate of the iliac crest with a rotational motion or by tapping the needle to a depth of approximately 6 cm.

− A heparinized 10 ml syringe is attached to the tip of the needle (5000 IU of heparin diluted in 5 ml of saline, with which the syringe is washed to prevent clotting of the aspirate).

− Bone marrow is taken by rotating the needle 45 degrees with each aspiration, gradually withdrawing the tip of the needle from the sampling area, gaining 1-2 ml per full turn. If the needle is fully rotated 180 degrees, it is removed by 1–2 cm and the procedure is repeated.

− For the next sampling, the needle is moved along the iliac crest approximately 2 cm from the previous sampling site and, while sampling, the needle is also rotated.

− Depending on the amount of bone marrow required, harvesting is performed from 2–5 points of the iliac crest.

− The aspirate is usually used in a raw form or concentrated using centrifugation.

− Under the control of an electro-optical converter, using the same needle that was used for sampling or a spinal needle, the obtained bone marrow is injected into the fracture site.

− Based on preoperative planning, the injection is made in the place of the greatest divergence of fragments or a bone defect.

− The aspirate is injected slowly at a rate of approximately 20 ml per minute until significant resistance is felt.

− Aspirate should be infused around peripheral fragments.

− The total aspirate volume can vary from 20 ml to 80 ml depending on the fracture location. The literature describes the

administration of up to 150 ml of aspirate, but great care should be taken when administering such volumes [34].

− Next, the needle is slowly removed and direct pressure (squeezing) is applied to the injection site.

− In the postoperative period, the limb is immobilized and the load is limited from 4 to 6 weeks to prevent mechanical disruption of the bone healing process. Further, after 6 weeks, they begin to allow a gradual load on the limb.

Choice of anatomical area for aspiration and recommended sites for bone marrow sampling

When the greatest number of bone marrow and progenitor cells is required, it is important to choose the right aspiration site with the highest content of these elements. In adults, these are the bones where hematopoiesis takes place (pelvis, sacrum, vertebral bodies, ribs, and sternum). Of all these bones, the pelvis, and especially the anterior and posterior iliac crests, are the most accessible.

A bone marrow aspirate from the posterior iliac crest has been found to have the highest concentration of MSCs that can be taken from anywhere along the crest from the anterior superior to the posterior superior iliac spine. On average, the distance from the anterior to posterior superior iliac spine along the iliac crest is approximately 24 cm [34]. Bone marrow can be taken from any point along this interval.

Using a scalpel with No. 11 blade, an incision/puncture is made parallel to the skin lines of force (Langer). For the anterior iliac crest, the incision is made approximately 4 to 5 cm posterior and outward to the anterior superior iliac spine to avoid damage to the lateral femoral cutaneous nerve and external iliac artery. For the posterior iliac crest, the incision is made approximately 4 cm outward from the posterior superior spine.

After making these two incisions, the needle can be advanced in different directions to perforate the outer cortical plate of the iliac crest above the acetabulum.

Regardless of the point of entry, aspiration should be performed no more than 3 to 4 cm below the iliac crest, because posteriorly the thickness of the ilium decreases and remains minimal in its central part. The bone marrow aspiration needle is placed perpendicular to the iliac crest and the tip of the needle is passed along with the obturator using slight rotational and advance movements to position the tip of the needle directly on the external cortical plate. After aspiration, the aspiration needle trocar is reinserted and the needle is advanced approximately 5 mm from the primary aspiration site to the new sampling site.

The introduction of the needle is limited to the internal cortical plate. By advancing the needle by 5–10 mm and changing the entry point into the cortical plates by 5–10 mm, samples are taken that differ significantly [35]. The procedure must be carried out with care to prevent the needle from falling through the internal cortical plate. To do this, you need to work with two hands, with one hand being on the patient. The needle is better controlled and does not fall through the internal cortical plate when using a rigid stop.

There is another way, parallel, to take bone marrow. With this method, the needle is inserted between the outer and inner edges of the anterior superior or posterior iliac spine. The needle can then be advanced using a trocar at 5 mm intervals through the flat part of the anterior or posterior edge of the iliac wing to a depth of 6–8 cm, after which the needle must be rotated to take fresh material.

An aspirate richer in connective tissue progenitor cells is obtained from the posterior iliac crest compared to that taken from the anterior iliac crest [34]. The method of fully inserting the needle and then turning it without re-inserting the needle theoretically reduces the risk of

infection, of local inflammation development, and bleeding from the aspiration site [34].

Selection of needle diameter

No difference was found when aspirate was taken with an 11 or 8 gauge needle. Eight gauge needles are stiffer and more controllable, allowing longer needle lengths to be used. The long needles (17.8 cm) are particularly suitable for obese patients. A wide variety of needles and aspirate collection systems are available, including single lumen, double lumen, with special holders, graduated and non-graded [34].

Syringe size

It may seem that the volume of the syringe for bone marrow aspiration does not matter much. In one of the conducted studies, it was proved that the syringe volume affects the number of collected MSCs. J.Hernigou et al. compared the concentration of stem cells in the aspirate when using a 10 ml and 50 ml syringe [34]. In 30 patients, bone marrow was taken from both wings of the ilium. On the one hand, multiple samplings were made at standard points using 10 ml syringes, on the other hand, according to the same protocol, 50 ml syringes were used; 1–2–4–10 ml of aspirate were taken into a 10 ml syringe, and 5–10–20–50 ml into a 50 ml syringe. Then the aspirate taken with two different types of syringes was compared and analyzed. It was found that the concentration of MSCs is 3 times higher in patients who underwent bone marrow aspiration with a 10 ml syringe with the same volume of aspirate sampling. It has been hypothesized that, with the same aspiration force, a piston of a smaller diameter forms a greater negative pressure. That is why it is possible to obtain mesenchymal cells in a higher concentration. Therefore, aspiration with small volume syringes and harvesting from different points is recommended [35].

Aspiration volume and dilution

Smaller aspiration volumes are better (1 to 2 ml each) for increasing the yield of nucleated cells and connective tissue precursors per milliliter [36].

The aspirate volume taken affects the concentration of obtained MSCs. G. F. Muschler et al. were the first who studied the effect of the volume of aspirate taken at one time, comparing 1, 2, and 4 ml samples [35]. They found that the total number of MSCs increased with a larger volume taken. The amount of peripheral blood in the sample also increased. The concentration of MSCs decreased by 28% (1451–1051 msc/mL) in 2 ml samples compared to 1 ml ones and by 38% (1418–882 msc/mL) in 4 ml samples compared to 2 ml samples. The authors recommended limiting the volume of aspirate to less than 1 ml from one sampling site, if methods of concentration of obtained MSC solutions are not used intraoperatively. Similar results were obtained by P. Hernigou et al. who compared the concentration of obtained MSCs by aspiration of different volumes using a syringe of the same volume from one sampling site [37]. They found that MSCs in high concentrations are obtained at low aspiration volumes. Thus, when using a 10 ml syringe, the concentration of MSCs decreased by a mean of 82% when the syringe was completely filled compared with a 1 ml intake, from 2062 to 376 msc/mL. The investigators concluded that filling 10–20% of the syringe is optimal when taking bone marrow. With an increase in the aspirated volume, the concentration of MSCs decreases due to dilution with peripheral blood.

Negative pressure suction

When receiving aspirate, it is recommended to use a 10 ml syringe with a needle fixed on it to control the volume taken and the aspiration

force. With the 10 mm syringe fully extended, underpressure of -441 mm Hg is created.

Although larger syringes may create a more considerable underpressure, they all generate approximately the same underpressure when the plunger is displaced and 1 ml is aspirated. When using a 20 ml syringe, where the maximum degree of underpressure reaches 517 mmHg, twice more force is required than when using a 10 ml syringe, so it is much more difficult to control.

Theoretically, a 360-degree rotation of the needle tip during aspiration can improve suction efficiency, but studies have not found a significant difference. It is necessary to create a soft tissue roll at the site of aspiration to prevent air intake into the syringe.

Most of the published papers regarding the clinical outcomes of using autologous bone marrow are of low evidence degree, small case series of nonunions, especially in the tibia. However, the bone marrow can be used not only in cases of slow-healing or nonunion fractures, but also in cases of fresh fractures.

The use of RBM aspirate in orthopedics is at its early stage of development [38]. Although good clinical results have been reported in an increasing number of publications on this topic, there is no established indication for the use of bone marrow [39]. Obvious advantages of using bone marrow aspirate are that it is readily available, has a low risk of complications at the donor site, and provides a simple and rapidly reproducible method in combination with an allograft or injection at a fracture site [34]. Currently, most publications describe in detail the use of bone marrow in the treatment of aseptic and atrophic nonunions without a significant displacement of fragments or large defect at the fracture site [40–41]. Further studies are needed to expand the indications for the use of bone marrow aspirate in the treatment of other types of nonunions, as well as its use in acute trauma. To determine the role of

bone marrow aspirate in the treatment of trauma patients, studies are needed to compare its use with conventional methods of treatment, such as using autograft from the iliac wing.

Conclusion

Based on the aforegoing, we can conclude that autologous platelet-rich plasma, red bone marrow, and autologous platelet lysate have a number of invaluable properties, such as osteostimulation, anti- and pro-inflammatory, angioprotective effects. These products are easy to be obtained in terms of a multidisciplinary hospital. The use of autologous bone marrow is the most readily available, since its preparation requires only a large diameter needle; and for the lysate and PRP, a centrifuge is needed, which implies setting up the appropriate parameters: rotation speed and centrifugation time. A freezer is also needed, which is also available in any large hospital. In order to use platelet rich plasma and platelet lysate, they need to be prepared in advance. In a certain percentage of cases, the need for bone grafting can be found out only intraoperatively. And then, autologous bone marrow, the most accessible plastic material in the operating room at that moment, is used. At the N.V. Sklifosovsky Research Institute of Emergency Medicine, for bone grafting, allogeneic bone is used and specially designed grafts based on or containing type 1 allogeneic collagen, which is a carrier for biologically active substances. For bone grafting and osteogenesis stimulation, it is planned to use the above materials with platelet lysate, platelet-rich plasma, and red bone marrow. There is a lack of evidence-base, i.e. well-designed randomized controlled trials, for the routine use of platelet-rich plasma in traumatology. It should be noted that platelet activity depends on the individual characteristics of the patient from whom blood was taken. Thus, further studies are needed to standardize the procedure,

determine the required volume and concentration of platelet-rich plasma to improve bone regeneration.

High-quality, large-scale clinical trials coming up in the near future will be critical to developing a detailed understanding of the properties of platelet-rich plasma, lysate, and bone marrow and their role in bone regeneration. The heterogeneity of platelet-rich plasma preparations, both at present and in historical terms, does not allow the creation of full-fledged clinical recommendations regarding its use. This heterogeneity has also made it difficult to interpret existing studies. Further studies are needed to determine the most effective stimulation method for different types of fracture or nonunion.

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