

USE OF BONE MARROW (BM) AND CONCENTRATED BONE MARROW ASPIRATE (CBMA) FOR TREATMENT OF CRITICAL LONG FRACTURES

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ABSTRACT

Bone growth is determined by many factors – immobility of bone ends, osteoblastic potency, contact of the bone margins and distance between them. In critically long defects, where the distance between the bone ends is greater than the diameter of the bone, healing processes are compromised because of the limited possibility of bone communication. Applying the gold standard - autotransplantation from a rib, iliac bone, or other donor organ is not always possible, and new healing possibilities are investigated. Synthetic osteoinductive biomaterials such as hydroxyapatite, tricalcium phosphate, collagen, composites, and others are successfully used as carriers, but their efficacy is limited without their activation by a biological organic autologous osteoinductor. Recent studies have shown that platelet-rich plasma, bone marrow aspirate, or tissue growth factors have an exceptionally high potential for autologous osteoinductors.

The purpose of this article is to compare the osteoinductive potential of bone marrow and concentrated bone marrow aspirate in the healing of critically long fractures without autotransplantation.

Key words: bone marrow, concentrated bone marrow aspirate, critical long fractures.

Introduction

Extensive bone defects are a real challenge for any surgeon. Such defects are observed in patients suffering from severe traumatism, neoplastic processes or hereditary deformities (C.M. Clokie et al. 2002; R. Mazzonetto et al. 2007). In such patients, rapid bone regeneration is a key point in repairing bone defects and impaired function in the pathological area. One big part of the fractures heal on their own, but in critically long bone defects they lack the necessary conditions for bone regeneration – we observe a large distance between the bone ends and too slow osteoinductive activity, necessitating to explore new methods for restoring bone integrity.

Autologous bone grafts are considered the "gold standard" in such patients (Yassine et al. 2017). Despite its success, the procedure is too limited due to the donor limit and the recovery period is too long, accompanied by pain and the risk of *complications* (Rose and Oreffo 2002; Spitzer et al. 2002). In allografts the major risks lie in the high rate of immune rejection and pathogen transmission to the recipient (Herberts et al. 2001).

Bone marrow aspirate (BMA) is an autologous bone marrow suspension that is aspirated directly from the iliac crest, humerus, or femur. Due to the fact that there are erythro- and myelopoietic cells, fibroblasts, macrophages, adipocytes, osteoblasts, osteoclasts, and endothelial cells in the bone marrow aspirate (Henning et al., 2017), a number of authors (Gianakos et al., 2016; Theophilus, 2018) recommend their use as an autologous biological aid in critically long bone fractures. Mesenchymal stem cells are involved in bone regeneration in many ways – they differentiate into tissue-specific cells, thus replacing the damaged cells in the traumatic area. These cells indirectly affect tissue repair by secreting soluble factors that accelerate vascularization, cell proliferation, while modulating inflammatory processes (Kalia, 1999). Bone marrow aspirate therapy is preferable to expanded and purified mesenchymal stem cells in relation with the possibility of a positive interaction between different cell types during tissue repair. Moreover, the need for pre-expansion of

mesenchymal stem cells *in vitro* in order to reach a sufficiently high number for transplantation delays therapy and is a prerequisite for culture contamination. In contrast, bone marrow aspiration is a safe, clean and effective method for the direct transplantation of osteoinductive cells into the defective area (Yassine et al., 2017). Moreover, bone marrow aspirate has been proposed for the new “*platinum standart*” in bone reconstruction (Soltan et al., 2005).

Concentrated bone marrow aspirate (cBMA) is a concentration of bone marrow aspirate, leading to an increase in the number of progenitor cells and growth factors per unit volume. Autologous bone marrow aspirate is concentrated by centrifugation, which increases not only the number of mesenchymal stem cells, but also platelets containing polypeptide growth factors such as PDGF, TGF β , IGF-1, VEGF, HGF, EGF, and bFGF (Aminkov et al., 2016) and hematopoietic stem cells per unit volume. The platelet component of cBMA releases growth factors and stimulates the migration of stem cells into the area of application, and increases adhesion between them (Holton et al., 2016). This, in turn, leads to an increased concentration of osteoinductive material within a single operation (Emara et al., 2018).

For the successful repair of critically long bone defects, 3 key elements must be provided: *osteoconductivity*, *osteoinductivity*, and *osteogenic cells*. The bone marrow stem and progenitor cells are the best sources of autologous bone regeneration cells (Quarto et al. 2001; Lee et al., 2010) with proven osteoinductive and osteogenic properties. Osteoconductivity can be ensured by incorporating inorganic biomaterials – *hydroxyapatite* (Yassine et al., 2017), *tricalcium phosphate* (Marvis et al., 2015), *collagen*, etc., which act as a carrier and scaffold for successful binding of fracture ends.

Materials and methods

Obtaining bone marrow aspirate (BMA)

There are several methods for obtaining bone marrow aspirate. What they have in common is that, in addition to the need for strict aseptic and antiseptic, patients must undergo general anesthesia with a good plan of analgesia. BMA can be obtained from the iliac crest (Zhang et al., 2014), humerus or femur. Yassine et al., 2017 describes a method for extracting bone marrow aspirate from the iliac crest in rabbits. The rabbit is fixed in the thoracic position, and after aseptic preparation of the field around the iliac crest, access is started (Fig. 1 – A, B).



Figure 1: A. – positioning for bone marrow aspiration (Piñero et al.)
B. – place for aspiration (no Piñero et al.)

A 18G puncture needle and 3 ml syringe were used, with the lack of anticoagulant requiring bone marrow aspiration to be applied immediately in the area of the bone defect, preceded by the filling of the defect with biocompatible and osteoconductive hydroxyapatite (Fig. 2).



Figure 2: (Yassine KA et al.).

In the absence of a bone marrow aspirate scaffold, it can be left in the syringe for 15 minutes until coagulum is formed, which would facilitate its insertion and retention in the pathological defect (Lim et al., 2019).

According to Takigami et al., 2007, describing therapy for critical bone defect in dogs, the most reliable source of hematopoietic bone marrow containing the maximum amount of osteoblastic progenitor cells is the proximal humerus. In this method, the patient is positioned in a lateral recumbence with the donor limb resting on top. The elbow is rotated medially, with access between tuberculum majus and caput humeri. The needle is inserted with slight rotational movements into the cortical bone of the shoulder bone. Take 10 ml. syringe containing anticoagulant (Acid-Citrate-Dextrose A) and a 17G needle that is introduced into the bone marrow and then aspirated (Theophilus et al., 2018). The aspirate thus obtained can be mixed with a scaffold (hydroxyapatite / calcium phosphate, etc.) and applied to the defect area.

Concentrated bone marrow aspirate (cBMA)

Bone-marrow aspiration is obtained as previously described, except that anticoagulant (heparin 5000 UI / ml) is pre-loaded into the syringe and after aspiration the material is transferred to a sterile vacutainer (containing heparin 5000 UI / ml). Immediately thereafter, the tube was centrifuged at 2000 RPM for 30 min. at room temperature (Piñero et al. 2009). Three fractions are formed in the tube: the top layer contains platelet-poor plasma, in the middle we have the so-called buffy-coat containing nuclear cells (due to their higher density) such as platelets, leukocytes, hematopoietic precursor cells, bone marrow stromal cells, mesenchymal and progenitor cells, with the lowest erythrocyte fraction. The supernatant is removed and the cBMA (above the erythrocyte fraction) is aspirated and ready for direct application in the pathological area (Ferreira D. et al., 2011).

Discussion

It is well known that autologous bone marrow aspirate has many osteoinductive factors such as mesenchymal stem cells, endothelial cells, osteoblasts, macrophages, platelets and others (Soltan et al., 2009; Kalén et al., 2008). Methods for bone marrow aspiration from the iliac crest, humerus, or femur of the patient are relatively fast, easy, and require no special equipment. Most authors recommend that the procedure be performed by the iliac crest (Gianakos et al., 2016; Raskin, 1998; Ryu et al., 2012; Bittencourt et al., 2016; Yassine et al., 2017) for ease of access, thinner bone cortex and the possibility of percutaneous aspiration, which in turn is a prerequisite for lower trauma, post-operative pain and bleeding (Phedy et al., 2011).

The level of bleeding is essential when selecting a bone marrow aspiration site, since osteostimulatory cells in the peripheral blood are significantly less than in the bone marrow and blood aspiration into the syringe may compromise the osteoblastic effect of the bone marrow (Phedy et al., 2011).

Administration of a bone marrow aspirate scaffold material (hydroxyapatite, calcium phosphate or collagen) facilitates the procedure and financial costs, with Lim et al., 2019 describing the increased osteogenetic effect of coagulated bone marrow aspirate in autologous bone graft fracture of New Zealand rabbits (Fig. 3 A, B).

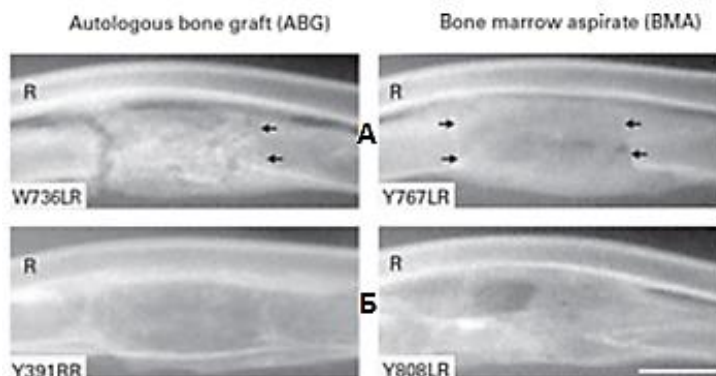


Figure 3: A – 4th week after surgery; B – 12th week after surgery (Lim et al., 2019)

The incorporation of inorganic materials such as hydroxyapatite (Yassine et al., 2017) and tricalcium phosphate (Marvis et al., 2015) is appropriate as these materials are characterized by excellent biocompatibility and osteoconductive properties (Dorozhkin, 2009). Despite the great advantages of calcium phosphate granules as a carrier, some authors observe physical disadvantages due to their granular nature - lack of mechanical strength and osteoinductive nature (Lin et al., 2012).

It is known that bone marrow aspirate also contain mesenchymal stem cells, but their concentration is too low (0.001% of nuclear cells) (Kasten et al., 2008).

The advantage of bone marrow aspirate over bone marrow aspirate concentrate is rapid retrieval, ease of administration, and fewer opportunities for complications (some anticoagulants such as EDTA can damage platelets and their alpha granules) (Farias et al., 2016). On the other hand, concentrated bone marrow aspirate has a significantly higher osteoinductive potential and a faster onset of osteogenetic stimulation (Emara K. et al., 2018). The choice of method of administration depends on the particular patient, the type of bone defect, and the presence or absence of an inorganic scaffold.

Conclusion

Due to its osteoinductive properties, cellular composition and autologous nature, the bone marrow aspirate positively affects the regeneration of critically long bone fractures. The addition of an inorganic carrier such as hydroxyapatite or calcium phosphate granules that act as a scaffold to form new bone tissue further accelerates the repair of the defect. Concentrated bone marrow aspirate has an increased platelet count (growth factors, respectively) and cytokines per unit volume, which determines its more pronounced reparative properties.

At the global level, research in these fields is extremely small, which opens a new area for research on the effects of this type of regenerative therapy in our country. Following positive results in animal models, this treatment approach could be implemented as a routine regenerative technique in both veterinary and human medicine.

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