Bone Marrow Aspirate Concentrate

Subjects: Orthopedics

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Human bone marrow (BM) has been highlighted as a promising source of mesenchymal stromal cells (MSCs) containing various growth factors and cytokines that can be potentially utilized in regenerative procedures involving cartilage and bone.

bone marrow | bone marrow aspirate concentrate | mesenchymal stromal cells

1. Introduction

Human bone marrow (BM) has been spotlighted as a promising source of mesenchymal stromal cells (MSCs) containing various growth factors and cytokines that are potentially utilized towards regenerative procedures involving cartilage and bone [1]. Following the harvesting and isolation process of the BM aspirate, the proportion of MSCs is only around 0.001% to 0.01% of the nucleated cell content of the bone marrow aspiration concentrate (BMAC) [2][3]. Hence, the quality and composition of the BM aspirate used in the preparation of the BMAC remain important to obtain optimal results upon its use in various regenerative procedures. Although the composition of the BMAC is largely dependent on the biological attributes of the patient concerned [4][5], the procedure variables such as location and technique of harvesting have a role in contributing to the variability in the yield of MSCs [6]. There is a wide range of devices and systems available to harvest and process BM aspirate, each using slightly different methods. However, the separation is usually based on the density gradient existing between the blood cells, platelets, nucleated cells, and serum proteins [7]. Based on the processing methods, the cellular and chemical composition of the BMAC may be altered, thereby leading to significant variation in their regenerative potential [8].

In BMAC, MSCs have the capacity to rebuild tissue by differentiating or inducing differentiation of native progenitors into a variety of cell types such as fibroblasts, chondroblasts, myocytes, and other forms of tissue-regenerating cells. BM also contains other multipotent cells, such as hematopoietic stem cells and vascular progenitors, which are likely to play a substantial role in the repair of damaged tissues. Various forms of clinical evidence exist to demonstrate the safety and efficacy of bone marrow-derived MSCs in an osteoarthritic (OA) knee [9][10][11][12][13]. The application of BMAC has been expanded to other indications such as a partial tear of ACL [14], meniscus injuries [15], tendon pathologies [16], bone defect in the form of delayed or non-union of fractures [17][18], chondral and osteochondral defects [19], and patellofemoral arthritis [3]. The components of BMAC are a significant amount of growth factors and cytokines in addition to MSCs and other multipotent cells. BMAC can be administered in isolation or as combination therapies in conjunction with platelet-rich plasma (PRP) [20], stromal vascular fraction (SVF) [21], or surgical procedures like core decompression [22], stress-relieving osteotomies [23], autologous chondrocyte implantation (ACI) [24], matrix-induced chondrocyte implantation (MACI) [24], and

osteochondral autograft transfer system (OATS) [25]. Being a minimally invasive procedure, it is tolerated well by patients with better compliance with the post-procedural rehabilitation program. The short- and long-term results of BMAC in cartilage regeneration are encouraging with good to excellent clinical, functional, radiological outcomes [10][12][13]. No major adverse effects were reported with BMAC therapy except for donor site pain [26].

A fresh, uncultured, and unreduced volume of autologous BMAC injectate containing stromal cells are the potential regenerative and proliferative elements due to the synergistic coordination between the cellular elements and the pool of extracellular matrix, growth factors, and cytokines [27][28]. The modality of BMAC application can be given in the form of either intra-articular, intra-osseous, subchondral injections or surgical implantation with a bio-scaffold [29][30]. Among all the forms of injection, intra-articular modality remains relatively simple, easy to perform under sterile conditions whereas intra-osseous and subchondral injections require hospitalization as a daycare procedure. Due to the difficulty in accurate delivery of stromal cells into the lesion, engineered chondrogenesis came into existence which delivers a stable construct of stromal cells loaded along with bio-scaffold and growth factors [31]. Such a bio-scaffold reduces the chondrocyte loss, maintains the equal distribution of cellular structure, and enhances chondrogenesis [32]. The most commonly used bio-scaffold in the published literature are tricalcium phosphate, hyaluronate, collagen derivatives, agarose, fibrin glue, and chitosan [33][34]. Additive technology of autologous PRP or allogenic homologous platelet lysate has been admixed with engineered chondrogenesis technology to improve the clinical and functional outcome in OA knee individuals [35][36][37][38][39][40].

Several studies with the usage of BMAC for focal cartilage defects and OA knees have reported favorable outcomes. A single intra-articular BMAC injection was found to be a safe and reliable treatment option for grade 3 and 4 OA knees at 30 months follow-up [9]. Keeling et al. demonstrated improvement in pain and patient-reported outcomes in OA knee patients with BMAC injections at short- to mid-term follow-up. In severe degenerative arthritis, BMAC has shown clinical benefits compared to PRP and hyaluronate [12]. In a 5-year follow-up study, intra-articular BMAC injection has proven clinical benefits in K-L grade 1 and 2 osteoarthritis knees [41]. When admixed with an adipose tissue graft, BMAC has not shown superior results in OA knee individuals compared to BMAC alone [21]. In elderly individuals with OA knees, intra-articular BMAC has the potential to slow the timing for the arthroplasty procedure [42]. Gobbi et al.; demonstrated the complete healing of grade 4 multiple chondral injuries of the knee treated with autologous BMAC admixed with either collagen type 1 or 3. In second-look arthroscopy, hyaline-like cartilaginous tissues were demonstrated. These patients have shown no adverse events in a long-term follow-up [43]. Enea et al., have shown that scaffold-based BMAC along with microfracture to be a single-stage technique for the focal chondral defects of the knee [44]. With the MRI evidence, Krych et al. demonstrated that demineralized bone graft (DBG) admixed with BMAC improved cartilage filling in the focal cartilage defects when compared with DBG admixed with autologous PRP [45]. Conversely, a few studies have shown no significant benefits with BMAC when compared with either saline, placebo, hyaluronate, PRP, SVF, or clinical grade MSCs. No statistically significant difference was observed with autologous BMAC in terms of pain relief and functional improvement in patients with bilateral OA knee when compared with saline injections yet they claimed that BMAC was a viable cellular product for pain relief in a short term follow-up of 6 months [46].

2. Variables in the Harvesting and Processing Technique of Bone Marrow Aspiration Concentrate (BMAC)

Researchers aim to discuss the variables in the harvesting and processing technique of BMAC as shown in **Figure 1** and their impact on the yield of MSCs in the final concentrate obtained.

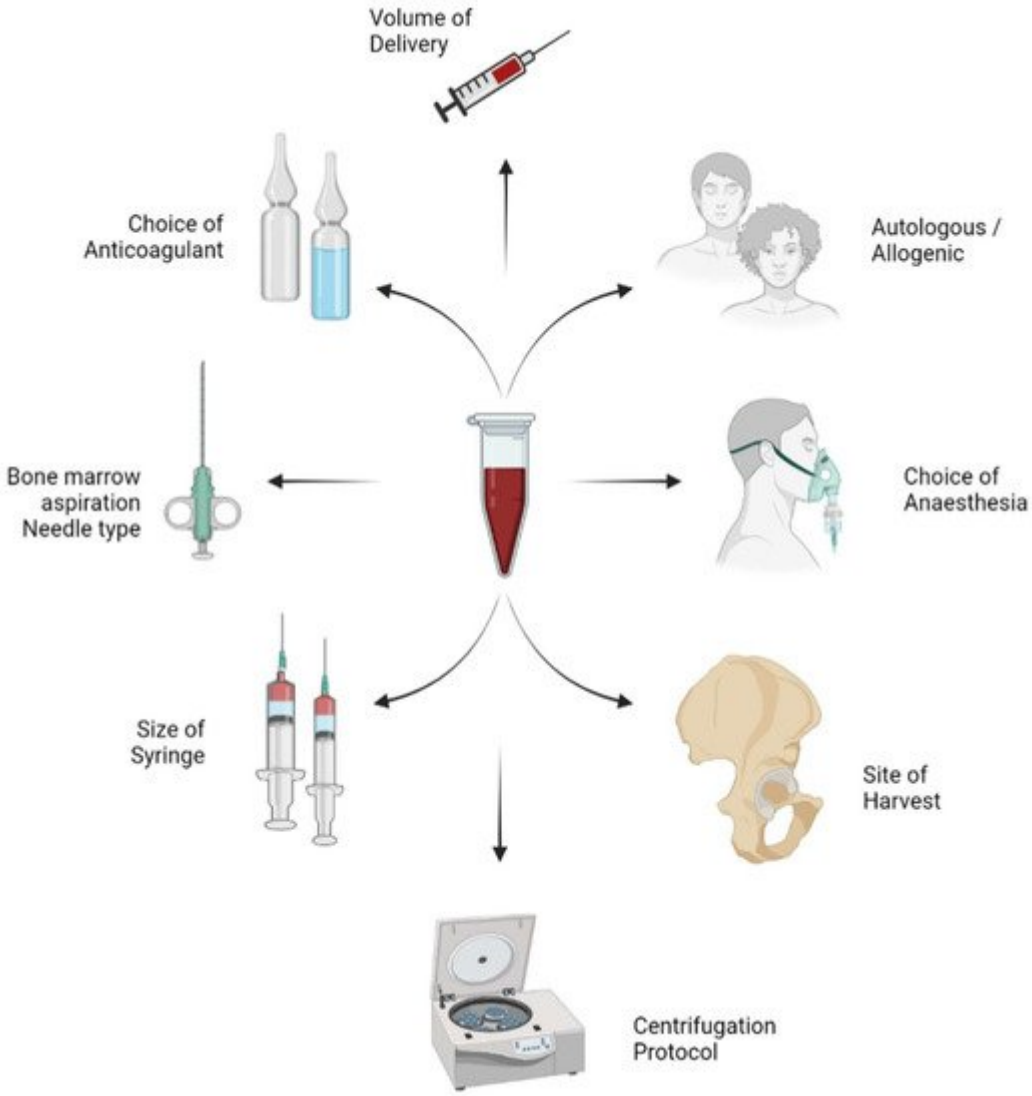

Figure 1. Variables in the harvesting and process technique of bone marrow aspiration concentrate (BMAC).

2.1. Autologous versus Allogenic Mesenchymal Stromal Cells (MSCs)

The efficacy of BMAC in cartilage regeneration depends upon the ability of the cells in the BMAC to withstand and restore the biochemical disharmony in the pathological milieu being evaluated [47][48]. Autologous sources of MSCs from bone marrow are relatively easy to harvest, cost-effective without the risk of any graft rejection or disease transmission [49]. However, autologous MSC products need a two-staged procedure for cartilage regeneration while planning for culture expansion whereas allogeneic MSC preparations can be delivered as a single staged procedure with the desired dosage of MSCs [50]. Allogeneic products namely CARTISTEM (allogeneic cord blood-

derived MSCs—2.5 × 10^6 cells/500 µL/cm^2 area of knee cartilage) [51], Stempeucell (allogeneic ex-vivo cultured pooled human BM-MSCs—2 × 10^8 cells cryopreserved and stored in 15 mL cryo-bags) [52], and JointStem (autologous AD-MSCs—10 × 10^7 cells) [53] launched the MSC-derived products with a definite dosage for cartilage injuries. Nonetheless, allogeneic MSC preparation lacks literature evidence in terms of long-term safety, and efficacy [54]. The dynamic fate of implanted allogeneic MSCs is under debate.

Due to cellular heterogeneity and the subjective characteristics of the MSCs harvested, autologous sources of the MSC cocktail generate inconsistent results upon the analysis in varied scenarios whereas the allogeneic pool of MSC contains homogeneous cells with theoretical grounds to deliver consistent results although they also suffer from immune reactions [54][55][56]. Hence, the clinical outcome depends on the harvesting and cultural characteristics of MSCs isolated either from the autologous or allogeneic source. As age progresses, the MSC count in autologous sources decreases [57]. To achieve the desired cartilage regeneration by MSCs, allogeneic sources of MSCs can be isolated and culture-expanded to provide more MSCs and to provide off-the-shelf products to allow for emergency application. To have the desired cartilage regeneration, the optimal dose of cells, adjuvants, and source of MSC harvest has not been standardized.

Cell-based regenerative therapies rely on consistent, potent, and effective sources of MSCs. Various researchers have conducted clinical trials on embryonic stem cells and induced pluripotent stem cells (iPSCs). The parameters affecting the commercialization of iPSCs are cost, long-term culture and storage, and tumorigenic potential [58]. Autologous cell sources avoid host immune rejection for cell engraftment and retention. Autologous cell therapy requires multi-stage procedures for cell isolation, expansion, and transplanting back to target sites. The variability in the clinical outcome by using the autologous cell source depends on the subjective patient difference which is a major obstacle for reliability and quality control of the product [49]. To overcome all these obstacles, an allogeneic source of cells can be attempted as promising next-generation cell therapy.

Before selecting any allogeneic MSC product for cartilage regeneration, certain parameters should be considered such as MSC passage number, the desired number of MSCs to be injected, the immunogenicity of allogeneic MSCs, the shelf life of allogeneic MSC product, and injection and rehabilitation protocols [56]. Allogeneic preparation of MSCs has to be carried out in a Good Manufacturing Practice (GMP)-certified laboratory with the due regulatory guidelines and protocols [56]. The double negative cell population (HLA-1 negative and HLA-2 negative) renders an allogeneic pool of MSCs as the preferential therapeutic product in the field of regenerative medicine [59]. The temporal relationship between the efficacy of the MSCs delivered in cartilage regeneration with the number of cellular passages, dosage and frequency, cell of origin, usage of scaffolds, and bio-micromolecules has yet to be established. Having discussed the yield, safety, and efficacy of both the autologous and allogenic sources of MSCs, the final choice is always taken upon discussion with the patient based on the affordability and personal preferences.

2.2. Choice of Anaesthesia

The available literature lacks the standard guidelines or consensus for reducing the pain during bone marrow aspiration (BMA) and the post-procedural period. The pain experienced by more than 50% of the individuals who are undergoing BMA was neglected [60]. There are both dependent and independent factors in experiencing pain during bone marrow aspiration namely age, gender, body mass index (BMI), information regarding the procedure, previous BMA, site of BMA, the experience of the physician or surgeon, duration of BMA, and the level of difficulty in performing BMA [61]. A temporal association has been documented between duration and the level of difficulty in performing BMA [62]. The patients undergoing repeated BMA procedures were reported to endure unbearable pain [63]. An adequate level of anesthesia and analgesia is essential for any orthopedic surgery to be successful. For successful harvesting of bone marrow, monitored anesthesia (conscious sedation), regional anesthesia, and general anesthesia are the most widely used anesthesia techniques [61][64][65].

Various studies have used different local anesthetic agents [lidocaine, chloroprocaine, bupivacaine, articaine, or mepivacaine) to aspirate BM but reported no significant difference in the reduction of pain [66][67]. The coupling of intravenous sedation (IVS, lorazepam, midazolam, or diazepam) with local anesthesia reduces anxiety and pain perception [68][69] and IVS drugs also cause retrograde amnesia in some patients [70]. Holdsworth et al. documented less BMA pain and distress in patients receiving propofol/fentanyl general anesthesia than the eutectic mixture of local anesthetics (EMLA) or oral midazolam/EMLA [71]. Pretreatment with oral tramadol 50 mg 1 hour before BMA reduces procedural pain significantly while compared with oral placebo [62]. General anesthesia by propofol and fentanyl offers a good choice for short-term painful procedures in children undergoing treatment for BMA as a daycare procedure [65]. Deep sedation with midazolam, fentanyl, and propofol has proven benefits in the form of pain reduction [72]. The literature lacks the association between the choice of anesthesia and the yield of BMAC. Further studies are warranted to validate the yield of BMAC with the different choices of anesthesia used for the BMA procedure.

2.3. Site of Aspiration

Bone marrow and adipose tissue remain the commonly investigated source of MSCs for cartilage regeneration. The number of MSCs present in bone marrow is less when compared with adipose tissue [73]. The anterior and posterior iliac crests [8][74][75], the ilium [76][77], the proximal humerus [77][78], the proximal tibia [64][79], the distal femur [80][81], the distal tibia [82], the sternum [83][84], the mandible [85][86], and the calcaneum [87][88] are the most common locations for BMAC harvest without significant morbidity to the donor site. The primary site recommended to harvest BMAC is the iliac crest. Hernigou et al., defined the “zone model” and “sector rule” in pelvic bone for choosing the entry point to draw bone marrow [89]. This zone model divides the iliac crest into six different zones as sectors. With the help of CT scans, the sectors were defined based on the bone thickness, the maximum available bone depth for trocar purchase in these different parts of the iliac crest, and the corresponding vascular structures at risk. Hernigou et al., determined that the safe entry point should be approximately 2.5 cm distally from the anterior superior iliac spine (ASIS) [89]. Various studies have stated that BMAC is harvested from ASIS or the iliac crest in the supine position and posterior superior iliac crest (PSIS) in the prone position, respectively [8]. In children, the sternum, anterior iliac crest, tibia, and spinous process of the vertebra are the most common sites for bone marrow aspiration [75]. Bierman et al., reported that the posterior iliac crest is the safest site for harvesting

bone marrow as it is the thickest portion of the posterior segment of the iliac crest with a huge amount of cancellous bony tissues [75]. The volume of bone marrow in the posterior iliac crest is more than the anterior iliac crest [75].

There is a longstanding ongoing debate on the harvesting technique of BMAC. Oliver et al. [90] stated that more BMAC cellular concentrations can be obtained by single insertion technique than multiple insertion technique whereas Peters et al. [91] commented that multiple insertions (up to four) resulted in a higher volume and concentration of BMAC cellular components. Kasashima et al. stated that the insertion of 5 mm of bone marrow needle into the equine sternum three times yielded more BM-MSCs with reduced peripheral blood contamination [92]. They further claimed that under USG guidance, the accurate placement of bone marrow needles into the medullary cavity facilitates the harvest of bone marrow with the least possible damage to the sternum [92].

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