17 Bone Marrow Aspirate Concentrate for the Treatment of Early Osteoarthritis

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17.1 Introduction

Osteoarthritis (OA) is a chronic degenerative disease, which affects articular cartilage. The prevalence of osteoarthritis ranges from 14% to 18% of the adult population aged over 60 years old, of which knee OA is the most prevalent, followed by hip and hand OA [1, 2]. Current conventional treatments for early osteoarthritis include medications such as nonsteroidal anti-inflammatory drugs, steroids, and supplements, which focus on managing pain and inflammation. The recent advancement to the use of orthobiologics such as platelet-rich plasma (PRP), mesenchymal stem cells (MSCs), and bone marrow aspirate concentrate (BMAC) aims to prevent disease progression by altering tissue homeostasis. As disease-modifying treatments are limited in clinical late-stage osteoarthritis, early intervention with biologics such as BMAC can be critical in preventing disease progression.

Bone marrow (BM)-derived cells are one of the commonly used biologics for the treatment of osteoarthritis. Bone marrow MSCs (BM MSCs) are a progenitor stem cell population found in the bone marrow that appear to be promising for the treatment of OA upon intra-articular injection [3, 4]. They act by three different mechanisms: (a) differentiation of MSCs into specific cell lineages, (b) secretion of exosomes and cytokines by MSCs to modulate inflammation, cell growth, and survival, and (c) direct MSC contact with host cells to modulate function [5]. However, since they need to be culture expanded before implantation, they are more than "minimally manipulated" and, as such, subject to regulatory approval. The clinical use of BM MSC therapies is currently not approved by the Food and Drug Administration (FDA) [6]. The use of BMAC is, thus, one of the few methods, by which progenitor cells such as BM MSCs can be implanted clinically, as it is currently approved by many regulatory bodies around the world, including the FDA. The processing of BMAC is typically done

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at the point of care in an entirely closed system, making it one of the safest and most feasible ways to implant bone-marrow-derived progenitors and growth factors.

Autologous BMAC has been used clinically in many studies for the treatment of early osteoarthritis. In this chapter, we discuss the methods and equipment used for the harvest and processing of BMAC, its cellular and growth factor components, and possible mechanisms of action. We review published clinical studies that have applied BMAC for the treatment of early osteoarthritis and discuss their findings, including the various factors that may affect treatment outcomes.

17.2 Harvest and Processing of BMAC

Bone marrow aspirate (BMA) is typically harvested from the iliac crest, femur, or tibia. The posterior iliac crest is the most common aspiration site, as it gives a better yield of BM MSCs compared to other sites such as the anterior iliac crest, femur, or tibia [7, 8]. However, the percentage of BM MSCs in BMA is extremely low, between 0.001% and 0.01% [9], and the delivery of large volumes of BMA to the treatment site is not feasible. The centrifugation of BMA overcomes this issue by achieving the concentration of most cell types and growth factors found in BMA into a small volume that can be directly implanted. The BMA is typically concentrated at the point of care using commercially available centrifuges to create BMAC. Most commercial systems utilize density gradient centrifugation to isolate and concentrate the mononuclear cell (MNC) or total nucleated cell (TNC) fraction along with platelets, which is separated from the red blood cells (RBCs) and plasma. Nearly all the supernatant plasma is then removed, and the total nucleated cell fraction and platelets are resuspended in the remaining plasma, resulting in a concentrated mixture of cells and growth factors (Fig. 17.1). A stepwise method for the harvest and processing of BMAC is described by Chahla et al. [10].

There are multiple commercial systems available today to achieve the concentration of bone marrow aspirate at the point of care. These include the Harvest Smart Prep system (Terumo BCT), the BioCUE (Zimmer Biomet), the Magellan (Isto Biologics), the Angel Bone Marrow Processing System (Arthrex), the Pure BMC device (Angel Corporation), the ART BMC device (Celling Biosciences), and Accelerate BMC (Exactech). The technical features and quality parameters of many of these point-of-care devices are reviewed in [11]. One prospective study compared the Harvest, Magellan, and BioCUE systems and found that the Harvest system achieved a significantly higher number and concentration of MSCs, after centrifugation, compared to the Biomet and Magellan systems [12]. This may indicate that the Harvest system achieves more efficient concentration compared to the other two systems studied. Another study that compared the Biomet, Harvest, and Arthrex systems noted that the Harvest system concentrated white blood cells (WBCs) more consistently than the Arthrex system. The Harvest system recovered the highest percentage of colony-forming units (CFU-Fs), indicating MSCs, CD34+ hematopoietic stem cells (HSCs), and WBCs, while the Biomet system recovered the highest percentage of platelets [13]. Thus, it seems that BMACs processed in different commercial systems show differences in cellular composition, which may lead to differences in clinical outcomes. Each system holds an advantage for the concentration of a particular cell type, indicating the clinical significance of the system used.

Some studies continue to utilize Ficoll–Paque-based density gradient centrifugation to isolate and concentrate the bone marrow mononuclear cell (BM MNC) fraction. This method eliminates platelets and granulocytes as well as red blood cells, leading to higher concentrations of uncommitted stem cells [14]. However, it has been shown that Ficoll–Paque density gradient centrifugation can compromise BM MNC yield [15], and that the use of a BMAC device improved total nucleated cell (TNC) count to 2.4 times that of the Ficoll method [16]. The Ficoll method is

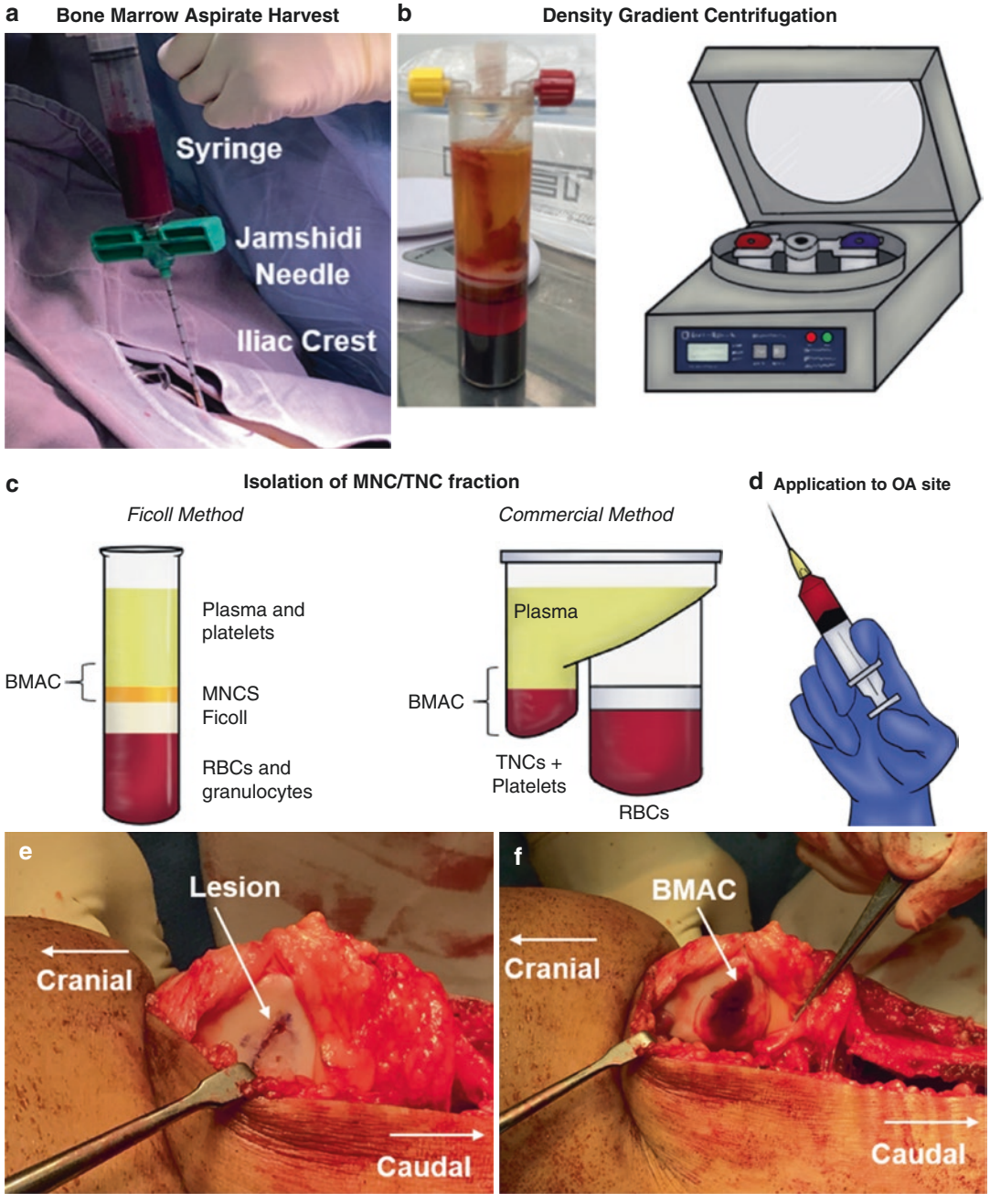


Fig. 17.1 Harvest, processing, and delivery of bone marrow aspirate concentrate (BMAC). (**a**) Bone marrow aspirate is harvested typically from iliac crest bone, (**b**) undergoes centrifugation either using a commercially available BMAC device or a Ficoll–Paque density gradient procedure to isolate (**c**) the mononuclear cell (MNC) or the total nucleated cell (TNC) fraction, which is then (**d**) applied to the osteoarthritic (OA) site for treatment. Images from our clinic showing a patellar cartilage lesion (**e**) before and (**f**) after application of BMAC treatment

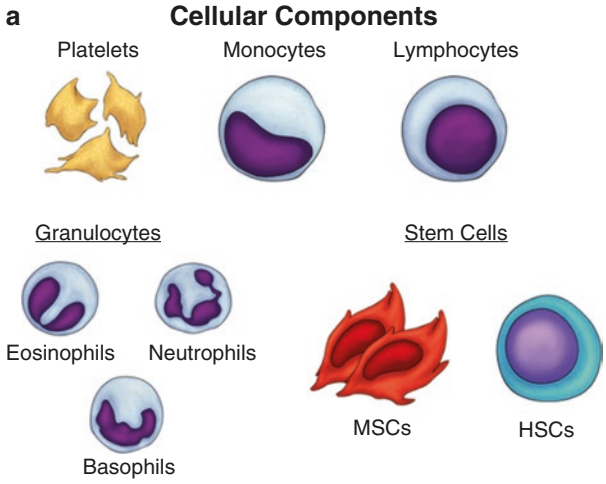
also not an entirely closed system unlike many commercial BMAC devices. It requires careful manual layering of the BMA over the Ficoll solution, making it investigator dependent, time-consuming, and requiring the use of a GMP facility.

17.3 BMAC Components and Possible Mechanism of Action

BMAC contains concentrated cells, including platelets, granulocytes, lymphocytes, monocytes, progenitor cells, and a small proportion of stem cells—MSCs and hematopoietic stem cells (HSCs) (Fig. 17.2a). A three- to fourfold increase in total nucleated cells was reported after bone marrow concentration compared to the same volume of bone marrow aspirate [16, 17], verifying that the systems used did concentrate nucleated cells. An increase of MSC concentration in BMAC compared to BMA has also been reported, with higher CD90+/CD73+/CD271+ MSC populations [18], and higher colony-forming unit (CFU) counts in BMAC [17–19]. MSCs have self-renewal capabilities and the ability to differentiate into osteocytes and chondrocytes upon implantation, to regenerate injured tissue. They also secrete a range of trophic factors, which can modulate inflammation, cell growth, and survival. CD34+ HSCs are also enriched in BMAC, making up 1–2% of cells [19, 20]. HSCs can promote angiogenesis and promote MSC osteogenesis [21, 22]. The platelet component of BMAC is rich in growth factors, which can aid in stem cell migration and provide stem cell adhesion sites [23].

BMAC also contains enriched levels of the growth factors such as platelet-derived growth factor-BB (PDGF-BB), vascular endothelial growth factor (VEGF), transforming growth factor- β1 (TGFβ1), bone morphogenic protein-2 (BMP2), and basic fibroblast growth factor (b-FGF) as well as cytokines such as interleukins (IL) IL-18 and IL-1β and the interleukin receptor antagonist IL-1ra (Fig. 17.2b). These growth factors can influence cell behavior upon implantation and promote MSC differentiation. TGFβ1 is known to promote MSC differentiation and chondrocyte proliferation [24, 25]. BMP-2 can have a synergistic effect along with TGFβ in promoting chondrogenesis [26]. PDGF functions to promote collagen synthesis and angiogenesis [27] and can suppress IL-1β cartilage degradation [28].

The growth factor and cellular components of BMAC differs significantly from those contained in other orthobiologics such as platelet-rich plasma (PRP), which is also commonly used in the treatment of OA. The most important distinc-



b Cytokines and Growth Factors

Growth Factors	Cytokines
PDGF-AB, AA, BB	IL-1ra
TGFβ1, 2	Il-8
VEGF	IL-1β
bFGF	
Activin A	
IGF-1	
BMP2	
EGF	

Fig. 17.2 The typical components of BMAC. (**a**) Cells including platelets, monocytes, lymphocytes, granulocytes, mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs). (**b**) Growth factors and cytokines

tion is that BMAC contains MSCs, while PRP does not have any. While the number of platelets was similar in BMAC and PRP, WBCs were enriched 11-fold in BMAC compared to PRP [17]. BMAC also contained higher levels of bFGF than PRP, but similar levels of TGFβ1, PDGF-BB, VEGF, and BMP2 [17, 19]. BMAC contained higher levels of pro-inflammatory cytokines IL-1β and IL-8 than PRP, but also a clinically relevant concentration of IL-1ra [17]. The presence of IL-1β and IL-8 in BMAC may cause an unintended effect of neutrophil migration and monocyte stimulation at the injection site, leading to a more inflammatory phenotype. However, this is offset by the high levels of IL-1ra found in BMAC, which may lead to an overall anti-inflammatory effect via the prevention of IL-1 catabolism. Importantly, the ratio of IL-1ra/IL-1 in the BMAC needs to be considered, and this may vary based on the donor and the centrifugation system. When BMAC was processed using the Angel Arthrex system, the average ratios of IL-1ra/IL-1β were 193.54 at a 2% hematocrit setting and 720.62 at a 15% hematocrit setting, indicating that the BMAC would have significant anti-inflammatory effects [29]. Advantageously, the presence of other inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interferon gamma (IFNγ), and IL-6 was undetectable in BMAC [17].

17.4 Clinical Outcomes

In this review, our focus is on the use of BMAC for the treatment of early stage OA. Studies that included only early OA patients or those that included patients of all OA severity were reviewed and are summarized in Table 17.1. Studies that primarily focused on patients with severe Kellgren–Lawrence (KL) Scale Grade 3–4 OA or severe OA were omitted during review. The KL scale ranks the severity of knee OA based on AP knee radiographs through the identification of five hallmark radiological features of OA: formation of osteophytes on joint margins or tibial spines, periarticular ossicles associated with the distal and proximal interphalangeal joints, narrowing of the joint cartilage associated with sclerosis of subchondral bone, small pseudocystic areas with sclerotic walls situated usually in the subchondral bone, and finally the altered shape of the bone ends, in particular, the head of the femur [41]. Out of the 12 studies reviewed, nine focused on knee OA, one on hip OA, and one on both knee and hip OA. One study by Centeno et al. in 2015 focused on OA and/or rotator cuff tears of the shoulder [31]. Here, shoulder pathology was assessed through magnetic resonance imaging (MRI) and physical examinations.

Across the studies listed in the table, there is a general trend of improvement in outcome scores that mainly pertain to function and pain regardless of the method used to assess treatment outcome. These improvements can be seen as early as 1-month post-treatment with BMAC and the effect persists in subsequent follow-ups of up to 2 years [37, 38]. One of these studies compared the effect of BMAC alone to exercise therapy, concluding that the injection of BMAC showed more benefits [34]. All patients who received exercise therapy converted to BMAC injection after 3 months and showed results that were comparable to the initial BMAC injection group.

To note, the time of follow-up post-treatment is typical across all studies with the maximum data available being a 2-year follow-up. While it is evident that the short-term effects of BMAC are beneficial, the long-term effects of BMAC have yet to be elucidated. Therefore, continuous follow-up will be fruitful to determine if a single BMAC injection is sufficient in mitigating OA progression.

Apart from these measurement outcomes, which are reliant on patient response, two separate studies have reported MRI scores to objectively quantify the local effect of BMAC on the treated knee. Goncars and colleagues utilized the Whole Organ MRI Scoring (WORMS) method to determine the degree of abnormality within the affected region [35]. Of the 14 different features measured by WORMS, 3 were identified to have a significant improvement. These features are articular cartilage integrity, bone marrow abnormality and synovitis, all of which demonstrated-

Table 17.1 Overview of clinical studies that used bone marrow aspirate concentrate (BMAC) for early OA treatment

Study	OA pathology	Groups and number of patients	Additional factors	Marrow harvest	Dosage	Follow-up duration	Results	Safety
Centeno et al., 2014 [30]	KL grade 1–4, knee OA	Two treatment groups: 681 patients, 840 procedures	PRP and PL added to BMAC in both groups	Posterior superior iliac crest	1–3 mL BMAC produced, dosage unspecified	1, 3, 6 months, 1 year	Treatment using BMAC with adipose graft did not show an advantage over BMAC alone	No serious adverse events
		BMAC only 518 patients, 616 procedures, 397 M, 219 F Mean age: 54.3 ± 14.1		Density gradient centrifugation	For patients treated with additional adipose graft, 5–10 mL of BMC and adipose graft was injected		Significant improvements in self-rated functional and pain scores post-procedure	Minor complications: Swelling and pain post-treatment
		BMAC with adipose graft 163 patients, 224 procedures, 119 M, 105 F Mean age: 59.5 ± 10.3						
Centeno et al., 2014 [31]	KL grade 1–4, hip OA	216 patients, 124 M, 92 F	PRP and PL added to BMAC	Posterior superior iliac crest	1–4 mL of BMAC, 1 mL of PRP, and 1 mL of PL injectate solution	1, 3, 6 months, annually after treatment	Significant improvements in NPS and OHS. Patients ≤55 years old were likely to report greater improvements than older patients	No serious adverse events
		Mean age: 57 ± 10.6		Density gradient centrifugation				Minor complications: Swelling and pain post-treatment
Centeno et al., 2015 [32]	KL grade 1–4, knee OA	Two groups received BMAC with PRP and PL: 373 patients, 424 procedures (cell count data available for 409 procedures)	PRP and PL added to BMAC	Posterior superior iliac crest	1–3 mL BMAC produced	1, 3, 6 months, 1 year	Significant improvement in IKDC and LEFS scores	No serious adverse events
		Lower cell count 188 patients, 224 procedures, 145 M, 81 F Mean age: 54.5 ± 12.8		Density gradient centrifugation	Lower nucleated cell count in BMAC: $<4 \times 10^8$		Higher cell count group reported lower pain scores in comparison to the lower cell count group; however, there are no significant differences using other metrics	
		Higher cell count 170 patients, 185 procedures, 140 M, 45 F Mean age: 50.2 ± 15.6			Higher nucleated cell count in BMAC: $>4 \times 10^8$			

Centeno et al., 2015 [33]	Symptomatic OA at the glenohumeral joint and/or rotator cuff tear <1.5 cm	Two groups (102 patients, 115 shoulders):	PRP and PL added to BMAC	Posterior superior iliac crest	1–3 mL BMAC produced, dosage unspecified	1, 3, 6, 12 months, and annually post-treatment	Significant improvement in DASH and NPS scores	No serious adverse events
		OA only 34 patients, 27 M, 7 F Mean age: 52.1 ± 14.3		Density gradient centrifugation				
		Rotator cuff disorder 81 patients, 53 M, 28 F Mean age: 59.5 ± 11.9						
Centeno et al., 2018 [34]	KL grade 2–3, knee OA	Two treatment groups (48):	PRP and PL added to BMAC	Posterior superior iliac crest	5–7 mL injectate solution (75% by volume of BMAC, 12.5% by volume PRP, and 12.5% by volume PL)	6 weeks, 3, 6 months, 1, 2 years	Significant improvement in KSS-knee, KSS-function, SF-physical, LEAS, and ROM after BMAC treatment	No serious adverse events
		Exercise therapy control 22 patients Mean age: 57 ± 8.5 All 22 patients received BMAC treatment after 3 months (crossover group)		Density gradient centrifugation			Significant improvement in KSS-knee, SF-12 physical, and LEAS scores in crossover group compared to exercise therapy alone	Minor complications: Swelling and grinding with pain post-treatment
		BMAC with PRP and PL 26 patients Mean age: 54 ± 8.9						
Goncars et al., 2019 [35]	KL grade 2–3, knee OA	32 patients, 16 M, 16 F Mean age: 53.96 ± 14.15	Only MNC fraction of BMAC was used	Iliac crest	Up to 5 mL of MNC suspension was used	1, 3, 6, 12 months	Significant improvement in KOOS and WORMS score. Improvement in KSS score	No serious adverse events
		Two patients received BM-MNC injections in both knee joints		Density-gradient centrifugation	An average of $45.56 \pm 34.94 \times 10^6$ MNCs were injected			Minor complications: Swelling and pain post-treatment

(continued)

Table 17.1 (continued)

Study	OA pathology	Groups and number of patients	Additional factors	Marrow harvest	Dosage	Follow-up duration	Results	Safety
Kim et al., 2014 [36]	KL grade 1–4, knee OA	41 patients, 75 knees, 17 M, 24 F	Adipose tissue was added to BMAC	Anterior superior iliac crest or posterior superior iliac crest	7 mL of BMAC and 10 mL of adipose tissue to affected knee	3, 6, 12 months	Significant improvement in IKDC, KOOS, LKQ, SF-36, and VAS scores post-procedure	No serious adverse events
		Mean age: 60.7 (range 53–80)		SmartPReP2 platelet concentration system (harvest technology)	Estimated 2.4×10^5 adult stem cells and 1.8×10^9 MNCs			Minor complications: Swelling and pain post-treatment for up to 4 weeks
Oliver et al. 2015 [29]	KL grade 2–4, knee OA	70 (21 M, 49 F)	Lipoaspirate added to BMAC	Posterior superior iliac crest	3 mL of BMAC and 2 mL of lipoaspirate to affected joint; 1 mL of BMAC and 1 mL of lipoaspirate to medial joint capsule	90 days, 180 days, 1 year	Significant improvement in KOOS scores post-procedure	No serious adverse events
		Mean age: 60 (range 28–83)		Angel centrifugation system (Arthrex)				Minor complications: Transient pain and short-term swelling post-injection (<7 days)
Oliver et al., 2018 [37]	KL grade 2–4, knee OA	254 patients, 98 M, 156 F	Lipoaspirate added to BMAC	Posterior superior iliac crest	3 mL of BMAC to affected joint, 1 mL of BMAC and 1 mL of lipoaspirate to medial joint capsule, 1 mL of BMAC and 1 mL of lipoaspirate to other soft tissue injury to the knee	3, 6 months, 1, 2 years	Significant improvement in VAS, WOMAC pain and WOMAC stiffness scores post-procedure.	No serious adverse events
		Mean age: 60 (range 29–83)		Angel centrifugation system (Arthrex)				Minor complications: Increased pain, swelling, and experienced noise post-injection

Rodriguez-Fontan et al., 2018 [38]	KL grade 1–2, knee and hip OA	19 patients, 3 M, 16 F 25 joints: 10 knees, 15 hips Mean age: 58 ± 12.7 years	None	Anterior iliac crest BioCUE platelet concentration system (Zimmer Biomet)	12 mL of final BMAC, dosage unspecified	6, 12, 18, 24 months	Significant improvement in WOMAC scores post-procedure	No serious adverse events Minor complications (11 patients): Mild pain at BMC extraction site (24 h post-operation), pain 2 weeks post-injection, swelling
Shapiro et al., 2017; Shapiro et al., 2019 [39, 40]	KL grade 1–3, bilateral knee OA	Two treatment groups: 25 patients, 7 M, 18 F Mean age: 60 (42–68)	Platelet-poor plasma added to BMAC	Superior iliac crest	5 mL of BMAC with 10 mL platelet-poor bone marrow plasma to one knee	1 week, 3, 6 months	Significant improvement in OARSI ICOAP and VAS scores post-procedure	No serious adverse events.
		BMAC on right knee and placebo on left 13 patients *BMAC on left knee and placebo on right* 12 patients		Magellan autologous platelet separator system (Arteriocyte)	15 mL saline as placebo Estimated median of 56% mononuclear cells in 1 mL BMAC: 1.5×10^8 WBCs, 8.0×10^7 MNCs, 4.4×10^6 HSCs, and 3.4×10^4 MSCs	MRI only: 6, 12 months	No significant change was seen in quantitative T2 MRI mapping in second study	Minor complications: Effusions seen post-procedure, likely due to residual 15 mL product than inflammation

(continued)

Table 17.1 (continued)

Study	OA pathology	Groups and number of patients	Additional factors	Marrow harvest	Dosage	Follow-up duration	Results	Safety
Upadhyay et al., 2014 [14]	KL grade 0–1, knee OA	Two treatment groups: 50 patients, 12 M, 38 F *BMAC only* 25 patients, 7 M, 18 F Mean age: 56.12 ± 9.311 *Sodium hyaluronate control* 25 patients, 5 M, 20 F Mean age: 55.72 ± 7.673	BMAC was diluted in phosphate buffered saline	Posterior iliac crest Ficoll–Paque density gradient centrifugation	2 mL diluted BMAC or sodium hyaluronate	1, 3, 6 months	Significant improvement in WOMAC and VAS scores compared to controls at 6 months of follow up	No serious adverse events Minor complications: Transient pain at injection site (<2 days)

DASH Disabilities of the arm, shoulder, and hand, *IKDC* International Knee Documentation Committee, *KL* Kellgren–Lawrence, *KOOS* Knee Injury and Osteoarthritis Outcome Score, *KSS* Knee Society Score of Assessment and Function, *LEAS* Lower Extremity Activity Scale, *LKQ* Lysholm Knee Questionnaire, *NPS* Numeric Pain Score, *OARSI ICOAP* Osteoarthritis Research Society International Intermittent and Constant Osteoarthritis Pain Questionnaire, *OHS* Oxford Hip Scores, *PL* Platelet Lysate, *PRP* Platelet-Rich Plasma, *ROM* Range of Motion, *SF-12* Short Form-12 Scales, *VAS* Visual Analog Scale, *WOMAC* Western Ontario and McMaster Universities Arthritis Index, *WORMS* Whole Organ MRI Scoring

vdecreases in abnormalities after 6 months of treatment. Compared to the rest of the clinical studies we have identified, only the MNC fraction of BMAC was used in Goncars' study. The absence of other factors normally present within BMAC may alter treatment outcomes, indicating that the improvements seen in WORMS were due solely to the MNCs.

In contrast, Shapiro et al. used quantitative T2 MRI mapping to determine the regenerative capacity of BMAC in bilateral knee OA [39]. Each patient in the study received a BMAC injection to one knee and saline to the other, allowing the saline-injected knee to act as a placebo control. While the same short-term benefits of BMAC were evident, no significant changes that indicated cartilage regeneration due to BMAC were seen a year post-treatment. This led to the conclusion by the authors that BMAC failed to show regenerative potential as results were similar to the saline-injected knees. Interestingly, patients in the study reported improvements in the placebo knee, which may be indicative of the systemic effect of the MSCs originally injected to the treated knee or instead might be indicative of a placebo effect [40].

Of the 13 studies that have been listed in Table 17.1, 3 have reported the need for total knee or hip arthroplasty (TKA or THA) after BMAC injection. Patients receiving these interventions were those that did not respond positively to BMAC and typically had higher severity of OA compared to the rest of the group. Nevertheless, the need for knee or hip replacement makes up a minority in each study. In Centeno's 2018 study, before receiving BMAC treatment, 52% of the patients were candidates for TKA as they had KL Grade III OA. However, only 3 of the 48 patients received TKA during the follow-up period [34], perhaps indicating the success of the BMAC intervention. Rodriguez-Fontan reported that while 7 of the 19 patients in the study were unsatisfied with BMAC, only 2 received THA after 8 months post-treatment [38]. Of note, one of these patients was 65 and had pre-existing comorbidities such as diabetes, obesity, and osteoporosis, which would have contributed to the need for THA. Finally, in Kim's study, 22 of the 75 knees treated showed unfavorable results, but only four of these knees underwent additional interventions such as TKA, high tibial osteotomy, and unicondylar knee arthroplasty [36]. However, the medical history was not indicated for most patients that received these additional treatments, so it is unknown what the patient's OA severity was and what other confounding conditions they might have had. As the time of follow-up across all these studies is only available for up to 2 years, long-term data on the number of patients requiring TKA after early-stage BMAC treatment are needed to better determine the long-term efficacy of BMAC.

The severity of OA at the time of therapy could possibly be an important predictor of clinical outcomes. In one study by Centeno et al. in 2014, it was found that patients with lower OA severity (KL Grade 2) were significantly more likely (2.2 times) to report ≥50% improvement on the subjective reported outcome scale than KL Grade 3 patients [30]. However, this correlation did not extend to other outcomes such as the lower extremity functional questionnaire (LEFS) or the numeric pain scale (NPS). Similarly, Kim et al. reported that as KL grade increased, the response to BMAC injection was poorer, implying that patients with early OA benefitted greatly to the treatment compared to more severe OA [36]. Unlike the studies that we have listed in Table 17.1, the study by Kim and colleagues included additional treatment of PRP post-injection of up to 4 weeks for patients that experience pain and swelling at the joint site. This may lead to confounding effects of the initial treatment, masking the true effect of BMAC. Contrary to these studies, Oliver et al. showed that the severity of OA did not affect treatment outcomes, reporting that Visual Analog Scale (VAS), Western Ontario and McMaster Universities Arthritis Index (WOMAC) pain, and WOMAC stiffness scores across KL Grades 2–4 showed similar improvements [29, 37].

Age may also be another important predictor of clinical outcomes. In 2014, Centeno et al. conducted a separate study on the efficacy of BMAC on hip OA. They found that patients younger than 55 years old were likely to answer

more favorably on the numeric pain scale (NPS) and Oxford Hip Scores (OHS) [31]. However, age did not have a strong correlation with positive treatment outcome. When grouping patients by age, Kim and colleagues found that older patients showed marginally inferior scores but were still statistically insignificant [36]. Thus, more investigation needs to be done to determine the effect of age on BMAC treatment efficacy. Moreover, other predictors such as gender and BMI were shown not to significantly affect outcome scores [29, 31–33].

Such variations in reporting especially with regard to whether KL grade correlates with favorable outcomes may suggest various possibilities. The method in which BMAC was processed and prepared to produce the final injectate may lead to differences in cellular composition that ultimately affect treatment outcomes. Another factor that is glaring across all the studies identified in Table 17.1 is the different measurement outcomes used to assess OA progression, leading to the inconsistencies across various studies. Furthermore, the follow-up surveys that are conducted in most studies are self-assessments by the patients. As these tests are subjective in nature, the response to and perception of these assessments, along with the degree of treatment satisfaction measured, will vary from patient to patient.

17.5 Perspectives

17.5.1 Augmentation of BMAC with Additional Factors

The combinatorial delivery of BMAC along with additional factors that could possibly enhance the therapeutic effects of BMAC has been trialled. In four studies, BMAC was injected along with PRP and platelet lysate (PL) [30, 32–34], while in one study, platelet-poor plasma was used [40]. In vitro, when added to culture medium, both PL and PRP improve MSC proliferation [42, 43] and differentiation into chondrocytes [43, 44]. Thus, they may improve the activity of MSCs when injected along with BMAC. Four studies reported the addition of adipose tissue (in the form of lipoaspirate) to the BMAC before injection. In one report, the addition of an adipose graft to BMAC did not produce any detectible benefits over the use of BMAC alone, upon intra-articular injection [30]. Other studies injected BMAC along with a few milliliters of lipoaspirate into involved soft tissue structures to utilize the inherent scaffolding properties of adipose tissue [29, 37]. However, as no control arm (BMAC without lipoaspirate) was included in these studies, the additional beneficial effects of lipoaspirate are unknown. Overall, it is undetermined whether the beneficial effects reported in these studies are due to the BMAC, the adjuvant factors, or a synergistic effect of the two. In the absence of further studies with appropriate controls, the optimal method to augment BMAC therapy remains undetermined.

17.5.2 Allogeneic or Autologous BMAC

All the studies reviewed in this chapter (Table 17.1) used autologous BMAC. While the use of autologous BMAC has obvious advantages due to the lack of immune response, it may not be as effective in older patients. The number of stem cells present in BMA and their efficacy is significantly lower in older patients than younger ones [45]. The use of allogeneic BMAC from younger patients may result in greater efficacy; however, there is currently a lack of studies utilizing allogeneic BMAC. This may be due to the potential safety concerns of graft versus host disease or secondary infection from the donor [46]. Until these concerns can be appropriately addressed, autologous BMAC may be the only suitable option for treatment as its benefits outweigh the risks that come with allogeneic BMAC.

17.5.3 Cellular Composition

The method of BMAC processing has a significant impact on the cellular composition of the BMAC (Fig. 17.1c). In two of the studies

reviewed, Ficoll–Paque density gradient centrifugation was used to concentrate BMA [14, 35]. Thus, in these studies, BMAC only contained the MNC fraction. In five studies by Centeno et al., the method of density gradient centrifugation (whether Ficoll–Paque or commercial device) was not mentioned. However, in these studies, BMAC was combined with PRP/PL before injection, introducing platelets, plasma, and growth factors. In the other five studies that used a commercial BMAC device, the BMAC included the TNC fraction, platelets, and a small amount of plasma. Varying efficacies of BMAC in these groups that used different methods could be due to the differences in cellular composition due to the BMAC processing method used and combination with other biologics.

17.5.4 Dosage

The amount of BMAC that was finally injected ranged from 1 to 12 mL in the studies reviewed (Table 17.1). However, only 4 of the 12 studies reviewed analyzed the cellular concentration of the BMAC product. It is probable that patient characteristics and the method of BMAC processing would have a significant effect on cellular concentration within a given amount of BMAC. Thus, it is difficult to compare dosages in different studies if the cellular concentration is not specified, even if the injection volumes were similar. The age of the patient may also affect the minimal effective dose of autologous BMAC. In older patients, larger volumes of BMA may need to be aspirated in order to achieve a similar stem cell yield as younger patients.

In one study where patients received either a low dose ($<4 \times 10^8$ nucleated cells) or a high dose ($>4 \times 10^8$ nucleated cells), both groups reported significant improvements in pain and function of the osteoarthritic knee joint. The only significantly improved outcome in the high cell dose group was a reported lower post-treatment pain scale value [32]. Although there were no differences in functional outcomes, the improved pain relief with a higher cell dose is an important finding. Another study that involved the injection of only BM MNCs used an average cell dose of $45.56 \pm 34.94 \times 10^6$ cells [35], which was effective in causing a significant improvement in Knee Injury and Osteoarthritis Outcome Score and WORMS scores after 6- and 12-month follow-ups.

Two studies estimated the number of MSCs injected—while one study injected a median of 4.4×10^6 HSCs and 3.4×10^4 MSCs (out of 8×10^7 total MNCs) [40], another estimated 2.4×10^5 adult stem cells and 1.8×10^9 MNCs were implanted when using a mixture of BMAC and adipose tissue [36]. These reported numbers of MSCs are far lower than the dosage of culture-expanded MSCs that appears to be effective for OA treatment [4]. This is expected, as other reports have noted that the amount of MSCs implanted using BMAC is several magnitudes lower than if culture-expanded MSCs are used [32, 47]. However, the presence of other cell types and concentrated growth factors in BMAC may lead to a combinatorial effect in the management of pain and inflammation, as well tissue regrowth. Overall, the dose of MNCs or TNCs required to achieve an effective clinical outcome for the treatment of OA is still unresolved.

More cell dose response studies are required in order to elucidate the appropriate BMAC dosage for the maximization of clinical outcomes. Future clinical studies should quantify cellular concentration in the BMAC before implantation. Understandably, there are difficulties in enumerating MSC numbers in BMAC by counting CFU-Fs or using flow cytometry, as these methods can be time consuming and require dedicated technical staff and equipment. However, the enumeration of nucleated cells within the BMAC using either a hematology analyzer or hemocytometer is both quick and feasible in a regular clinical setting.

17.5.5 Safety and Limitations of the BMAC Technique

Most of the clinical studies reviewed (Table 17.1) did not report any serious adverse effects after BMAC treatment. However, com-

mon minor adverse effects included short-term pain at the site of bone marrow harvest and transient swelling and pain at the site of injection up to 7-day post-injection. Centeno et al. reported 6% adverse events in the BMAC group (including two severe events) and 8.9% adverse effects in the BMAC + adipose graft group (including one severe event) [30]. However, they did not define what qualified as a severe adverse event. Overall, BMAC treatment appears to be a generally safe procedure, with few serious adverse events reported.

The invasive harvesting of autologous bone marrow aspirate from the iliac crest is a significant disadvantage of the BMAC technique, which can lead to pain at the harvest site. The presence of white blood cells such as monocytes and neutrophils in BMAC can cause the increased secretion of pro-inflammatory cytokines such as IL-1β and IL-8 [17], promoting inflammation at the injection site. However, the presence of IL-1β and IL-8 is offset by the high levels of IL-1ra found in BMAC, which may lead to an overall anti-inflammatory effect.

17.6 Conclusions

BMAC is one of the emerging orthobiologics that have shown promise for the treatment of early osteoarthritis. Several studies have evaluated BMAC as a treatment for OA, and it was found to be a generally safe treatment. Many studies have reported the reduction of pain and improved joint function after BMAC treatment. However, the regenerative effects of BMAC are still unresolved, and the varying efficacies of BMAC therapies reported indicate the need for the standardization of processing technique and dosage applied. The lack of information on cellular composition and concentration in many clinical studies makes it difficult to compare across studies and determine the true efficacy of BMAC, especially with regard to the minimum effective dose. Overall, longer term follow-up studies are required to determine the exact effects of BMAC treatment on disease progression.

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