

Evaluation of the Consistency and Composition of Commercially Available Bone Marrow Aspirate Concentrate Systems

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Background: Bone marrow aspirate (BMA) concentrate (BMAC) has gained popularity as a treatment modality for various orthopaedic conditions; however, there are still inconsistencies in its reported therapeutic efficacy. This may be because of the many different commercial BMAC preparation systems used clinically, which generate dissimilar concentrate products.

Purpose: To compare 3 commercially available BMAC preparation systems: Harvest SmartPrep 3, Biomet BioCUE, and Arthrex Angel. We evaluated the consistency of each of these systems and compared the composition of their concentrate products.

Study Design: Controlled laboratory study.

Methods: A total of 10 patients donated whole blood and BMA, which were combined and processed with the 3 different BMAC preparation systems. Samples were taken before and after processing for the measurement of white blood cells (WBC), platelets (PLT), CD34+ cells, and colony-forming unit–fibroblast (CFU-F). To evaluate consistency, the variances of cell yield and concentration increase from baseline for each cell type were compared between systems. To compare concentrate product composition, differences between the systems' mean cell yield and concentration increase from baseline for each cell type were evaluated.

Results: The Harvest system (variance, 0.25) concentrated WBC more consistently than the Arthrex system (variance, 3.25) ($P = .024$), but no other differences in consistency were noted between the 3 systems. The Harvest system recovered the greatest percentage of CFU-F ($82.4\% \pm 18.2\%$), CD34+ cells ($81.1\% \pm 28.5\%$), and WBC ($77.3\% \pm 8.6\%$), whereas the Biomet system recovered the greatest percentage of PLTs ($92.9\% \pm 27.3\%$). The Arthrex system concentrated PLT to the greatest degree (11.10 ± 2.05 times baseline), while the Biomet system concentrated WBC to the greatest degree (5.99 ± 1.04 times baseline).

Conclusion: The consistency of the 3 systems was similar for all but 1 of the evaluated cell types. However, the composition of the concentrate products differed across systems. This may grant each system unique advantages without having to sacrifice reproducibility.

Clinical Relevance: Understanding the consistency of different BMAC preparation systems and their product makeup may aid in determining optimal therapeutic doses of different cell types.

Keywords: bone marrow concentration; mesenchymal progenitor cells; autologous bone marrow harvesting; connective tissue healing; bone healing

As medical knowledge and technological innovation have progressed, the health care community has continued to explore the field of regenerative medicine and the many therapeutic interventions that it has to offer. Within the realm of orthopaedics, the therapeutic application of bone marrow aspirate (BMA) has been contemplated ever since it was discovered that this tissue stored cells with chondrogenic and osteogenic capacity.^{6,19,42} It was originally hypothesized by many health scientists that these cells

could be retrieved and employed to replace lost or damaged cartilage and bone in other diseased areas of the body. Several bones in the human body serve as a repository of these mesenchymal progenitor cells, which can be diverted through separate pathways to form adipocytes, chondrocytes, or osteocytes, but the iliac crest continues to be the preferred site of aspiration because of the ease of access, low rate of adverse events, and relatively higher concentration of progenitor cells.^{25,42} Furthermore, studies comparing the chondrogenic and osteogenic potential of bone marrow–derived stem cells versus adipose tissue–derived stem cells have indicated that bone marrow–derived stem cells may be more suitable for chondrogenesis and osteogenesis

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because of their greater synthesis of type 2 collagen, greater proliferative rate, and more native-like columnar arrangement after proliferation.^{1,24}

Within the past 2 decades, BMA has been applied to a variety of disease states, the most common being osteoarthritis of the knee.^{8,14,35,39,40,44} Although a well-defined mechanism of action is yet to be elucidated, many reports indicate significant improvements to physical examination and radiological findings even after long-term follow-up.^{8,35,39,40} Other studies, however, suggest that the results may not be sufficiently positive and that, despite some tissue regeneration, the new hyaline cartilage-like tissue may not fully imitate the properties of native cartilage.^{14,44}

A major obstacle for BMA therapy may be the low quantity of mesenchymal progenitor cells found in the bone marrow, which has been estimated to comprise between 0.001% and 0.02% of nucleated cells.^{2,25,42,43} To combat this issue, several companies have developed devices to concentrate the BMA, producing BMA concentrate (BMAC). Previous studies have found that these devices are capable of increasing the concentration of mesenchymal progenitor cells between 1.6 to nearly 5 times the concentration in bone marrow at baseline.^{4,22} Similarly, these devices have been found to increase the concentration of CD34+ cells, white blood cells (WBC), and platelets (PLT) up to 4.4, 5, and 11 times that of baseline measurements, respectively.^{4,15,23} However, previous studies lack data describing the consistency of these devices.

Currently, there exists an overwhelming consensus from the literature on the need to determine the optimum concentrations of cells for mesenchymal progenitor cell therapy.^{9,14,21,26} Because multiple commercial BMAC devices are available to the practitioner, it is critical to assess the consistency with which various cell types can be retrieved to better determine the effectiveness of BMAC and to quantify optimal progenitor cell therapy. The goal of this study was to compare 3 commercially available BMAC preparation systems and evaluate the consistency of their concentrate products.

METHODS

Sample Procurement

The informed consent form, as well as blood and marrow collection protocols, was approved by the LeukoLab Institutional Review Board (protocol No. 7000-SOP-078). Phlebotomy and bone marrow aspiration were completed at StemExpress (Western Institutional Review Board;

TABLE 1
Donor Demographics

Identification No.	Body Mass		Sex	Ethnicity	Smoker
	Age, y	Index, kg/m ²			
1801	20	26.4	Female	White	No
1802	18	21.1	Male	Hispanic	No
1803	35	27.7	Male	Hispanic	No
1804	46	24.2	Male	White	Yes
1805	29	26.5	Female	White	Yes
1806	23	26.1	Male	White	No
1807	20	27.1	Female	White	No
1808	30	22.3	Female	White	No
1809	28	19.7	Female	White	Yes
1810	27	29.4	Male	White	Yes
Mean	27.6	25.1			

No. 601-01). All donors were healthy and were not selected based on age, sex, or ethnicity. A total of 10 donors (Table 1) underwent venipuncture, for which 100 mL of whole blood was collected in 10% anticoagulant citrate dextrose solution–A (ACD-A), as well as bone marrow aspiration, for which a heparin rinse was performed and 100 mL was collected in 6% ACD-A. To perform bone marrow aspiration, patients were placed in a prone position, the posterior superior iliac spine and sacroiliac joint were palpated, local anesthetic was injected down to the periosteum, and an 11-gauge needle was inserted through the skin and subcutaneous tissues until reaching the posterior iliac crest. The needle was then drilled into the medullary cavity parallel to the iliac crest. Whole blood and BMA from each patient were combined to form a single sample of 200 mL of 8% ACD-A whole blood/BMA mix. A sample from each whole blood/BMA mix was taken to represent the baseline for each patient. All samples were stored at ambient temperature without ice and were shipped overnight for processing the following day.

There was 60 mL drawn from the freshly shaken whole blood/BMA mix to be processed by the Harvest system in accordance with the company's instructions for use (IFU; 4003170/0928). Then, 57 mL was drawn from the whole blood/BMA mix and added to 3 mL of ACD-A, creating a final solution with 13% ACD-A to be processed by the Arthrex system in accordance with the company's IFU (LM1-00003-EN_A). Finally, 54 mL was drawn from the whole blood/BMA mix and added to 6 mL of ACD-A, creating a final solution with 17% ACD-A to be processed by the Biomet system in accordance with the company's IFU (01-50-1465 2010-06).

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Ethical approval for this study was obtained from the New England Independent Review Board (No. 04-144).

Sample Processing

Processing and testing of samples were completed at BioSciences Research Associates. Samples were taken to be processed by 1 of 3 different separation systems: Harvest BMAC-60-07 procedure pack (catalog No. 51417; Harvest/Terumo BCT) with Harvest SmartPrep 3 system (serial No. SMP#-0213; Harvest/Terumo BCT), Arthrex Angel BMC kit (catalog No. ABS-10062; Arthrex) with Angel system (serial No. GB279; Arthrex), and BioCUE platelet concentration system (catalog No. 800-0611A; Biomet) with Drucker Model 755VES centrifuge (serial No. 600206-4; Biomet). Samples were aliquoted in a random sequence during each trial to ensure that there was no chance of one of the systems receiving a benefit of the first or last draw.

Each device was loaded according to the manufacturer's instructions. The Angel system samples were processed with a 25% hematocrit setting. All concentrate product volumes were adjusted to approximately 10 mL by adding back platelet-poor plasma from each respective donor collected by each respective system. Volumes of the concentrate product, added platelet-poor plasma, and final adjusted product were recorded.

Complete blood counts were performed for baseline samples and each of the device's concentrate products with a Coulter AcT diff2 hematology analyzer (serial No. 465208; Beckman Coulter). WBC, erythrocyte, and PLT counts and hematocrit were recorded. Complete blood counts were completed in duplicate. If the duplicates were not within 10% of each other, then a third replicate was performed.

Baseline samples and concentrate products were processed and analyzed for colony-forming unit–fibroblast (CFU-F) according to the following standard operating procedures from BioSciences Research Associates (BSR-TM 054) (N. A. Stephens, personal communication, August 22, 2018). Mesenchymal stem cell basal medium (catalog No. PT-3238; Lonza) was warmed, and mesenchymal stem cell growth supplement (hMSC SingleQuot Kit; catalog No. PT-4105; Lonza) was thawed in a 37°C water bath. Then, 50 mL of mesenchymal stem cell growth supplement (catalog No. PT-4106E; Lonza), 10 mL of L-glutamine (catalog No. PT-4107E; Lonza), and 0.5 mL of GA-1000 (catalog No. PT-4504E; Lonza) were added to 440 mL of mesenchymal stem cell basal medium to make mesenchymal stem cell growth medium (MSCGM). Next, 5 U/mL of heparin (catalog No. 401586D; APP Pharmaceuticals) was added to 500 mL of MSCGM, and the mixture was filtered. A 2-mL cell suspension with a concentration of 2e6 total nucleated cell count (TNC)/mL with MSCGM plus heparin was prepared, and 5 U/mL of heparin was added. Additionally, 3 Falcon T25 tissue culture flasks (catalog No. 353109; Corning) with 9 mL of MSCGM were prepared. Moreover, 1, 0.5, or 0.25 mL of cell suspension was added to their respectively labeled flask and cultured at 37°C and 5% CO₂ for 3 to 4 days. Medium was aspirated, and nonadherent cells were removed before 5 mL of fresh warm MSCGM was added. Flasks were allowed to continue culturing at 37°C and 5% CO₂ until day 10 to 14.

Once sufficient colonies were present, all of the MSCGM was aspirated from the T25 flasks, and each flask was

washed with 4 mL of 1X phosphate buffered saline (catalog No. 17-516F; Lonza). The 1X phosphate buffered saline was aspirated, and the flasks were allowed to dry at room temperature for 4 to 5 minutes. Then, 2 mL of Giemsa stain (catalog No. 3250-16; Ricca Chemical) was added to each flask and allowed to incubate for 3 minutes at room temperature, after which the Giemsa stain was aspirated. Next, 5 mL of distilled water was added to each flask and allowed to incubate for 4 minutes at room temperature. Distilled water was aspirated, and washing with water was repeated to get rid of excess staining. Colonies with more than 50 cells were considered a CFU-F. The total number of CFU-F in each flask was counted, and the mean number of CFU-F per 1e6 TNC for the 3 flasks was reported. The CFU-F measurement was performed to estimate the quantity of mesenchymal progenitor cells present in baseline samples and concentrate products.²⁰ Cytometric analysis for CD34+ cell count was also performed on baseline samples and concentrate products with an Accuri C6 flow cytometer (BSR No. 106; BD Biosciences). CD34+ cell count was performed to estimate the quantity of hematopoietic stem cells (HSC) present in baseline samples and concentrate products.¹¹

Statistical Analysis

Statistical analysis was performed with SPSS (IBM) and RStudio software (RStudio). Yields (ie, percentage of cells recovered) for WBC, PLT, CFU-F, and CD34+ were calculated as the ratio of the cell count in the concentrate product times the volume of the concentrate product to the cell count in the baseline sample times the volume of the sample processed. Concentration increases (ie, increase in cell concentration relative to baseline) for WBC, PLT, CFU-F, and CD34+ were calculated as the ratio of the cell concentration in the concentrate product to the cell concentration in the baseline sample. The baseline sample was the same for all devices, and the volume processed was 60, 57, and 54 mL for the Harvest, Arthrex, and Biomet systems, respectively.

Consistency was defined as a separation system's ability to repeatedly recover the same percentage of a particular cell type or its ability to repeatedly increase the concentration of a particular cell type to the same degree. Stated otherwise, greater consistency was reflected by a smaller variance in cell yields or concentration increases. The Levene test, which examines whether variances from different samples are equal, was performed to evaluate differences in consistency between the 3 separation systems with respect to yield and concentration increase of WBC, PLT, CFU-F, and CD34+. Pairwise comparisons were made using the Bonferroni correction if the Levene test result was significant. Although other methods such as the Bartlett, Hartley, or *F* test could be used to perform a similar analysis, the Levene test was chosen because of its greater robustness, especially with small sample sizes such as the ones used in this study, as well as its ability to handle comparisons between more than 2 groups.

Yields and concentration increases of WBC, PLT, CFU-F, and CD34+ were compared between the 3 separation

TABLE 2
Comparison of the Yield Variance (%²)^a

	CFU-F	CD34+	PLT	WBC
Arthrex (n = 9)	1.44	1.89	1.16	0.90
Biomet (n = 10)	2.70	5.97	7.45	2.60
Harvest (n = 10)	3.32	8.12	2.12	0.75
<i>P</i> value (Levene test)	.500	.520	.281	.083

^aCFU-F, colony-forming unit–fibroblast; PLT, platelets; WBC, white blood cells.

systems using 1-way analysis of variance (ANOVA), with a significance level of .05. The post hoc Tukey honest significant difference test was performed to compare the separation systems pairwise if ANOVA results were significant.

RESULTS

The comparison of yield consistency, concentration increase consistency, mean yield, and mean concentration increase between systems included 9 samples for the Arthrex system and 10 samples for both the Biomet and Harvest systems. The results of 1 concentrate product were discarded because of specimen clotting (CFU: 0.572; CD34+: 0.055; PLT: 14.5; WBC: 10.2).

The results of the yield consistency analysis can be seen in Table 2. The Levene test indicated that there were no significant differences in variance between the 3 systems with regard to the yield of WBC, PLT, CFU-F, or CD34+. Because there were seemingly large differences in some yield variances, it is important to note that the lack of statistical significance may be because of this study's small sample size.

The results of the concentration increase consistency analysis can be seen in Table 3. The Levene test indicated that there were no significant differences in variance between the 3 separation systems with regard to the concentration increase of CFU-F, CD34+, or PLT. However, there was a difference with regard to WBC ($P = .013$). After employing the Bonferroni correction, our analysis indicated that the Harvest system (0.25) had a significantly smaller variance than the Arthrex system (3.25) ($P = .024$) with regard to the concentration increase of WBC. As explained above, the study's sample size may have contributed to a lack of significance between groups with large differences in their concentration increase variance.

The results of the mean yield analysis can be seen in Table 4. Post hoc testing indicated that the Harvest system (82.4 ± 18.2) recovered a greater percentage of CFU-F than both the Arthrex (25.8 ± 12.0) ($P < .001$) and the Biomet systems (47.4 ± 16.4) ($P < .001$) and that the Biomet system recovered a greater percentage of CFU-F than the Arthrex system ($P = .018$). Post hoc testing also showed that the Arthrex system (36.6 ± 13.7) recovered a smaller percentage of CD34+ cells than both the Biomet (71.9 ± 24.4) ($P = .008$) and the Harvest systems (81.1 ± 28.5) ($P = .001$). Conversely, post hoc testing indicated that the Biomet system (92.9 ± 27.3) recovered a greater percentage of

TABLE 3
Comparison of the Concentration Increase Variance (\times Baseline²)^a

	CFU-F	CD34+	PLT	WBC
Arthrex (n = 9)	5.20	6.81	4.18	3.25
Biomet (n = 10)	2.06	4.78	5.31	1.09
Harvest (n = 10)	1.09	2.66	0.74	0.25
<i>P</i> value (Levene test)	.088	.258	.112	.013
With Bonferroni correction				
Arthrex-Biomet	—	—	—	.445
Arthrex-Harvest	—	—	—	.024
Biomet-Harvest	—	—	—	.142

^aDashes indicate no post-hoc testing was performed due to Levene test result > 0.05 . Bold values indicate statistical significance. CFU-F, colony-forming unit–fibroblast; PLT, platelets; WBC, white blood cells.

TABLE 4
Comparison of the Yield (%)^a

	CFU-F	CD34+	PLT	WBC
Arthrex (n = 9)	25.8 \pm 12.0	36.6 \pm 13.7	58.3 \pm 10.8	29.7 \pm 9.5
Biomet (n = 10)	47.4 \pm 16.4	71.9 \pm 24.4	92.9 \pm 27.3	66.4 \pm 16.1
Harvest (n = 10)	82.4 \pm 18.2	81.1 \pm 28.5	66.1 \pm 14.6	77.3 \pm 8.6
<i>P</i> value (ANOVA)	<.001	.001	.001	<.001
With Tukey HSD test				
Arthrex-Biomet	.018	.008	.002	<.001
Arthrex-Harvest	<.001	.001	.649	<.001
Biomet-Harvest	<.001	.658	.012	.123

^aData are shown as mean \pm SD unless otherwise indicated. Bold values indicate statistical significance. ANOVA, analysis of variance; CFU-F, colony-forming unit–fibroblast; HSD, honest significant difference; PLT, platelets; WBC, white blood cells.

PLT than both the Arthrex (58.3 ± 10.8) ($P = .002$) and the Harvest systems (66.1 ± 14.6) ($P = .012$). With regard to WBC, the Arthrex system (29.7 ± 9.5) recovered a smaller percentage than both the Biomet (66.4 ± 16.1) ($P < .001$) and the Harvest systems (77.3 ± 8.6) ($P < .001$).

The results of the mean concentration increase analysis can be seen in Table 5. No significant differences were found between the 3 systems with regard to CFU-F or CD34+ cells. However, post hoc testing indicated that the Arthrex system (11.10 ± 2.05) increased the concentration of PLT more than both the Biomet (8.39 ± 2.30) ($P = .010$) and the Harvest systems (3.85 ± 0.86) ($P < .001$) and that the Biomet system increased the concentration of PLT more than the Harvest system ($P < .001$). Furthermore, post hoc testing showed that the Biomet system (5.99 ± 1.04) increased the concentration of WBC more than the Harvest system (4.49 ± 0.50) ($P = .027$).

DISCUSSION

The primary purpose of this study was to compare the consistency, as well as to evaluate differences in the concentrate products, of 3 commercially available BMAC systems.

TABLE 5
Comparison of the Concentration Increase (\times Baseline)^a

	CFU-F	CD34+	PLT	WBC
Arthrex (n = 9)	4.91 \pm 2.28	6.95 \pm 2.61	11.10 \pm 2.05	5.64 \pm 1.80
Biomet (n = 10)	4.30 \pm 1.43	6.49 \pm 2.19	8.39 \pm 2.30	5.99 \pm 1.04
Harvest (n = 10)	4.79 \pm 1.05	4.71 \pm 1.63	3.85 \pm 0.86	4.49 \pm 0.50
P value (ANOVA)	.689	.073	<.001	.028
With Tukey HSD test				
Arthrex-Biomet	—	—	.010	.806
Arthrex-Harvest	—	—	<.001	.119
Biomet-Harvest	—	—	<.001	.027

^aData are shown as mean \pm SD unless otherwise indicated. Dashes indicate no post-hoc testing was performed due to ANOVA test result $>$ 0.05. Bold values indicate statistical significance. ANOVA, analysis of variance; CFU-F, colony-forming unit-fibroblast; HSD, honest significant difference; PLT, platelets; WBC, white blood cells.

To strengthen the validity of the results, a single-donor model was utilized in which each separation system processed a fraction of the same sample from each donor. This is in contrast to a prior investigation that used different samples for each separation system.²²

Prior studies have indicated varying effectiveness of BMAC therapies, and many have argued for the need to determine the optimum amount and type of cell required for best clinical therapy.^{9,14,21,26} To begin such determinations, it is vital for clinicians to make use of BMAC systems that will yield desirable concentrate products in a consistent manner. In general, our results indicate that, of the 3 systems that we assessed, differences in consistency were limited to 1 cell type between 2 of the systems. However, the concentrate products differed significantly in their composition across systems, revealing that each system holds particular advantages for the recovery and concentration of particular cell types.

An evaluation of each system's consistency was felt to be of substantial importance, as, to our knowledge, there is no currently available literature on the topic. Furthermore, it can indicate the reproducibility of a particular separation system's concentrate product with regard to repeatedly recovering the same percentage of a particular cell type or repeatedly increasing the concentration of a particular cell type to the same degree. To evaluate any differences in consistency, our study compared the variances of yield and concentration increase across the 3 separation systems. Our results indicate that although different systems may recover a different percentage of each cell type, they each perform this recovery with similar consistency. Similarly, while different systems increased the concentration of some cell types differently, they performed this concentration increase with similar consistency for 3 of the 4 cell types evaluated. In fact, only the Arthrex and Harvest systems showed any difference in consistency, which was limited to the concentration increase of WBC. These findings argue for the similar reproducibility of the 3 separation systems.

To identify any potential advantages of the different separation systems, we compared their ability to recover and concentrate 4 cell types with different biological

function and clinical relevance. Mesenchymal progenitor cells, which can differentiate through adipocytic, chondrocytic, and osteocytic lineages, have been isolated from human bone marrow.^{6,42} Moreover, cells with these properties have been discovered specifically in CFU-F and adherent and nonphagocytic cells, which replicate quickly and produce many of the proteins found in the bone matrix such as collagen type 1 and collagen type 3.^{7,12,20} Although CFU-F may contain a heterogeneous set of cells, they are used as a marker for mesenchymal progenitor cells within BMAC products, as they are believed to be positive predictors for therapy.²⁸ Furthermore, early studies have demonstrated host and exogenously administered mesenchymal progenitor cells' presence within acutely injured tissues, suggesting that they may play a role in the tissue repair process.²⁹ Clinically, they have shown promise as a treatment for a variety of diseases, especially osteoarthritis of the knee.^{8,31,35,39,40} For these reasons, an ability to recover and concentrate mesenchymal progenitor cells efficiently and consistently is desirable to clinicians and researchers. Our study indicates that the Harvest system recovers a greater percentage of the total CFU-F population than the other 2 systems but that there is no difference in concentrating ability between any of the systems. Because the optimal amount and frequency of BMAC therapy have not yet been determined, understanding the differences in each system's capabilities may lead to future optimization.¹⁰

Despite only comprising about 1.6% of bone marrow, CD34+ cells are critical to human health because of their role in hematopoiesis, as they have been shown to be markers of HSC.^{11,30,33} Increasing the quantity of CD34+ cells in BMAC products may be clinically useful, as it may provide the patient with a greater capacity to generate a host of myeloid and lymphoid lineage blood cells. Surprisingly, HSC have also been shown to display plastic behavior and an ability to change their differentiation pattern depending on their environment. This has been used to explore their possible application as a therapeutic approach for demyelinating and motor neuron diseases, which have shown some early promising results.³⁸ Other diseases for which CD34+ cell treatment has demonstrated encouraging results include Crohn disease and type 1 diabetes mellitus.^{13,17} Our results suggest that the Biomet and Harvest systems may be preferable to the Arthrex system if a greater absolute number of CD34+ cells is desired but that none of the systems is definitively preferable if the objective is to use the greatest concentration of CD34+ cells.

The hemostatic and wound-healing properties of PLT have long been observed and used in medical therapy.⁵ Furthermore, PLT have recently been observed to produce factors that aid in mesenchymal progenitor cell proliferation and regulate vascular growth and degeneration.^{27,34} These factors, which include fibroblast growth factor, platelet-derived growth factor- α and - β , vascular endothelial growth factor, and insulin-like growth factor-1 and -2, among others, may contribute to the beneficial effects observed when platelet-rich plasma is employed as a therapy for some musculoskeletal diseases, including but not limited to articular cartilage damage, osteoarthritis, and

rotator cuff tears.¹⁶ Despite encouraging results, the exact mechanism of action, as well as the optimal therapeutic quantity, of PLT continues to be uncertain.^{16,18} For this reason, knowledge of the amount of PLT given in BMAC therapies, based on the separation system used, can prove to be of substantial benefit. Our study suggests that the Biomet system is preferable when a greater absolute quantity of PLT is desired but that the Arthrex system should be employed if a greater concentration of PLT is necessary. Interestingly, our results also demonstrate that a system's ability to more effectively recover a particular cell type may not guarantee it the ability to more effectively increase the concentration of that same cell type and vice versa.

The role of WBC in a variety of orthopaedic therapeutic settings has been explored, and it has been suggested that their presence within concentrate products leads to the increased expression of inflammatory cytokines, resulting in inferior outcomes when compared with leukocyte-poor preparations.^{36,45,46} In particular, researchers have noted an increased presence of interleukin-1 β and tumor necrosis factor- α and an increased activation of the NF- κ B pathway.⁴⁶ Yet, others argue for the need for WBC to adequately elicit the body's natural healing responses. Specifically, it has been noted that the presence of neutrophils is necessary for the production of leukotrienes, which are then converted to lipoxin by PLT and serve a powerful anti-inflammatory role.^{32,41} Additionally, the role of monocytes has been shown to modulate between a proinflammatory one in the presence of active infections and injuries to an anti-inflammatory one that enhances the body's reparative processes when the initial threat has been dealt with.^{3,32,37} Because of these opposing views and the potential for advantages or deleterious effects, the quantification of WBC in a BMAC product is of great clinical relevance. Our study indicates that the Arthrex system allows for the greatest removal of WBC during processing and that the Biomet system contains a greater concentration of WBC than the Harvest system. Employment of these findings should be kept in mind when selecting between the different systems, especially if enhancing or limiting inflammation at the therapeutic site is of importance.

It cannot be overstated that this study does not suggest the definitive superiority of one technology over another (because any such claim would lack supporting evidence) but rather highlights the differing capabilities of 3 commercially available bone marrow separation systems. In fact, the scarcity of clinical evidence in this field limits our ability to comment on whether a greater yield or concentration increase of certain blood constituents, as well as the consistency with which this is obtained, is of clinical significance. Although all 3 systems rely on a similar basic process of centrifugation for the separation of BMA and whole blood components, each centrifuge and collection system is engineered differently, producing different separation results between the systems. It is imperative that researchers and clinicians consider all of these differing attributes when deciding which system to adopt for each of their varying objectives. Increased reporting by researchers and clinicians on the application and efficacy of each of these technologies is necessary if optimal therapeutic protocols are to

be established in the future. Without additional evidence of the efficacy of these varying concentrations of BMAC components, health care providers who utilize orthobiologics will be unable to fully and adequately appreciate the benefits and limitations of these BMAC separation systems.

Several limitations exist in our study, which could have implications for the interpretation of the results. The relatively small sample size of our study, which was largely because of financial constraints, increases the possibility of a type II error. Additionally, only 9 concentrate products from the Arthrex system were included in our statistical analysis of consistency and concentrate product composition. The Levene test, which was chosen for its greater robustness and ability to handle comparisons between multiple groups, is not without faults of its own. For example, this test performs best when comparing independent groups, which was not the case for our study because samples from the same donors were processed by the 3 different separation systems. It is possible that using an alternative statistical test to compare variances between groups would have resulted in more or less significant differences between the 3 systems. Furthermore, our analysis did not include erythrocyte, growth factor, or cytokine evaluations, which may be of substantial clinical relevance in BMAC therapy. Finally, this study is unable to propose or support a range of ideal concentrations for each component of BMAC therapy, as optimal therapeutic values have not yet been described with conclusive evidence by prior studies. However, our study did include a single-donor model, which allowed for better direct comparison between separation systems. The analysis also required duplicate measurements for the baseline and concentrate products, lending greater validity to our findings. Most importantly, our evaluation includes an analysis of the consistency of each system, which, to our knowledge, is currently absent in the available literature.

CONCLUSION

With regard to consistency, only the concentration increase of WBC of the Arthrex system was less consistent than that of the Harvest system. All other comparisons of consistency between the 3 systems failed to demonstrate any significant differences. Our study revealed that the Biomet system recovered a greater percentage of CFU-F, CD34+ cells, PLT, and WBC compared with the Arthrex system but that the Arthrex system concentrated PLT to a greater degree. Additionally, the Harvest system recovered a greater percentage of CFU-F, CD34+, and WBC than the Arthrex system, but once again, the Arthrex system returned a concentrate product with a greater concentration of PLT. When comparing the Harvest system with the Biomet system, the former achieved a greater recovery of CFU-F, while the latter achieved a greater recovery and concentration of PLT and a greater concentration of WBC. Further exploration of the optimal BMAC formulation as a therapeutic option for a variety of diseases is necessary to continue to improve patient outcomes. Such investigations

should utilize these results to help validate and strengthen their findings.

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REFERENCES

- Afizah H, Yang Z, Hui JHP, Ouyang HW, Lee EH. A comparison between the chondrogenic potential of human bone marrow stem cells (BMSCs) and adipose-derived stem cells (ADSCs) taken from the same donors. *Tissue Eng.* 2007;13:659-666.
- Alvarez-Viejo M, Menendez-Menendez Y, Blanco-Gelaz MA, et al. Quantifying mesenchymal stem cells in the mononuclear cell fraction of bone marrow samples obtained for cell therapy. *Transplant Proc.* 2013;45:434-439.
- Arnold L, Henry A, Poron F, et al. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J Exp Med.* 2007;204(5):1057-1069.
- Betsch M, Schnependahl J, Thuns S, et al. Bone marrow aspiration concentrate and platelet rich plasma for osteochondral repair in a porcine osteochondral defect model. *PLoS One.* 2013;8(8):e71602.
- Schultze Brewer DB. Max (1865), G. Bizzozero (1882) and the discovery of the platelet. *Br J Haematol.* 2006;133:251-258.
- Caplan AI. Mesenchymal stem cells. *J Orthop Res.* 1991;9:641-650.
- Castro-Malaspina H, Gay RE, Resnick G, et al. Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. *Blood.* 1980;56:289-301.
- Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M, Karli D. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. *Pain Physician.* 2008;11:343-353.
- Chahla J, Dean CS, Moatshe G, Pascual-Garrido C, Cruz RS, LaPrade RF. Concentrated bone marrow aspirate for the treatment of chondral injuries and osteoarthritis of the knee: a systematic review of outcomes. *Orthop J Sports Med.* 2016;4(1):2325967115625481.
- Chahla J, Mannava S, Cinque ME, Geeslin AG, Codina D, LaPrade RF. Bone marrow aspirate concentrate harvesting and processing technique. *Arthrosc Tech.* 2017;6(2):e441-e445.
- Civin CI, Strauss LC, Brovall C, Fackler MJ, Schwartz JF, Shaper JH. Antigenic analysis of hematopoiesis, III: a hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. *J Immunol.* 1984;133:157-165.
- Colter DC, Sekiya I, Prockop DJ. Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells. *Proc Natl Acad Sci U S A.* 2001;98:7841-7845.
- Dalal J, Gandy K, Domen J. Role of mesenchymal stem cell therapy in Crohn's disease. *Pediatr Res.* 2012;71(4):445-451.
- Davatchi F, Sadeghi Abdollahi B, Mohyeddin M, Shahram F, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis: preliminary report of four patients. *Int J Rheum Dis.* 2011;14:211-215.
- Degen RM, Bernard JA, Oliver KS, Dines JS. Commercial separation systems designed for preparation of platelet-rich plasma yield differences in cellular composition. *HSS J.* 2017;13:75-80.
- Dhillon RS, Schwarz EM, Maloney MD. Platelet-rich plasma therapy: future or trend? *Arthritis Res Ther.* 2012;14:219.
- El-Badawy A, El-Badri N. Clinical efficacy of stem cell therapy for diabetes mellitus: a meta-analysis. *PLoS One.* 2016;11(4):e0151938.
- Filardo G, Kon E, Roffi A, Di Matteo B, Merli ML, Marcacci M. Platelet-rich plasma: why intra-articular? A systematic review of preclinical studies and clinical evidence on PRP for joint degeneration. *Knee Surg Sports Traumatol Arthrosc.* 2015;23(9):2459-2474.
- Friedenstein AJ, Chailakhyan RK, Gerasimov UV. Bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffuse chambers. *Cell Tissue Kinet.* 1987;20:263-272.
- Friedenstein AJ, Deriglasova UF, Kulagina NN, et al. Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. *Exp Hematol.* 1974;2(2):83-92.
- Gianakos AL, Sun L, Patel JN, Adams DM, Liporace FA. Clinical application of concentrated bone marrow aspirate in orthopaedics: a systematic review. *World J Orthop.* 2017;8:491-506.
- Hegde V, Shonuga O, Ellis S, et al. A prospective comparison of 3 approved systems for autologous bone marrow concentration demonstrated nonequivalency in progenitor cell number and concentration. *J Orthop Trauma.* 2014;28(10):591-598.
- Hermann PC, Huber SL, Herrler T, et al. Concentration of bone marrow total nucleated cells by a point-of-care device provides a high yield and preserves their functional activity. *Cell Transplant.* 2008;16:1059-1069.
- Hui JHP, Li L, Teo YH, Ouyang HW, Lee EH. Comparative study of the ability of mesenchymal stem cells derived from bone marrow, periosteum, and adipose tissue in treatment of partial growth arrest in rabbit. *Tissue Eng.* 2005;11:904-912.
- Hyer CF, Berlet GC, Bussewitz BW, Hankins T, Ziegler HL, Philbin TM. Quantitative assessment of the yield of osteoblastic connective tissue progenitors in bone marrow aspirate from the iliac crest, tibia, and calcaneus. *J Bone Joint Surg Am.* 2013;95:1312-1316.
- Imam MA, Holton J, Ernstbrunner L, et al. A systematic review of the clinical applications and complications of bone marrow aspirate concentrate in management of bone defects and nonunions. *Int Orthop.* 2017;41:2213-2220.
- Italiano JE Jr, Richardson JL, Patel-Hett S, et al. Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha-granules and differentially released. *Blood.* 2008;111:1227-1233.
- Jäger M, Hernigou P, Zilkens C, et al. Cell therapy in bone healing disorders. *Orthop Rev (Pavia).* 2010;2:e20.
- Karp JM, Teo GSL. Mesenchymal stem cell homing: the devil is in the details. *Cell Stem Cell.* 2009;4(3):206-216.
- Krause DS, Fackler MJ, Civin CI, May WS. CD34: structure, biology, and clinical utility. *Blood.* 1996;87:1-13.
- Lamo-Espinosa JM, Mora G, Blanco JF, et al. Intra-articular injection of two different doses of autologous bone marrow mesenchymal stem cells versus hyaluronic acid in the treatment of knee osteoarthritis: multicenter randomized controlled clinical trial (phase I/II). *J Transl Med.* 2016;14:246.
- Lana JF, Macedo A, Ingrao ILG, Huber SC, Santos GS, Santana MHA. Leukocyte-rich PRP for knee osteoarthritis: current concepts. *J Clin Orthop Trauma.* 2019;10(suppl 1):S179-S182.
- Loken MR, Shah VO, Dattilio KL, Civin CI. Flow cytometric analysis of human bone marrow, II: normal B lymphocyte development. *Blood.* 1987;70:1316-1324.
- Lucarelli E, Beccheroni A, Donati D, et al. Platelet-derived growth factors enhance proliferation of human stromal stem cells. *Biomaterials.* 2003;24:3095-3100.
- Matsumoto T, Okabe T, Ikawa T, et al. Articular cartilage repair with autologous bone marrow mesenchymal cells. *J Cell Physiol.* 2010;225:291-295.
- McCarrel TM, Minas T, Fortier LA. Optimization of leukocyte concentration in platelet-rich plasma for the treatment of tendinopathy. *J Bone Joint Surg Am.* 2012;94:e143.
- Minutti CM, Knipper JA, Allen JE, Zaiss DMW. Tissue-specific contribution of macrophages to wound healing. *Semin Cell Dev Biol.* 2017;61:3-11.
- Moraleda JM, Blanquer M, Bleda P, et al. Adult stem cell therapy: dream or reality? *Transpl Immunol.* 2006;17:74-77.
- Orozco L, Munar A, Soler R, et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study. *Transplantation.* 2013;95:1535-1541.

40. Orozco L, Munar A, Soler R, et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: two-year follow-up results. *Transplantation*. 2014;97:e66-e68.
41. Parrish WR, Roides B. Physiology of blood components in wound healing: an appreciation of cellular co-operativity in platelet rich plasma action. *J Exerc Sports Orthop*. 2017;4(2):1-14.
42. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;284:143-147.
43. Veyrat-Masson R, Boiret-Dupré N, Rapatel C, et al. Mesenchymal content of fresh bone marrow: a proposed quality control method for cell therapy. *Br J Haematol*. 2007;139:312-320.
44. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage*. 2002;10:199-206.
45. Wang SZ, Fan WM, Jia J, Ma LY, Yu JB, Wang C. Is exclusion of leukocytes from platelet-rich plasma (PRP) a better choice for early intervertebral disc regeneration? *Stem Cell Res Ther*. 2018; 9:199.
46. Xu Z, Yin W, Zhang Y, et al. Comparative evaluation of leukocyte- and platelet-rich plasma and pure platelet-rich plasma for cartilage regeneration. *Sci Rep*. 2017;7:43301.