

High Variability of Mesenchymal Stem Cells Obtained via Bone Marrow Aspirate Concentrate Compared With Traditional Bone Marrow Aspiration Technique

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Background: Bone marrow aspirate (BMA) is a common source for harvesting mesenchymal stem cells (MSCs), other progenitor cells, and associated cytokines and growth factors to be used in the biologic treatment of various orthopaedic pathologies. The aspirate is commonly centrifuged into a concentrated volume that can be immediately administered to a patient using commercially available kits. However, the handling and efficacy of BMA concentrate (BMAC) are still controversial.

Purpose: To characterize BMA versus BMAC for MSC quantity, potency, and cytokine profile.

Study Design: Controlled laboratory study.

Methods: From 8 participants (age, 17-68 years), 30 mL of bone marrow was aspirated by a single surgeon from either the proximal humerus or distal femur and was separated into 2 equal samples. One sample was kept as BMA, and the other half was centrifuged into BMAC. The 2 samples then underwent flow cytometry for detection of MSCs, cell analysis for colony-forming units (CFUs), and cytokine profiling. A 2-tailed *t* test was used to detect differences between MSCs, CFUs, and cytokine density concentrations between BMA and BMAC.

Results: The average concentration of MSCs in both BMA and BMAC was 0.001%. Average MSC events detected by flow cytometry were significantly higher in BMA versus BMAC (15.1 and 8.1, respectively; $P < .045$). Expanded MSCs demonstrated similar phenotypes, but CFUs were significantly increased in BMA compared with BMAC (104 vs 68 CFUs, respectively; $P < .001$). Total protein concentration and cytokine profiling demonstrated great variability between BMA and BMAC and between patients. Most importantly, BMAC failed to concentrate MSCs in 6 of 8 samples.

Conclusion: There is great variability in MSC concentration, total protein concentration, and cytokine profile between BMA and BMAC.

Clinical Relevance: When studying the clinical efficacy of BMAC, one must also evaluate the sample itself to determine the presence, concentration, and potency of MSCs if this is to be considered a cell-based therapy. Further standard operating procedures need to be investigated to ensure reproducible results and appropriate treatments.

Keywords: BMAC; BMA; MSC; bone marrow

Recently, mesenchymal stem cells (MSCs) have been investigated as a primary treatment for, as well as augmentation to, numerous orthopaedic procedures. MSCs have the ability to differentiate into multiple musculoskeletal cell types, release cytokines and growth factors, and mitigate the immune response.⁵ As such, stem cell therapy is gaining popularity for difficult to treat orthopaedic

pathologies such as osteoarthritis, muscle and tendon injuries, and cartilage restoration.³⁴

One commonly described method of obtaining MSCs is from bone marrow aspiration.³ Bone marrow aspirate (BMA) not only contains MSCs but also includes platelets, red and white blood cells, and hematopoietic and nonhematopoietic precursors.⁵ It is believed that by centrifuging the aspirate, differences in density gradients allow for a concentration of MSCs in an appropriate injectable volume that can be immediately administered to a patient.⁵ Other bioactive cells including lymphocytes, neutrophils, monocytes, and platelets, along with growth factors including

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platelet-derived growth factor (PDGF), transforming growth factor- β (TGF- β), and bone morphogenic proteins, are also concentrated during this process.⁹ This process has been termed “bone marrow aspirate concentrate” (BMAC), and many companies have developed BMAC kits that meet U.S. Food and Drug Administration regulations.^{3,17,22}

Despite the interest in use of orthopaedic procedures, the use of either BMA or BMAC remains poorly understood, as do the harvesting and processing techniques used to obtain them. Previous studies have suggested that MSCs only represent about 0.001% to 0.01% of the cells in bone marrow concentrate,²⁷ making it unknown if processes such as BMAC provide enough viable MSCs to have clinical effectiveness. Moreover, often the use of BMA or BMAC in orthopaedic procedures has been improperly defined or advertised as stem cell therapy to render it more appealing.^{12,13,18,19,25,28,31}

The purpose of this study was to compare the concentration of MSCs, their potency, and the cytokine profile of BMA versus BMAC in a group of patients undergoing surgery. We hypothesized that MSCs obtained from BMA will have superior or equivalent quality and quantity compared with BMAC.

METHODS

This study took place from June 2017 through January 2018. A total of 8 consecutive patients between 17 and 68 years of age consented to participate in the study. All participants were under the care of a single surgeon (P.M.). Patients were included if they underwent surgery for anterior cruciate ligament (ACL) tear or rotator cuff surgery during the study period. Exclusion criteria were history of diabetes, blood disorders, hematological malignancy, use of immunosuppressive medications, or medications with bone marrow-suppressive effects. The study protocol received institutional review board approval, and all participants provided informed consent or informed assent for minors.

Bone Marrow Aspiration and Concentration

Using sterile techniques during the anticipated procedure, 30 mL of bone marrow was aspirated from either the

proximal humerus (n = 6) or distal femur (n = 2) using the manufacturer's described technique (BioCUE MINI Platelet Concentration Kit; Biomet Biologics). The aspiration trocar utilized was fenestrated, seated to the first laser line. The location used was based on convenience of the planned procedure. The bone marrow was aspirated from the greater tuberosity for cuff surgery, specifically at the site of anticipated anchor placement.²⁴ For ACL surgery, the BMA was aspirated from roof of the notch.^{8,20} The aspirate was collected into a 30-mL syringe containing 4 mL of anti-coagulant citrate dextrose-solution A (ACD-A) to prevent coagulation. The needle was advanced 1 cm and rotated 90° after each 7 mL was aspirated. Half of the volume was retained as the BMA sample for the study to be used as an internal control. The remainder was centrifuged at 3200 rpm for 15 minutes using the BioCUE MINI Platelet Concentration Kit, to be used as BMAC. A total of 3.5 mL of each BMA and BMAC was washed with phosphate-buffered saline and resuspended in alpha modified Eagle's medium with 16% fetal bovine serum and seeded in a T25 flask. The media was changed every other day until passage 10. Eight patient samples were cultured; each sample had a BMA and BMAC sample.

Flow Cytometry

We analyzed 100 μ L of BMA and BMAC with flow cytometry for MSCs markers, as defined by the International Society for Cellular Therapy.³⁷ This included positive (cluster differentiation [CD] 90, CD73, and CD105) and negative (human leukocyte antigen-DR isotype [HLA-DR], CD45, CD11b, CD19, and CD34) cocktail markers. Conjugated primary monoclonal antibodies and isotype controls were used as recommended by the manufacturer (BD Biosciences). Cells were analyzed on a FACS Fortessa flow cytometer (BD Biosciences) and analyzed using FCS Express (Denovo Software).

Cell Analysis

An equal number of cells from BMA and BMAC were used for colony-forming unit (CFU) assays. Four different seeding densities were chosen: 50, 100, 200, and 500 cells/cm². Various seeding densities were used to determine if CFUs were affected by initial plating density.³⁵ Samples of BMA

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Ethical approval for this study was obtained from Houston Methodist Research Institute (Pro00015718).

TABLE 1
Patient Characteristics^a

Patient	Surgery Date, Month/Day/Year	Location of Aspiration	Age, y	Sex	Race	BMI, kg/m ²
1	6/14/2017	Distal femur	17	Female	White	20.7
2	6/14/2017	Distal femur	41	Male	White	30.9
3	6/28/2017	Proximal humerus	52	Male	White	29.9
4	6/28/2017	Proximal humerus	55	Male	White	33.3
5	7/5/2017	Proximal humerus	48	Male	Declined to answer	25.8
6	7/5/2017	Proximal humerus	68	Male	White	31.7
7	9/20/2017	Proximal humerus	63	Female	White	21.6
8	1/31/2018	Proximal humerus	66	Male	White	21.8
Mean ± SD	—	—	51.3 ± 16.6	—	—	27.0 ± 5.1

^aBMI, body mass index.

and BMAC were cultured in triplicate using sterile media and standard laboratory techniques. After 2 weeks, CFUs were circled with a 1.8 cm-diameter self-inking marker. A colony was counted if it was ≥ 1 mm in diameter.^{23,26} Doubling time was calculated for cells derived from BMA and BMAC until passage 10.

Protein Profiling

To test any paracrine or downstream growth factor upregulation, protein quantification of the BMA and BMAC was investigated on all the samples using Bio-Rad protein assay (Bio-Rad Laboratories). After quantification, equal amounts of BMA and BMAC were loaded onto the Proteome Profiler Human XL Cytokine Array (R&D Systems) to analyze the presence of 102 different cytokines.

Statistical Analyses

Statistical analysis was performed utilizing a 2-tailed *t* test to detect differences between MSCs, CFUs, and cytokine density concentrations between BMA and BMAC. An alpha of 0.05 was set as statistically significant. SPSS Statistics (version 21; IBM Corp) was used for statistical processing.

RESULTS

The average age of patients was 51.25 ± 16.6 years (range, 17-68 years), with an average BMI of 26.96 ± 5.1 kg/m² (range, 20.7-33.3 kg/m²) (Table 1). There were 6 male and 2 female patients. Per the exclusion criteria, none of the included patients had a history of diabetes, blood disorders, or hematological malignancy, and none used immunosuppressive drugs or drugs with bone marrow-suppressive effects.

Flow cytometry demonstrated the heterogeneity of the analyzed samples. In Figure 1, there is representative scatterplot that highlights the difference in cell populations between BMA and BMAC samples. The quantification of MSCs as detected by flow cytometry for the first 1 million events in each 100- μ L sample was significantly higher in BMA versus BMAC samples (Figure 2). The overall concentration of MSCs in BMAC was 0.001%. There was a statistically higher number of stem cells isolated in the

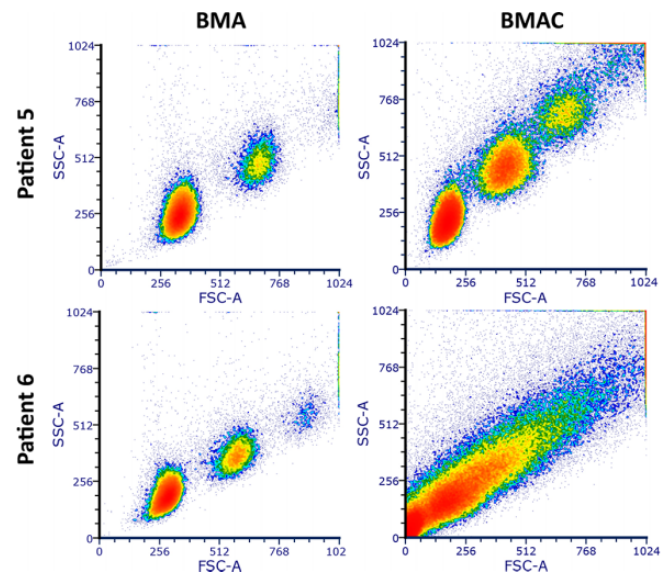


Figure 1. Representative side scatterplot of bone marrow aspirate (BMA) versus BMA concentrate (BMAC) samples. Red, highest cell density; blue, smallest cell density. Flow cytometry was used to identify the different cell populations among BMA versus BMAC samples. Patient 5 is displayed in the top row, and patient 6 is displayed in the bottom row. For patient 5, there are 2 distinct populations in the BMA sample, while 3 populations of cells are apparent in the BMAC. Conversely, for patient 6, there are 3 distinct populations in the BMA sample and a more heterogeneous distribution in the BMAC sample. FSC-A, forward scatter; SSC-A, side scatter.

aspiration compared with the concentrate (15.1 vs 8.1, respectively; $P = .045$). We were unable to concentrate MSCs utilizing the BMAC methodology in 6 of 8 samples, and in samples 4 and 5 we were unable to detect any MSC events in the concentrate.

Despite the difference in concentration, when MSCs were grown and expanded, the phenotypes were similar between BMA and BMAC with the typical spindle-like shape (Figure 3). Doubling times were similar until passage 4, after which they diverged (Figure 4B). However, there was no statistical significance between doubling time in BMA

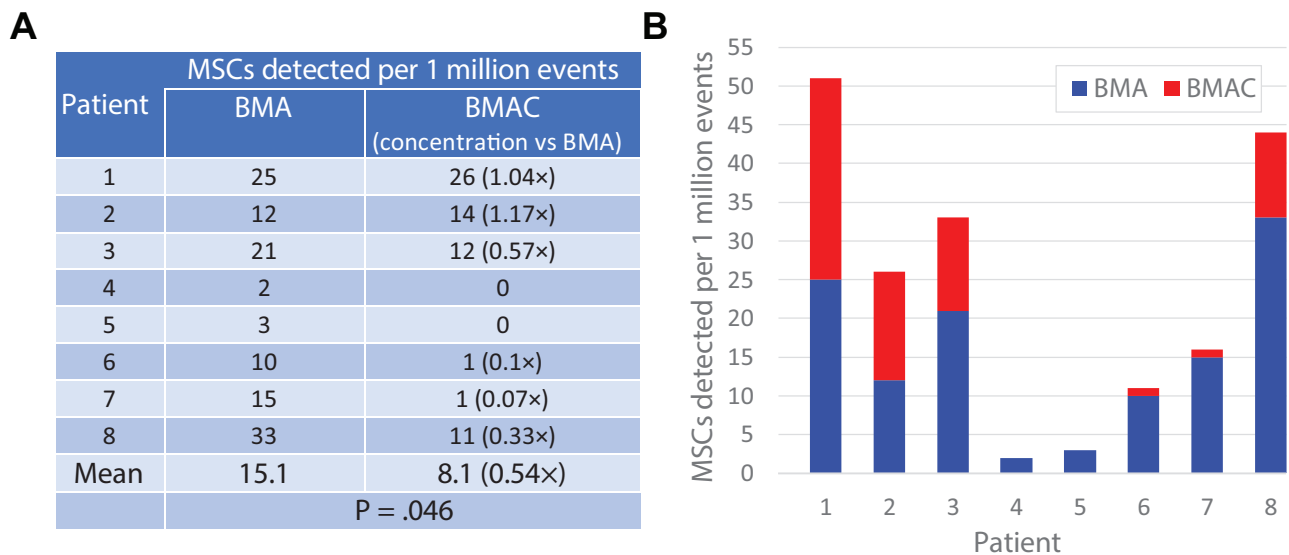


Figure 2. (A) Table and (B) bar graph depicting the number of mesenchymal stem cells (MSCs) detected by flow cytometry. The overall concentration of MSCs in bone marrow aspirate concentrate (BMAC) was 0.001%. There was a statistically higher number of stem cells isolated in the aspiration compared with the concentrate (15.1 vs 8.1, respectively; $P = .045$). (B) Quantification of MSCs as detected by flow cytometry for the first 1 million events was significantly higher in bone marrow aspirate (BMA) versus BMAC samples.

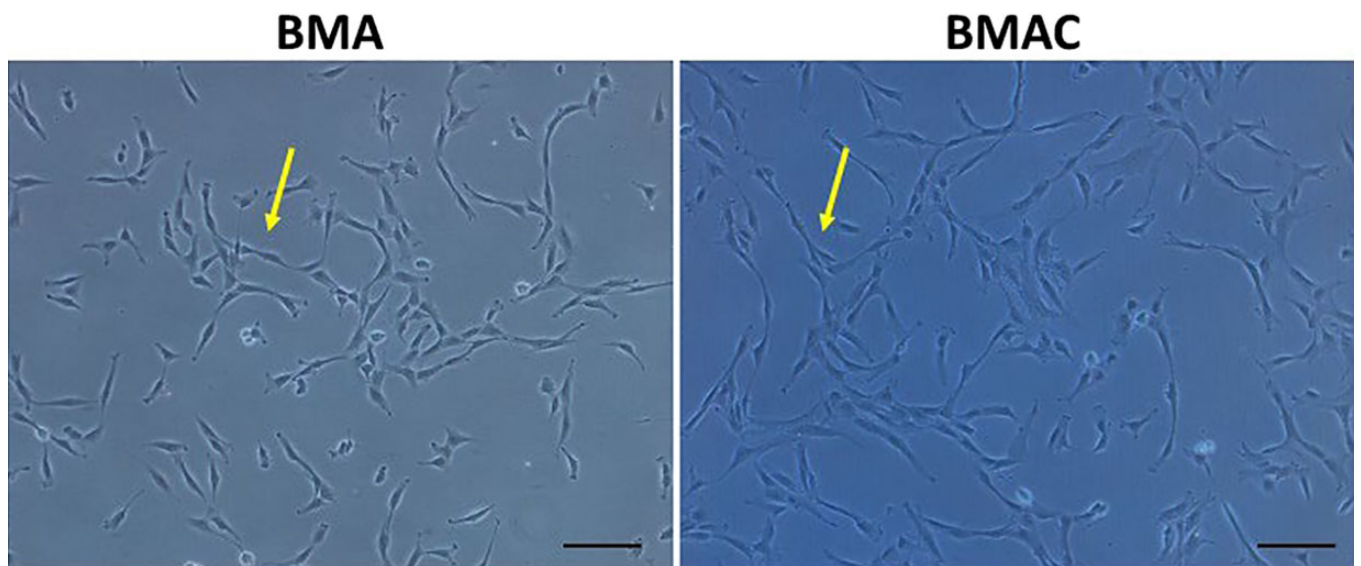


Figure 3. Morphology of mesenchymal stem cells (MSCs) using an inverted microscope. Phenotypes of expanded MSCs are similar between bone marrow aspirate (BMA) and BMA concentrate (BMAC). Both BMA and BMAC yielded MSCs in the typical spindle-like shape (arrows). Scale bar = 10 μ M.

and BMAC when the graphs did diverge ($P = .1274$). MSCs cultured with initial seeding densities of 100 and 500 cells/ cm^2 demonstrated significantly more colony-forming units (CFUs) after 2 weeks in BMA as compared with BMAC ($P < .05$) (Figure 4A).

The total protein concentration for each sample is quantified in Figure 5A. There is no correlation or clear pattern

of protein content between BMA and BMAC or between patients. Half of the patients demonstrated a higher protein concentration in BMAC compared with BMA (patients 2, 4, 6, and 8). We used the Proteome Profiler Human XL Cytokine Array to characterize the protein profile of patients 7 and 8 as a proof of concept (Figure 5B). For patient 7, we found that the protein content in BMAC was

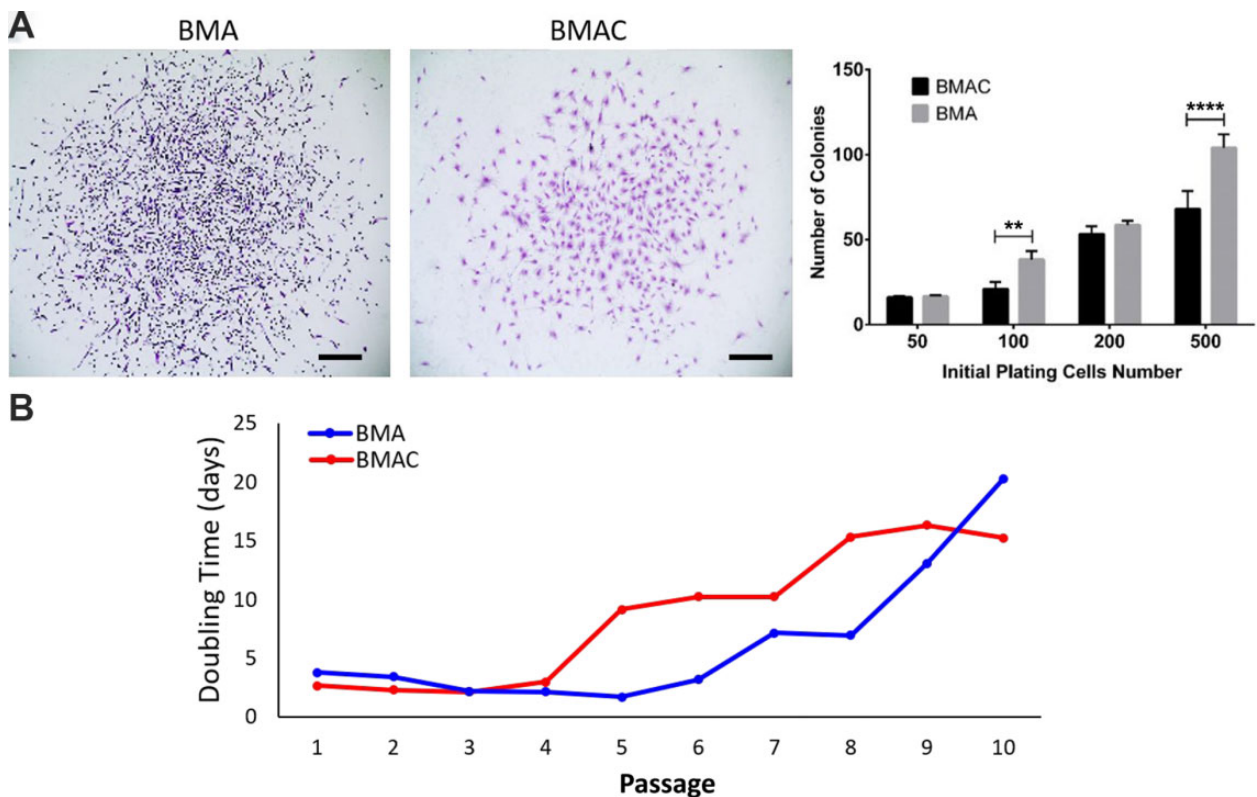


Figure 4. (A) Phenotype of bone marrow aspirate (BMA) versus BMA concentrate (BMAC) mesenchymal stem cells (MSCs) and number of colonies with different initial plating number, MSCs cultured with initial seeding densities of 100 and 500 cells/cm² demonstrated significantly more colony-forming units after 2 weeks in BMA as compared with BMAC (** $P < .05$, **** $P < .001$). Scale bar = 10 μ M. (B) Mean doubling time of BMAC versus BMA from passages 1 to 10. Doubling times were similar until passage 4, after which they diverged ($P = .1274$).

206.05 mg/mL versus 118.0 mg/mL in BMA. Despite this difference, and the considerable amount of protein present, only 5 of 102 screened cytokines were identified using the Proteome Profiler Human XL Cytokine Array: adiponectin, aggrecan, angiogenin, vitamin D binding protein, and tumor necrosis factor- α (TNF- α) (Figure 6A). Each cytokine demonstrated a significantly different concentration between BMA and BMAC. Furthermore, the presence of TNF- α , an inflammatory cytokine, was 50 times higher in BMAC than BMA.

Patient 8 demonstrated a very different profile. Twenty-three cytokines were identified in both BMA and BMAC (Figure 6B). While adiponectin was significantly higher in concentration in the BMAC of patient 7, it was significantly higher in the BMA of patient 8. Similarly, angiogenin was also significantly higher in the BMAC of patient 7, but higher in the BMA of patient 8. Other regenerative molecules, such as PF4, PFGF-AA, and osteopontin, were significantly higher in concentration in BMA in comparison with BMAC of patient 8.

DISCUSSION

In this study, we found that there is great variability in MSC concentration, total protein concentration, and

cytokine profile between BMA and BMAC techniques. Average MSC events detected by flow cytometry were significantly higher in BMA versus BMAC (15.1 vs 8.1, $P < .045$), and CFUs were significantly increased in BMA compared with BMAC (104 vs 68, respectively; $P < .001$). Additionally, total protein concentration and cytokine profiling demonstrated great variability not only between BMA and BMAC but also between patients. We found there was no correlation or clear pattern of protein content between BMA and BMAC or between patients.

It is well known that human BMA contains MSCs. However, the number of MSCs is very low per unit volume; MSCs make up only around 0.01% to 0.02% of the total cell volume.^{1,14} Because of the small number of MSCs per unit volume of aspiration, BMAC has been considered as a method to increase the concentration of MSCs in a short time, directly in the operating room.²⁸⁻³² BMAC involves using a density gradient centrifuge in order to concentrate MSCs, and many kits are available.^{18,28} However, there are conflicting findings regarding the superiority of BMAC over traditional BMA. Some studies^{1,14,30} have found that BMAC is an effective way to concentrate MSCs. Other studies, similar to ours, have found that BMAC does not result in a higher number of MSCs.^{29,30,31} In addition, our study, although limited with its small sample number,

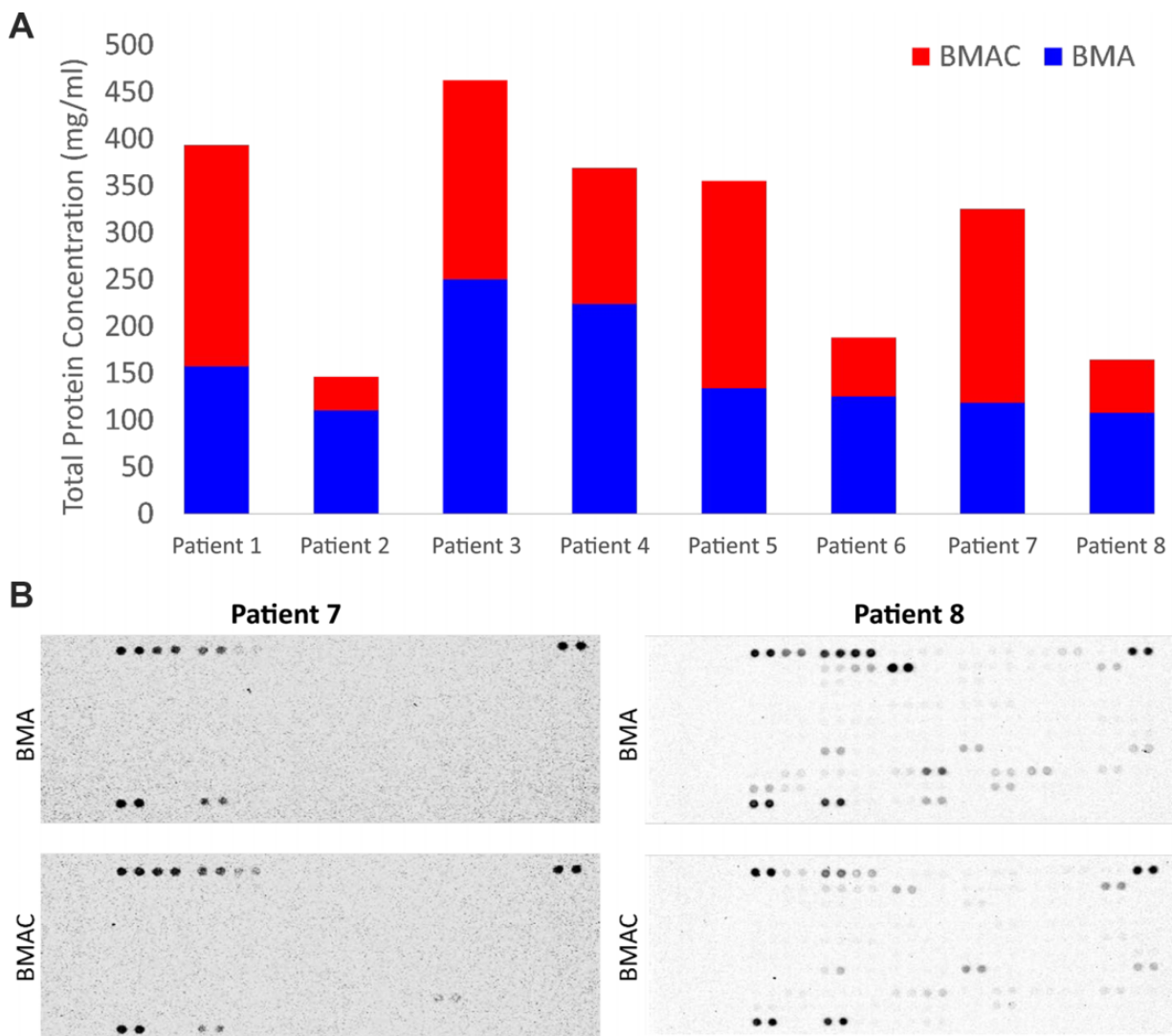


Figure 5. (A) Total protein concentration of bone marrow aspirate (BMA) versus BMA concentrate (BMAC) by patient. To test any paracrine or downstream growth factor upregulation, protein quantification of the BMA and BMAC was investigated in all the samples using the Bio-Rad protein assay. Patients 2, 4, 6, and 8 demonstrated higher protein concentration in BMAC compared with BMA. No correlation or clear pattern of protein content between BMA and BMAC or between patients was found. (B) Proteome Profiler Human XL Cytokine Array for patients 7 (left) and 8 (right). Equal amounts of BMA and BMAC were loaded onto the Proteome Profiler Human XL Cytokine Array to analyze the presence of 102 different cytokines.

found that the number of colonies was significantly higher in BMA samples when 100 or 500 cells were plated, suggesting that BMA MSCs have higher viability and are healthier to divide in cell culture. This may be due to the centrifugation process in BMAC having detrimental effects on MSCs in the aspirate. Although the doubling time for BMAC is initially higher, there is not a significant difference between BMA and BMAC MSCs.

In addition, the site of extraction of MSCs may influence the quality of MSCs. We extracted 75% of the MSCs from the proximal humerus, while 25% were extracted from the distal femur. It is possible that had the aspiration occurred at the iliac crest, the differences found between BMA and BMAC would have been less evident. Moreover, the site of

extraction may influence the relative quantity of hematopoietic stem cells, which could have an impact on the quantity of MSCs extracted.

In order to evaluate the efficacy of BMAC as a viable option to concentration MSCs, it must be determined if they have any efficacy clinically. There have been a few studies^{5,11,12} that have suggested improved outcomes with BMAC injections for various orthopaedic pathologies. In 2014, Gobbi et al¹¹ demonstrated improvement in radiographic and clinical outcome scores at 3 years of follow-up in 25 patients with grade 4 chondral lesions treated with single-stage BMAC covered by a collagen-based scaffold. The best results were seen in patients younger than 45 years and those with smaller lesions, and thus it was

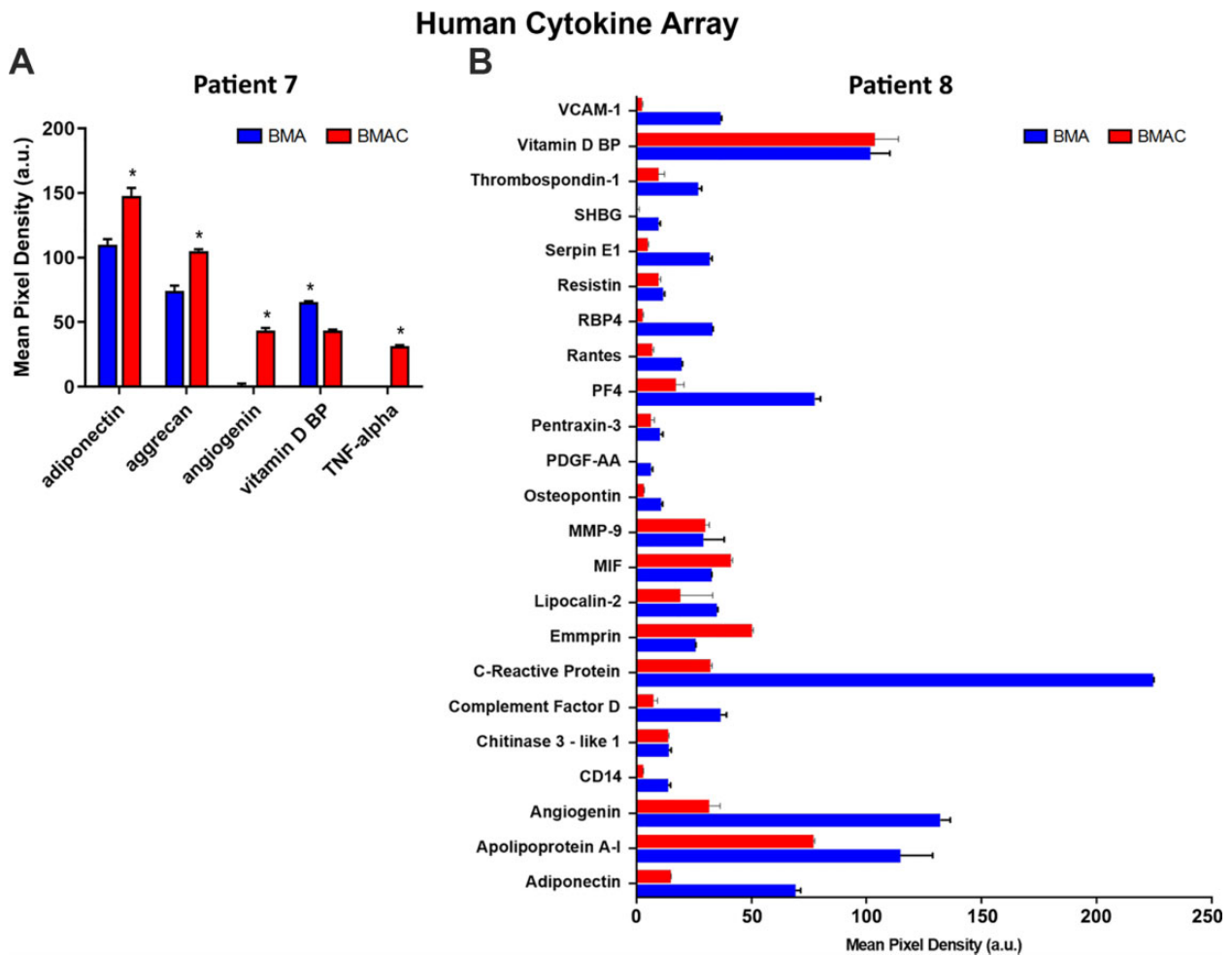


Figure 6. Proteome Profiler Human XL Cytokine Array for bone marrow aspirate (BMA) versus BMA concentrate (BMAC) in 2 patients. (A) Patient 7 had higher concentrations of protein in BMAC versus BMA, but only 5 of 102 screened cytokines were identified using the Proteome Profiler Human XL Cytokine Array: adiponectin, aggrecan, angiogenin, vitamin D BP, and tumor necrosis factor- α (TNF- α). In addition, each cytokine demonstrated a significantly different concentration between BMA and BMAC ($*P < .05$). (B) In patient 8, a very different cytokine profile was identified compared with patient 7. In this patient, significantly higher concentrations of regenerative molecules such as platelet factor 4 (PF4), platelet-derived growth factor-AA (PDGF-AA), and osteopontin were found in BMA in comparison with cytokines identified in the BMAC sample. a.u., arbitrary unit; BP, binding protein; MIF, macrophage migration inhibitory factor; MMP-9, matrix metalloproteinase-9; RBP4, retinol binding protein 4; SHBG, sex hormone binding globulin; VCAM-1, vascular cell adhesion molecule-1.

concluded that BMAC is a safe and effective treatment option with no significant complications.¹² Gobbi et al also later compared matrix-induced autologous chondrocyte implantation (MACI) to BMAC in patellofemoral chondral lesions with a minimum 3-year follow-up.¹² They found equivocal improvements in both groups in radiographic and clinical outcome scores at the 2-year follow-up mark. They noted more than 80% complete defect filling on magnetic resonance imaging in those treated with BMAC, and those who underwent second-look arthroscopy with concomitant biopsy revealed hyaline-like cartilage on histology.¹² However, these results are not easily extrapolated to other patient populations with larger lesions and have not been compared with the clinical use of BMA aspirations. Moreover, the combined use of a collagen-based scaffold also

introduced another variable, which can affect the final efficacy of BMAC itself.

Other studies^{2,13,19,31} were unable to demonstrate improved results with the use of BMAC. A systematic review by Filardo et al⁹ identified a number of preclinical and clinical trials looking at MSCs for the treatment of cartilage lesions. However, they concluded that further studies including randomized controlled trials were needed to support the potential of MSCs isolated from BMA or BMAC in cartilage repair. Shapiro et al³¹ looked at 25 patients with bilateral knee osteoarthritis. Using the contralateral knee as a control, patients were randomized to receive BMAC injection in one knee and saline placebo in the other. Although both knees saw improvements at 1 week, 3 months, and 6 months, there was no significant

difference in those treated with BMAC compared with placebo ($P > .09$).³¹

In this study, we compared the concentration of MSCs, doubling time in vitro, and cytokine analysis between equal samples of BMA versus BMAC collected from the distal humerus or proximal humerus of 8 patients in order to understand which could be of beneficial BMAC use.

Our overall hypothesis was that the concentration procedure might affect the efficacy of the MSCs as well as help concentrate regenerative growth factor, such as that present in platelet rich plasma (PRP), which can lead to better healing. We found that the overall concentration of MSCs in BMAC was consistent with previous literature at 0.001%.^{1,14,27} However, we found an inconsistent number of MSCs obtained from BMA between patients and were unable to identify any MSCs in 2 samples of BMAC. We failed to concentrate MSCs via BMAC in 6 of 8 patients. These findings emphasize the lack of consensus in terms of the number of MSCs injected, frequency of injections, volume of BMAC injected, and timing of injections when stem cells from BMAC are used in orthopaedic procedures. In addition, due to the low number of MSCs, it seems improper for us to name BMAC therapy as stem cell therapy.

Recently, Cotter and colleagues⁵ reviewed the current literature using BMAC for cartilage defects of the knee. They concluded that the low starting concentration of MSCs in the bone marrow necessitates further investigations as to whether the quantity of bone marrow-derived MSCs results in significant differences in clinical effect. Further higher-quality studies are needed to determine the optimal concentration of cells injected and the timing of injections.⁵ In order to effectively utilize BMA or BMAC in the clinic, standardization regarding isolation, amount of MSCs injected, frequency of injections, and volume injected is needed. Given the large variation surrounding the number of MSCs that are injected, standardization for clinical practice will most likely be difficult.

A different potential benefit surrounding BMAC treatment is that MSCs become “activated” during the centrifuge processing, allowing them to quickly differentiate into the desired cell lineage. This can also be induced by adding a collagen matrix.^{12,27} To test this theory, we cultured MSCs using 4 different initial seeding densities. However, after 2 weeks, we discovered a significantly higher proliferation of cells in the aspiration as compared with the concentrate for half of the cultures. This suggests that the cells may actually become less potent, or more stressed as they are processed and concentrated. Although the MSCs may be more concentrated, their ability to proliferate and differentiate may be affected, suggesting that their use in clinical practice would have little utility. The MSCs may in some way become damaged during the ultracentrifugation process, ultimately affecting their healing potential and clinical use.²⁹

Another theory is that the beneficial effect of BMAC treatment could be due to augmented protein content instead of the actual presence of MSCs.^{6,21} For this reason, we identified and compared the cytokine profile of BMA versus BMAC in order to determine if BMAC had a superior treatment effect not because of the quantity of MSCs

obtained, but because of the superior cytokine profile. We anticipated finding higher concentrations of regenerative growth factors such as aggrecan, vitamin D, or platelet derived growth factor (PDGF). It has been suggested the BMAC promotes regeneration by delivering MSCs in addition to platelets and noncellular growth factors such as PDGF and vascular endothelial growth factor (VEGF).³⁰ PDGF recruits cells involved in the musculoskeletal repair process (ie, MSCs, osteoblasts, osteocytes, and tenocytes) and upregulates angiogenesis.^{7,10,15,25,32,33,38} VEGF helps to initiate angiogenesis after tissue injury.¹⁶ However, we were unable to find any clear pattern or correlation because of the great variability both between BMAC and BMA and between patients. Although we would expect to see a higher concentration of these factors in BMAC because of the increased concentration of MSCs, we found no difference in the sample we analyzed. Moreover, in one instance, we unexpectedly found a higher concentration of TNF- α in BMAC, a proinflammatory cytokine. The increase in TNF- α may have kept the MSCs in the concentration from becoming stressed. However, this finding is particularly concerning because MSCs should have regenerative and anti-inflammatory properties, associated with a decrease in TNF- α . The large variability suggests that further investigated is warranted to determine the appropriate method to isolate MSCs.³⁶ In addition, the effect of the BMAC method on MSCs needs to be further investigated.

Moreover, the clinical efficacy of using a low versus high quantity of MSCs for treatment must be determined. Studies have not yet determined the minimal amount of MSCs necessary to result in a clinically significant difference in outcome. Although the quantity of MSCs needed for injection is still under investigation, our study suggests that the quality of the MSCs extracted also has importance. The variability and difference in doubling time and cytokine expression suggest that the quality of MSCs is just as important a factor to consider as the quantity of MSCs injected.

This study does come with limitations related to the small number of patients included in it. The average age was 51.25 years, and a previous study has shown a decrease in MSC quantity and quality with increasing age.⁴ There is also the potential for confounding in harvesting and processing techniques, which was minimized by having all procedures performed by a single surgeon. We were also unable to control for other patient factors, such as sex or harvest location, which has previously been shown to affect MSC concentration.³ Finally, this study does not evaluate the clinical efficacy of BMAC versus BMA MSCs, as it focused on the basic science of MSCs rather than clinical outcomes.

CONCLUSION

There is great variability in MSC concentration, protein concentration, and cytokine profile between BMA and BMAC and between patients. Because of the low inherent concentration of MSCs in bone marrow, it is difficult to reliably isolate and concentrate these cells. Furthermore, the significantly lower CFUs in BMAC suggest a lower potency of MSCs compared with BMA. Further studies are

needed to determine proper harvesting and processing techniques to reliably standardize the concentration of MSCs injected before they are introduced as “stem cell treatment” in orthopaedic procedures. However, this study strongly suggests that claims of BMAC’s superiority over BMA need to be questioned, and its use limited until additional research is performed to further delineate MSC viability, differentiation, and efficacy between the 2 different techniques. Finally, clinical trials need to be conducted to evaluate specific MSC populations and growth factors to clinical therapeutic effects among BMAC and BMA.

REFERENCES

- Cassano JM, Kennedy JG, Ross KA, et al. Bone marrow concentrate and platelet-rich plasma differ in cell distribution and interleukin 1 receptor antagonist protein concentration. *Knee Surg Sports Traumatol Arthrosc.* 2018;26(1):333-342.
- Centeno C, Pitts J, Al-Sayegh H, Freeman M. Efficacy of autologous bone marrow concentrate for knee osteoarthritis with and without adipose graft. *Biomed Res Int.* 2014;2014:370621.
- Chahla J, Mannava S, Cinque ME, et al. Bone marrow aspirate concentrate harvesting and processing technique. *Arthrosc Tech.* 2017;6(2):e441-e445.
- Chambers SM, Goodell MA. Hematopoietic stem cell aging: wrinkles in stem cell potential. *Stem Cell Rev.* 2007;3(3):201-211.
- Cotter EJ, Wang KC, Yanke AB, Chubinskaya S. Bone marrow aspirate concentrate for cartilage defects of the knee: from bench to bedside evidence. *Cartilage.* 2018;9(2):161-170.
- da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci.* 2006;119(pt 11):2204-2213.
- Doorn J, Moll G, Le Blanc K, van Blitterswijk C, de Boer J. Therapeutic applications of mesenchymal stromal cells: paracrine effects and potential improvements. *Tissue Eng Part B Rev.* 2012;18(2):101-115.
- Figueroa D, Calvo R, Vaisman A, et al. Arthroscopic intercondylar notch bone marrow aspiration during anterior cruciate ligament reconstruction. *Arthrosc Tech.* 2019;8(12):e1437-e1441.
- Filardo G, Madry H, Jelic M, et al. Mesenchymal stem cells for the treatment of cartilage lesions: from preclinical findings to clinical application in orthopaedics. *Knee Surg Sports Traumatol Arthrosc.* 2013;21(8):1717-1729.
- Friedlaender GE, Lin S, Solchaga LA, Snel LB, Lynch SE. The role of recombinant human platelet-derived growth factor-BB (rhPDGF-BB) in orthopaedic bone repair and regeneration. *Curr Pharm Des.* 2013;19(19):3384-3390.
- Gobbi A, Chaurasia S, Karnatzikos G, Nakamura N. Matrix-induced autologous chondrocyte implantation versus multipotent stem cells for the treatment of large patellofemoral chondral lesions: a nonrandomized prospective trial. *Cartilage.* 2015;6(2):82-97.
- Gobbi A, Karnatzikos G, Sankineani SR. One-step surgery with multipotent stem cells for the treatment of large full-thickness chondral defects of the knee. *Am J Sports Med.* 2014;42(3):648-657.
- Hauser RA, Orlofsky A. Regenerative injection therapy with whole bone marrow aspirate for degenerative joint disease: a case series. *Clin Med Insights Arthritis Musculoskelet Disord.* 2013;6:65-72.
- 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.
- Hernigou P, Poignard A, Manicom O, Mathieu G, Rouard H. The use of percutaneous autologous bone marrow transplantation in nonunion and avascular necrosis of bone. *J Bone Joint Surg Br.* 2005;87(7):896-902.
- Holmes K, Roberts OL, Thomas AM, Cross MJ. Vascular endothelial growth factor receptor-2: structure, function, intracellular signalling and therapeutic inhibition. *Cell Signal.* 2007;19(10):2003-2012.
- Indrawattana N, Chen G, Tadokoro M, et al. Growth factor combination for chondrogenic induction from human mesenchymal stem cell. *Biochem Biophys Res Commun.* 2004;320(3):914-919.
- Kim GB, Seo MS, Park WT, Lee GW. Bone marrow aspirate concentrate: its uses in osteoarthritis. *Int J Mol Sci.* 2020;21(9):3224.
- Kim JD, Lee GW, Jung GH, et al. Clinical outcome of autologous bone marrow aspirates concentrate (BMAC) injection in degenerative arthritis of the knee. *Eur J Orthop Surg Traumatol.* 2014;24(8):1505-1511.
- Lavender C, Johnson B, Kopiec A. Augmentation of anterior cruciate ligament reconstruction with bone marrow concentrate and a suture tape. *Arthrosc Tech.* 2018;7(12):e1289-e1293.
- Lin W, Xu L, Zwingerberger S, et al. Mesenchymal stem cells homing to improve bone healing. *J Orthop Translat.* 2017;9:19-27.
- McCarrel T, Fortier L. Temporal growth factor release from platelet-rich plasma, trehalose lyophilized platelets, and bone marrow aspirate and their effect on tendon and ligament gene expression. *J Orthop Res.* 2009;27(8):1033-1042.
- Muench LN, Berthold DP, Kia C, et al. Nucleated cell count has negligible predictive value for the number of colony-forming units for connective tissue progenitor cells (stem cells) in bone marrow aspirate harvested from the proximal humerus during arthroscopic rotator cuff repair. *Arthroscopy.* 2021;37(7):2043-2052.
- Muench LN, Kia C, Otto A, et al. The effect of a single consecutive volume aspiration on concentrated bone marrow from the proximal humerus for clinical application. *BMC Musculoskelet Disord.* 2019;20(1):543.
- Muschler GF, Nakamoto C, Griffith LG. Engineering principles of clinical cell-based tissue engineering. *J Bone Joint Surg Am.* 2004;86(7):1541-1558.
- Otto A, Muench LN, Kia C, et al. Proximal humerus and ilium are reliable sources of bone marrow aspirates for biologic augmentation during arthroscopic surgery. *Arthroscopy.* 2020;36(9):2403-2411.
- Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* 1999;284(5411):143-147.
- Ryu DJ, Jeon YS, Park JS, et al. Comparison of bone marrow aspirate concentrate and allogenic human umbilical cord blood derived mesenchymal stem cell implantation on chondral defect of knee: assessment of clinical and magnetic resonance imaging outcomes at 2-year follow-up. *Cell Transplant.* 2020;29:963689720943581.
- Scarpone M, Kuebler D, Chambers A, et al. Isolation of clinically relevant concentrations of bone marrow mesenchymal stem cells without centrifugation. *J Transl Med.* 2019;17(1):10.
- Schafer R, DeBaun MR, Fleck E, et al. Quantitation of progenitor cell populations and growth factors after bone marrow aspirate concentration. *J Transl Med.* 2019;17(1):115.
- Shapiro SA, Kazmerchak SE, Heckman MG, Zubair AC, O'Connor MI. A prospective, single-blind, placebo-controlled trial of bone marrow aspirate concentrate for knee osteoarthritis. *Am J Sports Med.* 2017;45(1):82-90.
- Siegel G, Kluba T, Hermanutz-Klein U, et al. Phenotype, donor age and gender affect function of human bone marrow-derived mesenchymal stromal cells. *BMC Med.* 2013;11:146.
- Soltan M, Smiler DG, Gailani F. A new “platinum” standard for bone grafting: autogenous stem cells. *Implant Dent.* 2005;14(4):322-325.
- Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. *Sports Health.* 2009;1(6):461-468.
- Staszkiwicz J, Frazier TP, Rowan BG, et al. Cell growth characteristics, differentiation frequency, and immunophenotype of adult ear mesenchymal stem cells. *Stem Cells Dev.* 2010;19(1):83-92.
- Taraballi F, Pastò A, Bauza G, Varner C, Amadori A, Tasciotti E. Immunomodulatory potential of mesenchymal stem cell role in diseases and therapies: a bioengineering perspective. *J Immunol Regen Med.* 2019;4:100017.
- Viswanathan S, Shi Y, Galipeau J, et al. Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT(R)) Mesenchymal Stromal Cell committee position statement on nomenclature. *Cytotherapy.* 2019;21(10):1019-1024.
- Zhong W, Sumita Y, Ohba S, et al. In vivo comparison of the bone regeneration capability of human bone marrow concentrates vs. platelet-rich plasma. *PLoS One.* 2012;7(7):e40833.