

Bone marrow concentrate and platelet-rich plasma differ in cell distribution and interleukin 1 receptor antagonist protein concentration

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Abstract

Purpose Bone marrow concentrate (BMC) and platelet-rich plasma (PRP) are used extensively in regenerative medicine. The aim of this study was to determine differences in the cellular composition and cytokine concentrations of BMC and PRP and to compare two commercial BMC systems in the same patient cohort.

Methods Patients (29) undergoing orthopaedic surgery were enrolled. Bone marrow aspirate (BMA) was processed to generate BMC from two commercial systems (BMC-A and BMC-B). Blood was obtained to make PRP utilizing the same system as BMC-A. Bone marrow-derived samples were cultured to measure colony-forming units, and flow cytometry was performed to assess mesenchymal stem cell (MSC) markers. Cellular concentrations were assessed for all samples. Catabolic cytokines and growth factors important for cartilage repair were measured using multiplex ELISA.

Results Colony-forming units were increased in both BMCs compared to BMA ($p < 0.0001$). Surface markers were consistent with MSCs. Platelet counts were not significantly different between BMC-A and PRP, but there were differences in leucocyte concentrations. TGF- β 1 and PDGF were not different between BMC-A and PRP. IL-1ra concentrations were greater ($p = 0.0018$) in BMC-A samples (13,432 pg/mL) than in PRP (588 pg/mL). The

IL-1ra/IL-1 β ratio in all BMC samples was above the value reported to inhibit IL-1 β .

Conclusions The bioactive factors examined in this study have differing clinical effects on musculoskeletal tissue. Differences in the cellular and cytokine composition between PRP and BMC and between BMC systems should be taken into consideration by the clinician when choosing a biologic for therapeutic application.

Level of evidence Clinical, Level II.

Keywords Bone marrow concentrate · MSCs · Platelet-rich plasma · PRP · Interleukin 1 receptor antagonist · IL-1ra

Introduction

Biologic solutions to address the pathologic process of osteoarthritis (OA) have been investigated as potential treatments for focal cartilage lesions, osteochondral lesions, and generalized OA [1]. Mesenchymal stem cells (MSCs) appear promising for the treatment of OA, but are subject to regulatory restrictions, and are not currently approved by the FDA or many other regulatory bodies for clinical use [6, 36]. Bone marrow concentrate (BMC) has been used to deliver MSCs to damaged cartilage [23], to augment microfracture [17, 26–28, 48], and as a direct joint injection method for cartilage repair and early OA [33]. BMC is generated by centrifugation of bone marrow aspirate (BMA) allowing for immediate administration to a patient, and many regulatory bodies have approved these products for clinical use. While BMC contains fewer MSCs than what can be obtained through culture expansion [11, 53], the number of MSC needed for effective therapy has yet to be determined. In addition to MSCs, BMC contains

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numerous bioactive molecules and cell types including lymphocytes, neutrophils, monocytes, and platelets in various stages of differentiation [21, 23, 34]. In orthopaedics, cytological analysis of BMC is rarely performed. In one study, a fourfold increase in platelets, total nucleated cells, and CD34+ cells in BMC compared to BMA was reported [34]. The concentration of growth factors in BMC has not been quantified, despite their role in the regenerative potential of BMC [40, 47].

Platelet-rich plasma (PRP) is gaining popularity as a biologic treatment for focal cartilage defects and OA [13, 20]. PRP is generated by centrifugation of peripheral blood resulting in increased platelet concentration. The platelets provide multiple growth factors with known roles in target cell activation, cell recruitment, cartilage matrix production, and modulation of the inflammatory response [22]. In current orthopaedic practice, it is commonly regarded that BMC is essentially PRP with additional stem cells; however, this has not been fully elucidated. Therefore, one aim of this study was to determine how BMC differs from PRP. There is known variability in PRP due to differences between patients and manufacturers, and the same premise is assumed for BMC, resulting in the second aim of this study: to compare BMCs generated from two different commercial systems.

Materials and methods

This study was prospective and supervised by the biologics committee as a quality assessment project. Between 11/2013 and 07/2014, 29 consecutive patients, within an age range of 18–70 years, consented for participation. All patients were under the care of a single surgeon (JGK). Patients were excluded if there was a history of blood disorders, haematological malignancy, use of immunosuppressive drugs, or drugs with bone marrow-suppressive effects.

Blood and bone marrow aspirate collection

Blood (25 mL) was drawn into a syringe containing 4 mL acid citrate dextrose (ACD). One millilitre was removed for the study and the remainder was processed to generate 3 mL PRP (Magellan[®], Arteriocyte Medical Systems Inc., Hopkinton, MA). One millilitre of PRP was removed for study purposes. Bone marrow was aspirated from the iliac crest into a 30-mL syringe containing 4 mL ACD. The needle was advanced 1 cm and rotated 90° after each 5 mL aspirate until a total of 120 mL was aspirated in four syringes. The total aspirate was mixed, 1 mL was retained as the BMA sample for the study, and the remainder was separated into two 60 mL samples and processed in two systems; Magellan[®] (BMC-A) (Arteriocyte Medical

Systems Inc.) and SmartPrep[®] 2 (BMC-B; Harvest Technologies Corp., Plymouth, MA). All aspirations were performed by the same surgeon (JGK). One millilitre of each BMC-A and BMC-B was retained for the study. Representatives from both BMC companies observed aspiration and concentration techniques. Samples were shipped de-identified to prevent bias in sample processing and analysis. All samples were processed within 24 h of collection.

Cytology

Automated counts were performed on whole blood (WB), PRP, BMA, BMC-A, and BMC-B samples to assess platelets, red blood cells (RBC), and nucleated cell counts (NCC), which included myeloid precursors as well as white blood cells (WBC: neutrophils, monocytes, lymphocytes, eosinophils, basophils). Cytological smears were evaluated of all samples to verify automated counts.

Colony-forming units and flow cytometry

Equal volumes of BMCs were used for CFU assays, rather than a known number of nucleated cells. This reflects clinical practice where volume, as opposed to cell count, would be the unit of BMC application. Samples of BMC were cultured in duplicate with DMEM containing 10 % FBS, penicillin/streptomycin, and 1 ng/mL basic fibroblastic growth factor [50]. Media were changed every 48 h. 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. This adapted technique [24, 33] allowed for retention of viable cells and further analyses.

Cells were then passaged once and then lifted with Accumax (Sigma-Aldrich, St. Louis, MO) when confluent at passage 1 for flow cytometry of MSCs markers [16], including positive (CD146, CD73, CD271) and negative markers (CD34, CD45). Conjugated primary monoclonal antibodies and isotype controls were used as recommended by the manufacturer (BD Biosciences, San Jose, CA). Cells were analysed on a FACS Canto II (BD Immunocytometry Systems) flow cytometer and using FlowJo software (Tree-Star Inc., Ashland, OR).

Growth factor and cytokine analyses

Multiplex assays were performed according to manufacturer directions to measure vascular endothelial growth factor (VEGF), interleukin 1 beta (IL-1 β), interleukin 6 (IL-6), interleukin 8 (IL-8), tumour necrosis factor- α (TNF- α), interleukin 1 receptor antagonist protein (IL-1ra), and interferon- γ (IFN- γ ; Fluorokine MAP Multiplex Human Cytokine Panel A, R&D Systems, Minneapolis, MN). Transforming growth factor beta 1 (TGF- β 1), TGF- β 2, and

Table 1 Comparison of cellular composition and differential cell count in WB, PRP, and BMC-A

	WB, PRP, BMC-A cytology			
	Mean \pm SD (range)			
	WB	PRP	BMC-A	<i>p</i> value
WBC (thou/ μ L)	4.2 \pm 1.6* (1.9–6.4)	3.1 \pm 1.2*† (0.7–5.1)	36.7 \pm 16.9† (16.8–77.2)	* 0.032 † 0.0001
Neutrophils (thou/ μ L)	2.6 \pm 1.3* (0.7–4.5)	0.9 \pm 0.4*† (0.1–1.8)	17.5 \pm 9.8† (5.4–42.7)	* 0.0003 † 0.0004
Monocytes (thou/ μ L)	0.2 \pm 0.09* (0.1–0.4)	0.3 \pm 0.2*† (0.1–0.7)	3.3 \pm 2.3† (0.8–10.5)	* 0.41 † 0.0010
Lymphocytes (thou/ μ L)	1.2 \pm 0.4* (0.6–2.2)	1.7 \pm 0.8*† (0.4–2.8)	12.6 \pm 5.4† (5.3–27.3)	* 0.07 † 0.0001
Eosinophils (thou/ μ L)	0.07 \pm 0.05* (0.0–0.1)	0.0 \pm 0.0*† (0.0–0.0)	0.6 \pm 0.4† (0.1–1.3)	* 0.0039 † 0.0015
Basophils (thou/ μ L)	0.01 \pm 0.04* (0.0–0.1)	0.04 \pm 0.05*† (0.0–0.1)	0.2 \pm 0.2† (0.04–0.7)	* 0.50 † 0.015
Platelets (thou/ μ L)	80.3 \pm 55.5* (14–178)	202.3 \pm 86.1*† (73–368)	151.6 \pm 111.2† (25–362)	* 0.0010 † 0.92

White blood cells (WBC) and all cell differential means are expressed with standard deviation to nearest whole unit, thousands of cells per microlitre (thou/ μ L), the range in the parentheses. A shared symbol (* or †) between two groups corresponds to the shared *p* value based on a two-sided paired *T* test

TGF- β 3 were measured using the Fluorokine MAP Multiplex TGF-beta 3-plex Kit (R&D Systems). Platelet-derived growth factor-BB (PDGF-BB) and acidic fibroblast growth factor (FGF-1) were quantified using the Fluorokine MAP Multiplex Human Angiogenesis Panel A (R&D Systems). Multiplex assays were performed using the Luminex 200 instrument (Luminex Corp., Austin, TX).

Institutional Review Board approval

This study was approved by the Hospital for Special Surgery's Institutional Review Board (ID #29055).

Statistical analyses

BMA was obtained from 29 patients. Blood was also obtained from the last 14 of the 29 patients. In BMC-B from the first six patients, no MSCs were observed in the cultures. The company was contacted, and they replaced the machine, which resulted in growth of MSCs in all subsequent samples. The authors regarded this as mechanical failure and elected to exclude these patients from the study. Four BMA and BMC samples were excluded because they were not processed within 24 h of collection due to a shipping delay as the result of inclement weather. Further inclusion/exclusion criteria for statistical analysis were set to confirm that BMC and PRP were generated. For BMC, this was defined as an increase in NCC or CFU compared to BMA, resulting in exclusion of 1 sample. For PRP, inclusion was defined as an increase in platelet or PDGF concentration compared to that measured in

whole blood, resulting in exclusion of two patients [13]. To account for the individual variability inherent in biologics, paired statistical methods were used. Data were tested for normality using a Shapiro–Wilk test. A two-sided paired *t* test was used for normally distributed differences between groups; otherwise, a Wilcoxon Signed Rank test was used, with an α of 0.05. There were no available studies using a multiplex of BMA/BMC data to perform a power analysis.

Results

The mean age of patients was 48.2 years with a range of 23–68. Ten were female and the remainder (9) male. There were no reported complications associated with collection of bone marrow or blood.

Cytology and platelet concentration in PRP compared to BMC

BMC-A was used for comparison to PRP because both were generated from the same system, thus allowing for a comparison based on biologic differences rather than preparation methods. The concentration of total WBCs and all subtypes of WBCs were greater in BMC-A compared to PRP (Table 1). There was an 11.8-fold increase in WBC concentration in BMC-A compared to PRP. Neutrophils were increased in BMC-A by 19-fold, monocytes by 11-fold, and lymphocytes by sevenfold compared to PRP. The platelet concentration in PRP was

Table 2 Comparison of cellular composition and differential cell count in BMA, BMC-A, and BMC-B

	BMA, BMC-A, BMC-B cytology			
	Mean \pm SD (range)			
	BMA	BMC-A	BMC-B	<i>p</i> value
NCC (thou/ μ L)	11.6 \pm 5.8* \dagger (4.9–23.2)	38.3 \pm 20.6* \ddagger (10.5–94.0)	47.4 \pm 20.1 \dagger \ddagger (15.2–81.4)	* <0.0001 \dagger <0.0001 \ddagger 0.14
WBC (thou/ μ L)	10.7 \pm 5.3* \dagger (4.7–21.4)	36.7 \pm 16.9* \ddagger (16.8–77.2)	44.3 \pm 18.6 \dagger \ddagger (14.4–77.6)	* <0.0001 \dagger <0.0001 \ddagger 0.19
Neutrophils (thou/ μ L)	7.1 \pm 4.1* \dagger (2.8–15.5)	17.5 \pm 9.8* \ddagger (5.4–42.7)	27.4 \pm 12.8 \dagger \ddagger (9.0–53.5)	* <0.0001 \dagger <0.0001 \ddagger 0.0023
Monocytes (thou/ μ L)	0.6 \pm 0.4* \dagger (0.2–1.6)	3.3 \pm 2.3* \ddagger (0.8–10.5)	2.9 \pm 1.2 \dagger \ddagger (0.3–5.0)	* <0.0001 \dagger <0.0001 \ddagger 0.33
Lymphocytes (thou/ μ L)	2.0 \pm 0.6* \dagger (1.0–3.5)	12.6 \pm 5.4* \ddagger (5.3–27.3)	9.7 \pm 3.9 \dagger \ddagger (1.6–20.8)	* <0.0001 \dagger <0.0001 \ddagger 0.054
Eosinophils (thou/ μ L)	0.2 \pm 0.2* \dagger (0.04–0.8)	0.6 \pm 0.4* \ddagger (0.1–1.3)	0.8 \pm 0.5 \dagger \ddagger (0.2–1.9)	* <0.0001 \dagger <0.0001 \ddagger 0.15
Basophils (thou/ μ L)	0.04 \pm 0.03* \dagger (0.0–0.1)	0.2 \pm 0.2* \ddagger (0.04–0.7)	0.2 \pm 0.2 \dagger \ddagger (0.0–0.9)	* <0.0001 \dagger 0.013 \ddagger 0.51
Platelets (thou/ μ L)	31.5 \pm 25.4* \dagger (3–94)	151.6 \pm 111.2* \ddagger (25–362)	111.1 \pm 97.4 \dagger \ddagger (11–300)	* <0.0001 \dagger <0.0001 \ddagger 0.031

Nucleated cell counts (NCC), white blood cells (WBC), and all cell differential means are expressed with standard deviation to nearest whole unit, thousands of cells per microlitre (thou/ μ L), the range in the parentheses. A shared symbol (*, \dagger , or \ddagger) between two groups corresponds to the shared *p*-value based on a two-sided paired *T* test

increased by 2.5-fold compared to WB; however, there was no difference in platelet concentration between BMC-A and PRP.

Cytology and platelet concentration in BMA compared to BMC

The NCC in both BMC-A and BMC-B was increased compared to BMA verifying that both systems concentrated nucleated cells (Table 2). The NCC in BMC-A was increased by 3.3-fold, and by 4.1-fold in BMC-B compared to BMA. There was no difference in NCC between BMC-A and BMC-B. The total WBC concentration was increased in both BMC-A and BMC-B compared to BMA, but there was no difference between BMC-A and BMC-B. All subtypes of WBCs were increased in BMC-A and BMC-B compared to BMA. The average neutrophil concentration in BMC-B was 1.6-fold greater than BMC-A. In contrast, the average concentration of lymphocytes was 1.3-fold greater in BMC-A compared to BMC-B. However, there was no significant difference between lymphocyte concentration in BMC-A and BMC-B. There were no statistically

significant differences in monocyte, eosinophil, or basophil concentrations between BMC-A and BMC-B. Platelet concentration was increased by 4.8-fold in BMC-A and 3.5-fold in BMC-B compared to BMA, resulting in a significantly greater platelet concentration in BMC-A compared to BMC-B.

Colony-forming units

The number of CFUs was not different between duplicates (*p* = 0.44), so duplicates were averaged for analyses. The mean number of CFUs was 7.8 \pm S.D. 12.3 (range 0–46) in BMA, 41.4 \pm 27.4 (range 9–90) in BMC-A, and 32.7 \pm 30.3 (range 2–90) in BMC-B. CFUs were significantly increased in both BMCs compared to BMA (*p* < 0.0001), and BMC-A and BMC-B were not significantly different (*p* = 0.079).

Flow cytometry of MSCs from BMC

The minimum number of 2.5×10^5 cells needed for flow cytometry of each molecule limited the ability to

measure all five MSC markers in every sample, so CD45 and CD271 were prioritized based on the literature [14, 45]. There were insufficient CFUs in BMA samples for comparison to BMC. All MSCs from BMC-A and BMC-B were negative for the lymphocyte marker CD45 (Fig. 1). The majority of BMC-A and BMC-B-derived MSCs were positive for CD271, and only a small percentage were positive for the haematopoietic stem cell marker CD34. All MSCs were positive for CD73 and variably positive for CD146.

Growth factors and cytokines

PDGF-BB

The concentration of PDGF was not significantly different between PRP and BMC-A (Table 3). As expected, PDGF was increased (1.7-fold) in PRP compared to WB. PDGF was also increased in both BMC-A and BMC-B compared to BMA, with concentrations greater in BMC-A compared to BMC-B (Table 4).

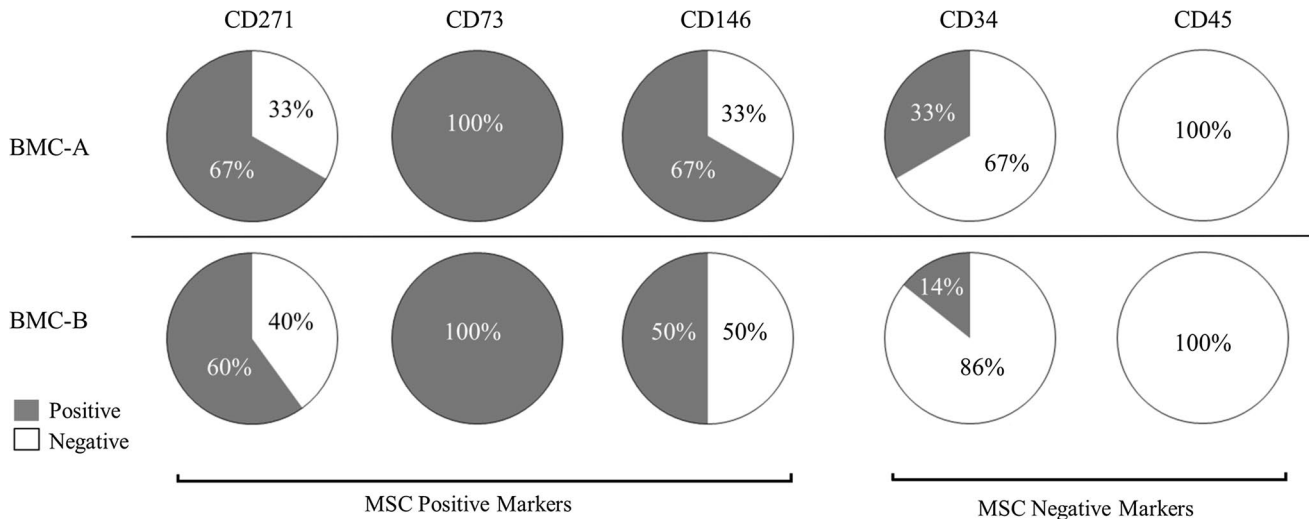


Fig. 1 Cell surface marker expression based on flow cytometry of cell samples cultured from bone marrow concentrate from commercial system A (BMC-A) and bone marrow concentrate from commercial system B (BMC-B). CD271, CD73, and CD146 are markers

expressed on mesenchymal stem cells (MSCs). MSCs are negative for CD34, a haematopoietic stem cell marker, and CD45, a leucocyte marker

Table 3 Comparison of growth factor and cytokine concentration in WB, PRP, and BMC-A

	WB, PRP, BMC-A cytokines			
	Mean ± SD (range)			
	WB	PRP	BMC-A	<i>p</i> value
PDGF (pg/mL)	3530 ± 2078* (842–7698)	5975 ± 2644*† (2277–10,680)	7549 ± 3601† (2433–15,056)	* 0.0075 † 0.51
TGF-β 2 (pg/mL)	<17.5*	65 ± 70*† (0–218)	219 ± 219† (0–779)	* N/A † 0.049
VEGF (pg/mL)	31 ± 40* (0–152)	10 ± 19*† (0–66)	276 ± 273† (5–941)	* 0.0093 † 0.0010
IL-8 (pg/mL)	<3.5*	<3.5*†	273 ± 300† (0–1176)	N/A
IL-1β (pg/mL)	<1.5*	<1.5*†	7 ± 13† (0–48)	N/A

Means are expressed with standard deviation to nearest whole unit, picograms per millilitre (pg/mL), the range in the parentheses

PDGF platelet-derived growth factor, TGF-β transforming growth factor beta, VEGF vascular endothelial growth factor, IL-1β interleukin 1 beta, IL-8 interleukin 8, N/A not able to be calculated or not applicable

A shared symbol (* or †) between two groups corresponds to the shared *p* value based on a two-sided paired *T* test

Table 4 Comparison of growth factor and cytokine concentration in BMA, BMC-A, and BMC-B

	BMA, BMC-A, BMC-B cytokines			
	Mean ± SD (range)			
	BMA	BMC-A	BMC-B	<i>p</i> value
PDGF (pg/mL)	1820 ± 976*† (380–3819)	7549 ± 3601*‡ (2433–15,056)	5036 ± 3645†‡ (618–16,030)	* <0.0001 † 0.0006 ‡ 0.0013
TGF-β 2 (pg/mL)	<17.5*†	219 ± 219*‡ (0–779)	33 ± 83†‡ (0–284)	* N/A † N/A ‡ 0.0023
VEGF (pg/mL)	68 ± 53*† (1–172)	276 ± 273*‡ (5–941)	502 ± 508†‡ (0–1958)	* 0.0001 † <0.0001 ‡ 0.081
IL-8 (pg/mL)	82 ± 110*† (0–448)	273 ± 300*‡ (0–1176)	404 ± 387†‡ (0–1328)	* 0.0003 † <0.0001 ‡ 0.24
IL-1β (pg/mL)	<1.5*†	7 ± 13*‡ (0–48)	31 ± 40†‡ (0–110)	* N/A † N/A ‡ 0.021

Means are expressed with standard deviation to nearest whole unit, picograms per millilitre (pg/mL), the range in the parentheses

PDGF platelet-derived growth factor, TGF-β transforming growth factor beta, VEGF vascular endothelial growth factor, IL-1β interleukin 1 beta, IL-8 interleukin 8, N/A not able to be calculated or not applicable

A shared symbol (*, †, or ‡) between two groups corresponds to the shared *p* value based on a two-sided paired *T* test

TGF-β1, 2, 3

There was no significant difference in TGF-β1 concentration between BMC-A and PRP (Fig. 2). Not surprisingly, TGF-β1 was increased in PRP compared to WB and in both BMC-A and BMC-B compared to BMA. BMC-A had significantly more TGF-β1 compared to BMC-B. TGF-β2 was below the limit of detection (17.5 pg/mL) in all BMA and WB samples. TGF-β2 was present in 15 out of 18 BMC-A samples, 4 out of 19 BMC-B samples, and 9 out of 12 PRP samples. The concentration of TGF-β2 was greater in BMC-A compared to PRP (Table 3) and BMC-B (Table 4). TGF-β3 concentrations were below the limit of detection (62.2 pg/mL) in all samples.

VEGF

VEGF concentrations were lowest in PRP, WB, and BMA samples (Tables 3, 4). In BMC-A, VEGF was increased compared to PRP (Table 3). Compared to BMA, VEGF concentration was increased fourfold in BMC-A and sevenfold in BMC-B (Table 4).

IL-8

IL-8 concentrations were below the limit of detection (3.5 pg/mL) in WB and PRP samples (Table 3). IL-8 was

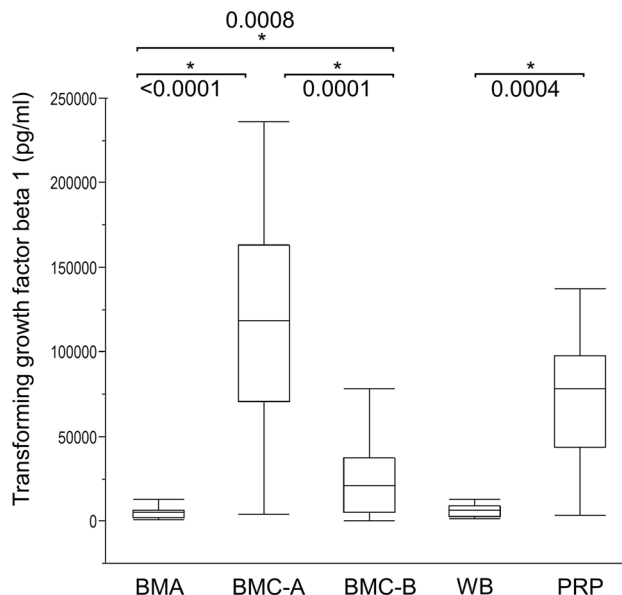


Fig. 2 Transforming growth factor beta 1 in BMA, BMC-A, BMC-B, whole blood (WB), and platelet-rich plasma (PRP). *Significant difference (*p* < 0.05) between two groups based on a two-sided paired *T* test. Data are presented in an outlier plot with whiskers extending to the farthest point within 1.5 interquartile range lengths

increased threefold in BMC-A and fivefold in BMC-B samples compared to BMA (Table 4). There was no difference in IL-8 concentration between the two BMC groups.

IL-1 β

There was no measurable IL-1 β in WB, PRP, or BMA (Tables 3, 4). Additionally, IL-1 β was undetectable in nine BMC-A and six BMC-B samples. In samples that were above the lower limit of detection (1.5 pg/mL), IL-1 β was greater in BMC-B compared to BMC-A (Table 4).

IL-1ra

All samples contained measurable IL-1ra. IL-1ra was significantly increased in BMC-A compared to PRP (Fig. 3). In WB, IL-1ra was greater than in PRP. Compared to BMA, IL-1ra was significantly increased in both BMC-A (threefold) and BMC-B (fivefold), and BMC-B was significantly greater than BMC-A. The ratios of IL-1ra to IL-1 β were calculated for all samples with detectable concentrations of IL-1 β (Fig. 4). The average IL-1ra/IL-1 β ratio for the six patients with BMC-A and BMC-B samples with detectable IL-1 β was not significantly different ($p = 0.09$).

TNF- α , IL-6, IFN- γ , FGF-1

All four of these cytokines were undetectable in all samples. Minimum detectable concentrations for the assays were: TNF- α (5.1 pg/mL), IL-6 (5.2 pg/mL), IFN- γ (2.9 pg/mL), and FGF-1 (5.7 pg/mL).

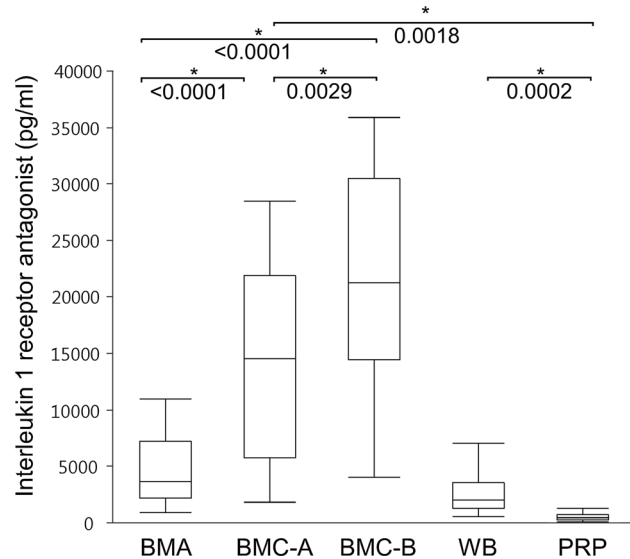


Fig. 3 Interleukin 1 receptor antagonist in BMA, BMC-A, BMC-B, WB, and PRP. *Significant difference ($p < 0.05$) between two groups based on a two-sided paired T test. Data are presented in an outlier plot with *whiskers* extending to the farthest point within 1.5 interquartile range lengths

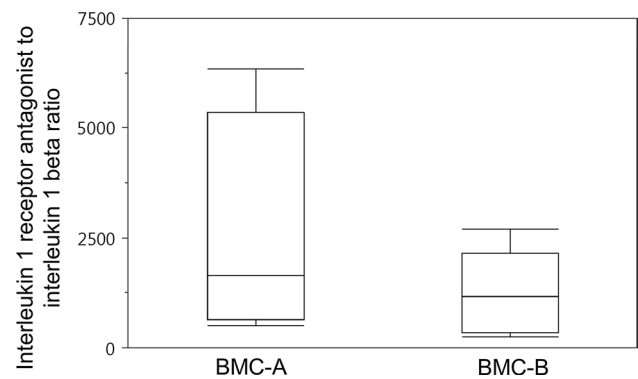


Fig. 4 Ratio of interleukin 1 receptor antagonist to interleukin 1 beta in BMC-A and BMC-B. Ratios represented are from the $n = 9/18$ BMC-A samples and $n = 13/19$ BMC-B samples that had detectable levels of IL-1b. The samples with IL-1b levels below the limit of detection (1.5 pg/mL) would have an even greater positive biological effect on competing with endogenous IL-1b in clinical use. *Significant difference ($p < 0.05$) between two groups based on a two-sided paired T test. Data are presented in an outlier plot with *whiskers* extending to the farthest point within 1.5 interquartile range lengths

Discussion

The most important finding of this study is the discovery that bone marrow-derived biologics contain clinically relevant concentrations of IL-1ra. This study also shows several significant differences in the cellular and molecular composition of PRP and BMC (Table 5), and differences in cytology and bioactive molecules between BMC manufacturing systems. Both BMC manufacturing systems effectively concentrated BMA as demonstrated by an increased total NCC in most BMC samples. BMC-B initially failed to generate BMC due to a malfunction of the centrifuge. Further, there was one failure to generate BMC in BMC-A and two failures to generate PRP from blood with no known machine malfunction. This might be expected based on similar results when generating PRP [10], but it has not been previously documented. Failure to generate BMC or PRP would not be visually recognized by the surgical team which underscores the importance of counting cells and platelets in biologics when reporting outcome data.

Although there was an increase in NCC in the BMC-B group compared to BMC-A, CFUs in BMC-A and BMC-B were not significantly different. This suggests that NCC alone may not be predictive of MSC concentration. In a previous study, higher NCCs and CFUs were found in BMC-B compared to BMC-A [33]. These conflicting results are likely explained by different study methodologies. In the current study, a uniform volume of BMC was used for culture rather than a defined number of nucleated cells [33]. This method was chosen to reflect clinical practice where a doctor would administer a specified volume of BMC rather than a defined number of nucleated cells. All MSCs had flow

Table 5 Summary of the defining characteristics of BMA, BMC-A, BMC-B, WB, and PRP

	BMA	BMC-A	BMC-B	WB	PRP
IL-1ra	++	+++	++++	++	+
IL-1ra/IL-1 β	–	++	+	–	–
MSC	+	+++	++	–	–
TGF- β 1, PDGF	+	+++	++	+	+++
IL-1 β , IL-8	+	++	++	–	–

The + symbols depict the presence of and relative concentration of the anabolic cytokine interleukin 1 receptor antagonist (IL-1ra), mesenchymal stem cells (MSCs), transforming growth factor beta-1 (TGF- β 1), platelet-derived growth factor (PDGF), and interleukin 1 beta (IL-1 β). A – symbol indicates the cytokine was below the limits of detection or MSCs were not assessed

cytometry markers consistent with MSCs. The heterogeneity in surface markers of MSCs observed in this study has been previously reported and is not surprising given the lack of cell selection processes and the short culture duration (analysis at passage 1) as some of the characteristic markers are not uniformly expressed until later passages [3, 38].

Several growth factors have positive effects on cartilage repair and in the treatment of OA. The role of PDGF in cartilage repair and homeostasis has been extrapolated from its function during chondrogenesis [2]. PDGF induces MSC proliferation [22, 25] and inhibits IL-1 β -induced chondrocyte apoptosis and inflammation [42]. PDGF was present in all samples in varying concentrations and corresponding to platelet concentrations. All three TGF- β isoforms examined have roles in chondrogenesis [7, 35, 39, 43, 44, 51, 56]. TGF- β 1 has been shown to stimulate chondrogenesis of synovium and bone marrow-derived MSCs [18, 37], inhibit IL-1 β -mediated inflammation [9], and enhance cartilage healing [12]. TGF- β 1 was increased in PRP and BMC, paralleling increased platelet concentrations. PRP and BMC-A did not differ in TGF- β 1 concentration; however, increased TGF- β 1 was present in BMC-A compared to BMC-B. These findings are likely related to platelet, monocyte, and neutrophil concentrations [31]. TGF- β 2 was present in most BMC-A and PRP samples, but only a few BMC-B samples. In OA chondrocytes, TGF- β 2 decreases collagen type 2 cleavage and chondrocyte hypertrophy through inhibition of IL-1 β and TNF- α [49]. Many cell types secrete TGF- β 2, thus differences in cellular distribution may be the source of the divergent TGF- β 2 concentrations.

In balance with growth factors, PRP and BMC products contain pro-inflammatory cytokines. IL-1 β and IL-8 are secreted by monocytes, neutrophils, and MSCs [19, 29, 52]. In WB and PRP, IL-1 β and IL-8 were undetectable, but they were present in BMA and increased in both BMC groups. This is likely a result of concentration of

neutrophils, monocytes, and MSCs during centrifugation [4]. IL-8 is a potent chemoattractant for neutrophils, which secrete IL-1, and in turn, can further stimulate monocytes to produce IL-8 [4, 8, 32]. This might suggest that PRP would be a less inflammatory, more anabolic biologic. However, the surprising and significant concentration of IL-1ra found in BMC and its absence in PRP needs to be taken into consideration when choosing between PRP and BMC for any therapeutic modality.

Interleukin 1 receptor antagonist (IL-1ra) is a naturally occurring antagonist that competitively binds to IL-1 β and IL-1 α cell surface receptors, thereby inhibiting IL-1 catabolism. IL-1ra is thought to be responsible for the beneficial effects of the biologic autologous conditioned serum (ACS) [41, 54]. Two randomized control trials in knee OA reported superiority of ACS over hyaluronan or saline injection [5, 55]. ACS is not approved for use in the USA, thus its implementation has been confined primarily to Europe. The original reported concentration of IL-1ra in ACS was 10,254 pg/mL [41], and subsequent studies documented concentrations of 2015 pg/mL [54] and <2000 pg/mL [46]. In the present study, the average concentration of IL-1ra in BMA was 4510 pg/mL, which was then increased threefold in BMC-A and fivefold in BMC-B. For effective therapy, an IL-1ra/IL-1 ratio of 10:1 to 100:1 [15, 30] has been reported as sufficient to block IL-1. The IL-1ra/IL-1 β ratio in BMC samples ranged from 249:1 to 17,568:1, indicating a net inhibitory effect on IL-1. The use of BMC products provides a patient-side method for generation of IL-1ra that would be allowed in regions such as the USA where ACS is restricted by regulatory agencies. While this study did not seek to correlate clinical outcomes with biologic components, due to the heterogeneity of surgical procedures involved, the variability of cellular and molecular components described can serve as a guide to design appropriately powered studies. Although PRP, BMC, and ACS are purported to alter the healing response through the actions of specific growth factors, stem cells, and IL-1ra, respectively, it is likely that the overall net effect of the various components will determine the clinical impact. Different tissues likely have different requirements for biologic agents, and the acute versus chronic disease progression will require distinct approaches. The ideal formulation for each tissue and disease state can only be determined through comparative clinical studies.

Conclusion

Differences in the cellular and molecular composition of PRP and BMC should be considered in light of the pathology being treated. BMC provides a patient-side method to

generate the potent anti-inflammatory therapy, IL-1ra, and deliver stem cells in addition to growth factors commonly found in PRP to patients where they might not be otherwise available due to regulatory restrictions.

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Compliance with ethical standards

Conflict of interest Author (JGK) is a consultant for Arteriocyte Medical Systems Inc. Author (LAF) is a consultant for Arthrex Inc. All samples were de-identified prior to shipment to prevent any potential biases from being introduced into the study analyses.

Ethical standards All procedures performed in studies involving human participants were approved by the institutional research review board and in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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