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Characterization of Growth Factors, Cytokines, and Chemokines in Bone Marrow Concentrate and Platelet-Rich Plasma

A Prospective Analysis

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Background: Platelet-rich plasma (PRP) and bone marrow concentrate (BMC) are orthobiologic therapies with numerous growth factors and other bioactive molecules. Before the clinical utility of PRP and BMC is optimized as a combined therapy or monotherapy, an improved understanding of the components and respective concentrations is necessary.

Purpose: To prospectively measure and compare anabolic, anti-inflammatory, and proinflammatory growth factors, cytokines, and chemokines in bone marrow aspirate (BMA), BMC, whole blood, leukocyte-poor PRP (LP-PRP), and leukocyte-rich PRP (LR-PRP) from samples collected and processed concurrently on the same day from patients presenting for elective knee surgery.

Study Design: Cross-sectional study; Level of evidence, 3.

Methods: Patients presenting for elective knee surgery were prospectively enrolled over a 3-week period. Whole blood from peripheral venous draw and BMA from the posterior iliac crest were immediately processed via centrifugation and manual extraction methods to prepare LR-PRP, LP-PRP, and BMC samples, respectively. BMA, BMC, whole blood, LR-PRP, and LP-PRP samples were immediately assayed and analyzed to measure protein concentrations.

Results: BMC had a significantly higher interleukin 1 receptor antagonist (IL-1Ra) concentration than all other preparations (all $P < .0009$). LR-PRP also had a significantly higher IL-1Ra concentration than LP-PRP ($P = .0006$). There were no significant differences in IL-1Ra concentration based on age, sex, body mass index, or chronicity of injury in all preparations. LR-PRP had significantly higher concentrations of platelet-derived growth factor AA (PDGF-AA) and PDGF-AB/BB than all other preparations (all $P < .0006$). LR-PRP also had significantly higher concentrations of matrix metalloproteinase 1 (MMP-1) and soluble CD40 ligand than all other preparations (all $P < .004$). LP-PRP had significantly higher concentrations of MMPs, namely MMP-2, MMP-3, and MMP-12, than all other preparations (all $P < .007$).

Conclusion: BMC is a clinically relevant source of anti-inflammatory biologic therapy that may be more effective in treating osteoarthritis and for use as an intra-articular biologic source for augmented healing in the postsurgical inflammatory and healing phases, owing to its significantly higher concentration of IL-1Ra as compared with LR-PRP and LP-PRP. Additionally, LR-PRP had a significantly higher concentration of IL-1Ra than LP-PRP. In cases where increased vascularity and healing are desired for pathological or injured tissues, including muscle and tendon, LR-PRP may be optimal given its higher overall concentrations of PDGF, TGF-β, EGF, VEGF, and soluble CD40 ligand.

Keywords: platelet-rich plasma (PRP); leukocyte-poor PRP (LP-PRP); leukocyte-rich PRP (LR-PRP); bone marrow aspirate (BMA); bone marrow aspirate concentrate (BMC); growth factors; cytokines; chemokines

Platelet-rich plasma (PRP) has become increasingly utilized as an adjunct to potentially promote musculoskeletal tissue healing, given the presence of growth factors and other bioactive molecules that perform a range of regenerative functions, including proliferation and recruitment of stem cells,

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activation of cellular pathways in fibroblasts, modulation of local inflammatory and fibrotic responses, and stimulation of neovascularization.[51,66] A growing number of randomized clinical studies have demonstrated the efficacy of PRP in the knee to reduce pain and improve functional outcomes in the treatment of osteoarthritis (OA).[§]

Likewise, bone marrow concentrate (BMC) has emerged as a minimally manipulated source of cells, growth factors, and cytokines that is not strictly regulated by the US Food and Drug Administration and does not require premarket approval. While there are substantially fewer clinical trials for BMC relative to PRP, good to excellent outcomes have been reported with BMC treatment of early knee OA[13,26,53,62,83] and moderate- to high-grade focal chondral defects.[16,31,32,42-46,64,96,97] Although the clinically relevant properties need to be defined, BMC—as compared with PRP—reportedly has a significantly greater concentration of interleukin 1 receptor antagonist (IL-1Ra), which inhibits IL-1β and its proinflammatory and catabolic effects (tissue degeneration).[12,83]

Another possible advantage of BMC over PRP is that it contains small numbers of mesenchymal stem cells (MSCs), which have demonstrated benefits in the regeneration of musculoskeletal tissues.[20,38,108,110] The anti-inflammatory and immunomodulatory properties of MSCs are a major area of scientific and clinical interest. MSCs were reported to improve clinical outcomes among patients undergoing high tibial osteotomy for genu varum with cartilage defects,[112] increase meniscus volume after partial meniscectomy,[106] and provide good to excellent outcomes in the treatment of chondral defects and early-stage OA.[16,18] Despite the growing focus in the lay press on the reported use of stem cells in orthobiologic injections, only 0.001% to 0.01% of mononuclear cells in bone marrow aspirate (BMA) represents MSCs.[71,88] Moreover, culture expansion may be necessary to increase stem cell numbers for any cellular-based biological effects to occur; however, no minimum therapeutic threshold concentration of stem cells currently exists. A recent study reported that while culture-expanded cells from BMC underwent chondrogenesis in vitro, minimally manipulated preparations from the same donors did not.[22] These data emphasize the importance of defining and quantifying the other clinically relevant factors that stimulate tissue healing and remodeling, such as cytokines, chemokines, and growth factors.

Before the clinical utility of PRP and BMC is optimized as a combined therapy or monotherapy in orthopaedics, an improved understanding of the components and their respective concentrations is first necessary to determine which biological treatments are best suited for specific pathological conditions. To date, the comparison of BMA and BMC composition has been limited.[12,37,58] Thus, the purpose of this study was to prospectively measure and compare anabolic, catabolic, anti-inflammatory, and proinflammatory growth factors, enzymes, cytokines, and chemokines in BMA, BMC, whole blood, leukocyte-rich PRP (LR-PRP), and leukocyte-poor PRP (LP-PRP) samples collected and processed concurrently from patients presenting for elective knee surgery. We hypothesized that there would be significant differences in the content of key bioactive molecules between BMC and PRP. Abbreviations used in this article are defined in Table 1.

TABLE 1
Abbreviations Used

	Definition
BMA	Bone marrow aspirate
BMC	Bone marrow concentrate
CD40	Cluster of differentiation 40
EGF	Epidermal growth factor
FGF	Fibroblast growth factor
GRO	Growth-regulated protein
IL	Interleukin
IL-1Ra	Interleukin-1 receptor antagonist
IP10	Interferon gamma-induced protein
LP-PRP	Leukocyte-poor platelet-rich plasma
LR-PRP	Leukocyte-rich platelet-rich plasma
MCP	Monocyte chemoattractant protein
MIP	Macrophage inflammatory protein
MMP	Matrix metalloproteinase
MSC	Mesenchymal stem cells
PDGF	Platelet-derived growth factor
PRP	Platelet-rich plasma
TGF-β	Transforming growth factor beta
TNFα	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor
WB	Whole blood

METHODS

Patient Enrollment and Demographics

After institutional review board approval (Vail Health Hospital), all consecutive patients (32 patients total) between 18 and 50 years of age presenting for elective knee surgery were screened and enrolled for combined LP-PRP and BMC injection therapy over a 3-week period. One patient

[§]References 14, 15, 23, 24, 48, 52, 60, 67, 85, 89, 90, 98, 107.

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TABLE 2
Patient Demographics and Type of Knee Surgery

	Mean (Range) or n
Age, y	34.8 (18-50)
Male:female	18:13
Body mass index, kg/m^2	26.4 (19.5-39.7)
Time from injury to surgery, wk	31.8 (0.5-200)
<6 wk	12
>6 wk	19
Knee surgery	
Ligament reconstruction	2
Meniscus repair	8
Cartilage procedure	4
Combined	17

was eliminated owing to a history of a chronic inflammatory condition (Sjögren syndrome), leaving 31 patients. Patient demographics, including sex, age, height, weight, and body mass index (BMI), were collected from each study participant (Table 2). Comorbidities were collected, including diabetes, blood dyscrasias, and inflammatory conditions, as well as anti-inflammatory medication use and tobacco smoking and/or marijuana use. Patients were excluded from the study if they had a current or previous history of blood dyscrasia or immunosuppression disorders, cancer, osteonecrosis or avascular necrosis, chronic inflammatory disease, rheumatoid arthritis, nonsteroidal anti-inflammatory drug use within 5 days of surgery, a hemoglobin level <11 g/dL, or a platelet concentration $<150 \times 10^3$/mL. This study followed the Minimum Information for Studies Evaluating Biologics in Orthopaedics (MIBO) guidelines[79] (Appendix Table A1, available in the online version of this article). This study was funded through a research endowment (see funding). Patients were not financially obligated for these injections.

Hematologic Analysis

Platelet concentration was significantly higher in LR-PRP than in all other preparations ($P < .0002$). BMC had a significantly higher concentration of leukocytes (all $P < .00002$) and monocytes ($P < .0002$) than all other preparations (Table 3).

Whole Blood Collection

Preoperatively, a certified phlebotomist cleansed the intravenous port with an alcohol swab, and 80 mL of whole blood was slowly and steadily drawn into 2 separate 60-mL disposable syringes with Luer-Lok tips (Becton, Dickinson and Company) prefilled with 3 mL of anticoagulant citrate dextrose solution formula A (ACD-A) with a 20-gauge 1.5-inch needle (Insyte Autoguard; Becton, Dickinson and Company). These syringes were capped and taken immediately to a separate on-site laboratory for processing. Sixty milliliters of whole blood was processed into 6 mL of LP-PRP for reinjection into the patient's operative knee. The remaining 20 mL of whole blood was processed into LR-PRP for research testing and analysis only.

Whole Blood Processing Technique

All whole blood samples were processed with centrifugation and manual extraction methods. Under a biosafety hood, the 80 mL of whole blood was distributed into two 30-mL samples and one 20-mL sample in separate 50-mL conical centrifuge tubes (Falcon). A 0.8-mL sample of whole blood was pipetted into a sterile microcentrifuge tube (1.5 mL; Seal-Rite) for hematology analysis with a Cell-Dyn Ruby hematology analyzer (Abbott Diagnostic Division) to perform a complete blood count (CBC), allowing measurement of platelets, leukocytes, and 6 differentials, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils in $\times 10^3/\mu L$ and erythrocytes in $\times 10^6/\mu L$ (Table 3). Then, 200 μL of whole blood sample was pipetted into a microcentrifuge tube for multiplex immunoassay and analysis.

LP-PRP Processing Technique

The two 30-mL samples of whole blood were then centrifuged at 500*g* for 10 minutes with a Sorvall ST 8 benchtop centrifuge (ThermoFisher Scientific). After the completion of this initial "separating" centrifugation, the conical tubes were taken under a biosafety hood, and a pipette (pipet controller; Falcon) was used to manually extract the excess platelet-poor plasma (PPP) layer lying just above the leukocyte layer. The leukocyte layer was purposely disrupted to release the platelets that comingle in the leukocyte layer and the top fraction of the red blood cell layer. The plasma was transferred to a 50-mL conical tube, and a second "condensing" centrifugation was performed at 3000*g* for 6 minutes to further concentrate the platelets. After the completion of this second centrifugation, the excess plasma and leukocyte layer were extracted and discarded. The cell pellet was resuspended in the resultant 6 mL of PPP, providing 6 mL of LP-PRP. Next, 0.8 mL of LP-PRP was transferred into a microcentrifuge tube for hematology analysis of the final product. Of the 0.8 mL of LP-PRP, 200 μL was pipetted into a microcentrifuge tube for immunoassay and analysis.

LR-PRP Processing Technique

Twenty milliliters of whole blood was centrifuged at 1500*g* for 10 minutes. After the initial "separating" centrifugation step, the PPP layer, leukocyte buffy coat layer, and top fraction of the erythrocyte layer were extracted and transferred into a 15-mL conical centrifuge tube (Falcon). The leukocyte-rich contents were then concentrated by centrifugation at 3000*g* for 6 minutes, followed by final manual extraction of the excess top fraction of PPP. Approximately 1 to 1.5 mL of PPP, the leukocyte layer, and a minute fraction of erythrocytes that adhere to the leukocyte layer were extracted and transferred to a

TABLE 3
Hematology Results for Blood and Bone Marrow Sources[a]

	WB	LR-PRP	LP-PRP	BMA	BMC	*P* Value
Platelets, $10^3/\mu L$	212	**1450**	599	164	901	<.0002
	(185, 236)	(1221, 1653)	(504, 737)	(135, 190)	(596, 1073)	<.002[b]
Total leukocytes, $10^3/\mu L$	5.5	26	1.6	15.9	**85.7**	<.00002
	(4.6, 6.2)	(22.6, 32)	(1, 2.4)	(13.5, 21)	(63.2, 94.8)	<.003[b]
Monocytes, $10^3/\mu L$	0.4	3.3	0.2	1.1	**6.8**	<.0002
	(0.4, 0.5)	(2.6, 4.1)	(0.1, 0.4)	(0.8, 1.5)	(5.9, 9)	<.0002[b]
Neutrophils, $10^3/\mu L$	3.0	9.1	0.2	10.6	**45.1**	<.00002
	(2.4, 3.9)	(5.6, 12.8)	(0.1, 0.6)	(8.7, 14)	(35.9, 57.5)	<.00002[c]
Erythrocytes, $10^6/\mu L$	**4.3**	2	0.1	3.9	2.4	<.00002
	(4.1, 4.6)	(1.7, 3)	(0.1, 0.3)	(3.7, 4.3)	(2.1, 2.9)	<.00002[d]

[a]Results are given as median (first quartile, third quartile). Values given in bold are significantly higher than all other preparations.
[b]All pairwise comparisons were significant.
[c]All pairwise comparisons were significant except LR-PRP vs BMA.
[d]All pairwise comparisons were significant except LR-PRP vs BMC.

15-mL conical tube. This resulted in approximately 2 mL of LR-PRP. Approximately 0.8 mL of LR-PRP was transferred into a microcentrifuge tube for hematology analysis of the final product. Of the 0.8 mL of LR-PRP, 200 μL was pipetted into a microcentrifuge tube for immunoassay and analysis.

All PRP samples were processed within 1 hour of the blood draw. Hematology analysis included quantification of the final CBC, which was used to quantify the platelet fold difference from baseline CBC. Platelets were not activated with calcium chloride or thrombin, because these factors were reported to possibly influence the biological effects of PRP.[1] Moreover, in previous quantitative analysis of platelet-derived growth factor AB (PDGF-AB), PDGF-BB, and transforming growth factor β1 (TGF-β1), no significant differences in concentrations were reported between inactivated and activated platelets.[68] No other diluents were added that could change the viscosity of the cell suspension or induce Rouleau-formation among erythrocytes. The PRP samples were then immediately assayed and analyzed for quantification of cytokine, chemokine, and growth factor composition and concentration. Whole blood and PRP were in liquid form at room temperature for processing and analysis. No samples were frozen because freezing was demonstrated to alter factor profiles (unpublished data from our center). Moreover, it was reported that bioactive growth factor and cytokine concentrations remain stable in whole blood, LR-PRP, and LP-PRP up to 4 hours from blood draw (K.E. Whitney, BS, unpublished data, 2019).[111]

Bone Marrow Aspiration Technique

BMA was harvested with a previously reported technique.[17] Each patient was positioned prone. Light conscious sedation was administered and monitored by an anesthesiologist. After palpation of the bony landmarks of the posterior iliac crest and sacroiliac joint, the procedural site was sterilely prepared and draped. The skin and soft tissue were then injected down to and including the periosteum of the harvest site with approximately 6 to 7 mL of 1% lidocaine without epinephrine with a 22-gauge needle (BD SafetyGlide; Becton, Dickinson and Company). The BMC aspiration kit (MarrowStim; Biomet Biologics) and the BMC aspiration drill (Powered Bone Marrow Biopsy System; Arrow OnControl) were sterilely draped. The provided 18-gauge BMA trocar and 18-gauge needle were percutaneously inserted until they reached the posterior iliac crest. The drill was then used to insert the trocar and needle into the medullary cavity of the posterior iliac crest. A syringe preloaded with 1 mL of ACD-A was injected into the inserted trocar to minimize the risk of coagulation. Approximately 60 mL of BMA was steadily aspirated into two 30-mL syringes (BD Luer-Lok tip; Becton, Dickinson and Company) preloaded with 5 mL of ACD-A. Syringes were capped and directly taken to an on-site laboratory for processing and analysis. For testing purposes, 200 μL of BMA was collected into a microcentrifuge tube with a pipette.

Bone Marrow Processing Technique

Centrifugation and manual extraction methods were used to isolate BMC. Under a biosafety hood, BMA was transferred into two 50-mL conical tubes, and 0.8 mL was pipetted into a microcentrifuge tube for hemanalysis with the Cell-Dyn Ruby hematology analyzer. A CBC with differential was automatically recorded before processing. The BMA was centrifuged with a Sorvall ST 8 benchtop centrifuge at 1500*g* for 10 minutes. After the completion of the soft spin centrifugation, the top fraction of PPP was extracted until 5 mL of PPP remained. All remaining levels were resuspended with the PPP layer and transferred to a 60-mL syringe preloaded with 2 mL of ACD-A. This sample was then filtered through an 18-μm blood filter (Hemo-Nate; Utah Medical) into a 50-mL conical tube to filter potential clots. A second centrifugation was performed at 3000*g* for 6 minutes. After this hard spin centrifugation, excess PPP from the top fraction was discarded, and the

remaining 5 mL of PPP and leukocyte layer were extracted together, resulting in 6 mL of BMC. A 0.8-mL sample of BMC was transferred into a microcentrifuge tube used for hematology analysis of the final BMC product. Of the 0.8 mL of BMC, 200 μL was pipetted into a microcentrifuge tube for immediate immunoassay and analysis. BMA was processed within 1 hour of the blood draw. Hematology analysis included quantification of the final CBC. No other diluents were added that could change the viscosity of the cell suspension or induce Rouleau formation among erythrocytes. BMA and BMC were in liquid form at room temperature for processing, immunoassay, and analysis.

Quantification of Growth Factor and Cytokine/Chemokine Composition

The LR-PRP and LP-PRP from the separate 200-μL samples were pipetted into microcentrifuge tubes for Luminex multiplex immunoassays (EMD Millipore Corp) that measured the concentrations of growth factors, cytokines, and chemokines. Specific immunoassay kits (EMD Millipore Corp) used were a human cytokine/chemokine magnetic bead panel, TGF-β magnetic bead panel, MMP magnetic bead panel 1, and MMP magnetic bead panel 2. Factors analyzed are listed in Tables 3 to 5 and Appendix Table A2 (available online). A standard manufacturer's protocol for the Luminex 200 (Luminex Corp) multiplex instrument was utilized as previously published.[78] All reagents were prepared and stored according to the manufacturer's instructions. Briefly, background, standards, and controls were added in duplicate to the appropriate wells with serum matrix solution. The unknown samples were subsequently added in duplicate, with premixed growth factor, cytokine, and chemokine antibody-immobilized magnetic beads. The plate was sealed and covered with foil during incubation with agitation at 600 rpm. With a handheld magnet, the plate was washed 2 times with the 1× wash buffer provided. Detection antibodies were added to the plate and incubated at room temperature for 30 minutes at 600 rpm. Streptavidin-phycoerythrin solution was added and incubated at room temperature for 30 minutes at 600 rpm. After 2 plate washes, drive fluid was added to resuspend the beads at 300 rpm for 5 minutes. Finally, the plate was analyzed with the Luminex 200 xPONENT 3.1 system (Luminex Corp) with xPonent software (EMD Millipore Corp), which created a standard curve for each analyte via a 5-parameter logistic curve-fitting method with the median fluorescent intensity. Growth factor, cytokine, and chemokine concentrations in the unknown samples were calculated with this method.

Quantification Metrics for BMC and PRP Relative to Starting Samples

The composition, total volume, and concentration of nucleated cells, platelets, and erythrocytes in the BMC and PRP samples were compared with the baseline samples of BMA and whole blood, respectively (Appendix Table A1, available online). The fold change in cell concentration in BMA, BMC, whole blood, LR-PRP, and LP-PRP was determined. The growth factors and proteins with approximately ≥80% detectable data were delineated (Table 3). Finally, the growth factor and protein composition and concentrations of BMC, LR-PRP, and LP-PRP were compared (Tables 4-6).

Biologics Delivery

After each patient's elective knee surgery, 6 mL of LP-PRP and 6 mL of BMC were injected into the operative knee while the patient was still under anesthesia. A 10-mL 1:1 dual-injection syringe and FibriJet applicator assembly (Nordson Medical) were used, and no additional media were injected.

Statistical Analysis

An a priori power and sample size calculation was made as informed by data from a similar previous study.[12] For the primary comparison of IL-1Ra level in BMC versus BMA, an estimated observed effect size of $d = 1.0$ was reported. To make this estimation, the approximation SD ≈ (Q3-Q1) / 1.35 was used, and a correlation of 0.5 was assumed between IL-1Ra in BMC and BMA samples in the same patient. For this study, we conservatively assumed a Bonferroni correction among all 10 pairwise comparisons, 2-tailed testing, and an overall alpha level of .05. Eighteen patients were sufficient to detect this magnitude of effect size with 80% statistical power. In the same study, the comparison between BMC and PRP exhibited a larger effect size; thus, we assumed correspondingly higher statistical power to test this co–primary hypothesis. Our plan was to prospectively enroll all patients over a 3-week period such that at least 18 patients would be included.

Many factors exhibited varying degrees of skew and missing or nondetectable values. We report only the factors with ≥80% detectable values (within the detectable range for each factor, measured in pg/μL). Factors that had <80% detection (values that were above or below the standard curve) were excluded from the final analysis. We tested an array of factors and cytokines with multiple bead panels specific for certain factors. Nondetect results were believed to be due to bead aggregation (clumping), which occurs more often when there are several bead panels present. There were also several factors with low concentrations that were out of range of the standards but were still largely within detectable limits of the fitted curve. In these factors, nondetect results may have been due to low concentrations and/or bead aggregation. Among the factors exhibiting highly detectable data, subthreshold values, or values not in the detectable range, were excluded from final analysis. The Skillings-Mack test, a nonparametric method for block designs with missing observations and reserved for repeated measures, was used to test the overall null hypothesis of equivalency among the 5 preparations. When that was statistically significant, pairwise comparisons were conducted with the dependent-samples Wilcoxon signed rank tests and Holm method to account for multiple comparisons. Secondarily, Spearman correlation and Mann-Whitney *U*

TABLE 4
Inflammatory/Catabolic Factors, Enzymes, and Cytokines in Whole Blood, LR-PRP, LP-PRP, BMA, and BMC[a]

	Preparation, pg/μL					
	WB	LR-PRP	LP-PRP	BMA	BMC	*P* Value
Eotaxin	**351.8**	253.3	121.2	232	177.6	<.00006
	(316.5, 449.8)	(197.3, 305)	(103.7, 175.9)	(179, 291.3)	(158.9, 214.7)	<.002[b]
FGF-2	61.7$^{+\omega\#}$	100.7$^{-\#}$	90.4$^{\omega}$	176.2$^{\wedge}$	229.6$^{\wedge-+}$	.010$^{\wedge}$
	(35.7, 211.6)	(57.9, 208.5)	(49.3, 294.7)	(68.5, 272.4)	(166.6, 541.1)	.006^{-}
						.013^{+}
						.0009$^{\omega}$
						.006$^{\#}$
GRO	423.4$^{+\omega}$	976.1$^{\wedge\omega}$	944.7^{-+}	246.7[c]	440.5$^{\wedge-}$	<.04[c]
	(306.7, 704.5)	(539.5, 1795.5)	(655.7, 2143)	(197.8, 331.9)	(270.6, 611.9)	.004$^{\wedge}$
						.0003^{-}
						.00004^{+}
						.0003$^{\omega}$
IL-8	9.3^{+}	12.5^{-}	9.7$^{\wedge}$	7.6$^{\wedge-+}$	10.2	.03$^{\wedge}$
	(6.3, 15.8)	(5.6, 23)	(4.7, 17.2)	(3, 10.2)	(4.9, 13.9)	.04^{-}
						.0006^{+}
IP10	166.4^{-}	188.4	194.2$^{\wedge-}$	164.7$^{\wedge}$	204.5	.008$^{\wedge}$
	(126.2, 231.5)	(146.2, 266.3)	(158.6, 292)	(132.8, 224.2)	(140, 297.6)	.002^{-}
MCP1	**903.2**	145.9^{-}	196.9^{+}	476$^{\wedge-+}$	283.6$^{\wedge}$	<.00006
	(748.9, 1312)	(108.3, 362.6)	(158.4, 263.9)	(351.5, 717.5)	(119.7, 377.1)	.001$^{\wedge}$
						.0007^{-}
						.00002^{+}
MIP1b	20.8$^{\omega\#\&}$	31.7$^{+\#}$	24$^{-\omega}$	11.2$^{\wedge-+\&}$	32.2$^{\wedge}$	.03$^{\wedge}$
	(13.1, 37.8)	(25.8, 42)	(12.5, 69.2)	(6.6, 20.9)	(21.8, 54.9)	.0004^{-}
						.003^{+}
						.03$^{\omega}$
						.02$^{\#}$
						.001$^{\&}$
MMP-1	1157$^{+\omega}$	**3125**	1731$^{-\omega}$	1104$^{\wedge-+}$	1501$^{\wedge}$	<.004
	(746.6, 2817)	(2595, 6987)	(1117, 3081)	(448.4, 2199)	(619.9, 2622)	.010$^{\wedge}$
						.0004^{-}
						.005^{+}
						.012$^{\omega}$
MMP-2	57,222	63,288	**95,962**	29,842	12,045	<.00006
	(40,168, 75,359)	(23,999, 107,452)	(58,876, 132,642)	(11,604, 48,581)	(3575, 34,557)	<.0002[d]
MMP-3	11,171$^{-\omega}$	9909$^{\wedge+}$	**19,292**	6678$^{+\omega}$	6176$^{\wedge-}$	<.007
	(8997, 15,708)	(6759, 14,791)	(8457, 26,915)	(3096, 9122)	(2130, 8464)	.001$^{\wedge}$
						.001^{-}
						.017^{+}
						.013$^{\omega}$
MMP-12	231.4$^{-\omega}$	264.8$^{\wedge+}$	**582.7**	126.5$^{+\omega}$	97.6$^{\wedge-}$	<.003
	(159.1, 363)	(196, 337.3)	(332.2, 927.7)	(95.5, 138.4)	(88.5, 160.1)	.005$^{\wedge}$
						.006^{-}
						.002
						.002$^{\omega}$
sCD40L[e]	1809$^{\omega}$	**5523**	2187^{-+}	458.6$^{\wedge+\omega}$	1669$^{\wedge-}$	<.005
	(1251, 2468)	(2833, 8022)	(1218, 3698)	(310.1, 1024)	(1233, 2615)	.0003$^{\wedge}$
						.040^{-}
						.00003^{+}
						.00003$^{\omega}$
TNFα	7.1$^{\omega\#\times}$	8.9$^{+\#}$	10.7$^{\wedge-\times}$	4.6$^{-+\omega}$	4.2$^{\wedge}$	.031$^{\wedge}$
	(4.5, 10.7)	(7, 14.5)	(7, 15.9)	(3.6, 5.4)	(2.5, 7.7)	.023^{-}
						.031^{+}
						.031$^{\omega}$
						.031$^{\#}$
						.0008$^{\times}$

[a]Results are given as median (first quartile, third quartile). Values given in bold are significantly higher than all other preparations in pairwise comparisons. Additional shared symbol between groups corresponds to the shared *P* value based on significant pairwise comparison.
[b]All pairwise comparisons significant ($P < .002$) except LR-PRP vs BMA.
[c]BMA was significantly lower in GRO than all other preparations ($P < .04$).
[d]All pairwise comparisons significant ($P < .0002$) except LR-PRP vs whole blood.
[e]sCD40L is also angiogenic.

TABLE 5
Anabolic Growth Factors in Whole Blood, LR-PRP, LP-PRP, BMA and BMC[a]

	Preparation, pg/μL					
	WB	LR-PRP	LP-PRP	BMA	BMC	*P* Value
EGF	34.2$^{\omega}$ (18.5, 53)	69.7$^{\wedge\sim\omega}$ (35.5, 126.6)	34.5^{+} (22.4, 75.8)	23.5$^{\sim+}$ (14.4, 41.2)	26.5$^{\wedge}$ (14.9, 50.3)	.007$^{\wedge}$ .013$^{\sim}$ .028^{+} .007$^{\omega}$
PDGF-AA	5152 (4261.5, 7717)	**20,347** (14,159, 32,861)	11,535 (7383, 15,137)	2942 (1770, 3921)	11,358 (8825, 14,421)	<.0004 <.001[b]
PDGF-AB/BB	103,666$^{\sim\omega}$ (68,012, 146,308)	**376,949** (243,203, 645,125)	170,374$^{+\omega}$ (92,479, 297,433)	86,008$^{\wedge+}$ (57,097, 105,234)	190,942$^{\wedge\sim}$ (123,493, 293,249)	<.0006 .0006$^{\wedge}$ .0005$^{\sim}$ .003^{+} .003$^{\omega}$
TGF-β1	51,902$^{\sim\#\times}$ (28,779, 71,182)	226,959$^{+\#}$ (154,992, 323,105)	184,077$^{\omega\times}$ (125,981, 240,741)	46,679$^{\wedge+\omega}$ (17,943, 69,419)	230,974$^{\wedge\sim}$ (126,265, 286,380)	.0001$^{\wedge}$ .00008$^{\sim}$ .00007^{+} .00004$^{\omega}$ .00004$^{\#}$ .00004$^{\times}$
TGF-β2	9770$^{+\perp\partial}$ (5632, 14,319)	42,698$^{\omega\times\perp}$ (26,764, 54,318)	28,685$^{\sim\#\times\partial}$ (16,307, 37,604)	9002$^{\wedge\omega\#}$ (3859, 12,544)	36,093$^{\wedge\sim+}$ (20,737, 51,453)	.00003$^{\wedge}$ .027$^{\sim}$ .00002^{+} .00004$^{\omega}$ .00003$^{\#}$ .008$^{\times}$ .00002$^{\perp}$ .00002$^{\partial}$
VEGF	55.2$^{\sim}$ (28.6, 250.5)	126.7 (60.3, 250.2)	138.1$^{\wedge\sim}$ (53.2, 508)	51.6$^{\wedge}$ (36.5, 298)	91.1 (64.2, 237.9)	.025$^{\wedge}$ .0004$^{\sim}$

[a]Results are given as median (first quartile, third quartile). Values given in bold are significantly higher than all other preparations in pairwise comparisons. Additional shared symbol between groups corresponds to the shared *P* value based on significant pairwise comparison.

[b]All pairwise comparisons were significant ($P < .001$) except LP-PRP vs BMC.

tests were used to test for associations between factors and demographic covariates. The statistical software R was used for all analyses.[84]

RESULTS

Patient Demographics

Thirty-two patients were enrolled in the study. One patient was eliminated owing to a history of a chronic inflammatory condition (Sjögren syndrome), leaving 31 patients included in the analysis. Patient demographics are listed in Table 2.

All pairwise comparisons for leukocyte concentration were significantly different between preparations (all $P < .003$). All pairwise comparisons for monocyte concentration were significantly different between preparations (all $P < .0002$). Finally, all pairwise comparisons of erythrocytes were significantly different (all $P < .00002$), except between LR-PRP and BMC ($P = .06$).

There was a median (first quartile, third quartile) 2.9-fold increase (2.5, 3.3) in platelet concentration and a median 0.3-fold decrease (0.2, 0.4) in leukocyte concentration in LP-PRP as compared with whole blood. There was a median 7.2-fold increase (5.7, 7.8) in platelet concentration in LR-PRP and median 5.1-fold increase (4.2, 6.0) in leukocyte concentration in LR-PRP as compared with whole blood.

Proinflammatory Factors, Cytokines, and Chemokines Analysis

Appendix Table A2 (available online) lists the catabolic/proinflammatory factors and cytokines as well as MMPs. IL-1β is an important and clinically relevant proinflammatory cytokine that was largely nondetectable, with a high subthreshold nondetect rate (66.4%). Because of these missing data, we did not include this cytokine in our final analysis. For eosinophil chemotactic protein (eotaxin) and monocyte chemoattractant protein 1, whole blood had significantly higher respective concentrations than all other preparations (all $P < .00006$). BMA was significantly lower in growth-regulated oncogene than all

TABLE 6
IL-1Ra Concentration Across Biologic Preparations and Results Based on Age, Sex, and Injury Chronicity[a]

	Preparation, pg/μL					
IL-1Ra	WB	LR-PRP	LP-PRP	BMA	BMC	*P* Value
Overall	143 (72.4, 195.4)	257.8 (100.7, 408.6)	48.9 (34.3, 76.8)	463 (328.7, 560.2)	**796.6** (579.2, 1288)	<.0009 <.003[b]
Age, y						>.440
<35 (n = 15)	124.9 (74.2, 163.3)	257.8 (92.2, 408.6)	55.1 (28.1, 77.1)	492.3 (362.1, 602.9)	842.1 (619.1, 1050)	
>35 (n = 16)	172.4 (66.3, 255.6)	239.2 (113, 389)	41.5 (35.7, 71.7)	419.3 (313.1, 541.4)	679.6 (463.5, 1353)	
Sex						>.15
Male (n = 18)	145.9 (73.4, 200.1)	267.7 (111.6, 426.9)	55.3 (36, 104.7)	478.1 (377, 626.8)	973.4 (683.2, 1375)	
Female (n = 13)	142.4 (58.7, 176.3)	176.5 (85.2, 374.6)	35.8 (31.6, 67.9)	352.6 (173.4, 499.1)	616.3 (366, 1006)	
BMI, kg/m^2						>.20
<25 (n = 11)	124.9 (56.4, 177)	281.2 (67.5, 396.8)	48.9 (35.8, 77.1)	383 (216.5, 511)	790.9 (295.6, 1050)	
>25 (n = 20)	155.6 (78.3, 217.1)	229.3 (110.6, 407.8)	46.2 (34.1, 75)	463.4 (334.1, 730.1)	796.6 (605.2, 1462)	
Chronicity						>.21
Acute (n = 12)	152.7 (87.1, 338.9)	188.7 (64, 349.9)	47.5 (34.4, 94.3)	494.3 (307.8, 605.9)	1063 (506.9, 1505)	
Chronic (n = 19)	124.9 (72.4, 177)	281.2 (107.1, 417.3)	48.9 (34.7, 70.8)	383 (328.7, 544.1)	784 (607, 1040)	

[a]Results are given as median (first quartile, third quartile). Values given in bold are significantly higher than all other preparations. There are no significant differences based on age, sex, BMI, or chronicity of injury, with the lowest *P* value among the preparations listed in the table. Acute, <6 weeks; chronic, >6 weeks.
[b]All pairwise comparisons were significant ($P < .003$).

other preparations (all $P < .04$). For MMP-1 and sCD40L, LR-PRP had significantly higher concentrations than all other preparations (all $P < .004$). For 3 of 4 MMPs (ie, MMP-2, MMP-3, and MMP-12), LP-PRP had significantly higher concentrations than all other preparations (all $P < .007$). BMC had a significantly lower concentration of MMP-2 than all other preparations (all $P < .00006$) (Table 4).

Growth Factor Analysis

Appendix Table A2 (available online) lists the growth factors. For epidermal growth factor (EGF), LR-PRP had a significantly higher concentration than whole blood, BMA, and BMC (all $P < .05$). LR-PRP also had significantly higher concentrations of PDGF-AA and PDGF-AB/BB than all other preparations (all $P < .0006$). There were no significant differences among BMC, LP-PRP, and LR-PRP for TGF-β1. For TGF-β2, LR-PRP and BMC had significantly higher concentrations than whole blood, LP-PRP, and BMA (all $P < .04$) (Table 5).

Interleukin 1 Receptor Antagonist

BMC had a significantly higher IL-1Ra concentration than all other preparations ($P < .0009$). All pairwise comparisons among preparations were significant ($P < .003$). BMA had a significantly higher concentration of IL-1Ra than whole blood, LR-PRP, and LP-PRP ($P < .003$). LR-PRP also had a significantly higher IL-1Ra concentration than LP-PRP ($P = .0006$). There were no significant differences in IL-1Ra concentration based on age, sex, BMI, or chronicity of injury (Table 6, Figure 1).

DISCUSSION

The most important finding of this study was that there were specific growth factors, cytokines, and chemokines present in variable concentrations depending on the biologic preparation. In particular, BMC provided a significantly higher concentration of IL-1Ra as compared with whole blood, LR-PRP, LP-PRP, and BMA. There were no significant differences in IL-1Ra when grouped by age, sex, BMI, or chronicity of injury for all preparations. LR-PRP also had a significantly higher IL-1Ra concentration than LP-PRP. In addition, our study demonstrated that LP-PRP had significantly higher concentrations of MMP-2, MMP-3, and MMP-12 than all other preparations, while BMC had a significantly lower concentration of MMP-2 than all other preparations. LR-PRP had significantly higher concentrations of MMP-1, sCD40L, PDGF-AA, and PDGF-AB/BB than all other preparations. Moreover, LR-PRP and BMC had significantly higher concentrations of TGF-β2 than whole blood, LP-PRP, and BMA.

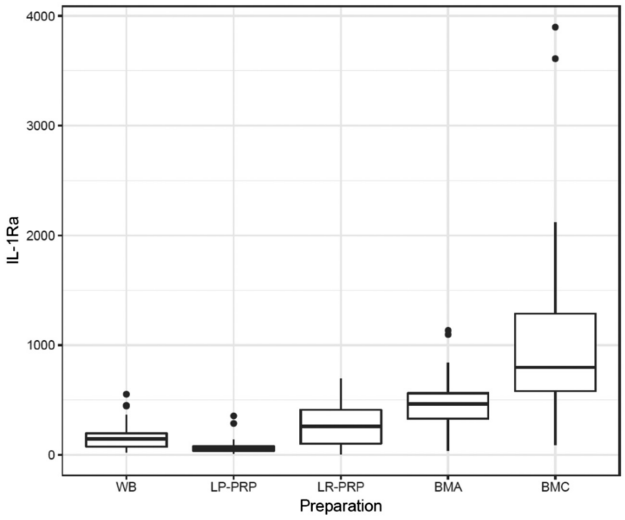

Figure 1. Interleukin 1 receptor antagonist (IL-1Ra) concentration (pg/μL) in whole blood (WB), leukocyte-poor platelet-rich plasma (LP-PRP), leukocyte-rich platelet-rich plasma (LR-PRP), bone marrow aspirate (BMA), and bone marrow concentrate (BMC). All pairwise comparisons were significant ($P < .003$), with BMC being significantly higher in IL-1Ra than all other preparations ($P < .0009$). Boxplots represent median, interquartile range, and extreme values.

In the current study, BMC had significantly higher concentrations of monocytes, neutrophils, and total leukocytes versus the other biologic preparations. The higher concentration of monocytes in BMC is intuitive given that it represents one of the most readily available minimally manipulated sources of MSCs used in common orthopaedic practice.[16] MSCs are a type of monocyte that constitutes only a limited component of the total monocytes present in BMC.[71,88] Other clinically relevant factors and cytokines, including IL-1Ra, have been implicated in the anti-inflammatory and regenerative processes.[12,16] To date, the degree to which MSCs secrete growth factors and anti-inflammatory molecules in response to inflammatory molecules is still being defined.

We found that BMC had a significantly greater concentration of IL-1Ra when compared with LR-PRP and LP-PRP. Similarly, Cassano et al[12] compared IL-1Ra in BMC and PRP; however, they did not compare BMC with LR-PRP and LP-PRP. Oliver et al[83] also assessed IL-1Ra concentration in BMC from a small subset of patients but made no comparisons with other biologics products. IL-1Ra is a competitive antagonist that binds to IL-1β and IL-1α (isoforms of IL-1) cell surface receptors, thereby inhibiting IL-1 catabolism and its proinflammatory effects.[12] IL-1Ra represents a target to reduce IL-1β-mediated inflammation and matrix degradation.[104] IL-1β reportedly induces several inflammatory and catabolic effects, including MMP-3 and tumor necrosis factor gene expression,[100] nitric oxygen production and prostaglandin E2 release,[47] chondrocyte apoptosis and inflammation,[76] and inhibition of proteoglycan and collagen synthesis.[34,99] In particular, IL-1β is a major inflammatory cytokine promoting the catabolic activity and intra-articular destructive effects associated with OA.[6] IL-1Ra functions to neutralize IL-1β and is theorized to be responsible for the beneficial effects and pain relief with use of BMC.[109] Animal studies reported less articular cartilage erosions associated with IL-1Ra administration through intra-articular injection of the protein[11] and via gene transfer.[86] In an experimental equine OA model, in vivo delivery of the IL-1Ra gene reportedly resulted in significant improvement in clinical parameters of pain and disease activity, preservation of articular cartilage, and beneficial effects on histological parameters of the synovial membrane and adjacent articular cartilage.[39] Moreover, a prospective randomized controlled double-blind trial of human patients reported that IL-1Ra is more effective than placebo and/or hyaluronic acid for the treatment of knee OA.[4]

Therefore, BMC may serve as a more appropriate anti-inflammatory biologic to use clinically given the greater concentration of IL-1Ra versus LR-PRP and LP-PRP, especially for intra-articular knee injections for OA. Likewise, clinical studies reported promising results with the intra-articular use of BMC for the treatment of knee OA[13,26,53,62,83,95] and as augmented treatment with concurrent cartilage procedures for moderate- to high-grade focal chondral deficits.[16,31,32,42-46,64,96,97] Thus, the anti-inflammatory benefit of BMC appears to have applications in settings of acute inflammation as well as after the postsurgical inflammatory and healing phases.

Regarding inflammatory cytokines, we found no significant difference among preparations in IL-8, a particularly potent inflammatory cytokine with increased expression during times of inflammation.[5,91] This was similar to the finding of Sugaya et al,[101] who reported no significant difference in IL-8 between BMC and PRP, but dissimilar to that of Cassano et al,[12] who reported greater IL-8 concentration in BMC as compared with undetectable levels in PRP. Similar to the Cassano et al[12] work, the current study found that IL-1β was largely nondetectable in samples with a high subthreshold nondetect rate of 66.4% in our data. There were no significant differences among preparations with respect to this nondetect rate. Given the high nondetection rate, we chose not to include IL-1β in our final data analysis. However, with the available detectable IL-1β data, the IL-1Ra:IL-1β ratio was 443:1. Cassano et al[12] reported an IL-1Ra:IL-1β ratio in BMC ranging from 249:1 to 17,568:1. Likewise, Oliver et al[83] reported an IL-1Ra:IL-1β ratio in BMC ranging from 193.5:1 to 720.6:1. For effective therapy, an IL-1Ra:IL-1 ratio of 10:1 to 100:1 was reported to be sufficient to inhibit IL-1.[27,50] These data suggest that the concentration of IL-1Ra in BMC could have a strong net inhibitory effect on IL-1β and further support its use as an anti-inflammatory for knee OA or in the postsurgical inflammatory and healing phases.

Our results demonstrated that LP-PRP had significantly higher concentrations of catabolic proteases, including MMP-2, MMP-3, and MMP-12, than all other preparations, while LR-PRP was significantly higher in MMP-1 versus all other preparations. Prior studies reported higher concentrations of MMP-2, MMP-3, and/or MMP-9 in LR-PRP than in

LP-PRP.[87,102] The contrasting results observed with MMP-2 and MMP-3 may be partly due to differences in preparation and activation methods (no platelet activation in our study); however, higher MMP concentrations are reported to correlate with higher leukocyte and platelet concentrations.[87,102] MMPs are implicated in normal wound healing but also chronic nonhealing wounds/ulcers when present in excessive amounts.[9,41] These proteases regulate endothelial extracellular matrix turnover by maintaining physiological balance between pro- and antiangiogenic factors through the activation of numerous growth factors, including TGF-β and VEGF. It was reported that MMPs are also involved in the recruitment and mobilization of progenitor cells from bone marrow and their incorporation at sites of injury during vascular repair.[105] When compared with all other preparations, including LP-PRP, LR-PRP also had a significantly higher concentration of sCD40L, which was reported to be both inflammatory and prothrombotic,[3,30,55,92] as well as angiogenic.[8,25,29,75] The CD40L/CD40 axis has an important role in inflammation primarily by increasing expression of cell adhesion molecules, proinflammatory cytokines, chemokines, and MMPs.[2,19,36]

In the current study, LR-PRP had significantly higher concentrations of PDGF-AA and PDGF-AB/BB than all other preparations and a significantly higher concentration of EGF than BMA, BMC, and whole blood. PDGF induces MSC proliferation and angiogenesis[37,40] and inhibits IL-1β-induced chondrocyte apoptosis and inflammation.[76] EGF reportedly plays a major role in cell proliferation, differentiation, and growth and is involved in promoting wound healing by stimulating epidermal and dermal repair, thereby demonstrating its powerful effect on the formation of granulation tissue.[93] LR-PRP and BMC also had significantly higher concentrations of TGF-β2 than whole blood, LP-PRP, and BMA. Regarding TGF-β1, there were no significant differences in concentration among LR-PRP, LP-PRP, and BMC. In contrast, Sundman et al[102] reported significantly higher TGF-β1 concentration in LR-PRP versus LP-PRP. PDGF and TGF-β are reported to have anabolic and anti-inflammatory effects[57,73,94] and are important factors in tendon healing.[94] TGF-β is known for its recruitment of fibroblasts and macrophages during wound healing,[63] regulation of MSC proliferation and differentiation,[49,59] MMP inhibition and downregulation of proteinase activity[63] to increase production of collagen types I and III,[63] and it was identified as a key growth factor involved in the development of fibrosis, with TGF-β1 and TGF-β2 being the profibrotic isoforms.[56,69,77] TGF-β1 reportedly stimulates chondrogenesis of synovium and bone marrow–derived MSCs,[33,65] enhances cartilage healing,[21] and inhibits IL-1β-mediated inflammation.[7] Likewise, TGF-β2 is reported to decrease collagen type 2 cleavage and chondrocyte hypertrophy in human OA cartilage through inhibition of IL-1β and tumor necrosis factor α, thereby demonstrating its anti-inflammatory effect.[103]

LR-PRP is frequently used in the treatment of tendon and muscle injury,[66] and our results of significantly increased PDGF-AA, PDGF-AB/BB, and TGF-β2 support its use for these applications. Higher concentrations of PDGF in LR-PRP versus LP-PRP are consistent with prior literature.[102] In addition, LR-PRP and LP-PRP had the highest concentrations of VEGF, which is a promoter of angiogenesis and thus tissue healing[10,35]; however, VEGF reportedly negatively affects articular cartilage healing by stimulating vascular invasion and ossification.[74] With its influence on angiogenesis already substantiated,[10,35] VEGF was reported to improve healing and biomechanical tensile properties over saline injection in a rat Achilles tendon rupture model.[61] Therefore, in addition to its significantly greater concentration of proangiogenic sCD40L, our results provide support for the use of LR-PRP as an overall source of PDGF, TGF-β, EGF, and VEGF growth factor delivery to diseased tendon or muscle.

The necessity of standardized preparation protocols and composition reporting that allow comparison among studies and provide reproducibility has been established for PRP[15] and BMC.[80] The minimum reporting requirements for biological outcomes (MIBO) evaluating PRP and MSCs are also established[79] and were adhered to in this study. In contrast to standard pharmaceuticals with an established bioactive substance concentration administered to accomplish a specific biological effect, most biologics form an extremely complex milieu of varying composition that is difficult to characterize. The cumulative effect of the various constituents in BMC and PRP determines the overall clinical effect. The composition of BMA obtained also depends on the clinical and biological attributes of the patient, including sex, age, harvest location, BMA processing technique, and BMA volume obtained.[28,54,70,81,82,113] In addition, similar variability is reported for PRP.[15,72] The substantial heterogeneity in procedural designs and reporting standards by studies conducted with different outcome measures, generally poor methodology, and a lack of randomized controlled trials[15,18,66,80] provides further limitations of the current literature. We strove to follow the MIBO guidelines to provide the best recommended current methodology and reporting on the harvesting and preparation of these biological agents.

Limitations to this study primarily center on the exclusion of some factors and cytokines owing to a high rate of nondetectable, typically subthreshold values. We reported only the factors/cytokines with approximately ≥80% detectable data (within the detectable range for each factor measured in pg/μL). We tested for an array of factors and cytokines using multiple bead panels specific for certain factor/cytokine groups. As a result, nondetectable data, primarily subthreshold values, were most likely due to bead aggregation (clumping), which occurs more often when multiple bead panels are present. We noticed bead aggregation primarily in the 38-plex kit, where more beads were present. Certain kits (ie, 3-plex or TGF-β) had fewer analytes and were less likely to aggregate. Furthermore, we expect that the cellular content within the BMA aspirated is not uniform, with the first 2 to 5 mL of BMA aspirated being rich in cell content, with that in the remaining BMA lower. Further studies are required to develop a methodology to provide a uniform cellular content with the BMA aspiration. Another area for improvement in future studies is more

frequent needle adjustment during aspiration. Muschler et al[81] reported that needle adjustment every 1 to 2 mL of aspirated volume improves osteoblast progenitor cell yield. Although we did adjust the position of the needle when aspirating between 30-mL syringes, we did not do so every 2 mL. As much as individual patient factors are important in the quality and content of the BMA, frequent needle adjustment could improve cell and growth factor yield and may influence the yield results. Likewise, we had some variability in blood cell concentrations—specifically, the total white blood cell counts. There was heterogeneity among BMC preparations and a lack of blood cell concentration references to follow, thus highlighting the importance of having preparation protocols and blood cell–referenced values, which need to be further investigated and standardized for these products.

Furthermore, we used inactivated samples, which is the standard for clinical use at our center. When biologic products are introduced to human tissues, platelets degranulate and released growth factors and cytokines. The growth factor and cytokine concentrations, including MMPs, and the observed relationships among different products could vary from measured levels after platelet degranulation.

CONCLUSION

BMC is a clinically relevant source of anti-inflammatory biologic therapy that may be more effective in treating OA and for use as an intra-articular biologic source for augmented healing in the postsurgical inflammatory and healing phases, owing to its significantly higher concentration of IL-1Ra as compared with LR-PRP and LP-PRP. Additionally, LR-PRP had a significantly higher concentration of IL-1Ra than LP-PRP. In cases where increased vascularity and healing are desired for pathological or injured tissues, including muscle and tendon, LR-PRP may be optimal because of its higher overall concentrations of PDGF, TGF-β, EGF, VEGF, and sCD40L.

REFERENCES

1. Anitua E, Sanchez M, Nurden AT, Nurden P, Orive G, Andia I. New insights into and novel applications for platelet-rich fibrin therapies. *Trends Biotechnol*. 2006;24(5):227-234.
2. Antoniades C, Bakogiannis C, Tousoulis D, Antonopoulos AS, Stefanadis C. The CD40/CD40 ligand system: linking inflammation with atherothrombosis. *J Am Coll Cardiol*. 2009;54(8):669-677.
3. Aukrust P, Muller F, Ueland T, et al. Enhanced levels of soluble and membrane-bound CD40 ligand in patients with unstable angina: possible reflection of T lymphocyte and platelet involvement in the pathogenesis of acute coronary syndromes. *Circulation*. 1999;100(6):614-620.
4. Auw Yang KG, Raijmakers NJ, van Arkel ER, et al. Autologous interleukin-1 receptor antagonist improves function and symptoms in osteoarthritis when compared to placebo in a prospective randomized controlled trial. *Osteoarthritis Cartilage*. 2008;16(4):498-505.
5. Baggiolini M, Clark-Lewis I. Interleukin-8, a chemotactic and inflammatory cytokine. *FEBS Lett*. 1992;307(1):97-101.
6. Barreto A, Braun TR. A method to induce interleukin-1 receptor antagonist protein from autologous whole blood. *Cytokine*. 2016;81:137-141.
7. Blaney Davidson EN, van der Kraan PM, van den Berg WB. TGF-beta and osteoarthritis. *Osteoarthritis Cartilage*. 2007;15(6):597-604.
8. Bou Khzam L, Boulahya R, Abou-Saleh H, Hachem A, Zaid Y, Merhi Y. Soluble CD40 ligand stimulates the pro-angiogenic function of peripheral blood angiogenic outgrowth cells via increased release of matrix metalloproteinase-9. *PLoS One*. 2013;8(12):e84289.
9. Caley MP, Martins VL, O'Toole EA. Metalloproteinases and wound healing. *Adv Wound Care (New Rochelle)*. 2015;4(4):225-234.
10. Carmeliet P, Collen D. Molecular basis of angiogenesis: role of VEGF and VE-cadherin. *Ann N Y Acad Sci*. 2000;902:249-262.
11. Caron JP, Fernandes JC, Martel-Pelletier J, et al. Chondroprotective effect of intraarticular injections of interleukin-1 receptor antagonist in experimental osteoarthritis: suppression of collagenase-1 expression. *Arthritis Rheum*. 1996;39(9):1535-1544.
12. Cassano JM, Kennedy JG, Ross KA, Fraser EJ, Goodale MB, Fortier LA. 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.
13. 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.
14. Cerza F, Carni S, Carcangiu A, et al. Comparison between hyaluronic acid and platelet-rich plasma, intra-articular infiltration in the treatment of gonarthrosis. *Am J Sports Med*. 2012;40(12):2822-2827.
15. Chahla J, Cinque ME, Piuzzi NS, et al. A call for standardization in platelet-rich plasma preparation protocols and composition reporting: a systematic review of the clinical orthopaedic literature. *J Bone Joint Surg Am*. 2017;99(20):1769-1779.
16. Chahla J, Dean CS, Moatshe G, Pascual-Garrido C, Serra Cruz R, 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.
17. 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.
18. Chahla J, Piuzzi NS, Mitchell JJ, et al. Intra-articular cellular therapy for osteoarthritis and focal cartilage defects of the knee: a systematic review of the literature and study quality analysis. *J Bone Joint Surg Am*. 2016;98(18):1511-1521.
19. Chakrabarti S, Blair P, Freedman JE. CD40-40L signaling in vascular inflammation. *J Biol Chem*. 2007;282(25):18307-18317.
20. Chen FH, Tuan RS. Mesenchymal stem cells in arthritic diseases. *Arthritis Res Ther*. 2008;10(5):223.
21. Chen J, Chen H, Li P, et al. Simultaneous regeneration of articular cartilage and subchondral bone in vivo using MSCs induced by a spatially controlled gene delivery system in bilayered integrated scaffolds. *Biomaterials*. 2011;32(21):4793-4805.
22. Chu CR, Fortier LA, Williams A, et al. Minimally manipulated bone marrow concentrate compared with microfracture treatment of full-thickness chondral defects: a one-year study in an equine model. *J Bone Joint Surg Am*. 2018;100(2):138-146.
23. Cole BJ, Karas V, Hussey K, Pilz K, Fortier LA. Hyaluronic acid versus platelet-rich plasma: a prospective, double-blind randomized controlled trial comparing clinical outcomes and effects on intra-articular biology for the treatment of knee osteoarthritis. *Am J Sports Med*. 2017;45(2):339-346.
24. Dai WL, Zhou AG, Zhang H, Zhang J. Efficacy of platelet-rich plasma in the treatment of knee osteoarthritis: a meta-analysis of randomized controlled trials. *Arthroscopy*. 2017;33(3):659-670, e651.
25. Danese S, Scaldaferri F, Vetrano S, et al. Critical role of the CD40 CD40-ligand pathway in regulating mucosal inflammation-driven angiogenesis in inflammatory bowel disease. *Gut*. 2007;56(9):1248-1256.
26. Davatchi F, Sadeghi Abdollahi B, Mohyeddin M, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis: 5 years follow-up of three patients. *Int J Rheum Dis*. 2016;19(3):219-225.
27. Dinarello CA, Thompson RC. Blocking IL-1: interleukin 1 receptor antagonist in vivo and in vitro. *Immunol Today*. 1991;12(11):404-410.
28. do Amaral RJ, da Silva NP, Haddad NF, et al. Platelet-rich plasma obtained with different anticoagulants and their effect on platelet

numbers and mesenchymal stromal cells behavior in vitro. *Stem Cells Int*. 2016;2016:7414036.
29. Dormond O, Contreras AG, Meijer E, et al. CD40-induced signaling in human endothelial cells results in mTORC2- and Akt-dependent expression of vascular endothelial growth factor in vitro and in vivo. *J Immunol*. 2008;181(11):8088-8095.
30. Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immunol Rev*. 2009;229(1):152-172.
31. Enea D, Cecconi S, Calcagno S, Busilacchi A, Manzotti S, Gigante A. One-step cartilage repair in the knee: collagen-covered microfracture and autologous bone marrow concentrate. A pilot study. *Knee*. 2015;22(1):30-35.
32. Enea D, Cecconi S, Calcagno S, et al. Single-stage cartilage repair in the knee with microfracture covered with a resorbable polymer-based matrix and autologous bone marrow concentrate. *Knee*. 2013;20(6):562-569.
33. Fan J, Ren L, Liang R, Gong Y, Cai D, Wang DA. Chondrogenesis of synovium-derived mesenchymal stem cells in photopolymerizing hydrogel scaffolds. *J Biomater Sci Polym Ed*. 2010;21(12):1653-1667.
34. Fernandes JC, Martel-Pelletier J, Pelletier JP. The role of cytokines in osteoarthritis pathophysiology. *Biorheology*. 2002;39(1-2):237-246.
35. Ferrara N, Gerber HP. The role of vascular endothelial growth factor in angiogenesis. *Acta Haematol*. 2001;106(4):148-156.
36. Ferroni P, Santilli F, Guadagni F, Basili S, Davi G. Contribution of platelet-derived CD40 ligand to inflammation, thrombosis and neoangiogenesis. *Curr Med Chem*. 2007;14(20):2170-2180.
37. Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. The role of growth factors in cartilage repair. *Clin Orthop Relat Res*. 2011;469(10):2706-2715.
38. Fortier LA, Nixon AJ, Williams J, Cable CS. Isolation and chondrocytic differentiation of equine bone marrow–derived mesenchymal stem cells. *Am J Vet Res*. 1998;59(9):1182-1187.
39. Frisbie DD, Ghivizzani SC, Robbins PD, Evans CH, McIlwraith CW. Treatment of experimental equine osteoarthritis by in vivo delivery of the equine interleukin-1 receptor antagonist gene. *Gene Ther*. 2002;9(1):12-20.
40. Gharibi B, Hughes FJ. Effects of medium supplements on proliferation, differentiation potential, and in vitro expansion of mesenchymal stem cells. *Stem Cells Transl Med*. 2012;1(11):771-782.
41. Gibson D, Cullen B, Legerstee R, Harding KG, Schultz G. MMPs made easy. *Wounds International Journal*. 2009;1(1):1-6.
42. Gigante A, Cecconi S, Calcagno S, Busilacchi A, Enea D. Arthroscopic knee cartilage repair with covered microfracture and bone marrow concentrate. *Arthrosc Tech*. 2012;1(2):e175-e180.
43. 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 non-randomized prospective trial. *Cartilage*. 2015;6(2):82-97.
44. 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.
45. Gobbi A, Karnatzikos G, Scotti C, Mahajan V, Mazzucco L, Grigolo B. One-step cartilage repair with bone marrow aspirate concentrated cells and collagen matrix in full-thickness knee cartilage lesions: results at 2-year follow-up. *Cartilage*. 2011;2(3):286-299.
46. Gobbi A, Whyte GP. One-stage cartilage repair using a hyaluronic acid-based scaffold with activated bone marrow-derived mesenchymal stem cells compared with microfracture: five-year follow-up. *Am J Sports Med*. 2016;44(11):2846-2854.
47. Goldring MB, Berenbaum F. The regulation of chondrocyte function by proinflammatory mediators: prostaglandins and nitric oxide. *Clin Orthop Relat Res*. 2004;427:S37-S46.
48. Gormeli G, Gormeli CA, Ataoglu B, Colak C, Aslanturk O, Ertem K. Multiple PRP injections are more effective than single injections and hyaluronic acid in knees with early osteoarthritis: a randomized, double-blind, placebo-controlled trial. *Knee Surg Sports Traumatol Arthrosc*. 2017;25(3):958-965.
49. Grafe I, Alexander S, Peterson JR, et al. TGF-beta family signaling in mesenchymal differentiation. *Cold Spring Harb Perspect Biol*. 2018;10(5):A022202.
50. Granowitz EV, Clark BD, Mancilla J, Dinarello CA. Interleukin-1 receptor antagonist competitively inhibits the binding of interleukin-1 to the type II interleukin-1 receptor. *J Biol Chem*. 1991;266(22):14147-14150.
51. Hall MP, Band PA, Meislin RJ, Jazrawi LM, Cardone DA. Platelet-rich plasma: current concepts and application in sports medicine. *J Am Acad Orthop Surg*. 2009;17(10):602-608.
52. Hart R, Safi A, Komzak M, Jajtner P, Puskeiler M, Hartova P. Platelet-rich plasma in patients with tibiofemoral cartilage degeneration. *Arch Orthop Trauma Surg*. 2013;133(9):1295-1301.
53. 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.
54. 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.
55. Henn V, Slupsky JR, Grafe M, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature*. 1998;391(6667):591-594.
56. Huard J, Li Y, Fu FH. Muscle injuries and repair: current trends in research. *J Bone Joint Surg Am*. 2002;84(5):822-832.
57. 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.
58. Johnson RG. Bone marrow concentrate with allograft equivalent to autograft in lumbar fusions. *Spine (Phila Pa 1976)*. 2014;39(9):695-700.
59. Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. In vitro chondrogenesis of bone marrow–derived mesenchymal progenitor cells. *Exp Cell Res*. 1998;238(1):265-272.
60. Kanchanatawan W, Arirachakaran A, Chaijenkij K, et al. Short-term outcomes of platelet-rich plasma injection for treatment of osteoarthritis of the knee. *Knee Surg Sports Traumatol Arthrosc*. 2016;24(5):1665-1677.
61. Kaux JF, Janssen L, Drion P, et al. Vascular endothelial growth factor-111 (VEGF-111) and tendon healing: preliminary results in a rat model of tendon injury. *Muscles Ligaments Tendons J*. 2014;4(1):24-28.
62. 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.
63. Klein MB, Yalamanchi N, Pham H, Longaker MT, Chang J. Flexor tendon healing in vitro: effects of TGF-beta on tendon cell collagen production. *J Hand Surg Am*. 2002;27(4):615-620.
64. Krych AJ, Nawabi DH, Farshad-Amacker NA, et al. Bone marrow concentrate improves early cartilage phase maturation of a scaffold plug in the knee: a comparative magnetic resonance imaging analysis to platelet-rich plasma and control. *Am J Sports Med*. 2016;44(1):91-98.
65. Kurth T, Hedbom E, Shintani N, et al. Chondrogenic potential of human synovial mesenchymal stem cells in alginate. *Osteoarthritis Cartilage*. 2007;15(10):1178-1189.
66. LaPrade RF, Geeslin AG, Murray IR, et al. Biologic Treatments for Sports Injuries II Think Tank—current concepts, future research, and barriers to advancement, part 1: biologics overview, ligament injury, tendinopathy. *Am J Sports Med*. 2016;44(12):3270-3283.
67. Laudy AB, Bakker EW, Rekers M, Moen MH. Efficacy of platelet-rich plasma injections in osteoarthritis of the knee: a systematic review and meta-analysis. *Br J Sports Med*. 2015;49(10):657-672.
68. Lee JW, Kwon OH, Kim TK, et al. Platelet-rich plasma: quantitative assessment of growth factor levels and comparative analysis of activated and inactivated groups. *Arch Plast Surg*. 2013;40(5):530-535.
69. Li Y, Foster W, Deasy BM, et al. Transforming growth factor-beta1 induces the differentiation of myogenic cells into fibrotic cells in injured skeletal muscle: a key event in muscle fibrogenesis. *Am J Pathol*. 2004;164(3):1007-1019.

70. Luangphakdy V, Boehm C, Pan H, Herrick J, Zaveri P, Muschler GF. Assessment of methods for rapid intraoperative concentration and selection of marrow-derived connective tissue progenitors for bone regeneration using the canine femoral multidefect model. *Tissue Eng Part A*. 2016;22(1-2):17-30.
71. Martin DR, Cox NR, Hathcock TL, Niemeyer GP, Baker HJ. Isolation and characterization of multipotential mesenchymal stem cells from feline bone marrow. *Exp Hematol*. 2002;30(8):879-886.
72. Mazzocca AD, McCarthy MB, Chowaniec DM, et al. Platelet-rich plasma differs according to preparation method and human variability. *J Bone Joint Surg Am*. 2012;94(4):308-316.
73. 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.
74. Medeiros Da Cunha CM, Perugini V, Bernegger P, et al. Vascular endothelial growth factor sequestration enhances in vivo cartilage formation. *Int J Mol Sci*. 2017;18(11):e2478.
75. Melter M, Reinders ME, Sho M, et al. Ligation of CD40 induces the expression of vascular endothelial growth factor by endothelial cells and monocytes and promotes angiogenesis in vivo. *Blood*. 2000;96(12):3801-3808.
76. Montaseri A, Busch F, Mobasheri A, et al. IGF-1 and PDGF-BB suppress IL-1beta-induced cartilage degradation through down-regulation of NF-kappaB signaling: involvement of Src/PI-3K/AKT pathway. *PLoS One*. 2011;6(12):e28663.
77. Moyer AL, Wagner KR. Regeneration versus fibrosis in skeletal muscle. *Curr Opin Rheumatol*. 2011;23(6):568-573.
78. Mukherjee S, Katki K, Arisi GM, Foresti ML, Shapiro LA. Early TBI-induced cytokine alterations are similarly detected by two distinct methods of multiplex assay. *Front Mol Neurosci*. 2011;4:21.
79. Murray IR, Geeslin AG, Goudie EB, Petrigliano FA, LaPrade RF. Minimum Information for Studies Evaluating Biologics in Orthopaedics (MIBO): platelet-rich plasma and mesenchymal stem cells. *J Bone Joint Surg Am*. 2017;99(10):809-819.
80. Murray IR, Robinson PG, West CC, et al. Reporting standards in clinical studies evaluating bone marrow aspirate concentrate: a systematic review. *Arthroscopy*. 2018;34(4):1366-1375.
81. Muschler GF, Boehm C, Easley K. Aspiration to obtain osteoblast progenitor cells from human bone marrow: the influence of aspiration volume. *J Bone Joint Surg Am*. 1997;79(11):1699-1709.
82. Muschler GF, Nitto H, Boehm CA, Easley KA. Age- and gender-related changes in the cellularity of human bone marrow and the prevalence of osteoblastic progenitors. *J Orthop Res*. 2001;19(1):117-125.
83. Oliver KS, Bayes M, Crane D, Pathikonda C. Clinical outcome of bone marrow concentrate in knee osteoarthritis. *Journal of Prolotherapy*. 2015;7(2):937-946.
84. *Package Skillings-Mack: The Skillings-Mack Test Statistic for Block Designs With Missing Observations*. Version 1.10. R Package Documentation; 2015.
85. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. *Am J Sports Med*. 2013;41(2):356-364.
86. Pelletier JP, Caron JP, Evans C, et al. In vivo suppression of early experimental osteoarthritis by interleukin-1 receptor antagonist using gene therapy. *Arthritis Rheum*. 1997;40(6):1012-1019.
87. Pifer MA, Maerz T, Baker KC, Anderson K. Matrix metalloproteinase content and activity in low-platelet, low-leukocyte and high-platelet, high-leukocyte platelet rich plasma (PRP) and the biologic response to PRP by human ligament fibroblasts. *Am J Sports Med*. 2014;42(5):1211-1218.
88. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;284(5411):143-147.
89. Raeissadat SA, Rayegani SM, Hassanabadi H, et al. Knee osteoarthritis injection choices: platelet- rich plasma (PRP) versus hyaluronic acid (a one-year randomized clinical trial). *Clin Med Insights Arthritis Musculoskelet Disord*. 2015;8:1-8.
90. Rayegani SM, Raeissadat SA, Taheri MS, et al. Does intra articular platelet rich plasma injection improve function, pain and quality of life in patients with osteoarthritis of the knee? A randomized clinical trial. *Orthop Rev (Pavia)*. 2014;6(3):5405.
91. Roberts CS, Pape HC, Jones AL, Malkani AL, Rodriguez JL, Giannoudis PV. Damage control orthopaedics: evolving concepts in the treatment of patients who have sustained orthopaedic trauma. *Instr Course Lect*. 2005;54:447-462.
92. Roselli M, Mineo TC, Basili S, et al. Soluble CD40 ligand plasma levels in lung cancer. *Clin Cancer Res*. 2004;10(2):610-614.
93. Salajegheh A. Epidermal growth factor. In: Salajegheh A, ed. *Angiogenesis in Health, Disease and Malignancy*. Berlin, Germany: Springer; 2016:61-68.
94. Schnabel LV, Mohammed HO, Miller BJ, et al. Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. *J Orthop Res*. 2007;25(2):230-240.
95. 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.
96. Skowronski J, Rutka M. Osteochondral lesions of the knee reconstructed with mesenchymal stem cells—results. *Ortop Traumatol Rehabil*. 2013;15(3):195-204.
97. Skowronski J, Skowronski R, Rutka M. Large cartilage lesions of the knee treated with bone marrow concentrate and collagen membrane—results. *Ortop Traumatol Rehabil*. 2013;15(1):69-76.
98. Smith PA. Intra-articular autologous conditioned plasma injections provide safe and efficacious treatment for knee osteoarthritis: an FDA-sanctioned, randomized, double-blind, placebo-controlled clinical trial. *Am J Sports Med*. 2016;44(4):884-891.
99. Smith RL, Allison AC, Schurman DJ. Induction of articular cartilage degradation by recombinant interleukin 1 alpha and 1 beta. *Connect Tissue Res*. 1989;18(4):307-316.
100. Stove J, Huch K, Gunther KP, Scharf HP. Interleukin-1beta induces different gene expression of stromelysin, aggrecan and tumor-necrosis-factor-stimulated gene 6 in human osteoarthritic chondrocytes in vitro. *Pathobiology*. 2000;68(3):144-149.
101. Sugaya H, Yoshioka T, Kato T, et al. Comparative analysis of cellular and growth factor composition in bone marrow aspirate concentrate and platelet-rich plasma. *Bone Marrow Res*. 2018;2018:1549826.
102. Sundman EA, Cole BJ, Fortier LA. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. *Am J Sports Med*. 2011;39(10):2135-2140.
103. Tchetina EV, Antoniou J, Tanzer M, Zukor DJ, Poole AR. Transforming growth factor-beta2 suppresses collagen cleavage in cultured human osteoarthritic cartilage, reduces expression of genes associated with chondrocyte hypertrophy and degradation, and increases prostaglandin E(2) production. *Am J Pathol*. 2006;168(1):131-140.
104. Thampatty BP, Li H, Im HJ, Wang JH. EP4 receptor regulates collagen type-I, MMP-1, and MMP-3 gene expression in human tendon fibroblasts in response to IL-1 beta treatment. *Gene*. 2007;386(1-2):154-161.
105. van Hinsbergh VW, Koolwijk P. Endothelial sprouting and angiogenesis: matrix metalloproteinases in the lead. *Cardiovasc Res*. 2008;78(2):203-212.
106. Vangsness CT Jr, Farr J 2nd, Boyd J, Dellaero DT, Mills CR, LeRoux-Williams M. Adult human mesenchymal stem cells delivered via intra-articular injection to the knee following partial medial meniscectomy: a randomized, double-blind, controlled study. *J Bone Joint Surg Am*. 2014;96(2):90-98.
107. Vaquerizo V, Plasencia MA, Arribas I, et al. Comparison of intra-articular injections of plasma rich in growth factors (PRGF-Endoret) versus Durolane hyaluronic acid in the treatment of patients with symptomatic osteoarthritis: a randomized controlled trial. *Arthroscopy*. 2013;29(10):1635-1643.
108. Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. *Muscle Nerve*. 1995;18(12):1417-1426.

109. Wehling P, Moser C, Frisbie D, et al. Autologous conditioned serum in the treatment of orthopedic diseases: the orthokine therapy. *BioDrugs*. 2007;21(5):323-332.
110. Wilke MM, Nydam DV, Nixon AJ. Enhanced early chondrogenesis in articular defects following arthroscopic mesenchymal stem cell implantation in an equine model. *J Orthop Res*. 2007;25(7):913-925.
111. Wilson BH, Cole BJ, Goodale MB, Fortier LA. Short-term storage of platelet-rich plasma at room temperature does not affect growth factor or catabolic cytokine concentration. *Am J Orthop (Belle Mead NJ)*. 2018;47(4):10-12.
112. Wong KL, Lee KB, Tai BC, Law P, Lee EH, Hui JH. Injectable cultured bone marrow-derived mesenchymal stem cells in varus knees with cartilage defects undergoing high tibial osteotomy: a prospective, randomized controlled clinical trial with 2 years' follow-up. *Arthroscopy*. 2013;29(12):2020-2028.
113. Xiong G, Lingampalli N, Koltsov JCB, et al. Men and women differ in the biochemical composition of platelet-rich plasma. *Am J Sports Med*. 2018;46(2):409-419.