Clinical application of concentrated bone marrow aspirate in orthopaedics: A systematic review

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Aim
To examine the evidence behind the use of concentrated bone marrow aspirate (cBMA) in cartilage, bone, and tendon repair; establish proof of concept for the use of cBMA in these biologic environments; and provide the level and quality of evidence substantiating the use of cBMA in the clinical setting.

Methods
We conducted a systematic review according to PRISMA guidelines. EMBASE, MEDLINE, and Web of Knowledge databases were screened for the use of cBMA in the repair of cartilage, bone, and tendon repair. We extracted data on tissue type, cBMA preparation, cBMA concentration, study methods, outcomes, and level of evidence and reported the results in tables and text.

Results
A total of 36 studies met inclusion/exclusion criteria and were included in this review. Thirty-one of 36 (86%) studies reported the method of centrifugation and preparation of cBMA with 15 (42%) studies reporting either a cell concentration or an increase from baseline. Variation of cBMA application was seen amongst the studies evaluated. Twenty-one of 36 (58%) were level of evidence IV, 12/36 (33%) were level of evidence III, and 3/36 (8%) were level of evidence II. Studies evaluated full thickness chondral lesions (7 studies), osteochondral lesions (10 studies), osteoarthritis (5 studies), nonunion or fracture (9 studies), or tendon injuries (5 studies). Significant clinical improvement with the presence of hyaline-like values and lower incidence of fibrocartilage on T2 mapping was found in patients receiving cBMA in the treatment of cartilaginous lesions. Bone consolidation and time to bone union was improved in patients receiving cBMA. Enhanced healing...
rates, improved quality of the repair surface on ultrasound and magnetic resonance imaging, and a decreased risk of re-rupture was demonstrated in patients receiving cBMA as an adjunctive treatment in tendon repair.

CONCLUSION
The current literature demonstrates the potential benefits of utilizing cBMA for the repair of cartilaginous lesions, bony defects, and tendon injuries in the clinical setting. This study also demonstrates discrepancies between the literature with regards to various methods of centrifugation, variable cell count concentrations, and lack of standardized outcome measures. Future studies should attempt to examine the integral factors necessary for tissue regeneration and renewal including stem cells, growth factors and a biologic scaffold.

Key words: Concentrated bone marrow aspirate; Bone; Cartilage; Osteochondral lesion; Osteoarthritis; Tendon

INTRODUCTION
With the widespread use of orthobiologics in everyday practice, attention must be directed to substantiate the evidence for their current use and to direct future practice guidelines. The use of concentrated bone marrow aspirate (cBMA) has become an increasingly popular alternative and adjunct in the treatment of cartilaginous lesions, bony defects, and tendinous injuries. This systematic review demonstrates the potential benefits of utilizing cBMA for the repair of different tissue types in the clinical setting. This systematic review also highlights discrepancies between the literature with regards to various methods of centrifugation, variable cell count concentrations, variable methods of application of cBMA, and the lack of standardized outcome measures.

MATERIALS AND METHODS
A systematic review was conducted according to PRISMA guidelines[3]. The following search terms were used in MEDLINE, EMBASE, and Web of Science databases on November 22, 2016: “cBMA OR concentrated bone marrow aspirate OR BMC OR bone marrow concentrate OR bone marrow derived mesenchymal stem cells”. This was paired with one of the following search strategies: (1) “cartilage OR chondrocytes OR chondrogenesis OR arthritis OR osteoarthritis OR osteochondral OR chondral”; (2) “tenocytes OR tendon OR tendinosis OR tendinopathy”; or (3) “bone OR bone healing OR malunion OR delayed union OR osteocyte OR osteogenes”. Inclusion criteria were: (1) clinical studies demonstrating the effect of cBMA in cartilage, bone; or tendon (2) published in peer-reviewed journal; and (3) written in English. Exclusion criteria included review articles, case reports, basic science studies, and studies evaluating additional pathologic processes. Two independent reviewers performed the literature search screening both title and abstract for all results. Potentially
eligible studies received a full text review. The reference list of the identified articles in the results were manually screened for additional articles. A senior author was consulted if a consensus could not be reached. The following information was extracted and recorded from the included studies: Number of patients, preparation method of cBMA, cell count, treatment groups, adjunctive therapies/scaffolds, follow-up, objective and subjective outcomes, and level of evidence.

RESULTS
The initial literature search resulted in 1202 total studies. Once duplicates were removed and articles were screened for inclusion/exclusion criteria, 135 were included and full texts were assessed for eligibility. A total of 36 studies met inclusion/exclusion criteria and were included in this review.

Study characteristics
Thirty-one of 36 (86%) studies reported the method of centrifugation and preparation of cBMA. Fifteen of 36 (42%) studies reported either a cell concentration or an increase from baseline. There were no studies that reported on the minimal number of colony forming units in which below that number, cBMA did not provide significant benefit. Twenty-one of 36 (58%) were level of evidence IV, 12/36 (33%) were level of evidence III, and 3/36 (8%) were level of evidence II. Two studies were industry funded while 37 declared no conflict of interest.

cBMA in full thickness cartilage lesions
Seven studies evaluated the effect of cBMA in the treatment of full thickness cartilage defects in the knee and all reported significant clinical improvement post-operatively summarized in Table 1[4-10]. Three studies evaluated the effect of cBMA combined with microfracture and demonstrated improved clinical outcomes with reconstitution of original cartilage on magnetic resonance imaging (MRI). All three studies reported bone marrow edema and/or subchondral irregularities[4-6]. One study evaluated the effects of cBMA when compared with matrix-induced autologous chondrocyte implantation (MACI) and found that patients receiving cBMA had a significantly improved IKDC subjective score ($P = 0.015$) with 81% complete cartilage filling on MRI[7]. One study compared the effects of cBMA to PRP and reported that patients who received cBMA had T2 values closer to that of superficial hyaline cartilage ($P = 0.01$)[10]. Variation of cBMA application was seen amongst the studies evaluated. Several studies used cBMA in isolation, while other studies combined cBMA with either a collagen or hyaluronic acid scaffold. Many of these studies prepared the defect site and implanted cBMA through arthroscopic techniques.

cBMA in osteochondral lesions
Ten studies evaluated the effect of cBMA in the treatment of osteochondral defects in the talus (7/10) and the knee (3/10) summarized in Table 2[2,11-19]. All ten studies reported both clinical and radiologic improvements post-operatively after receiving cBMA. Six studies evaluated the effects of cBMA with no concomitant procedure and reported good clinical outcome scores including AOFAS, IDKS, and KOOS. For studies that utilized either a collagen or a hyaluronic acid scaffold, no significant difference was reported between the two groups. Buda[11] evaluated cBMA compared to autologous chondrocyte implantation (ACI) and reported no clinical difference between the two treatment strategies but found a higher presence of hyaline like values and lower incidence of fibrocartilage on T2 mapping in the cBMA group. One study favored treatment with cBMA when comparing cBMA to microfracture reporting 100% and 28% normal IDKC values at 5-year follow up, respectively[18]. Lastly, one study reported higher MOCART scores and T2 relaxation values with measurements resembling those of native cartilage in groups that received both microfracture with cBMA compared to groups that received microfracture alone[19]. cBMA had also been used as an adjunctive treatment to autologous osteochondral transplantation and resulted in overall improved FAOS scores post-operatively[2]. Variation of cBMA application was seen amongst the studies evaluated. These included the use of either a collagen powder or hyaluronic acid scaffold, with the majority of studies using arthroscopic techniques for cBMA implantation.

cBMA in osteoarthritis
Five studies evaluated cBMA in the treatment of knee osteoarthritis (OA) summarized in Table 3[20-24]. Only two studies evaluated the efficacy of cBMA without an adjunctive procedure. One reported better clinical outcomes at one week and three months in patients who received cBMA but found no difference in these scores after six months[24]. One study reported significant clinical improvements but found that 76% of patients had abnormal International Cartilage Repair Society repair scores[23]. Three studies evaluated cBMA combined with either PRP or PRF and found functional and clinical improvements in the cBMA groups with improvement in cartilage repair, although not significant[20-22]. Variation of cBMA application was seen amongst the studies evaluated, which utilized ultrasound or fluoroscopy for needle placement or was performed under arthroscopic guidance.

cBMA in bone healing
Nine studies evaluated the use of cBMA in bone healing summarized in Table 4[25-33]. Eight of nine studies reported on the use of cBMA in either non-union or delayed union. One study demonstrated initial radiographic and functional improvements in the cBMA group, but reported similar outcomes after one year post-operatively[31]. All studies reported either lower or similar complication rates post-operatively in groups that received cBMA compared to groups receiving no additional treatment. Bone
Table 1  Studies evaluating concentrated bone marrow aspirate in the treatment of full thickness chondral lesions

| Ref.        | Tissue | BMAC preparation | Concentration | Study design/methods/follow up | Outcomes measured | Results | LOE |
|-------------|--------|------------------|---------------|---------------------------------|-------------------|---------|-----|
| Enea et al. | Knee   | 60 mL of BMA from iliac crest processed with MarrowStim Concentration Kit (Biomet) resulting in 3-4 mL of BMAC. Chondral lesion debrided and microfracture performed. Biocollagen MeRE collagen membrane (Biotech) cut to match shape and immersed in BMAC until implantation. 10:1 mixture of 1-2 mL fibrin glue and BMAC laid on lesion. Membrane inserted and placed. 2-3 mL of fibrin glue-BMAC injected over and left to solidify | n = 9. Arthroscopic microfracture covered with collagen membrane immersed in autologous BMAC from iliac crest. Follow up: 29 mo | Biopsy cartilage evaluated by surgeon using criteria of international cartilage repair society. The following items were utilized: Cartilage repair assessment, MRI, IKDC, Lysholm, VAS (pre and post op), Tegner (pre and post op). Four patients had second look arthroscopy and biopsy | Significant clinical improvement (P < 0.005). Cartilage macroscopic assessment at 12 mo revealed all repairs appeared almost normal. Histo-analysis showed hyaline-like cartilage repair in 1 lesion, fibrocartilage repair in 2 lesions and a mixture of both in 1 lesion. Post op MRls (6-9 mo out) all showed reconstitution of original cartilage. Bone marrow edema and/or subchondral irregularities observed in all cases. Non-homogeneous cartilage signal and fissuring observed in 2 of 3 cases | IV |
| Enea et al. | Knee   | 60 mL of BMA from the iliac crest was obtained and processed with MarrowStim Concentration Kit (Biomet) to obtain 3-4 mL of BMAC. Cartilage was treated with arthroscopic microfracture and the defect was covered with PGA-HA scaffold matrix (Chondrotissue) seeded with autologous BMAC. 10:1 mixture of 1-2 mL of fibrin glue and BMAC was then applied to lesion bed. PGA-HA soaked in BMAC was then applied with 2-3 mL additional fibrin glue-BMAC mixture dispersed over the matrix until solidification at 2-3 min | n = 9. (Outerbridge type III/IV) Consecutively treated with arthroscopic Polyglycolic acid/hyaluronan-covered microfracture and BMAC. Follow up: 22 mo | Clinical scoring, IKDC, Lysholm, VAS, Tegner, cartilage microscopic examination at 12 mo, MRI at 8-12 mo post op. 5 patients underwent second look and 2 had biopsy | All patients but one showed improvement in clinical scoring from pre-op to last follow-up (22 mo). All other variables increased from baseline to latest follow-up. Nineteen cartilage exams appeared normal, three almost normal, and one abnormal at 12 mo. Histo showed hyaline-like cartilage repair tissue formation in one case. MRI showed complete defect filling | IV |
| Gigante et al. | Knee | NA | NA | n = 5. MACI augmented with BMAC | Second look arthroscopy biopsy, CRA, ICRS II Visual Histological Assessment Scale | Normal ICRS/CRA at arthroscopic evaluation and had mean overall histological ICRS II of 59.8 ± 14.5. Hyaline-like matrix only found in one case. Mixture of hyaline/fibrocartilage was found in one case and fibrocartilage was found three cases | IV |
| Gobbi et al. | Patellofemoral | 60 mL of BMA from isipilateral iliac crest concentrated by BMAC Harvest Smart PreP2 system to obtain concentration of BMC 4-6 times baseline value | 4-6 x baseline | (1) MACI n = 19; (2) BMAC n = 18. Both with HYAFFI scaffold. Follow up: 3 yr | XR, MRI, IKDC score, KOOS score, VAS, Tegner | All groups showed significant improvements in all scores from preop to final follow up (P = 0.002). There was no difference between the two groups except in the IKDC subjective scores which favored BMAC group (P = 0.015). MRI showed complete filling of defect in 76% of MACI and in 81% of BMAC. Significant improvement at follow up across all measures. < 45-year-old and smaller lesions = better results. MRI = good stability of implant, hyaline-like cartilage found is histo analysis of biopsied tissue | III |
| Gobbi et al. | Knee | 60 mL of BMA from ipsilateral iliac crest concentrated by BMAC Harvest Smart PreP2 system to obtain concentration of BMC 4-6 times baseline value. Activated using batroxobin enzyme to form sticky clot. Implanted and covered with collagen-based membrane scaffold (ChondroGide) and sealed with fibrin glue (Tissucol) | 4-6 x baseline | n = 25. Cartilage transplantation with multipotent stem cells and collagen type I/III matrix | XR, MRI, VAS, IKDC, KOOS, Lysholm, Marx, Tegner | | IV |
BMA: Bone marrow aspirate; NS: Not significant; CRA: Cartilage repair assessment; MRI: Magnetic resonance imaging; MACI: Matrix-induced autologous chondrocyte implantation; PRP: Platelet-rich plasma.

### Table 2  Studies evaluating concentrated bone marrow aspirate in the treatment of osteochondral defects

| Ref.          | Tissue          | BMAC preparation                                                                 | Concentration | Study design/methods/Follow up measured | Outcomes | Results | LOE |
|---------------|-----------------|----------------------------------------------------------------------------------|---------------|----------------------------------------|----------|---------|-----|
| Buda et al.   | OCL of talus    | Scaffold was a hyaluronic acid membrane loaded with previously cultured chondrocytes (ACI) or with BMAC. Platelet rich fibrin gel was produced the day before surgery using Vivostat System 1 (vivolution A/S). Harvested and processed 120 mL of the patient’s venous blood to obtain 6 mL of platelet rich fibrin gel. 60 mL BMAC was harvested from posterior iliac crest using Smart PRepI to obtain 6 mL of BMAC. 1 g powder mixed with 2 mL BMAC and 1 mL platelet rich fibrin gel. The hyaluronic acid membrane was cut and loaded with 2 mL BMAC and 1 mL platelet rich fibrin gel. A layer of platelet rich fibrin gel was placed over the implant once in place to provide additional stability | n (total) = 80: (1) n = 40 - autologous chondrocytes implantation; (2) n = 40 with BMAC. Follow up: 48 mo | MRI, T2 mapping | Clinical scores, XR, MRI Mocart score, T2 mapping | Groups had similar results at 48 mo. No statistically significant difference in clinical outcomes. Return to sport was slightly better with BMAC. MRI MOCART score was similar in both groups. T2 mapping highlighted a higher presence of hyaline like values and lower incidence of fibrocartilage in BMAC group | IV |
| Buda et al.   | OCL of knee     | Combined with either MAST or HA matrix                                           | NS            | Clinical inspection, MRI, IKDC, KOOS | AOFAS scale score, radiographic, scaffold type, lesion area, previous surgery, lesion depth | Good clinical outcome and osteochondral regeneration on MRI and biopsies in both groups | IV |
| Buda et al.   | OCL of talus    | Scaffolds either: (1) porcine collagen powder Spongostard Powder (J and J) mixed with autologous cell concentrate and platelet gel; or (2) hyaluronic acid membrane (fidia advanced biopolymers) with addition of platelet gel. Platelet rich fibrin gel was produced the day before surgery using Vivostat System 1 (vivolution A/S). Harvested and processed 120 mL of the patient’s venous blood to obtain 6 mL of platelet rich fibrin gel. 60 mL BMAC was harvested from posterior iliac crest using Smart PRepI to obtain 6 mL of BMAC. 1 g powder mixed with 2 mL BMAC and 1 mL platelet rich fibrin gel. The hyaluronic acid membrane was cut and loaded with 2 mL BMAC and 1 mL platelet rich fibrin gel. A layer of platelet rich fibrin gel was placed over the implant once in place to provide additional stability | n = 30. One step arthroscopic BMAC transplant with scaffold. Follow up: 29 mo | MRI, T2 mapping | Clinical, MRI, IKDC, KOOS | Mean preop AOFAS was 65.2. Regardless of scaffolding type all patients showed similar pattern of clinical improvement at each follow-up. No correlation between area of lesion and preop AOFAS score but did observe relationship between area and AOFAS at each follow up post-operatively. No relationship between AOFAS score and depth of lesion | IV |
consolidation and time to bone union was improved in patients receiving cBMA, with faster healing rates when

| Authors          | OCL of bone | Scaffold | n   | Follow-up | Clinical, MRI outcomes                                                                 |
|------------------|-------------|----------|-----|-----------|---------------------------------------------------------------------------------------|
| Buda et al. [14] | Knee        | MAST or HA matrix + PRF | 20  | 24 mo     | All outcomes improved at 12 and 24 mo, satisfactory MRI AOFAS improved P < 0.0005. T2 mapping showed regenerated tissue with T2 values similar to hyaline cartilage in a mean of 78% of the repaired lesion area |
| Giannini et al. [15] | Talus | Porcine collagen powder (J and J) or hyaluronic membrane scaffold. 60 mL of bone marrow harvested from posterior iliac crest and concentrated by SmartPrep to 6 mL of BMC. One step delivery system | 49  | 48 ± 6 mo | AOFAS, radiograph, MRI AOFAS improved, histology showed regenerative tissue in various degrees of remodeling |
| Giannini et al. [16] | Talus | Porcine collagen powder (J and J) or hyaluronic membrane scaffold. 60 mL of bone marrow harvested from posterior iliac crest and concentrated by SmartPrep to 6 mL of BMC. One step delivery system | 25  | 29 mo (24-35) | AOFAS improved, histology showed regenerative tissue in various degrees of remodeling |
| Hannon et al. [17] | Talus | Hyaluronic acid-based scaffold was used with BMAC | 12 | 3 mL of BMAC | FAOS, SF-12, MOCART Mean FAOS and SF-12 PCS scores improved pre to post operatively (P < 0.01) for both groups. MOCART score significantly higher in cBMA + BMS (P = 0.023). T2 relaxation values in cBMA + BMS group significantly higher with measurements of adjacent cartilage FAOS, SF-12 significantly improved from pre to post-op |

KOOS: Knee injury and Osteoarthritis Outcome Score; NS: Not significant; OCL: Osteochondral lesions; BMA: Bone marrow aspirate; MRI: Magnetic resonance imaging.

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### Notes
- **WJO**: WJGNet.com
- **June 18, 2017**: Volume 8 Issue 6
60 mL of BMA from iliac crest was concentrated from 50 cc of heparinized IV venous blood drawn to be used for isolating PRP and platelet lysate. Lipoaspirate - miniliposuction of the posterior superior buttocks or lateral thigh was performed under ultrasound and minimally processed (centrifuged) adipose tissue was injected into the articular space. Preparations were injected into the articular space of the knee together (5-10 cc) between the meniscus on the most painful side and over lying collateral ligament.

10-15 cc whole bone marrow aspirate harvested from 6-8 sites on posterior iliac crest (3-4 each side). Centrifuged and cells isolated. Patient heparinized blood for PRP and PL. Aspirates mixed together and injected into joint. Cell counts were counted four times and average was taken under microscope for total nucleated cell count.

20 mL BMA from iliac crest isolated with density gradient (Ficoll-Paque), supplemented with 10% fetal bovine serum and penicillin streptomycin. Microfracture performed and sclerotic bone curetted. Autologous periosteal flap harvested from anteromedial ipsilateral proximal tibia to fit defect size and stuffed into place. 1 mL platelet concentrate and 1 mL fibrinogen and 1 mL thrombin placed with BMAC PR fibrin glue

60 mL of BMA from iliac crest was obtained to produce 1-3 mL of BMAC. 60 cc of heparinized IV venous blood drawn to be used for isolating PRP and platelet lysate. Lipoaspirate - miniliposuction of the posterior superior buttocks or lateral thigh was performed under ultrasound and minimally processed (centrifuged) adipose tissue was injected into the articular space. Preparations were injected into the articular space of the knee together (5-10 cc) between the meniscus on the most painful side and over lying collateral ligament.

**Table 3** Studies evaluating concentrated bone marrow aspirate in the treatment of osteoarthritis

| Ref.          | Tissue                | BMAC preparation                        | Concentration | Study design/methods/follow up | Outcomes measured                  | Results                                                                 | LOE  |
|---------------|-----------------------|-----------------------------------------|---------------|--------------------------------|------------------------------------|-------------------------------------------------------------------------|------|
| Centeno et al | Knee                  | 60 mL of BMA from iliac crest was      | NS            | Data from registry. (1) n = 616 - BMAC + PRP vs (2) BMAC + PRP + adipose graft. Outcomes and complication questionnaires at 1, 3, 6, 12 mo completed. 2 groups (A-BMAC and PRP protocol, B BMAC and PRP plus adipose fat graft (lipoaspirate) | LEFS, NPS, subjective percentage improvement rating, frequency and type of adverse events | Mean LEFS score increased in both groups and mean NPS decreased in both groups. AE rates were 6% without graft and 8.9% with graft. No difference between groups. Addition of adipose graft did not provide a detectible benefit over BMAC alone | N/   |
| Haleem et al  | Femoral condyle       | 20 mL BMA from iliac crest isolated with density gradient (Ficoll-Paque), supplemented with 10% fetal bovine serum and penicillin streptomycin. Microfracture performed and sclerotic bone curetted. Autologous periosteal flap harvested from anteromedial ipsilateral proximal tibia to fit defect size and stuffed into place. 1 mL platelet concentrate and 1 mL fibrinogen and 1 mL thrombin placed with BMAC PR fibrin glue | NS            | n = 5, treated with BMAC + PRF | Clinical scales assessed at baseline, 1, 3, 6, 12 and annually thereafter. NPS, LEFS, pain and functional outcome measures | Significant positive results with treatment for all pain and functional metrics. Higher cell group reported lower post treatment numeric pain scale values (P < 0.001). No significant difference detected for other metrics | N/   |
| Koh et al     | Knee                  | 60 mL BMA from iliac crest processed with MarrowStim Concentration Kit (Biomet) to obtain 3-4 mL of BMAC. Adipose tissue harvested from buttocks through liposuction. All fluid removed from knee arthroscopically. Lesion filled with cell suspension and held stationary for 10 minutes with defect facing upwards. Adherence of MSC confirmed. No narrow stimulation procedures were performed | Average of 3.8 × 10^6 (2.5-6.1 × 10^6) | n = 37 knees using second-look arthroscopy after mesenchymal stem cell implantation for cartilage lesions done 12 mo post op | IKDC, Tegner, cartilage repair scored using ICRS grading | All patients had statistically significant improvement at 6 and 12 mo (P < 0.005). No statistically significant difference between 6 and 12 mo post op in clinical scores. ICRS were near normal for 2 patients who consented to arthroscopy. MRI of 3 patients at 12 mo showed complete defect filling and complete surface congruity with native cartilage. Two patients showed incomplete congruity. BMAC on platelet rich fibrin gel as a scaffold may be effective to promote repair of articular cartilage defects | N/   |
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BMA: Bone marrow aspirate; MRI: Magnetic resonance imaging; NS: Not significant; OA: Osteoarthritis; BMI: Body mass index; VAS: Visual analogue scale; OARSI: Osteoarthritis Research Society International.

Table 4  Studies evaluating concentrated bone marrow aspirate in bone healing

| Ref.        | Tissue                  | BMAC preparation | Concentration | Study design/methods/ follow up | Outcomes measured                                                                 | Results                                                                 | LOE   |
|-------------|-------------------------|------------------|---------------|---------------------------------|----------------------------------------------------------------------------------|------------------------------------------------------------------------|-------|
| Bastos Filho et al[25] | Tibia/femur nonunion | 11G × 10 cm bone marrow aspiration needle into posterior iliac crest to obtain a total of 100 to 110 mL for each patient - concentrated to 20 mL with Sepax system | NS             | n = 6 patients with nonunion of tibia or femur. Four received percutaneous infusion of autologous bone marrow aspirated without Sepax processing. Two received with processing. Follow up to 6 mo | Clinical examination and radiographic evaluation at 2, 4, 6 mo. Clinical criteria included full weight bearing tolerance and absence of pain upon palpation at the fracture site. Radiographic healing checked with AP, lateral and oblique films to look for bone callus. Patient satisfaction questionnaire scale from 0-10 | Bone consolidation obtained in all the patients. Bone callus observed in the radiographic between 3 and 24 wk, average 13.8 wk in group without processing. Mean satisfaction increased in all patients | II    |
| Desai et al[24] | Nonunion/ delayed union of tibia | Total of 60 cc bone marrow aspirated from iliac crest with 16 gauge Jamshidi needle (Harvest system). Concentrated to 10 cc for injection | 101.48 ± 64.13/cc | n = 49 patients with tibial nonunion had BMAC injection with DBM and/ or rhBMP-2. Follow up until radiographic union or another procedure was performed | Radiographic healing (bridging of 3 out of 4 cortices on AP and lateral films) | No difference in healing rate between patients with fracture gaps less than and greater than 5 mm | III   |
| Garnavos et al[27] | Humeral shaft delayed union | With the use of a 10 cm long and 3 mm wide biopsy needle, 60 mL of bone marrow was aspirated from each patient’s iliac wing and was centrifuged to provide 10 mL of concentrated mesenchymal stem cells. The concentrated bone marrow mixed with 10 cc of DBM putty | NS             | n = 5. Intramedullary nailing with antegrade/ unreamed technique was performed for 4 patients. One patient was treated previously with retrograde/ unreamed nailing left in situ. The concentrated mixture was infused percutaneously in the area of nonunion with a biopsy needle under fluoroscopy. Patients were followed up every 4-6 wk for 12 mo | Patients were assessed for union process, discomfort, level of activities and functional improvement | There were no peri- or postoperative complications. Sound union was obtained in all cases from 12 to 20 wk after the operation. At final followup, all patients had regained a satisfactory range of shoulder and elbow motion. They had also returned to pre-injury level of activities and were happy with their treatment and outcome | IV    |
| Guimaraes et al[26] | Femoral shaft nonunion | 11G × 10 cm needle used for aspiration from iliac crest. The marrow samples were harvested in small amount (2 mL) and the contents of each syringe were pooled in the container of the bone-marrow-collection kit containing anticoagulant solution. The final volume of bone marrow aspirate (200 mL) was then filtered through a sequence of successively | 9.8 ± 4.3 × 10³ vs 20.2 ± 8.6 × 10⁷ | n = 16 patients with aseptic nonunion of femur were treated with injection of BM-MSCs who had locked IMN. Follow up: 3-8 mo | Radiographic RUST scores | Bone union occurred in 8 of 16 patients according to RUST. The grafts used in patients whom treatment failed contained significantly lower number of total nucleated cells (9.8 ± 4.3 × 10³ vs 20.2 ± 8.6 × 10⁷) | IV    |
smaller-diameter mesh filters. The cells were finally collected in a blood transfer pack unit. The aspirated material was reduced to a final volume of 40 mL by removing most of the RBCs by centrifugation.

**Hernigou et al.**

| Study | Tissue | Prognostic Factor | Treatment | Outcome |
|-------|--------|-------------------|-----------|---------|
| **Ankle nonunion** | Bone marrow aspirate obtained from anterior portion of the ipsilateral iliac crest | Total of 300 mL then concentrated to 50 mL | BMAC injected into noninfected atrophic nonunion of tibia | Follow up until union |
| **Tibial shaft nonunion** | Bone marrow aspirated from anterior iliac crest | 40 mL of bone marrow was aspirated from posterior iliac crest and transferred into a container prefilled with 5000 U/mL of heparin. Aspirate was diluted with phosphate-buffered saline at a ratio of 1:1 and centrifuged at room temperature at 3000 rpm for 30 min. The collected buffy coat was washed and transferred into a culture flask containing Dulbecco’s Modified Eagle Medium supplemented with 10% fetal bovine serum. Cells were incubated at 37 °C at 5% CO₂ with a routine culture medium change every two to three days. Subculture was performed between 14-18 million BMSCs. | 18 ± 7 million | Radiographic union; healing time; volume of callus |

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| Study | Tissue | Prognostic Factor | Treatment | Outcome |
|-------|--------|-------------------|-----------|---------|
| **cBMA in orthopaedics** | | | | |
enhanced healing rates, improved quality of the repair the use of cBMA during rotator cuff repair and reported at a mean follow up of 29.7 mo.

Five studies evaluating cBMA in tendon repair were included and summarized in Table 5.

- **Le Nail et al.**
  - **Study**: Open tibia fracture
  - **Method**: Use of cBMA during rotator cuff repair and reported excellent functional outcomes, early evaluated open Achilles tendon repair augmented with cBMA and reported negative correlation between the time needed to obtain progenitor cells in patients who did not achieve union as well as a greater number of progenitor cells compared to patients in the autograft group.
  - **Results**: Bone consolidation was obtained in 88.9% and mean interval between cell transplantation and union was 4.6 ± 1.5 months in autograft group. Bone union rate was 94.4% in group of composite BMAC-ACB transplantation. The time to union in BMAC-ACB grafting group was 3.3 ± 0.9 mo, and led to faster healing when compared to the autograft.

- **Thua et al.**
  - **Study**: Long bone nonunion
  - **Method**: Variation of cBMA application was seen amongst the studies evaluated. These methods utilized cBMA in isolation or in combination with DBM/rhBMP-2, freeze-dried allograft, or cancellous bone chips. Application of cBMA to the site of nonunion was accomplished by either fluoroscopic visualization or percutaneous injection.
  - **Results**: Bone consolidation was achieved in 23 successes (53.5%) within 17 wk after BMAC implantation. The time to union in BMAC-ACB grafting group was 3.3 ± 0.9 mo, and led to faster healing when compared to the autograft.

**DISCUSSION**

**cBMA in cartilage repair**

Articular cartilage injury presents orthopedic surgeons with a difficult challenge as its inherent avascularity and poor healing potential can hinder its self-regenerative capacity. This poor repair capacity has been implicated in the development of post-traumatic osteoarthritis (PTOA) and osteochondral lesions (OCL). Traditional techniques for surgical stimulation of cartilage repair include microfracture and micropicking. These techniques penetrate the subchondral bone in order to stimulate blood flow and allow MSCs access to the cartilage defect. In addition,
| Ref.          | Tissue                        | BMAC preparation                                                                 | Concentration | Study design/methods/follow up                                                                 | Outcomes measured                                                                 | Results                                                                 | Level of evidence |
|--------------|-------------------------------|-----------------------------------------------------------------------------------|---------------|-----------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|------------------------------------------------------------------------|------------------|
| Hernigou et al. [34] | Rotator cuff                 | 150 mL BMA from iliac crest mixed with an anticoagulant solution (citric acid, sodium citrate, dextrose). MSCs were injected in the tendon at the junction between the bone and tendon (4 mL), and in the bone at the site of the footprint (8 mL). Each patient in the MSC-treated group received a total of 12 mL of bone marrow concentrate. | n = 125 symptomatic patients. n = 75 control patients. Assessed the level of MSCs in the tuberosity of the shoulder of patients undergoing a rotator cuff repair. | RTC healing and re-tear rate confirmed by ultrasound and MRI.                  | 45/45 repairs with MSC augmentation had healed by six months vs 30/45 repairs without MSC treatment by 6 mo. Intact rotator cuffs were found in 39/45 patients in the MSC-treated group, but just 20/45 patients in the control group. Patients with a loss of tendon integrity at any time up to the ten-year follow-up milestone received fewer MSCs as compared with those who had maintained a successful repair during the same interval. | III |
| Hernigou et al. [35] | Tendon–bone interface rotator cuff | NS                                                                                | n = 11 patients undergoing arthroscopic RCR. After the determination of the optimal dose of insulin, MSCs were (1) exposed to the hormone insulin; (2) exposed to the growth factors IGF-1, bFGF, and GDF-5, which served as a positive control for MSCs’ differentiation into a tendon; or (3) left untreated to serve as a negative control. In the growth factor group, MSCs were treated with a 1-time dose, 10 ng/L, of IGF-1, bFGF, and GDF-5 or 10-10-mol/L insulin.                                                                 | Cell count, gene expression, protein analysis, and immunocytochemical analysis. Confirmation of protein levels was verified on immunocytochemistry analysis by 4 independent evaluators blinded to group assignment. | Mesenchymal stem cell content at the tendon–bone interface tuberosity was evaluated by bone marrow aspiration collected in the humeral tuberosities of patients at the beginning of surgery. | II |
| Mazzoneca et al. [36] | Rotator cuff                 | MSCs were exposed to either insulin or tendon-inducing growth factors or were left untreated to serve as a control. The BMA was overlaid onto a 17.5% sucrose gradient and centrifuged for 5 min at 1500 rpm (205 g), and the resulting pink middle layer was obtained. After the isolation of bone marrow, MSCs were exposed to a 1-time dose of 10-9-mol/L, 10-10-mol/L, 10-12-mol/L, or 10-13-mol/L insulin from bovine pancreas or were left untreated to serve as a control. | n = 23 BMAC harvested through the anchor tunnel of the humeral head during arthroscopy. n = 23 matched controls. Mean time to follow-up was | Reverse transcription polymerase chain reaction analysis, Single Assessment Numeric Evaluation score | Reverse transcription polymerase chain reaction analysis and cellular staining confirmed the osteogenic potential of the connective tissue progenitor cells. There was no statistically significant difference in cell count, gene expression, protein analysis, and immunocytochemical analysis. | II |
| Mazzoneca et al. [37] | Rotator cuff                 | Isolation 1: one 5 min centrifugation at 1500 rpm in which BMA was overlaid onto a 17.5% sucrose gradient in a 50-mL conical tube followed by extraction of CTPs in the fractional layer. Isolation 2:30 min Nucleated cells harvested from fractionated layer were counted and plated. n = 23 BMAC harvested through the anchor tunnel of the humeral head during arthroscopy. n = 23 matched controls. Mean time to follow-up was | | Reverse transcription polymerase chain reaction analysis, Single Assessment Numeric Evaluation score | Reverse transcription polymerase chain reaction analysis and cellular staining confirmed the osteogenic potential of the connective tissue progenitor cells. There was no statistically significant difference in cell count, gene expression, protein analysis, and immunocytochemical analysis. | II |
mosaicplasty and autologous chondrocyte implantation (ACI) have been utilized to repair chondral damage. First and second-generation ACI procedures, as well as mosaicplasty, have several concerns including donor site morbidity, cost, and lack of availability to all surgeons due to FDA restrictions. The inability of chondrocytes to self-regenerate and self-renew has directed surgeons to investigate alternative biologic augments in the traditional surgical treatment for cartilage defects. CBMA is a rich source of mesenchymal stem cells and has emerged as a treatment strategy to regenerate cartilage defects in OCL and PTOA.

Several in vivo models have demonstrated production of type II collagen and hyaline-like repair tissue when introducing MSCs to a cartilage defect, therefore the use of CBMA may provide further stimulation of chondrogenesis when addressing cartilaginous lesions. There have been a number of studies evaluating the use of CBMA in cartilage regeneration and repair in the animal model. Saw et al. investigated the use of CBMA combined with hyaluronic acid in the treatment of full-thickness chondral defects in a goat model and reported hyaline regeneration after 24 wk. Fortier et al. evaluated the treatment of full-thickness cartilage defects with CBMA combined with microfracture in the equine model. Improvements in both macroscopic and histologic scores in tissue treated with CBMA were reported with MRI demonstrating an increase in defect filling and improved repair tissue integration with normal surrounding cartilage.

The current literature demonstrates the potential benefits of utilizing CBMA for the repair of cartilage injury in the clinical setting. Significant clinical improvement in functional scores was demonstrated with the use of CBMA in the treatment of full thickness cartilage injury, post-traumatic osteoarthritis, and osteochondral lesions. Improved clinical and histologic results were reported when CBMA was used as an adjunctive procedure with either microfracture or MACI in the treatment of full thickness chondral lesions. On MRI, groups treated with CBMA demonstrated superior cartilage ingrowth with T2 values closer to that of superficial hyaline cartilage when compared to either a control scaffold or MACI alone. These positive results were also demonstrated when utilizing CBMA in the treatment of OCLs. Gobbi et al. compared with microfracture with CBMA in the treatment of OCLs and found that microfracture resulted in improved repair tissue integration with normal surrounding cartilage when compared to either a control scaffold or MACI.

| Stein et al. | Achilles Tendon 
|-------------|------------------|
| **30 to 60 mL of BMA, combined with a standard mixture of anticoagulant citrate dextrose solution A and separated by centrifugation at 3200 rpm for 15 min. The aspirate was concentrated to yield a volume of 6-9 mL of BMAC** | **Calf atrophy, maximum dorsi- and plantarflexion, and fatigue limit during single-limb heel raise. Functional and activity status was measured in terms of time to walking, light activity (such as cycling or jogging) and return to sport, as with the validated Achilles Total Rupture Score. Self-reported functional status, activity level and ATRS.** | **n = 28 open repairs with BMAC. Mean follow up: 29.7 mo. Patients were followed postoperatively at two weeks, six weeks, six months, one year and annually thereafter.** | **All patients achieved good or excellent outcomes postoperatively by attaining functional use or return to sport. At final follow-up of 29.7 ± 6.1 mo, mean calf circumference for paired operative and nonoperative extremities was 37.7 ± 2.0 and 38.2 ± 2.0 (difference -0.5 ± 1.3) cm, respectively, for the 26 patients with single Achilles tendon repair. Walking without a boot was at 1.8 ± 0.7 mo, and participation in light activity was at 3.4 ± 1.8 mo. Overall, 92% (25 of 27) patients returned to their preferred sport successfully at 5.9 ± 1.8 mo. Mean ATRS at final follow-up was 91 (range 72-100) points, with no single mean item score below 8 points. All patients were able to achieve a ROM of neutral dorsiflexion or greater and were able to successfully perform a single-limb heel raise at final follow-up.** |
in 65% normal IKDC at 2 years with decline to 27% at 5 years vs 100% normal at 2 years and no decline at 5 years for patients treated with cBMA. Buda et al[31] reported a higher presence of hyaline like values and lower incidence of fibrocartilage on T2 mapping in patients who received cBMA when compared to those who received ACI. Hannon et al[30] also demonstrated better T2 relaxation values with higher measurements of adjacent cartilage in patients treated with bone marrow stimulation (BMS) with cBMA than those treated with BMS alone. Surprisingly, these positive results were not translated as effectively when evaluating cBMA in the treatment of knee OA. Overall, studies demonstrated positive results with improved pain and clinical scores initially but after one-year follow-up, there was no significant difference between groups receiving cBMA and those that did not.

**cBMA in bone regeneration**

Nonunion is a catastrophic failure of bone healing, which has gained increased attention over the last two decades. It is estimated that 5% to 10% of fractures will result in delayed union or nonunion resulting in prolonged treatment and repeated hospitalizations, longer rehabilitation protocols, and increased overall morbidity[41]. The financial burden posed by nonunion remains a challenge for orthopedic surgeons with a total estimated cost of these complications ranging between $23000 and $60000 per patient[42]. Numerous techniques of treating nonunion have been described in the literature including invasive interventions such as open reduction internal fixation with the use of bone graft or bone graft substitutes. Autologous cancellous bone graft derived from the iliac crest is still considered the gold standard graft option due to its high potentials of osteoconduct, osteoinduction, and osteogenesis. However, there is a limit to the amount of bone graft from iliac crest donor site that can be harvested in the reconstruction of large osseous defects. In addition, there are disadvantages of chronic donor site pain, cosmetic concern, and nerve injury, which have been documented in the literature[28].

The use of cBMA as an adjunctive procedure has gained attention in the treatment of nonunions[30]. The current literature demonstrates faster healing with greater than 94% union rate when using cBMA combined with allograft compared with conventional autologous cancellous bone graft[33]. Ismail et al[31] reported similar union rates and outcomes when comparing cBMA and iliac crest autograft. The benefits of cBMA as an adjunctive therapy has also been demonstrated in the treatment of upper extremity long bone nonunion. Garnavos et al[37] described successfully using a minimal invasive approach by injecting cBMA to address humeral diaphyseal fractures, thereby avoiding potential complications associated with the conventional compression plating technique for treating humeral nonunions. Hernigou et al[39] utilized the same minimally invasive technique to treat diabetic ankle fractures nonunion. The diabetic population poses a challenge for orthopedic surgeons with well-documented increased complications and increased time to bony union.

Hernigou et al[39] also reported a union rate of 82.1% with minimal complications in patients who received cBMA compared to a union rate of 62.3% with major complications in patients who received iliac bone graft alone.

Several studies evaluated the effect of BMA concentration on functional outcomes when treating long bone nonunions. Hernigou et al[30] demonstrated that improved time to union with the use of cBMA was potentially related to the number of progenitors in the graft. The amount of bone healing may be directly related to the concentration of cells and the time to union may be indirectly related to the number of cells[30]. This finding was also supported by Guimaraes et al[38] demonstrating that grafts used in patients whom treatment failed contained significantly lower number of total nucleated cells. Bastos Filho et al[35] compared using cBMA vs whole volume BMA reporting no significant difference in time to union and patient satisfaction score. Although no significant difference was reported, this may be attributed to the small sample size in the cBMA group \((n = 2)\) and minimal follow up. In addition, this study highlighted that unprocessed cBMA contains larger volume and fatty content in the graft increasing the risk of pulmonary embolism, therefore the smaller volume of cBMA may in fact be a safer alternative.

**cBMA in tendon repair**

Tendon injuries typically result from repetitive motions or overuse and can be difficult to treat as many patients either present late or after a prolonged period of non-operative management making treatment challenging due to the chronicity of the injury. It has been well documented that delayed presentation of rotator cuff tears decreases the MSC content and healing potential in patients[23]. A study by Hernigou et al[25] reported a significant reduction in the number of MSCs at the tendon-bone interface of the greater tuberosity in patients with a rotator cuff injury. In addition, they found that the severity of the decrease in MSC content correlated to increasing patient age, delay between onset of symptoms and surgery, fatty infiltration stage of muscle, and the number of involved tendons[35]. It has been demonstrated that MSCs have the potential to develop into tenocytes and can be a source of growth factors to establish an environment conducive to tendon tissue regeneration. MSCs in the form of cBMA have been shown to improve the strength and quality of tissue formed when used in tendon repair[34,36,38].

The current literature has demonstrated that the addition of cBMA can help to heal tendon injuries and at times may decrease the healing time and rate of re-rupture. Hernigou et al[35] reported enhanced healing and improved quality of the repair surface on ultrasound and MRI in patients receiving cBMA during rotator cuff repair. They reported that 100% of the rotator cuff repairs healed by six months compared to 67% in the control group. Furthermore, 87% of the study group had an intact rotator cuff repair compared to 44% of the control at ten year follow up indicating superior outcomes in the longer term[34]. The benefits of cBMA in tendon repair
have also been demonstrated in the Achilles tendon model. Stein et al.\(^{[38]}\) reported excellent results with no re-ruptures, decreased calf atrophy, early mobilization, a 92% return to sport, and better ankle range of motion in patients receiving anisotropic cBMA during Achilles tendon repair compared to those who received no additional treatment.

One of the difficulties in analyzing BMA literature is the variable methods of harvesting, preparing, and concentrating cBMA. Mazzocca et al.\(^{[37]}\) devised a novel technique for harvesting BMA in patients undergoing rotator cuff repair with no donor site morbidity. BMA was harvested using the anchor tunnel of the humeral head during routine arthroscopic rotator cuff repair. No additional complications during the procedure, no significant delay in the procedure, and no difference in functional patient outcomes were reported when using this harvest technique\(^{[37]}\). Lee et al.\(^{[43]}\) studied the use of two different concentrations of allogenic cBMA in patients with lateral epicondylitis. They found no significant differences in the changes of elbow pain and performance between the two groups on follow up visits but they did note faster pain improvement and an earlier plateau of performance scores in the group that received a higher concentration of MSCs\(^{[43]}\). Lastly, Mazzocca et al.\(^{[46]}\) showed that MSCs treated with insulin showed statistically significant increase in gene expression of tendon-specific markers, increase in content of tendon-specific proteins, and increase in receptors on the cell surface. Therefore, these studies demonstrate that there are many factors that can increase the potential for tenocyte differentiation and enhanced tendon repair and regeneration.

Level of evidence
Although the literature highlights the potential benefit of cBMA as either a primary or adjunctive treatment strategy in the treatment of cartilaginous lesions, bony defects, and tendon injury, the majority of these studies were of clinical level of evidence III or IV. This review demonstrates the need for future randomized clinical trials with larger numbers of subjects and standardization of harvesting and application. Although several studies evaluated the effect of cell concentration on healing potential, an effective therapeutic range has yet to be established for each tissue environment.

Summary of MSC mechanism
Adult BMSCs have two primary functions: (1) to differentiate into distinctive end-stage cell types such as bone, cartilage, and tendon; and (2) to secrete bioactive macromolecules that are both immunoregulatory and regenerative\(^{[44]}\). Every cell has a half-life with a turnover sequence mechanism that gives rise to the phenotypes in complex tissues. This allows for both replacement of cells, as well as, the capacity for differentiation into bone, cartilage, and tendon. BMSCs also have characteristic markers of pericytes, which are smooth muscle vascular support cells that may play an important role in stem cell differentiation\(^{[44,45]}\). MSCs also demonstrate trophic activity through secretion of both cytokines and growth factors\(^{[46]}\). The intrinsic secretory activity of MSCs affords a regenerative environment for the repair of injured or damaged tissues\(^{[44]}\). Tissue-specific scaffolds have also been utilized in tissue engineering to reform tissues when MSCs are implanted into different tissue sites. The capacity for cell regeneration and repair relies on several additional factors including patient age, extent of injury/damage, and the functional ability of MSCs to grow and repair. Tissue engineering allows for the manipulation of both the delivery of MSCs to targeted tissue sites and the microenvironment for which cells grow in order to enhance differentiation\(^{[44]}\). Future investigations will continue to focus on harnessing the therapeutic potential of MSCs in tissue specific environments to enhance regeneration and repair of cartilage, bone, and tendon.

Conclusion
The current literature demonstrates the potential benefits of utilizing cBMA for the repair of cartilaginous lesions, bony defects, and tendon injuries in the clinical setting. The studies have demonstrated using cBMA as an adjunctive procedure can result in cartilage healing similar to that of native hyaline tissue, faster time to bony union, and a lower rate of tendon re-rupture. This systematic review also demonstrates discrepancies between the literature with regards to various methods of centrifugation, variable cell count concentrations, and lack of standardized outcome measures. Although several studies evaluated the effect of cell concentration on healing potential, an effective therapeutic range has yet to be established for each tissue environment. Future studies should attempt to examine the integral factors necessary for tissue regeneration and renewal including stem cells, growth factors and a biologic scaffold.

COMMENTS

Background
Bone marrow aspirate (BMA) has been utilized as a source of bone marrow-derived mesenchymal stem cells (BM-MSC) with its relative ease of harvest, low morbidity, and feasible cost. BMA alone has a relatively low percentage of MSCs and therefore concentrated bone marrow aspirate (cBMA) has gained increased attention. cBMA stimulates tissue regeneration and repair and has become an increasingly popular alternative and adjunct in the treatment of cartilaginous lesions, bony defects, and tendinous injuries.

Research frontiers
Current research has focused on the use of cBMA in cartilage, bone, and tendon regeneration and repair. The available literature regarding the use of cBMA in different tissue environments is highly heterogeneous with regards to indications, concentrations and overall functional outcomes. This systematic review attempts to establish proof of concept for the use of cBMA in these biologic environments.

Innovations and breakthroughs
This systematic review demonstrates the potential benefits of utilizing cBMA for the repair of different tissue types in the clinical setting based on the most up-to-date published clinical studies. This systematic review also highlights
discrepancies between the literature with regards to various methods of centrifugation, variable cell count concentrations, variable methods of application of cBMA, and the lack of standardized outcome measures.

**Applications**

The current literature demonstrates the potential benefits of utilizing cBMA for the repair of cartilaginous lesions, bony defects, and tendon injuries in the clinical setting. The studies have demonstrated using cBMA as an adjunctive procedure can result in cartilage healing similar to that of native hyaline tissue, faster time to bony union, and a lower rate of tendon re-rupture.

**Terminology**

cBMA: Concentrated bone marrow aspirate; BMA: Bone marrow aspirate concentrated by centrifugation in order to increase the ratio of MSCs.

**Peer-review**

The authors present a well written systematic review examining the use of BMA in the management of cartilage, bone, and tendon injuries. Overall, the paper is very well organized and reads well.

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