Enhanced Subchondroplasty Treatment for Post-Traumatic Cartilage and Subchondral Bone Marrow Lesions in a Canine Model

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ABSTRACT: This study characterizes outcomes associated with subchondroplasty (SCP) versus SCP enhanced with platelet-rich plasma (PRP) or bone marrow aspirate concentrate (BMC) treatment of impact-induced subchondral bone marrow lesions (BML) using a validated preclinical canine model. With IACUC approval, purpose-bred research hounds ($n = 24$) underwent arthroscopic impact injury (40 N) to both medial femoral condyles. At 3 months, functional assessments, arthroscopy, and magnetic resonance imaging (MRI) were performed. One knee in each dog ($n = 24$; $n = 12$ per endpoint) was randomly assigned to SCP with the other knee randomly assigned to SCP + PRP, SCP + BMC or sham injection (control) ($n = 8$ per group; $n = 4$ per endpoint). Dogs were evaluated at 6 and 12 months after treatment using functional assessments, radiography, arthroscopy, and MRI and humanely euthanized at 6 or 12 months after treatment for histologic assessments. At 6 months post-treatment, comfortable range-of-motion (CROM) was higher ($p < 0.04$) in SCP + PRP and SCP + BMC knees compared with controls. At 1 year post-treatment, %Total Pressure Index was higher ($p = 0.036$) in SCP + BMC compared with controls, pain was lower ($p < 0.05$) in SCP + BMC and SCP + PRP compared with SCP and controls, and CROM was higher ($p < 0.05$) in SCP + BMC and SCP + PRP compared with SCP and controls. Knees treated with SCP + PRP and SCP + BMC had better ($p < 0.05$) MRI grades than SCP and controls. No statistically significant differences in arthroscopic or histologic pathology were noted. Clinical significance: Biologics added to SCP treatment may further enhance its beneficial effects by improving range-of-motion, pain severity, and limb loading through 1 year after treatment. However, these benefits must be considered alongside cost, logistics, and treatment availability. © 2019 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 38:740–746, 2020

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Osteoarthritis (OA) creates a significant burden on our healthcare system. Millions of patients will be affected in their lifetime, resulting in decreased quality of life for patients and significant economic impact in terms of related healthcare costs and lost productivity. Post-traumatic OA (PTOA) accounts for approximately 12% of the total prevalence of symptomatic OA.[1] PTOA is unique in that, unlike heritable or aging-related OA, a known traumatic event has occurred to an otherwise healthy joint. In cases of PTOA, the traumatic event leads to a cascade of deleterious effects resulting in the development and progression of the whole-joint inflammatory and degenerative changes that define OA.

Because OA and PTOA are whole-joint disorders, we take a "joint as an organ" approach to diagnosis, prevention, and treatment. While articular cartilage and synovium receive the attention warranted based on their roles in OA, subchondral bone plays vital roles in OA and PTOA that are often overlooked.[2–5] Alterations in subchondral bone can affect the integrity and function of the whole joint organ.[6–9] As such, the subchondral bone plays an important role in the development and progression of OA and should be considered when implementing comprehensive treatment plans for patients with OA.

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Bone marrow lesions (BMLs) as seen on magnetic resonance imaging (MRI) currently provide the most sensitive and specific clinical measure of symptomatic subchondral bone pathology. These MRI findings correspond histologically with edema, trabecular fracture, disruption and necrosis, cysts, fibrosis, and cartilage fragmentation.[10] The association of subchondral BMLs and symptomatic OA pain has been well established. Previous studies reported that patients with painful knee OA were 2.5 times more likely to have BML compared with asymptomatic patients, and an increase in BML volume has been correlated with more severe pain.[11–13] Further, BMLs are often present in knees with early and pre-radiographic OA, which is thought to be due to subchondral remodeling and microfractures from impact injury and/or chronic overloading.[14,15]

Currently, BMLs are often therapeutically neglected in PTOA with the majority of treatment strategies focused on addressing the most apparent damage to the joint (i.e., cartilage, meniscus, or ligament) without consideration of whole-joint involvement. This current gap in effective interventions for progressive joint deterioration and the need for therapeutic strategies to address subchondral bone pathology are apparent.[16,17] An intervention with the capacity to relieve symptoms, repair subchondral bone, and alter the natural history of joint deterioration is needed.

Subchondroplasty® (SCP) is a procedure with the potential to meet this clinical need. SCP is performed by injecting a flowable, synthetic, calcium phosphate (CaP) bone void filler in the subchondral region of a persistent BML.[18–21] In bone, an endothermic reaction allows the CaP to crystallize such that it has the potential to improve the structural integrity of damaged subchondral bone through effective remodeling.[18,22,23]

While SCP has reported clinical benefits in treating subchondral bone pathology and can be considered an orthobiologic, it has been associated with significant morbidity when used at higher volumes, and alone, does not fully address the current gap in treatment.[21,24–26] As such, combination therapy to include other orthobiologics with CaP bone void filler may be beneficial. The two primary candidates for combination therapy are platelet-rich plasma (PRP) and bone marrow aspirate concentrate (BMC) based on their safety profiles, regulatory compliance, and known biologic effects on bone. PRP provides a source of platelet-derived growth factors with angiogenic and osteoinductive potential.[27] BMC also contains angiogenic and osteoinductive proteins as well as osteoprogenitor and pluripotent mesenchymal stem cells that provide osteogenic and chemotactic capabilities in stimulating bone healing, integration, and remodeling.[27] Therefore, this study was designed to use a valid preclinical model of PTOA[21,28] to assess the longitudinal effects of the addition of PRP or BMC to SCP for treatment of femoral condylar BMLs with respect to clinical, diagnostic imaging, and microstructural outcome measures. We hypothesized that BMC-augmented SCP would be associated with superior outcomes when compared with all other treatment groups.

MATERIALS AND METHODS

All procedures were approved by our institution's Animal Care and Use Committee. Purpose-bred research hounds (2–3 years of age, 20.1–24 kg; Marshall Farm BioResources, North Rose, NY, 145 USDA #21-A-008) ($n = 24$) were used. Orthopaedic examination was performed by a board-certified veterinary orthopaedic surgeon prior to inclusion to assess limb function, knee pain, comfortable knee range of motion (CROM), and knee effusion as previously described.[21] Briefly, knee CROM was measured using a standard goniometer placed along the lateral axis of the tibia and the other arm placed along the lateral axis of the femur with the hinge point centered over the knee joint line. The knee was then manually extended to the highest angle the dog tolerated without showing resistance or pain to determine and record the extension angle (degrees). The knee was then manually flexed to the most acute angle the dog tolerated without showing resistance or pain to determine and record the flexion angle (degrees). The flexion angle was subtracted from the extension angle to determine CROM for each knee. Clinical lameness scores were determined for each dog based on visual examination of gait by a board-certified veterinary orthopedic surgeon using a 10 cm visual analog scale (VAS) and a validated grading system: 0—No observable lameness; 1—Intermittent, mild weight-bearing lameness with little, if any, change in gait; 2—Moderate weight-bearing lameness, obvious lameness with noticeable gait change; 3—Severe weight-bearing lameness, "toe-touching" only; 4—Non-weight-bearing. Knee pain and knee effusion were assessed subjectively based on a VAS and recorded for each hindlimb of each dog.

On the day of surgery, dogs were premedicated, anesthetized, and prepared for aseptic surgery of both hindlimbs. Standard anterolateral scope and anteromedial instrument portals were created in each knee ($n = 48$ knees). Arthroscopic-assisted impact injury (40 N at a rate of 100 mm/s) was delivered to each medial femoral condyle using a custom-designed, spring-driven impactor with an 8 mm diameter tip inserted through the anteromedial portal as previously described.[28]

The dogs were recovered from surgery and received analgesics (intramuscular morphine followed by non-steroidal anti-inflammatory) for a minimum of 48 h postoperatively and then as needed based on physical parameters indicating the presence of pain. All dogs were restricted to their kennels with monitored daily out-of-kennel exercise, which included on-leash walks by an individual handler for 30 min and group enrichment in a confined area for 15–20 min for the entire study period.

Dogs were assessed at 12 weeks after impact injury using assessments of lameness, limb function, knee pain, comfortable knee range of motion, and effusion in the knees, as previously described.[21] For longitudinal measurements, loss in CROM was determined by subtracting the measured CROM at the given time point from the pre-inclusion CROM for each knee.

Limb kinetics were performed as previously described.[29–31] Briefly, kinetics assessment was performed by having a dedicated handler trot dogs across a pressure-sensing walkway (GAITFour, Haverton, PA) on-leash in each direction with the handler attempting to maintain a consistent velocity. Passes were included for analysis when the dogs walked at a steady pace with all four footfalls recorded for at least three gait cycles. At least three acceptable passes (3–5 gait cycles), with video documentation, were obtained for each dog at each time point. The software program was used to distinguish the paw print for each footfall, which was then identified manually as left front, right front, left hind, or right hind, accordingly. Mean percent body weight distribution (%BW) was determined for each limb using the three complete data sets based on the total pressure index (TPI). The %TPI for the operated limb was chosen a priori as the measure to report and compare.

Three months after injury, functional assessments, arthroscopy, and MRI were performed. Under general anesthesia, MRI of each knee was performed using a 1.5 T magnet (Sigma Horizon LX1.5 T; GE Health Care, Milwaukee, WI). The sequences performed included a sagittal T1 two-dimensional fast spin-echo (2D FSE), a sagittal T1, a sagittal T2 fat saturation (FAT-SAT) 2D, a coronal proton dense (PD) 2D, and a coronal PD (FAT-SAT, 2D FSE) using a dedicated four-channel phased array knee coil (Medrad, Inc., Warrendale, PA). A board-certified veterinary radiologist (blinded to treatment and time point) evaluated all MRIs to subjectively assess each MRI for the presence and severity of BMLs, as previously described.[21] Briefly, BML severity was graded using the following classification system: Grade 1—BML comprising <25% of condylar volume; Grade 2—BML comprising 25–50% of condylar volume; Grade 3—BML comprising >50% of condylar volume.

Arthroscopic evaluation of each knee joint was then performed to assess whole-joint pathology, as described previously.[32] Briefly, the entire joint was subjectively evaluated based on visualization and probing of suprapatellar, patellotrochlear, medial and lateral femorotibial, and intercondylar notch structures to assess for synovitis and/or cartilage, meniscal, and/or ligament pathology, which was described and documented, if present. Specifically, synovial pathology was graded as normal, slight, mild, moderate, marked, or severe.[32] Articular cartilage pathology was graded as smooth, slightly fibrillated, partial thickness lesions, deep lesions, or diffuse severe damage.[32] Meniscal pathology was graded as none, fibrillation, incomplete tear, complete tear, or complete disruption.[32]

Using fluoroscopic guidance, a custom-designed Jamshidi-type cannulated needle with end and side ports were inserted from proximal to distal within the condyle of each knee (Fig. 1), as previously described.[28] One randomly assigned knee of each dog was injected with 1 ml of AccuFill® Bone Substitute Material (BSM) into the BML bone defect such that the injectate filled the entire BML and the subchondral bone (Subchondroplasty® [SCP]) ($n = 24$; 12 per endpoint), and the other knee was then randomly assigned to one of the following treatment groups:

- SCP plus autogenous platelet-rich plasma (SCP + PRP) ($n = 8$; 4 per endpoint): 30 ml of whole blood were obtained from each dog via aseptic jugular venipuncture and processed using a commercially available system (GPS; Biomet Biologics, Warsaw, IN) to obtain PRP per manufacturer's instructions. One milliliter of PRP was first injected and then followed by 1 ml of AccuFill BSM, as described above.
- SCP plus autogenous bone marrow aspirate concentrate (SCP + BMC) ($n = 8$; 4 per endpoint): 30 ml of bone marrow were aspirated from the proximal humerus of each dog and processed using a commercially available system (BioCUE™; Biomet Biologics) to obtain BMC per manufacturer's instructions. one milliliter of BMC was first injected and then followed by 1 ml of AccuFill BSM, as described above.
- Sham injection (control) ($n = 8$; 4 per endpoint): The needle was inserted in the same manner as for all other treatments and then removed without injection being performed.

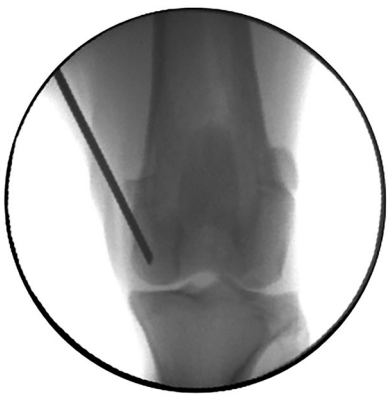

Figure 1. Representative image showing needle placement for fluoroscopically guided percutaneous injection into the area of bone marrow lesion in the medial femoral condyle.

To limit any bias, needles were inserted into both knees prior to the surgeon being told which would receive SCP treatment and which would receive the other treatment. Post-treatment radiographs were obtained to document the intended injection.

Dogs were assessed 6 and/or 12 months after treatment using functional assessments, radiographic evaluation, arthroscopy, and MRI as described above. Dogs were humanely euthanized at 6 or 12 months after treatment for histologic assessments. Necropsy was performed by a board-certified veterinary pathologist, who was blinded to treatment and clinical findings. Both knees from each dog were carefully dissected to assess the gross pathology of each joint and to collect tissues for whole joint histologic assessments, as previously described.[28] Briefly, synovial tissue was routinely processed for hematoxylin and eosin (H&E) staining. Medial menisci were divided into three sections, softened with 10% ethylenediaminetetraacetic acid (EDTA), and processed for H&E and toluidine blue staining. Medial femoral condyles were each divided into four coronal sections approximately 2–3 mm thick. The bone sections were alternated between decalcified and nondecalcified processing such that two sections were processed per knee in each manner for each dog. Medial tibial plateaus of both knees were divided into three sections approximately 2–3 mm thick. After decalcification with 10%, EDTA was complete, bone sections were processed, and stained (H&E and toluidine blue). For nondecalcified processing, tissues were dehydrated through a series of graded ethyl alcohol solutions, embedded in poly(methyl methacrylate), and sectioned (150 μm thickness) using a diamond saw and grinder. The sections were stained with Goldner's trichrome. Histologic scoring of the joint tissues were performed by two board-certified veterinary pathologists, blinded to treatments, using the Osteoarthritis Research Society International (OARSI) histologic scoring system for canine OA.[32] Categorical scores for femoral condyles, tibial plateau, meniscus, and synovium were determined, and a total score for each joint was derived by adding all category scores.

Descriptive statistics were performed to determine means and standard deviations (SD). Data were compared for statistically significant ($p < 0.05$) differences between groups using a one-way analysis of variance (ANOVA) for continuous data or ANOVA on ranks for categorical data. Repeated measures ANOVA or repeated measures on ranks were performed to determine within-group differences over time.

RESULTS

All dogs underwent impact, designated treatment, and all outcome measures as intended and described above. All dogs survived for the intended duration of the study. Three months after impact and prior to treatment, all knees showed clinical dysfunction (Fig. 2) and had focal articular cartilage defects with associated subchondral BMLs based on arthroscopic, radiographic, and MRI assessments.

At the 6-month post-treatment time point, clinical function measures were highest in the SCP + BMC group with CROM being significantly ($p < 0.04$) better in SCP + PRP and SCP + BMC treated knees compared with controls (Fig. 2). No statistically significant differences in arthroscopic pathology (Fig. 3), MRI (Fig. 4),

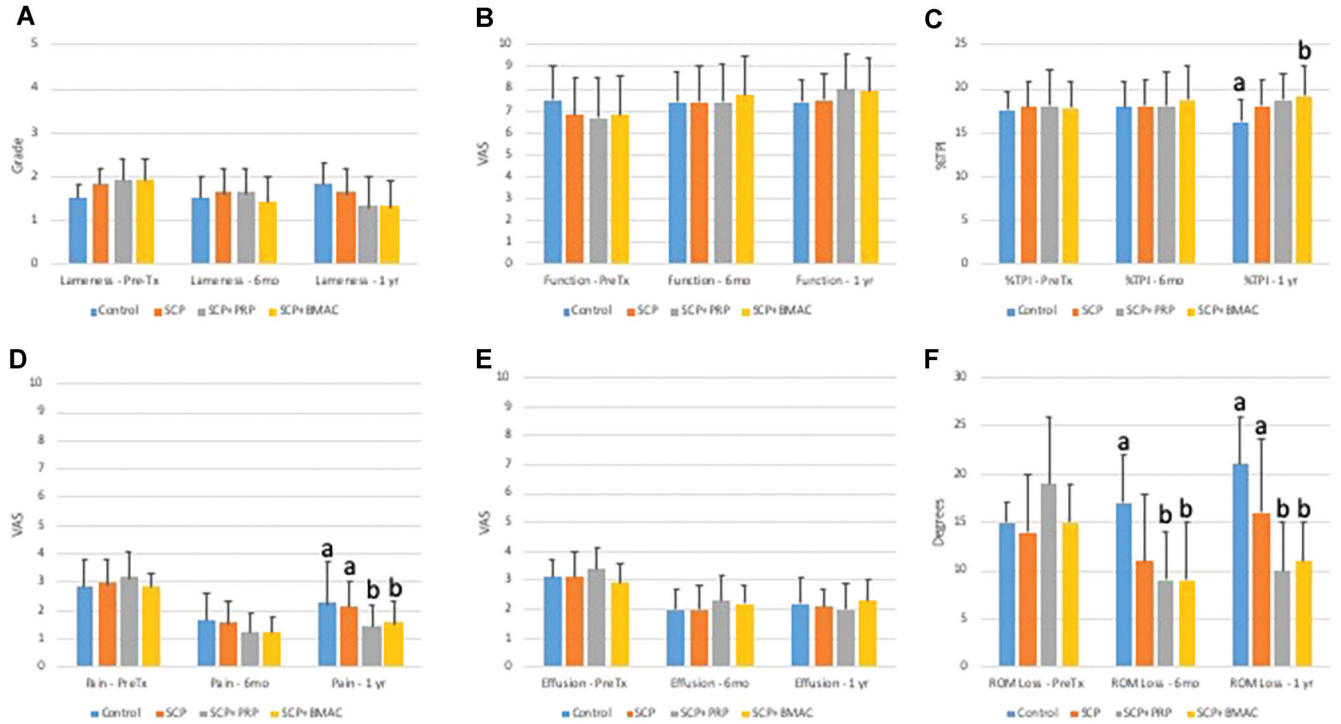

Figure 2. Mean ± standard deviation (SD) values for lameness (A), level of function (B), %TPI (C), pain (D), effusion (E), and range of motion loss (F). Lameness, normal is 0 and higher grades are worse (more lameness); function, normal is 10 and lower scores are worse (less function); expected normal %TPI in dogs is ~20% for each hindlimb. Pain, normal is 0 and higher scores are worse (more pain); effusion, normal is 0 and higher scores are worse (more effusion); loss in comfortable range-of-motion (CROM) was determined by subtracting the measured CROM at the given time point from the pre-inclusion CROM for each knee such that normal would be 0 and greater CROM loss values are worse (less ROM). Different letters denote statistically significant differences at the time point. [Color figure can be viewed at wileyonlinelibrary.com]

or histologic pathology scores (Fig. 5) were noted between groups at 6 months after treatment.

At 1-year post-treatment, clinical function measures were highest in the SCP + BMC group with %TPI being significantly ($p = 0.036$) higher in SCP + BMC compared with controls, pain being significantly ($p < 0.05$) lower in SCP + BMC and SCP + PRP compared with SCP and controls, and CROM being significantly ($p < 0.05$) better in SCP + BMC and SCP + PRP compared with SCP and controls (Fig. 2). Arthroscopic (Fig. 3) and histologic pathology scores (Fig. 5) revealed no statistically significant ($p > 0.05$) differences between treatment groups at 1 year; however, knees treated with SCP + PRP and SCP + BMC had significantly ($p < 0.05$) better MRI grades (Fig. 4) than SCP and controls. All SCP-treated medial femoral condyles showed remaining BSM injectate at the 1-year endpoint, however, the majority of the BSM material was resorbed, especially in the PRP-augmented and BMC-augmented groups (Fig. 5).

DISCUSSION

While BMC-augmented SCP was associated with significant benefits for limb kinetics, knee ROM, pain, and MRI grade after treatment, the results of the present study did not allow us to accept our hypothesis

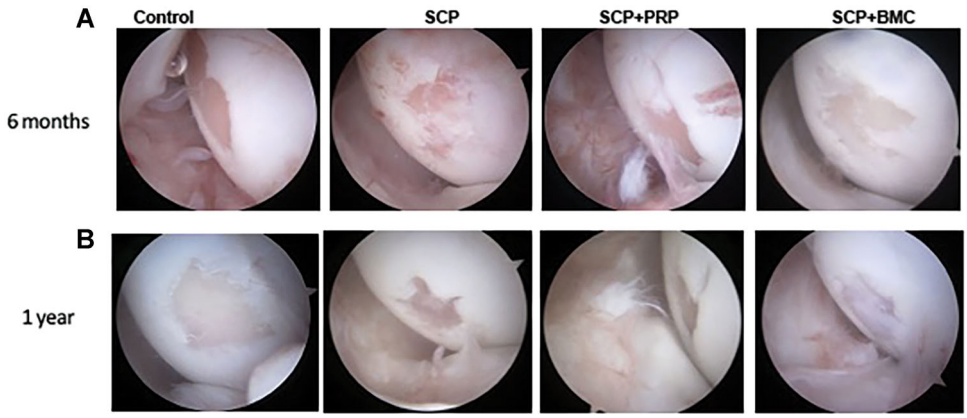

Figure 3. Representative arthroscopic images of each treatment group at 6 months and 1 year. [Color figure can be viewed at wileyonlinelibrary.com]

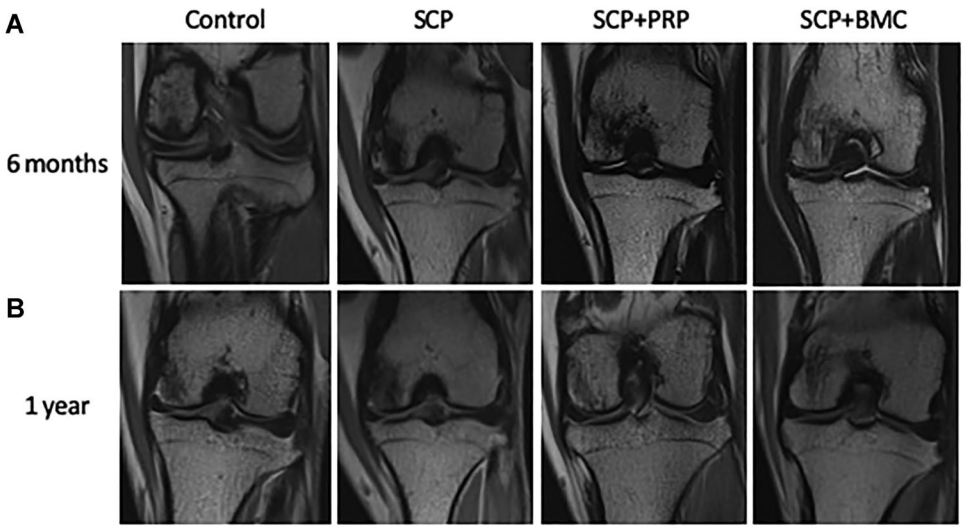

Figure 4. Representative magnetic resonance images showing a key areas of interest for each knee at 6 months and 1 year.

with respect to comprehensive superiority of BMC-augmented SCP treatment of femoral condyle BMLs in this preclinical model of PTOA.[21,28] Importantly, all of the SCP treatments evaluated in this model were safe in terms of related complications, and morbidity was appropriately mitigated by the smaller volume of synthetic, CaP bone void filler used in the present study when compared with a previous study using this model.[21] In addition, each of the SCP treatments was associated with some long-term benefits compared with controls, however, only knee ROM and knee pain in the BMC-augmented and PRP-augmented groups, and limb kinetics and MRI grading of BMLs in the BMC-augmented group, were shown to be statistically significant at the 6-month and/or 1-year time points.

CaP-based bone void filler has been used clinically as an option for treating bone defects based on its documented osteoinductive and osteoconductive properties.[33–37] These properties likely contributed to the beneficial effects of treatment noted in the present and previous studies through moderation of pain associated with subchondral BMLs.[21,28] SCP has been reported to help restore the structural integrity of cancellous and subchondral bone, promoting fracture healing and bone remodeling in the areas of BMLs and altering the symptomatic course of the disease to moderate knee pain and function.[21,28] Interestingly, the data from the present study suggest that BMC, and to a lesser extent PRP, further augmented these beneficial effects. In a previous study,[21] SCP treatment of impact-induced femoral condyle BMLs was associated with significantly better knee ROM (5°–7°) compared with untreated controls at 1 year and 2 years after treatment. In the present study, PRP-augmented and BMC-augmented SCP treatments were associated with between 10° and 12° improvements in knee ROM and significant improvements in knee pain at 1 year compared with controls.

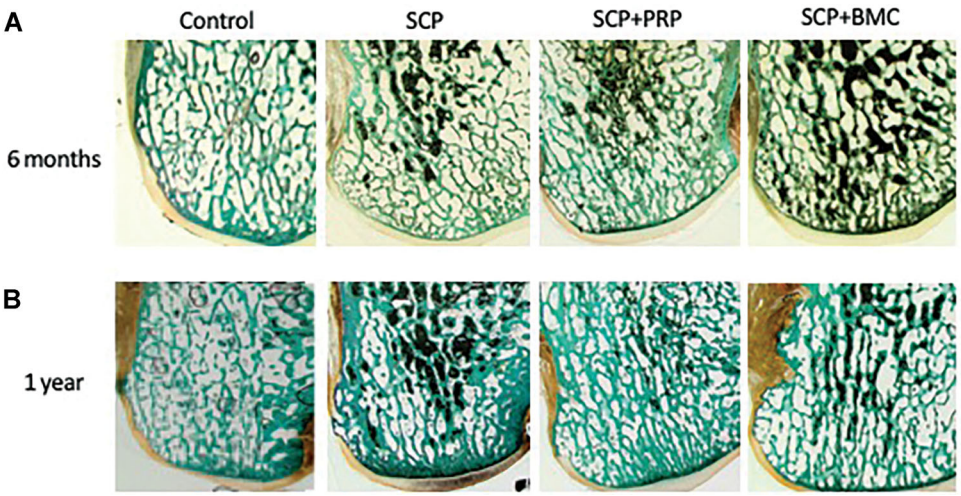

Figure 5. Tissues were collected for whole-joint histologic assessments using the Osteoarthritis Research Society International (OARSI) system.[32] Sections of medial femoral condyles with Goldner's Masson trichrome stain. Black material within the trabecular bone/bone marrow space of the subchondroplasty (SCP) groups is the injected Subchondroplasty® material. [Color figure can be viewed at wileyonlinelibrary.com]

In addition, BMC-augmented SCP treatment was associated with a significant ~18% improvement in limb loading compared with controls at 1 year after surgery. Taken together, these data suggest that these orthobiologics can improve knee pain and function in conjunction with SCP treatment of BMLs with BMC having the most potential benefit. However, cost, logistics, and availability may limit the clinical application of orthobiologics in conjunction with SCP for this indication.

Beneficial clinical outcomes associated with SCP treatment for BMLs including significantly less pain and improved functional scores through 2 years after treatment have been reported.[19,24] There are currently no studies evaluating PRP or BMC treatment of BMLs. However, there are studies that have looked at intraosseous (IO) injection of PRP in patients with more advanced knee OA. A pilot study in 2016 by Sánchez et al.[38] demonstrated improved KOOS pain and function scores in 14 patients treated with a combination of IO and intraarticular (IA) PRP. In a 2018 observational study by Sánchez et al., they compared IA PRP alone versus IO + IA PRP for severe knee OA in 60 patients. At 2, 6, and 12 months after treatment, the IO + IA group had a significant improvement in all KOOS and WOMAC subscales ($p < 0.05$), while the IA group did not improve in any of the scores.[39] Finally, in 2018, Su et al.[40] compared IA PRP, IA + IO PRP, and IA hyaluronic acid injections and found the combination of IA + IO PRP resulted in sustained lower VAS and WOMAC scores and improvement in the quality of life at 18 months. The patients included in the aforementioned studies consisted of patients with documented advanced knee OA. Acute trauma to the knee can result in alterations of the biomechanical and biochemical environment disrupting joint homeostasis. If the structural integrity of cancellous and subchondral bone can be restored, the healing response can be optimized and inflammation associated with BMLs subsides.[21] Therefore, it seems reasonable to suggest that SCP, in combination with PRP or BMC, could play a role in the restoration of joint function and health via mechanical support of the subchondral bone from SCP, and by improving the post-traumatic biochemical milieu with PRP or BMC. To our knowledge, this is the first study to evaluate PRP or BMC enhanced SCP treatment for BMLs of the knee.

The present study is not without limitations. Even with the use of a randomized experimental design, many of the differences noted among groups did not reach statistical significance. However, the study was also designed to address the refine, reduce, and replace principles (the 3Rs) by using preliminary data obtained from previous studies. Sample sizes for this preclinical translational animal model study were based on robust statistical analyses to determine minimum effective number of animals to be used to test the hypothesis (sample sizes of 5–12 dogs per group to achieve a power >0.8 with $p < 0.05$ based on previous data for CROM and histologic scoring[21]) and both statistically and clinically significant differences were realized using at least eight dogs per group. Another limitation involves the lack of characterization of PRP and BMC samples for each dog, however, canine PRP and BMC have been previously characterized and reported to have direct relevance for human clinical applications.[41,42] Similarly, while the endothermic nature of the crystallization process of SCP is a proposed advantage with respect to effects on cells and tissues, the effects from other interactions between SCP and PRP or BMC are unknown and require further study.

CONCLUSIONS

In the present study using a preclinical large animal model for evaluating treatment of impact-induced cartilage and subchondral BMLs, SCP was considered safe with lower injectate volumes resulting in less morbidity than higher volumes previously used.[21] The data suggest that biologics (PRP or BMC) added to SCP treatment may further enhance its beneficial effects by improving knee ROM, and limb loading through 1 year after treatment, possibly due to improved trabecular remodeling of subchondral bone. Taken together, the results of this study support further investigation towards clinical use of orthobiologic-augmented SCP treatment of BML-associated subchondral fractures with BMC having the most potential benefit based on best current evidence.

AUTHORS' CONTRIBUTION

All authors have read and approved the final submitted manuscript. The following is the author contribution: H.A.O., C.C.B., C.R.C., K.K., A.M.S., J.L.C.: substantial contributions to research design, acquisition, analysis and interpretation of data; H.A.O., C.C.B., C.R.C., K.K., S.L.S., A.M.S., J.L.C.: drafting the paper and revising it critically; H.A.O., C.C.B., C.R.C., K.K., S.L.S., F.M.P., A.M.S., J.L.C.: approval of the submitted and final versions.

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