Enhanced pain reduction and hyaline cartilage regeneration using micronized human bone marrow stroma/parenchyma compared to BMAC in a nude rat PTOA model

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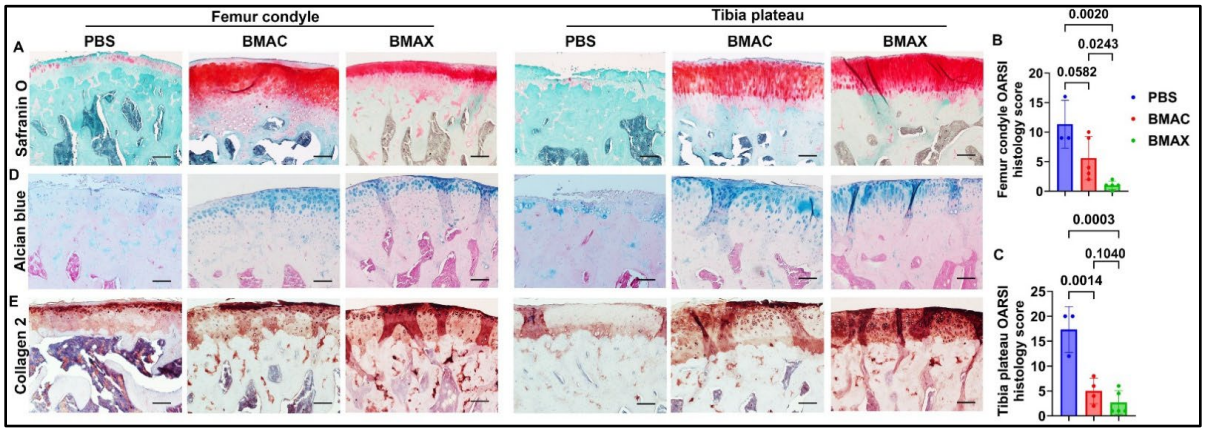
INTRODUCTION: Bone marrow aspirate concentrate (BMAC) is commonly employed to manage various musculoskeletal conditions or injuries. However, BMAC lacks essential bone marrow extracellular matrix (ECM) components pivotal for supporting cell proliferation, migration, differentiation, and communication among marrow-resident cells. We previously reported that MSCs cultivated using a novel processing technique from micronized bone marrow retained ECM components and functional pro-regenerative phenotypes in vitro. The objective of this study was to compare the efficacy of a unique processing technique that retains ECM components and mechanically generates micronized bone marrow (BMAX™) to BMAC in a rodent model of osteoarthritis (OA) using samples from a donor having an elevated senescent cell concentration to better represent an underserved population undergoing an autologous cell treatment.

METHODS: Four prospective (male and female) human donors were patients who underwent BMAC treatment for various orthopedic conditions and who also agreed to participate in the study preprocedurally. Samples were taken from each patient for analysis for senescent cells from BMA and also peripheral blood. Of the two (female) patients with the highest concentration of senescent cells one agreed to donate both a bone marrow core sample and a bone marrow aspirate sample (Informed consent under WCG-IRB# 20230413). Both BMAX™ and BMAC were collected and processed simultaneously in-office during the same surgical procedure. The samples were cultivated and colony-forming unit–fibroblast (CFU-F) assays were performed to quantify clonogenic mesenchymal stromal cell (MSC) precursors obtained from samples. MSCs obtained from both BMAX™ and BMAC cultures were identified via flow cytometry. All animal work was performed at Colorado State University under an approved IACUC protocol (#1322). Adult nude rats were used for 3 treatment groups: BMAX™, BMAC, and PBS controls. Treatments were administered intra-articularly at 4 weeks after induction of OA with destabilization of the medial meniscus (DMM) surgery. Rats were then evaluated for pain (Von Frey and knee-bend test), swelling (knee diameter) and for histological assessment of the affected joint. Statistical analysis was performed using two-way ANOVA. A p-value less than 0.05 was considered indicative of statistical significance.

RESULTS SECTION: Mechanically processed bone marrow MSCs using the BMAX™ device showed significantly higher CFU-F formation and yielded a higher relative percent of MSCs (87%) versus BMAC samples (50.3%,). Pain scoring for both tests was measured at baseline, after DMM injury, and at 4 and 8 weeks following injection treatments. Following DMM injury, both BMAC and BMAX™ treatments resulted in improved pain scores compared to controls at 4 and 8 weeks post-injection for both pain test modalities. Specifically in the knee bend test, BMAX™ treatment seemed to reduce direct pain perception to a higher degree than BMAC. Knee swelling was also evaluated by measuring the differences in knee diameter between right and left knee joints. BMAX™ injected rats exhibited significantly less swelling compared to both BMAC and PBS groups. These data suggest the micronized bone marrow processed through the BMAX™ system has more pronounced anti-inflammatory, or inflammation resolving, properties compared to BMAC. At 8 weeks post treatment, the PBS group showed obvious Safranin O positive matrix loss and superficial zone and midzone cartilage loss. In contrast, both BMAX™ and BMAC groups were found to retain strong Safranin O positive matrix staining which indicated more GAG-rich proteoglycans matrix in both femur condyle and tibial plateau region (**Fig. 1A**). Both groups also had significantly improved OARSI scores (low score indicate better cartilage repair) in cartilage of the femur condyle and tibia plateau (**Fig. 1B-C**). Importantly, BMAX™ was found to have a significantly higher level of improvement in OARSI scores versus BMAC in the femur condyle region (**Fig. 1B**). The OARSI histology scores of BMAX™ groups also showed a trending lower score compared to BMAC in the tibia plateau cartilage (**Fig. 1C**). Improved cartilage repair was also found in BMAX™ and BMAC groups in both femur condyle and tibia plateau regions using Alcian blue staining which showed an obvious retention of proteoglycan content (**Fig. 1D**). Consistent with these results, both BMAX™ and BMAC groups had more intense COL2 staining than the PBS group in both femur condyle and tibia plateau regions at 8 weeks post treatment with BMAX™ group being more uniformed COL2 intensity (**Fig. 1E**).



DISCUSSION: The findings reported here combined with previous reports from our group suggest BMAX™ generation of micronized bone marrow may provide multiple benefits over common BMAC products including 1) the ability to contain MSCs at the injection/delivery location 2) Enhanced in vivo survival 3) a preferred ECM microenvironment for proliferation and cell signaling and 4) optimized ability to maintain plasticity/stemness due to undisturbed coupling of MSCs with their native scaffold. Overall, novel preparations using micronized ECM-rich bone marrow derived therapeutics may better address the pathological inflammatory and destructive processes and improve clinical outcomes.

SIGNIFICANCE/CLINICAL RELEVANCE: Micronized human bone marrow stroma/parenchyma from a donor with elevated senescent cell concentrations can regenerate hyaline cartilage and reduce pain and swelling in a rat OA model.