ISAKOS CONGRESS 2023

Boston Massachusetts June 18–June 21

Updated Protocols to Enhance the Regenerative Capacity of Bone Marrow

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Disclosures:

Shareholder & President BMAX Medical LLC

Medical Director Elite Regenerative Institute

Introduction

Bone marrow aspirate concentrate (BMAC) is commonly used as a therapeutic agent to resolve orthopedic injuries using its unique biologic properties to reduce inflammation and prime the region for repair. Various factors impact the quality of autologous point-of-care biologics including their source and methods of harvest, processing, and re-administration to the patient. Previous research has demonstrated the importance of the location and method of bone marrow harvest for obtaining high yields of mesenchymal stem/stromal cells (MSCs) and that contrast agents[1,2] and the use of local anesthetics[3] can decrease MSC survival. In the current study we found *in vitro* that: 1) soluble factors differ in platelets derived from separate niches of peripheral blood and bone marrow[4]; 2) red blood cells and their releasates compromise bone marrow derived human MSC survival[5]; 3) the choice of anticoagulants influences the characteristics of BMAC and MSC bioactivity[6]; and 4) MSCs in their native parenchymal niche may have improved regenerative value.

Objective

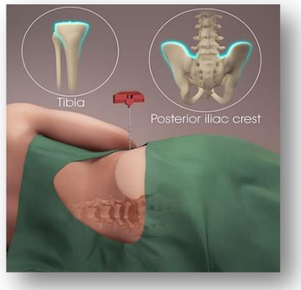
The purpose of these studies was to rigorously evaluate the cellular and acellular components of bone marrow which may have implications on regenerative capacity of autologous therapies. Differences in platelet characteristics in bone marrow and peripheral blood were examined along with soluble factors and MSC functional phenotypes derived from bone marrow using different harvest techniques. To better understand niche effects on bone marrow derived products, we additionally compared the secretome of bone marrow parenchyma versus BMAC fluid.

Methods

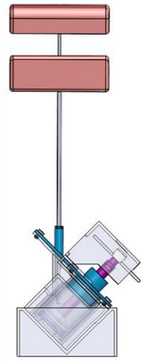
- Leukocyte-poor peripheral blood-derived platelets in plasma (LPP) and leukocyte-poor bone marrow platelets in plasma (BMP) were prepared, activated, incubated and sampled at various time points.
- Growth and immunomodulatory factors were quantitated in LPP and BMP. Differences in BMCs produced using SC and HS at various concentrations were measured *in vitro* including TNC, viability, MSCs CFU-f counts, and cytokine expression profiles.
- For RBC effects, bone marrow-derived human MSCs in early passage were grown under conditions of various HCT and RBCrel concentrations. The percentage of viable, apoptotic and necrotic MSCs was determined via flow cytometry.
- A novel prototype device (**BMAX™**) was used to create a bone marrow-derived MSC product containing native stroma components with a low HCT. **BMAX™** product including cells and stroma were plated in MSC culture media (MilliporeSigma) and incubated at 5% CO2 for 3-14 days (P0-P1) prior to evaluation with flow cytometry for cell phenotyping and immunoassays for secretome profiling.

BMAX™ Bone Marrow Processing Workflow

Harvest Bone Marrow Plug

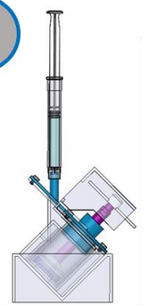


1



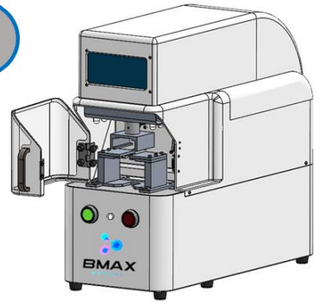
Insert Plug Into Device

2



Inject Saline or Plasma Into Device

3



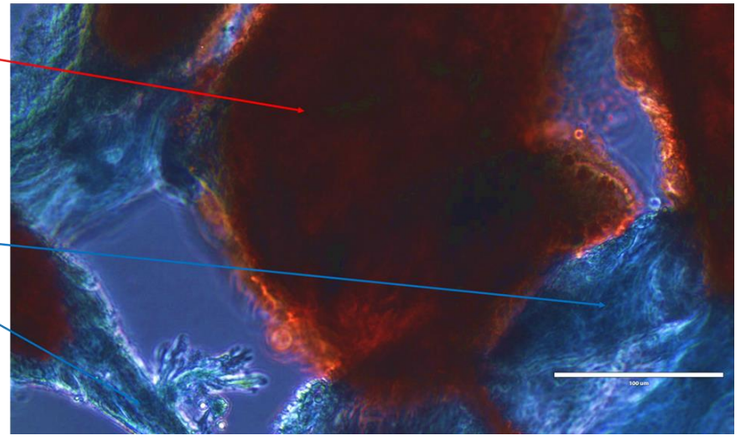
Instrument senses Device RFID and initiates appropriate processing program

4

Extract Processed Marrow

Bone is stained red with Alizarin Red

Glycoaminoglycan (GAG)-rich matrix within sections of trabecular cavities are stained blue with Alcian Blue
***This is where we are collecting the MSCs from in the BMAX™ system**



Results

Bone Marrow Derived Platelets Have a Unique Secretome Profile Compared to Peripheral Blood Platelets[1]

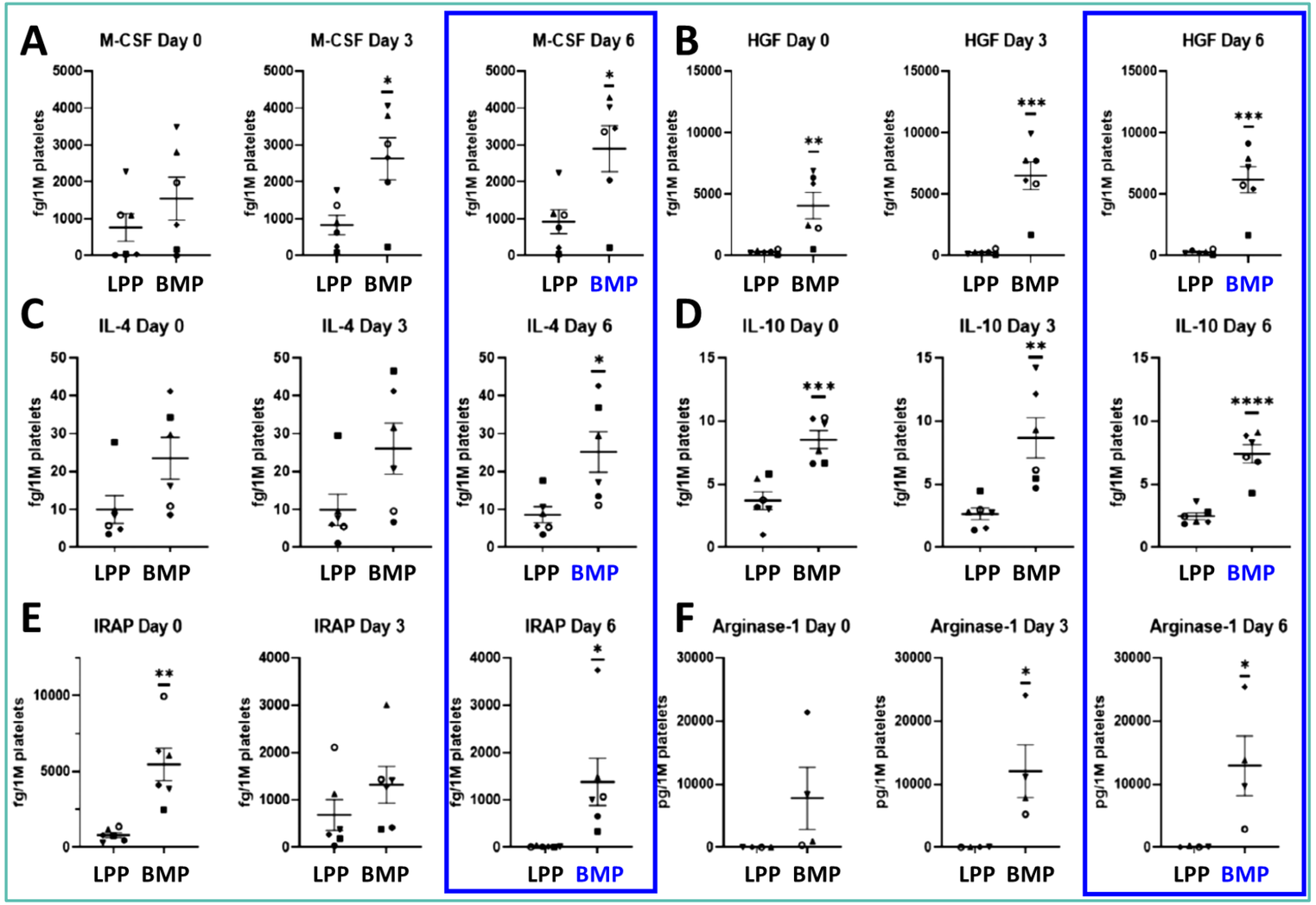


FIGURE 1. Relative levels of the growth factors (A) M-CSF and (B) HGF and immunomodulatory factors (C) IL-4, (D) IL-10 (E) IRAP, (F) Arginase-1 from six donors over a 6-day time course. Symbols represent each donor. Graphs show mean and standard error of the mean. Blue boxes highlight significant changes in all factors after 6 days incubation. *P < 0.05, **P < 0.01, ***P < 0.001. 1M, 1 million.

Results

Hematocrit (HCT) and RBC Releasate (RBCrel) Compromise Bone Marrow Derived MSC Health[2]

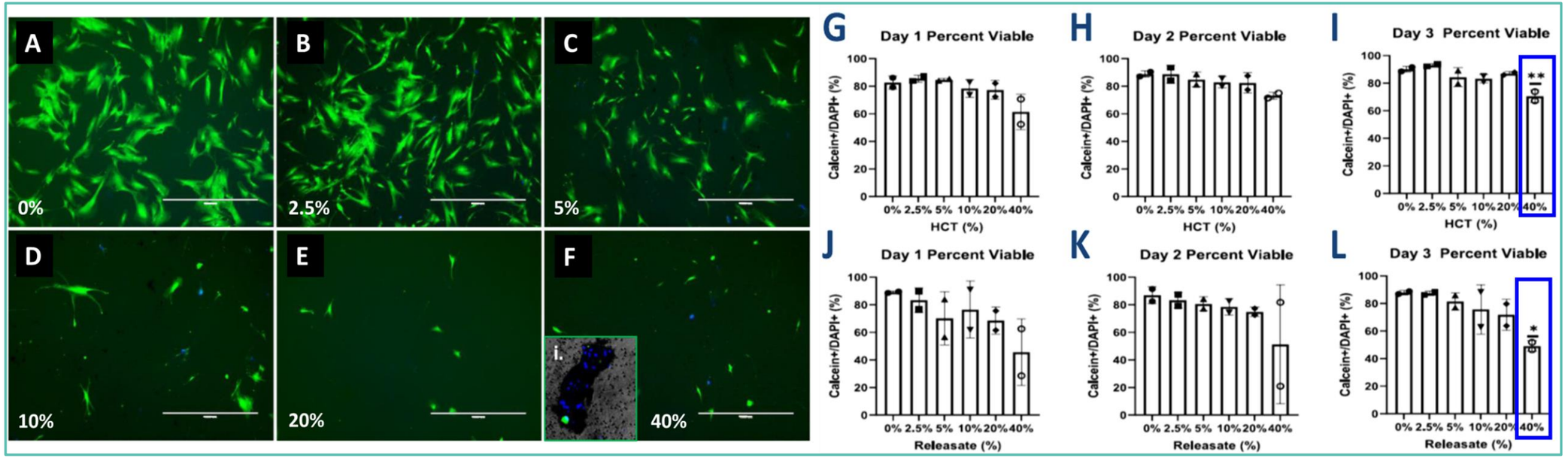


FIGURE 2. Fluorescent imaging of MSCs in HCTs at Day 3 (A–F) and quantitation of MSC viability Day 1–3 for HCT (G-I) and RBCrel (J–L). MSCs in culture were stained with calcein-AM (green, live) and DAPI (blue, dead) and imaged prior to harvesting for viability analysis. Day 1 (A). 0% HCT control (B). 2.5% HCT (C). 5% HCT (D). 10% HCT (E). 20% HCT, (E)(i). 20% HCT with fragmentation of nuclei (subpanel is enlarged, not to scale) (F). 40% HCT (arrow shows microparticle formation from MSC). For all micrographs: Scale bar = 860 microns; Total magnification = 104x (excluding F)(i). Error bars represent the standard deviation of the data set. Blue boxes indicating significant reductions in viability at Day 3 for MSCs co-cultured with RBCrel. Levels of significance: *p < 0.05, **p < 0.01.

Results

Anticoagulant Choice Influences Characteristics of BMAC and MSC Bioactivity *in vitro*[3]

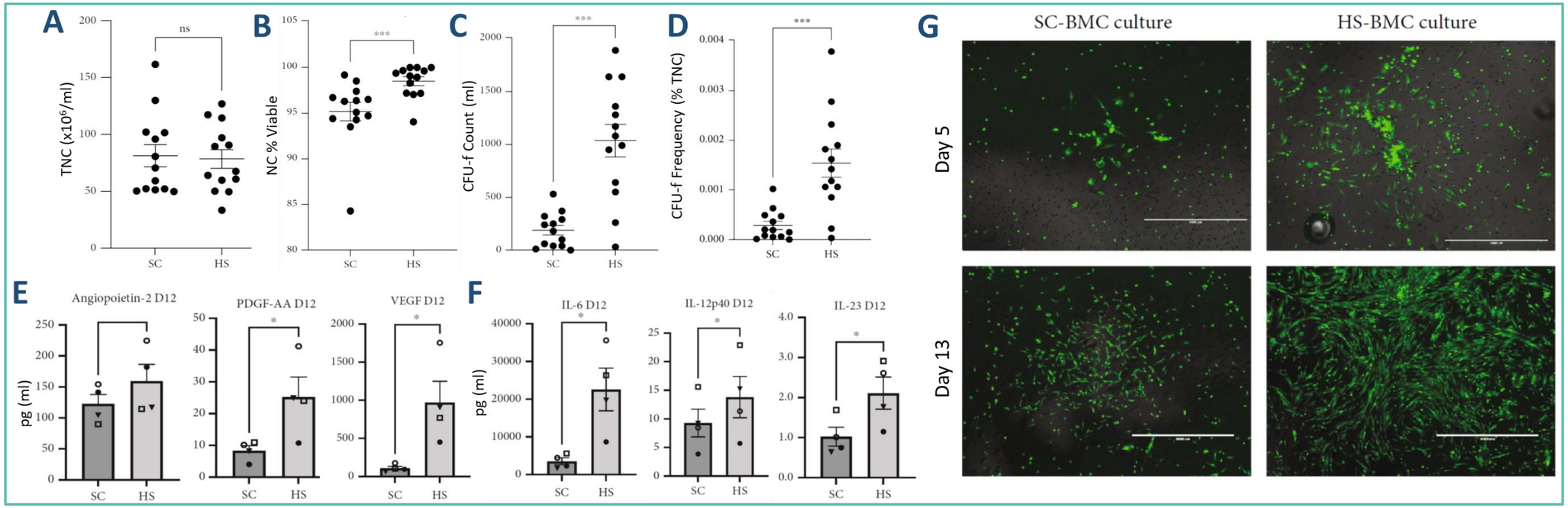


FIGURE 3. Comparison of BMC characteristics derived from 15% sodium citrate (SC) and 100 U/mL heparin sodium (HS): (A) total nucleated cell (TNC) count per milliliter, (B) nucleated cell (NC) viability, (C) colony-forming units with fibroblast morphology (CFU-f) counts per milliliter, and (D) frequency of CFU-f amongst the TNC population. (E) Growth factor concentrations and (F) immunomodulatory cytokine concentrations in cell culture media with BMCs at day 12 (D12). (G) Calcein-AM-stained CFU-fs in culture from SC and HS at days 5 and 13. Graphs display the population mean and standard error of the mean; shapes indicate donor-matched BMC products. ∗P < 0:05, ∗∗P < 0:01, and ∗∗∗P < 0:001.

Results

Isolation of Bone Marrow MSCs Embedded in Native Tissue Stroma (BMAX™) Yields Differential MSC Phenotypes with Enhanced Pro-Regenerative Characteristics

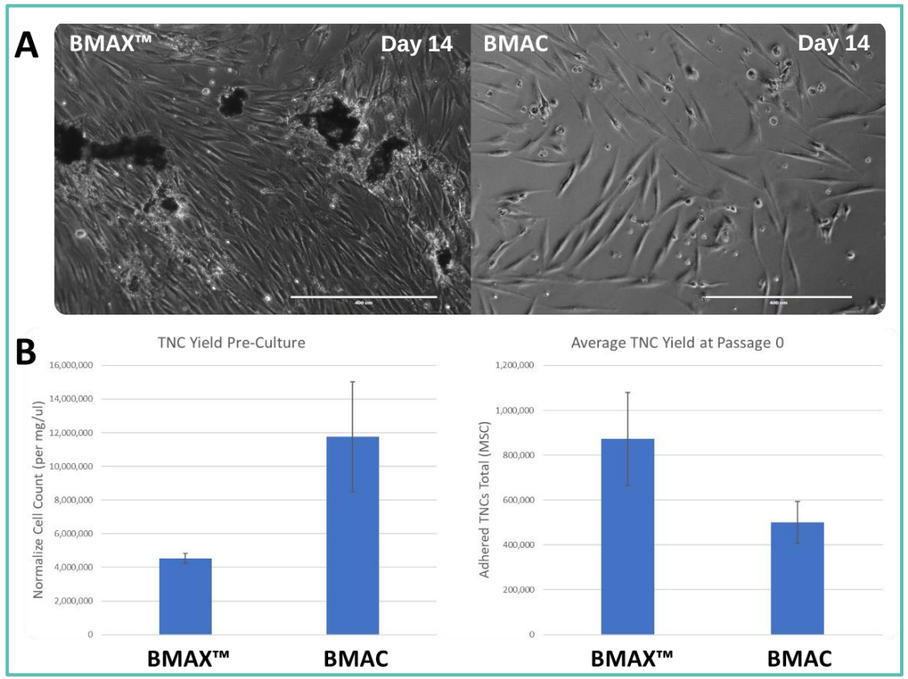


FIGURE 4. (A) High cell density associated with GAG-containing tissue from BMAX™ derived cultures versus more dispersed BMAC derived cultures. Total Magnification = 260x. (B) BMAX™ harvest yields lower TNC counts but more adherent MSC-like cells.

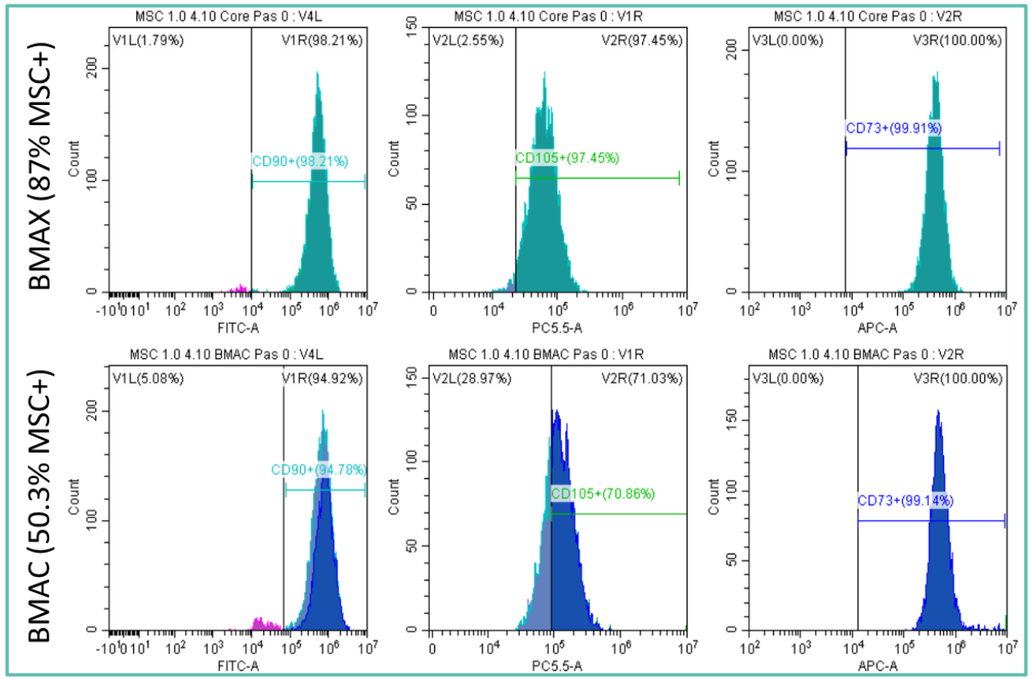


FIGURE 5. Flow cytometry results showing increased MSCs in BMAX™ processed bone marrow samples at passage 0. Adherent MSCs were stained for ISCT standard MSC epitopes. CD90+, CD105+ and CD73+ cells were gated to exclude population of cells staining for WBC markers.

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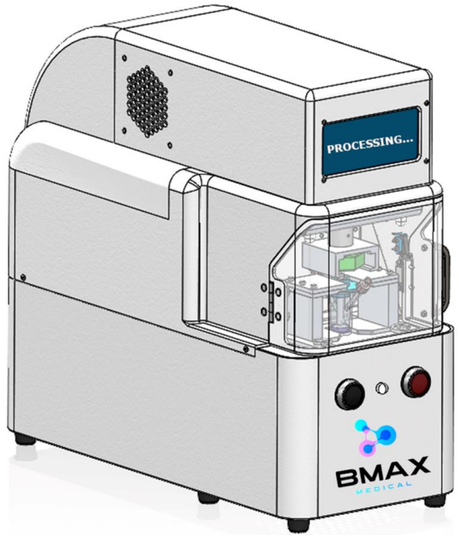
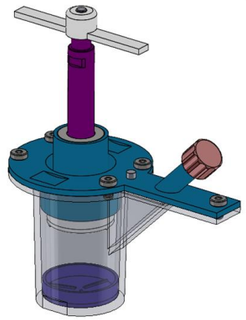
Results

Isolation of Bone Marrow MSCs Embedded in Native Tissue Stroma (BMAX™) Yields Differential MSC Phenotypes with Enhanced Pro-Regenerative Characteristics



BMAX™ Benchtop Unit



BMAX™ Bone Marrow Disposable

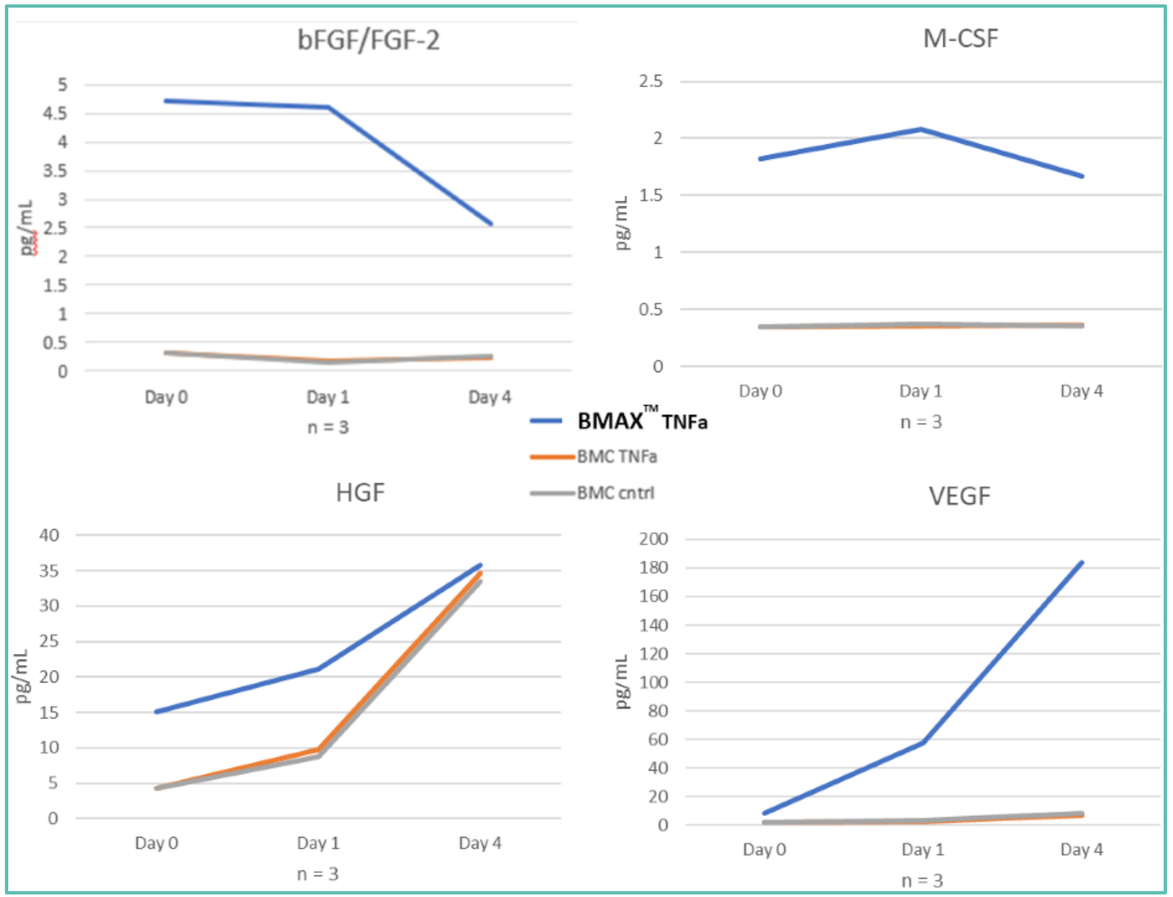


FIGURE 6. Cells Derived from the BMAX™ system have a unique secretory phenotype compared to BMAC when challenged with TNF-α. Cultures were challenged with 2.5 ng/mL TNF-α. Data is normalized per 1 million cells in culture.

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Conclusions & Future Directions

Autologous point-of-care biologics should be harvested, processed and handled in a manner which sources the biologic from tissues or fluids having the best secretome profile, reduces the number of RBCs to the greatest extent possible, retains as much of the native stroma for maximum growth potential and anti-inflammatory properties and excludes anticoagulants having negative impacts on cellular health. The selection of Heparin as an anticoagulant during harvesting may also improve the regenerative capacity of BMAC through retention of the native MSC secretome. Interestingly, BMAX™ derived cells *in vitro* exhibited distinct morphology and enhanced adherence, increased percent MSCs at passage 0, and a unique more pro-regenerative secretome profile versus traditional BMAC preparations. Factors such as platelet source, HCT percent, and anticoagulant effects should be considered for autologous bone marrow therapies. Furthermore, the retention of the native stroma may improve bone marrow-derived MSC health and function which the BMAX™ device may promote. Future studies will explore improved symptomology and outcomes *in vivo* for various orthopedic indications.

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COLORADO STATE UNIVERSITY

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