Stem Cell Yield after Bone Marrow Concentration

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Introduction

Background

- The number of mesenchymal stem/stromal cells (MSCs) in the human bone marrow (BM) is small compared to other cell types, estimated at 0.01-0.02%.
- Bone marrow aspirate concentration (BMAC) may be used to increase numbers of MSCs, but the efficacy of BMAC, as well as the composition of MSC subpopulations after processing, is currently unknown.
- The purpose of this study is to assess the enrichment of MSC subpopulations in bone marrow aspirate by two different BMAC devices versus standard BM aspiration from single donors.

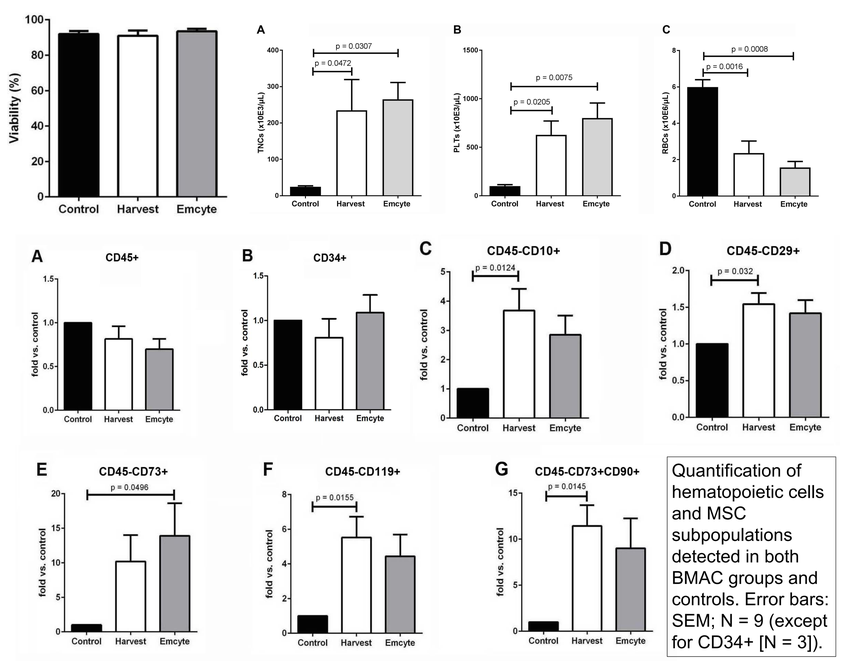
Methods

- 120 mL of BM was aspirated from multiple punctures of the iliac crest in 9 human male donors.
- Each sample was divided into 3 partitions and processed by either Emcyte GenesisCS® (Emcyte) or Harvest SmartPReP2 BMAC (Harvest) and compared to untreated BM aspirate as an internal control.
- Samples were quantitatively analyzed with flow cytometry for viability, expression of MSC subpopulation markers.
- Stem cell content was verified by quantification of colony forming unit-fibroblasts (CFU-F).
- Trophic factors were analyzed with enzyme-linked immunosorbent assays.

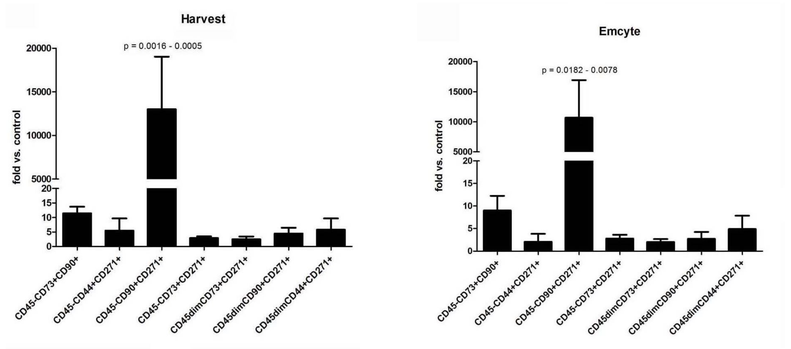
Antibodies used for flow cytometry

Specific antibody	Rationale for analysis
anti-human CD10	identifies MSC subpopulation *in vitro* and *in vivo*
anti-human CD29	identifies MSC *in vitro* (ISCT consensus)
anti-human CD34	identifies HSC; absent on MSC *in vitro* (ISCT consensus)
anti-human CD44	identifies MSC *in vitro*
anti-human CD45	identifies WBC and HSC; absent on MSC *in vitro* (ISCT consensus)
anti-human CD73	identifies MSC *in vitro* (ISCT consensus) and *in vivo*
anti-human CD90	identifies MSC *in vitro* (ISCT consensus) and *in vivo*
anti-human CD119	identifies MSC subpopulation *in vitro*
anti-human CD271	identifies MSC subpopulation *in vitro* and *in vivo*

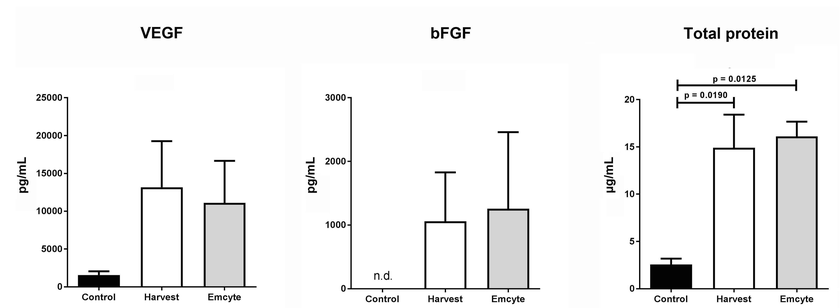
Cell Count Data



Quantification of hematopoietic cells and MSC subpopulations detected in both BMAC groups and controls. Error bars: SEM; N = 9 (except for CD34+ [N = 3]).



Trophic Factors



Results

- Cell viability after processing was over 90% in all groups
- Harvest BMAC contained more CD45-CD73+CD90+ (11.44 fold vs control), CD45-CD10+ (3.68 fold vs control), CD45-CD29+ (1.54 fold vs control), and CD45-CD119+ cells (5.52 fold vs control).
- Emcyte BMAC concentrated more CD45-CD73+ cells (13.90 fold vs control).
- Both BMAC devices mainly enriched the CD45-CD90+CD271+ MSC subpopulation (Harvest 13,011.84 fold vs control; Emcyte 10,669.11 fold vs control) compared to other MSC subpopulations.
- BM concentration also resulted in higher numbers of CFU-F (Harvest: 2,692/mL, Emcyte: 4,336/mL, control: 183/mL) total nucleated cells, platelets, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and total protein.
- Neither system concentrated red blood cells, CD34+ hematopoietic stem cells or bone morphogenetic protein 2 (data not shown)

Conclusion

- Both BMAC devices led to enrichment of MSC subpopulations with distinct phenotypes, without significant loss of cell viability.
- Quantifying the **CD45-CD10+, CD45-CD29+, CD45-CD119+, CD45-CD73+CD90+, and CD45-CD90+CD271+ MSC subpopulations** after BM aspiration is a first step toward quality control parameters for BMAC.
- Further studies correlating these phenotypes to therapeutic efficacy are suggested to assess their clinical applicability.

References

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