Marrow Cellution™ & An Intact Bone Matrix:

The Power of Synergy

Bone marrow cells reside deep inside bone cavities in the most protected part of the body and are redundant throughout the organism. This preferential status reflects the primary role these cells play in the survival of the organism. Stem cells from marrow naturally home to, thrive and proliferate in hypoxic tissue.[10] In response to trauma, which creates a hypoxic environment, marrow cells mobilize into the vasculature from the medullary space where the cells aggregate themselves into the recently created hypoxic damaged tissue.[3-10] Once resident, marrow stem cells are capable of functioning in the hostile hypoxic environment to orchestrate the tissue regeneration process.[10]

By capturing all of the nucleated cells through a proper marrow aspiration, you are maintaining the same relative proportion of cells that naturally aggregates at the defect site. The following quote captures the essence of this insight: "*These data demonstrate that removing BMSC's (bone marrow stem cells) from their normal environment of complimentary cells reduces their osteoblastic capacity and that to achieve their maximal differentiation, BMSC's require direct physical contact with accessory cells.*"[2]

Consequently, the potential of marrow-sourced nucleated cells should be thought of as a group of different cells that is able to:

1) Home to and self organize at the defect site
2) Release the appropriate levels of various growth factors to influence the function and cytokine production of resident cells based on the stage of the healing cascade and
3) Cooperate with and influence resident cells to accomplish the steps of the healing cascade that culminates in repair.[1, 10, 19-38]

Each nucleated cell type contributes to the process of tissue repair.[2] For example, granulocytes in the area of bone regeneration release large amounts of VEFG which up regulates the production of BMP-2 and BMP-6 by sub-populations of CD34+ cells.[37, 38]

Properly aspirating marrow, and appropriately administering the cells is significantly enhancing and exactly mimicking the body's natural healing process.[10] This simple explanation is fundamental to the use of marrow cells in damaged tissue. In the case of bone grafting, the cells need to be combined with a functional bone matrix. In the case of osteonecrosis or heart disease and limb ischemia, the ischemic bone or muscle serves as the scaffold and the cells are directly injected to the site.[14, 16, 30] Trauma that does not heal is often the result of an inadequate number of mobilized marrow cells at the site of the defect.[11, 12]

Autograft exactly mimics and supplements the natural process in that resident in the harvested bone are the living cells of the marrow that are cut from one part of the body and transplanted to another. Autograft is especially suited for bone repair as the bone from the autograft serves as an appropriate scaffold for the transplanted cells.

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A marrow aspirate that is efficient at capturing nucleated cells has the same number and types of nucleated cells as autograft. In fact, by collagenase digesting human autograft and counting the cells, Muschler et al demonstrated that autograft has approximately the number of nucleated cells per cc and CFU-F as the Marrow Cellution™ needle aspirate.[29, 42] By combining the nucleated cell rich fraction with an intact matrix, you create the same therapeutic composition (types and ratio of cells), as autograft without the morbidity.

By examining all of the cellular components of autograft, we see that those same cells are contained marrow aspirate.[29] When we look at the role of those different cells in bone formation, we see that each of the cellular components of autograft, and thus by extension, marrow, play a positive role in the micro-environment of bone formation. For example, T-cells and other lymphocytes improve the mobility and functionality of EPC's.[39, 40] In addition, some of the most potent stem cells reside in the deeper granulocytic layer.[28] This is likely because cycling progenitor cells increase the density of their nucleus just before going through mitosis. So, capturing all of the nucleated cells, similar to autograft, is the optimal strategy.

A review of the literature demonstrates similar clinical outcomes to autograft when using high concentrations of nucleated cells from marrow as measured by CFU-F. For example, Hernigou et al in non-union, Gan et al in multi-level lumbar spine fusion, Jager et al in orthopedic bone defects, Gangji et al and Hernigou et al in osteonecrosis, Velardi et al for pediatric skull defects, and Sauerbier et al for sinus lift augmentation.[13-18, 35]

Each cell in a different micro-environment can provide a different function; for example, granulocytes are inflammatory in the micro-environment of infection compared to pro-regeneration in the micro-environment of building new bone or tissue.[1, 41] Just because a cell has what could be construed to be a negative impact such as pro-inflammatory in a different micro-environment (i.e. granulocytes in an area of infection), does not mean that the cell will have that impact in another micro-environment such as tissue repair. Recent insights has focused on the role that marrow stem cells play in regulating other cells during the immune reparative processes at the sight of trauma.[43]

Aspirating high quality marrow and combining it with a functioning matrix for transplantation into a bone defect is exactly mimicking and supplementing the body's natural response to that surgical trauma site. Clinical results are positively correlated to the number of CFU-F contained in the graft.[15-18, 35]

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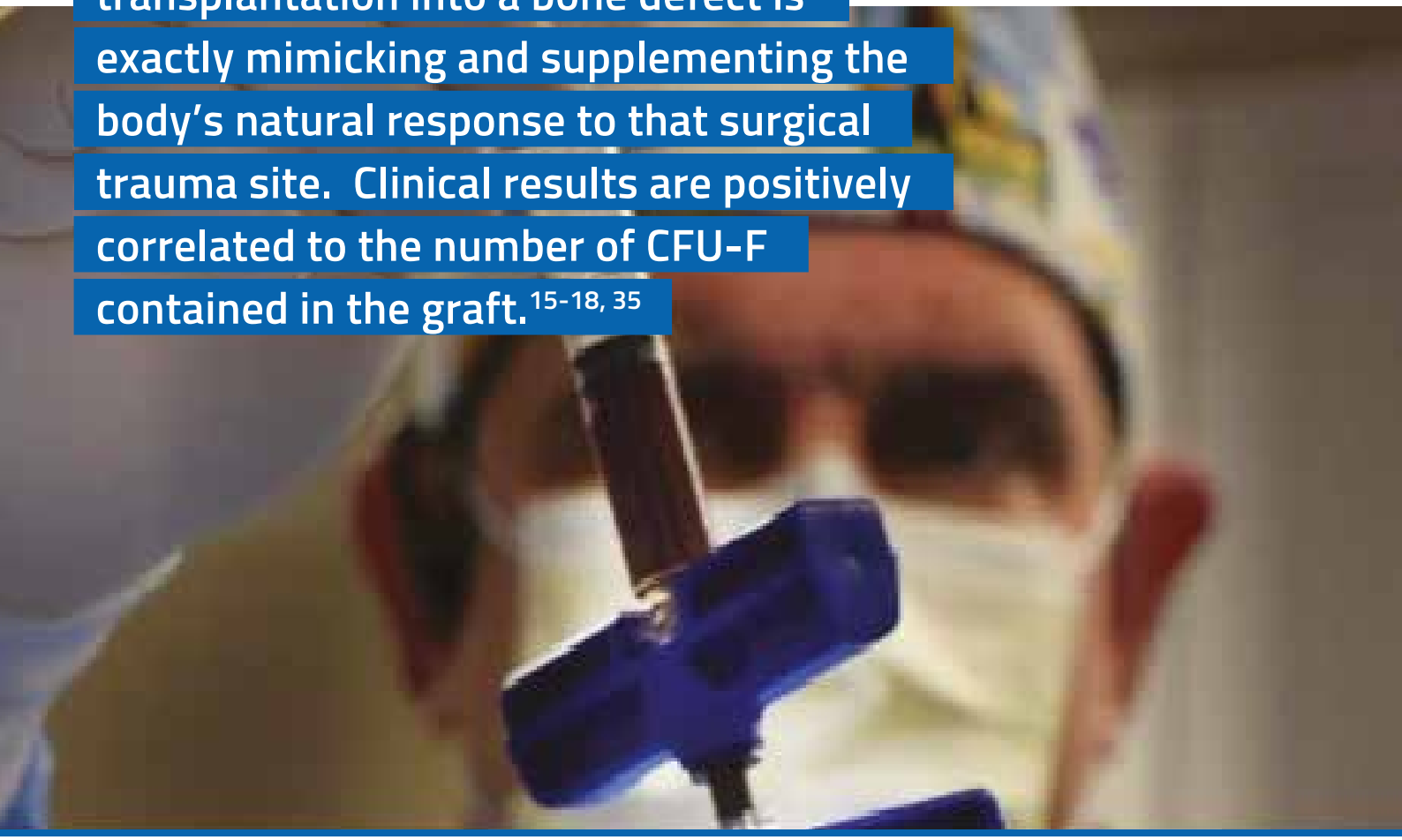

Bibliography

1 Autocrine angiogenic vascular prosthesis with bone marrow transplantation.
Noishiki Y, et al. Nat Med. 1996 Jan;2(1):90-3.

2 Megakaryocyte-bone marrow stromal cell aggregates demonstrate increased colony formation and alkaline phosphatase expression in vitro.
Miao D, et al. Tissue Eng. 2004 May-Jun;10(5-6):807-17.

3 Adult vasculogenesis occurs through in situ recruitment, proliferation, and tubulization of circulating bone marrow-derived cells.
Tepper OM, et al. Blood. 2005 Feb 1;105(3):1068-77.

4 Circulating bone marrow-derived osteoblast progenitor cells are recruited to the bone-forming site by the CXCR4/stromal cell-derived factor-1 pathway.
Otsuru S, et al. Stem Cells. 2008 Jan;26(1):223-34.

5 A systemic provascular response in bone marrow to musculoskeletal trauma in mice.
Laing AJ, et al. J Bone Joint Surg Br. 2007 Jan;89(1):116-20.

6 Stem cells and distraction osteogenesis: endothelial progenitor cells home to the ischemic generate in activation and consolidation.
Cetrulo CL Jr, et al. Plast Reconstr Surg. 2005 Sep 15;116(4):1053-64.

7 Expression of vascular antigens by bone cells during bone regeneration in a membranous bone distraction system.
Lewison D, et al. Histochem Cell Biol. 2001 Nov;116(5):381-8.

8 Endochondral ossification in fracture callus during long bone repair: the localisation of 'cavity-lining cells' within the cartilage.
Ford JL, et al. J Orthop Res. 2004 Mar;22(2):368-75.

9 Circulating Mesenchymal Stem Cells In The Fracture Non-Union Patients.
Chao Wan, et al. J Bone Joint Surg Br. Vol 88-B, Issue SUPP_III, 403-404.

10 Homing to hypoxia: HIF-1 as a mediator of progenitor cell recruitment to injured tissue.
Ceradin DJ, et al. Trends Cardiovasc Med. 2005 Feb;15(2):57-63.

11 Neurological and functional recovery in human stroke are associated with peripheral blood CD34+ cell mobilization.
Dunac A, et al. J Neurol. 2007 Mar;254(3):327-32

12 Circulating endothelial progenitor cells, vascular function, and cardiovascular risk.
Hill JM, et al. N Engl J Med. 2003 Feb 13;348(7):593-600.

13 Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells.
Hernigou P, et al. J Bone Joint Surg Am. 2005 Jul;87(7):1430-7.

14 Treatment of osteonecrosis with autologous bone marrow grafting.
Hernigou P, Beaujean F. Clin Orthop Relat Res. 2002 Dec;(405):14-23.

15 Bone marrow concentrate: a novel strategy for bone defect treatment.
Jäger M, et al. Curr Stem Cell Res Ther. 2009 Jan;4(1):34-43.

16 Treatment of osteonecrosis of the femoral head with implantation of autologous bone-marrow cells. A pilot study.
Gangji V, et. al. J Bone Joint Surg Am. 2004 Jun;86-A(6):1153-60.

17 O.629 Sinuslift with chair-side processed mesenchymal stem cells.
Sauerbier S, et al. J Craniomaxillofac Surg. 2008, Vol. 36(S):158.

18 Osteogenesis induced by autologous bone marrow cells transplant in the pediatric skull.
Velardi F, et al. Childs Nerv Syst. 2006 Sep;22(9):1158-66.

19 Therapeutic potential of non-adherent BM-derived mesenchymal stem cells in tissue regeneration.
Zhang ZL, et al. Bone Marrow Transplant. 2009 Jan;43(1):69-81.

20 Skeletal stem/osteoprogenitor cells: current concepts, alternate hypotheses, and relationship to the bone remodeling compartment.
Mödder UI, Khosla S. J Cell Biochem. 2008 Feb 1;103(2):393-400.

21 Hematopoietic stem cells regulate mesenchymal stromal cell induction into osteoblasts thereby participating in the formation of the stem cell niche.
Jung Y, et al. Stem Cells. 2008 Aug;26(8):2042-51.

22 Comparison of mesenchymal stem cell from bone marrow and adipose tissue for bone regeneration in a critical size defect of the sheep tibia and the influence of platelet rich plasma.
Niemeyer P, et al. Biomaterials. 2010 May;31(13):3572-9.

23 Mesenchymal stem cells derived from CD133-positive cells in mobilized peripheral blood and cord blood: proliferation, Oct4 expression, and plasticity.
Tondreau T, et al. Stem Cells. 2005 Sep;23(8):1105-12.

24 Local delivery of granulocyte colony stimulating factor-mobilized CD34-positive progenitor cells using bioscaffold for modality of unhealing bone fracture.
Mifune Y, et al. Stem Cells. 2008 Jun;26(6):1395-405.

25 Therapeutic potential of vasculogenesis and osteogenesis promoted by peripheral blood CD34-positive cells for functional bone healing.
Matsumoto T, et al. Am J Pathol. 2006 Oct;169(4):1440-57.

26 Fracture induced mobilization and incorporation of bone marrow-derived endothelial progenitor cells for bone healing.
Matsumoto T, et al. J Cell Physiol. 2008 Apr;215(1):234-42.

27 Hematopoietic origins of fibroblasts: I. In vivo studies of fibroblasts associated with solid tumors.
LaRue AC, et al. Exp Hematol. 2006 Feb;34(2):208-18.

28 Isolation of Bone Marrow Derived Stem Cells Using Density Gradient Separation.
Juopperi TA, et al. Exp Hematol. 2007 Feb;35(2):335-41.

29 Comparison of bone marrow aspiration and bone core biopsy as methods for Harvest and Assay of Human Connective Tissue Progenitors.
Rozic, et al. Cleveland Clinic NIH grant RO1 AR049686.

30 Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial.
Tateishi-Yuyama E, et al. Lancet. 2002 Aug 10;360(9331):427-35.

31 Midterm clinical result of tissue-engineered vascular autografts seeded with autologous bone marrow cells.
Shin'oka T, et al. J Thorac Cardiovasc Surg. 2005 Jun;129(6):1330-8.

32 Efficacy of bone marrow mononuclear cells to promote bone regeneration compared with isolated CD34+ cells from the same volume of aspirate.
Yasuhara S, et al. Artif Organs. 2010 Jul;34(7):594-9.

33 Comparison of different adult stem cell types for treatment of myocardial ischemia.
van der Bogt KE, et al. Circulation. 2008 Sep 30;118(14 Suppl):S121-9.

34 Concentration of bone marrow total nucleated cells by a point-of-care device provides a high yield and preserves their functional activity.
Hermann PC, et al. Cell Transplant. 2008;16(10):1059-69.

35 The clinical use of enriched bone marrow stem cells combined with porous beta-tricalcium phosphate in posterior spinal fusion.
Gan Y, et al. Biomaterials. 2008 Oct;29(29):3973-82.

36 Fate of Bone Marrow Stromal Cells in a Syngenic Model of Bone Formation.
Boukhechba F, et al. Tissue Eng Part A. 2011 Sep;17(17-18):2267-78.

37 Platelets and granulocytes, in particular the neutrophils, form important compartments for circulating vascular endothelial growth factor.
Kusumanto YH, et al. Angiogenesis. 2003;6(4):283-7.

38 Hypoxia and VEGF up-regulate BMP-2 mRNA and protein expression in microvascular endothelial cells: implications for fracture healing. Plastic & Bouletreau PJ, et al. Plast Reconstr Surg. 2002 Jun;109(7):2384-97.

39 Identification of a novel role of T cells in postnatal vasculogenesis: characterization of endothelial progenitor cell colonies.
Hur J, et al. Circulation. 2007 Oct 9;116(15):1671-82.

40 CD8+ T lymphocytes regulate the arteriogenic response to ischemia by infiltrating the site of collateral vessel development and recruiting CD4+ mononuclear cells through the expression of interleukin-16.
Stabile E, et al. Circulation. 2006 Jan 3;113(1):118-24.

41 Tissue augmentation by white blood cell-containing platelet-rich plasma.
Kawazoe T, et al. Cell Transplant. 2012;21(2-3):601-7.

42 Techniques and Its Impact on Cell Counts and CFU Counts.
Lecture: Scarpone MA, Kuebler D. Annual Orthopedic Update 2016, Allegheny Health Network.

43 Bone-derived stem cells repair the heart after myocardial infarction through transdifferentiation and paracrine signaling mechanisms.
Duran JM, et al. Circ Res. 2013 Aug 16;113(5):539-52.

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