MARROW, BLOOD AND INFLAMMATION

The Healing Cascade

INTRODUCTION

When determining the optimal intervention, clinicians should consider a host of variables such as age, inflammatory profile, current medications, health history, and the biology of the micro-environment. Often one, or a combination of biologics, is considered to include PRP, bone marrow, fat or placental tissue. This paper summarizes the differences between PRP and marrow and highlights possible resulting clinical implications such as aspiration technique and centrifugation.

BACKGROUND: MARROW, BLOOD AND INFLAMMATION

Bone marrow cells reside deep inside bone cavities in the most protected part of the body and are redundant throughout the organism. (68) This preferential status reflects the primary role these cells play in the survival of the organism. Trauma initiates the inflammatory phase of the healing cascade and causes the up regulation of the cytokines SDF-1, ATP, and VEGF released from platelets and inflammatory peripheral blood cells that participate in the formation of and migrate into the clot. (17,18,25,29,31,50,55,57) These inflammatory cytokines are what primarily stimulate stem cell migration from marrow into the vasculature where these mobilized cells aggregate themselves into the recently damaged tissue (i.e vasculogenesis) (55,58,59,60) Tissue repair is a dynamic self-organizing process that relies on marrow stem cell and marrow complimentary cell mediated vasculogenesis, cell-to-cell contact between marrow cells, cell mobility and growth factor production. Trauma and the resulting tissue and cellular damage creates a hypoxic environment. (19) Unlike mature cells, immature marrow cells function and thrive in areas of hypoxia. (19,27,52) Once resident, marrow stem cells orchestrate the transition from the inflammatory phase of the healing cascade to the proliferation and remodeling phase. (19,27) Mobilization of stem cells from the marrow space through the process of vasculogenesis is positively correlated with better clinical outcomes. (53)

HEALING CASCADE

The combination of Scaffold, Cells, and Signals sets the Environment for the Healing Cascade
Generally, a combination of a functional matrix, living cells, and growth factors produced by those living cells are required for tissue regeneration. The evolving types and amounts of growth factors produced by cells during the regeneration process drives the changing micro-environment during the healing cascade progression. (12,37)

Platelets and Cells from Peripheral Blood Initiate the Inflammatory Process of the Healing Cascade

Normal physiologic wound healing undergoes three overlapping phases 1) hemostasis and inflammation, 2) proliferation and 3) remodelling. Trauma causes peripheral blood platelets, white cells, and granulocytes to migrate by chemotaxis to the injury, form a platelet fibrin clot to end bleeding, and start the beginning inflammatory stage of the healing cascade. (12,38) CD184, also known as CXCR4, is widely expressed on peripheral blood cells, including B and T cells, monocytes, macrophages, dendritic cells, granulocytes,
platelets, lymphoid, myeloid precursor cells, endothelial progenitor cells and mature endothelial cells. (12,31,77) Similar to marrow stem cells, CD184 is the receptor for CXC chemokine SDF-1. Granulocytes, which include neutrophils, are important inflammatory cells for fighting infection and removal of cellular debris. (12) Their activities in the wound bed can cause cell death. (12) Inflammatory blood cells release cytokines in response to the hypoxic condition of the wound (i.e. HIF-1) that act as a signal for marrow stem cells to migrate to the area. (12,27) Once resident, these stem cells drive the remodelling and regeneration phases of the healing cascade. (9,27,46)

**Immune System Stem Cells and the Transition of The Healing Cascade from the Inflammatory Phase to the Proliferation and Remodelling Phase.**

At different stages of healing, immune system driven inflammatory and anti-inflammatory states are driven by cytokines that are variably expressed by resident and migratory cells. (11,12,34) A local increase in immune regulatory cells is required for suppression of the initial inflammatory response to begin the proliferation and remodelling phase. (14,22)

Regulatory immune cells at the site of injury are generated in response to contact with mesenchymal stem cells. (MSC’s) (9,10,11,14) MSC’s actively maintain a hypoimmunogenic state through the production of immunosuppressive paracrine factors or through direct cell-to-cell contact on immune cell populations, including t-cells and macrophages. (13,16,28,41,46) Such induced immune regulatory cells accumulate and converge their regulatory pathways to halt the inflammatory process. (15) MSC’s impart immune tolerance through the early stages of remodelling and provide protection to the developing tissue by upregulating t-regulatory cells (T-Reg) while suppressing inflammation. (20,83) T-Reg attenuate the accumulation of M1 pro inflammatory macrophages while up regulating the production of M2 anti-inflammatory macrophages along with tissue specific growth factors. (46,80)

**CHRONIC CONDITIONS AND AGE**

Successful repair after tissue injury requires resolution of the inflammatory response. (38) Different in-vitro and in-vivo studies have reported conflicting results regarding the use of blood sourced platelets and white cells for tissue regeneration. (32,63, 64, 67, 69,70) Controlling for platelet content of the biologic, certain research has demonstrated that leukocytes and their related growth factors contribute significantly to tissue regeneration while other research has documented the excess inflammation caused by the leukocytes hampered tissue regeneration. (32, 69) However, a consistent theme in the literature is the age-related increase in low-grade systemic inflammation defined as inflammation. (39,81,82) Blood monocyte changes with age result in a different survival profile and a shift towards a pro-inflammatory
phenotype and reduced function. (39,81) These age altered peripheral blood monocytes and macrophages from the innate immune system contribute significantly to inflammation through production of inflammatory cytokines and prolongation of the immune response to tissue injury. (39,46,81) The peripheral blood of older people has significantly larger proportion of inflammatory CD16+ cells than younger people. (82) Furthermore, the CD16+ population have increased adherence and migrate towards endothelial lesions via CX3CR1. (82)

In addition, the age of the actual cell in the blood impacts its inflammatory profile with aged neutrophils from peripheral blood having a significantly higher inflammatory profile than young neutrophils from marrow. (84,85) Neutrophils have been implicated in both driving tissue regeneration as well as mediating the tissue damage associated with a variety of chronic inflammatory diseases. (63,84,85) The abundant presence of neutrophils at the wound site corresponds to the elevated levels of proteolytic enzymes found in non-healing tissue. (86) Unbalanced proteolytic activity is a primary feature of non-healing wounds. (86) Mediators that are crucial for repair become targets of wound proteases that contribute to the overwhelming of local tissue protective mechanisms. (86) For example, growth factors pivotal for repair such as platelet-derived growth factor or vascular endothelial growth factor are targets of wound proteases, and they are inactivated by proteolytic cleavage. (86) The combination of a shift toward an inflammatory profile of blood monocytes combined with the action of neutrophils contributes to a diminishment of the body's tissue regeneration capability that correlates directly with age. (81,84,85,86)

Autophagy is a cellular housekeeping mechanism that is responsible for the removal of neutrophils and dysfunctional intracellular proteins (for example, dead organelles, damaged scaffold proteins).

Efficient autophagy prevents excess stimulation of the inflammatory response by eliminating proteins that occur as a consequence of either tissue injury or necrosis. (76) Macrophages from older patients have significantly impaired autophagy capability that exacerbates the inflammation phase of the healing cascade. (40,81) However, marrow sourced macrophages retain their phagocytosis capability despite age which assists in removing neutrophils and inflammatory debris and transition the wound into the proliferation and re-modelling phase. (40,80) The above research suggests that in older patients, increasing the ratio of anti-inflammatory marrow cells to inflammatory peripheral blood cells in a treating biologic is desirable. Cells capable of forming a CFU-f or megakaryocytes are found in marrow but not peripheral blood. (71) Thus, a high CFU-f or megakaryocyte count to total nucleated cell count, often reported as the number of CFU-f per million cells, will be correlated to a higher ratio of marrow cells to peripheral blood cells. (66,78,79) This ratio may be more meaningful than the overall number of cells in a treating biologic.
REFERENCES

5 Pettine K et al Percutaneous injection of autologous bone marrow concentrate cells significantly reduces lumbar discogenic pain through 12 months. Stem Cells 2015;33:146-56.
9 Maxson S et al Concise Review: Role of Mesenchymal Stem Cells in Wound Repair Stem Cells Translational Medicine February 2012, vol 1 no 2 142-149
11 El-Jawahri et al Interactions Between Multi potential Stromal Cells (MSC's) and Immune Cells During Bone Healing; Stem Cell Biology and Regenerative Medicine September 2016, pp 179-211
12 Frieri, M et al Wounds, burns, trauma, and injury Wound Medicine, 13 (2016) 12-17
13 Prevosto C et al Generation of CD4+ or CDB+ regulatory T cells upon mesenchymal stem cell – lymphocyte interaction Haematologica 2007; 92:881-888
14 Einhorn T et al Fracture healing: mechanisms and interventions Nat. Rev. Rheumatol. 2015 Jan 11 (1) 45-54
16 Phinney D et al Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNA’s Nature Communications 6:8472, 03, October 2014.
17 Zhang W et al; VEGF and BMP-2 Promote Bone Regeneration by Facilitating Bone Marrow Stem Cell Homing and Differentiation European Cells and Materials Vol 27, 2014 pg 1-12
21 Shoji T et al Comparison of fibrin clots derived from peripheral blood & bone marrow Connective Tissue Research July 2016
22 Stable et al CD+8 Lymphocytes Regulate the Arteriogenic Response to Ischemia by Infiltrating the Site of Collateral Vessel Development & Recruiting CD4+ Mononuclear Cells Through Expression of Interleukin 16 Circulation 2006;113; 118-124

23 Assmus B. et al Long-term clinical outcome after intracoronary application of bone marrow-derived mononuclear cells for acute myocardial infarction: migratory capacity of administered cells determines event-free survival European Heart Journal, February 2014, 1275-1283


25 Aiuti A et al. The Chemokine SDF-1 is a Chemoattractant for Human CD34+ Hematopoietic Progenitor Cells and Provides a New Mechanism to Explain the Mobilization of CD34+ Progenitors to Peripheral Blood. The Journal of Experimental Medicine Vol. 185 no1: 111

26 Dengshun D et al Megakaryocyte-Bone Marrow Stromal Aggregates Demonstrate Increased Colony Formation and Alkaline Phosphatase Expression in Vitro Tissue Engineering; Vol 10 No. 5/6 200424)


28 Sudeepa A et al Human mesenchymal stem cells modulate allogeneic immune cell responses, Blood 2005 105:1815-1822

29 Aceves J et al CXCR4+, and SDF-1 Bone Marrow Cells Are Mobilized into the Blood Stream in Acute Myocardial Infarction and Acute Ischemia World Journal of Cardiovascular Diseases, 2014, 4, 361-367

30 Sidney L et.al Concise Review: Evidence for CD34 as a Common Marker for Diverse Progenitors Stem Cells Volume 32 June 2014;

31 Laupheimer M et al Selective Migration of Subpopulations of Bone Marrow Cells along and SDF - 1a and ATP Gradient Bone Marrow Res. December 2014


33 Korf-Klingebiel et al Bone marrow cells are a rich source of growth factors and cytokines: implications for cell therapy trials after myocardial infarction European Heart Journal October 2008

34 Sadiik et al Lipid-cytokine-chemokine cascades orchestrate leukocyte recruitment in inflammation Journal of Leukocyte Biology February 2012 vol. 91 no. 2 207-215

35 Seeger F et al CXCR4 Expression Determines Functional Activity of Bone Marrow-Derived Mononuclear Cells for Therapeutic Neovascularization in Acute Ischemia Arteriosclerosis, Thrombosis, and Vascular Biology November 1, 2009

36 Smiler D et al Growth factors and gene expression of stem cells: bone marrow compared with peripheral blood Implant Dentistry 2010: Jun; 19(3) : 229-40

37 Fuchs, Etal Socializing with the Neighbors: Stem Cells and Their Niche Cell Volume 116, issue 6, March pg 769-778

38 Schmidt-Bleep, K et al Inflammatory phase of bone healing initiates the regenerative healing cascade Cell Tissue Research March 2012, volume 347, issue 3, pp 567-573

39 Gibon E et al, Aging, inflammation, stem cells, and bone healing Stem Cell Research & Therapy 2016 7:44


41 Gonzalez R et al Stem Cells Targeting Inflammation as Potential Anti-aging Strategies and Therapies Cell & Tissue Transplantation & Therapy 2015: 7 1-8

42 Lam Y et al. Aging impairs ischemia-induced neovascularization by attenuating the mobilization of bone marrow-derived angiogenic cells International Journal of Cardiology Metabolic Endocrine September 2016 Vol 12 pg 19-29


Aspire Medical Innovation GmbH
Prinzregentenweg 167
D-81611 München

Geschäftsführer/CEO: Scott Shea
Amtsgericht München
HRB 220207
USt-Id Nr.: DE 302 148 636

Commerzbank AG, München
BLZ: 700 400 48 - Konto: 730506000
IBAN: DE12 7004 0048 0735 0960 00
BIC: COBADEFFXXX
Fibrin and Activated Platelets Cooperatively Guide Stem Cells to a Vascular


46 Li D et al. Bone Marrow Mesenchymal Stem Cells Inhibit Lipopolysaccharide-Induced Inflammatory Reactions in Macrophages and Endothelial Cells Mediators of Inflammation Volume 2016, Article ID 2631439

47 Reale A et al. Functional and Biological Role of Endothelial Precursor Cells in Tumor Progression: A New Potential Therapeutic Target in Haematological Malignancies Stem Cells Int. 2016;

48 Lin C et al. Is CD34 truly a negative marker for mesenchymal stromal cells? Cytotherapy Vol 14 No 10 page 1159-1163


50 Menocal L et al. Role of whole bone marrow, whole bone marrow cultured cells, and mesenchymal stem cells in chronic wound healing. Stem Cell Research & Therapy 2015 6:24

51 Seebach C et al. Cell-Based Therapy by Implanted Human Bone Marrow-Derived Mononuclear Cells Improved Bone Healing of Large Bone Defects in Rats. Tissue Engineering Part A. May 2015, 21(9-10)


58 Massberg et al. "Platelets secrete stromal cell-derived facto alpha 1 and recruit bone marrow derived progenitor cells to arterial thrombi in vivo" JEM, vol 203, No5, May 15, 2006 1221-1233


60 Rafii D et al. Regulation of Vasculogenesis by Platelet Mediated Recruitment of Bone Marrow Derived Cells” Aterioscler, Thromb. Vasc Biol. 2008; 28; 217-222


63 Zhang Y et al. PKM2 released by neutrophils at wound site facilitates early wound healing by promoting angiogenesis. Wound Repair and Regeneration vol. 2 Issue 2 March / April 2016 pg 328-336

64 Duerschmied D et al. Platelet serotonin promotes the recruitment of neutrophils to sites of acute inflammation in mice blood.
December 12 2012


71 Cassano J et al. Bone marrow concentrate and platelet-rich plasma differ in cell distribution and interleukin 1 receptor antagonist protein concentration Knee Surgery, Sports Traumatology, Arthroscopy pp 1–10

72 Scarpone M et al. Annual Orthopedic Update 2016, Allegheny Health Network; "Marrow Cellution Bone Marrow Aspiration System and Related Concentrations of Stem and Progenitor Cells". Lecture - Michael A Scarpone MD, Daniel Kuebler


75 Dante D et al. Ultrasound-Guided Injection of Platelet-Rich Plasma and Hyaluronic Acid, Separately and in Combination, for Hip Osteoarthritis A Randomized Controlled Study Am J Sports Med March 2016 vol. 44 no. 3 664–671


82 Baylis D et al; Understanding how we age: insights into inflammation; Longevity & Healthspan May 2013; DOI: 10.1186/2046-2395-2-8©

83 Fontaine M et al. Unravelling the Mesenchymal Stromal Cells’ Paracrine Immunomodulatory Effects Transfusion Medicine Reviews Vol 30 issue 1 Jan 2016 pg. 37-43

84 Bordon Y et al. Neutrophils, Growing old disgracefully? Nature Reviews Immunology October 2015, 15, 665
85 Sabine A et al Inflammation in Wound Repair: Molecular and Cellular Mechanisms Journal of Investigative Dermatology 2007; 127, 514-525
86 Yager D et al The proteolytic environment of chronic wounds Wound Repair And Regeneration; The international Journal of Tissue Repair and Regeneration; Volume 7 Issue 6, November 1999 433-441

Address correspondence and reprint requests to: info@aspire-medical.eu