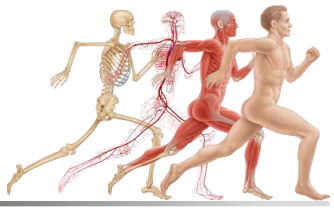
ASPIRE MEDICAL INNOVATION
NEWSLETTER



3/2017

CFU-F, ENDOTHELIAL CELLS & CD34+ CELLS
Understanding the Relationship

RELATIONSHIP BETWEEN CFU-F, ENDOTHELIAL PROGENITOR CELLS (EPC'S) AND CD 34+ CELLS

Stem Cell Marker CD34+ and CFU-f Test

CD34+ are a heterogeneous population of cells that are found in both marrow and blood and include mature endothelial cells, certain monocytes and macrophages, hematopoietic stem cells and endothelial progenitor cells. (30) A majority of these cells are committed blood lineage cells. (62,87) Cells that mark for CD34+ typically account for 1 - 2 % of nucleated cells from a marrow aspirate. Various medications, such asstatins, can influence the number and types of these cells found in marrow and blood. (88) Trauma causes endothelial progenitor cells (EPC's), that are a small sub set of the overall CD 34+ population, to mobilize from marrow and home to the site of injury. (47) Combination markers that include CD34+, as well as CD133+, CD 184+, ckit, VEGF-2 denote a smaller sub population of cells within the overall population of CD 34+ cells that have a greater proportion of endothelial progenitor cells. (35,49) However, markers used in flow cytometry that are based on CD 34 do not identify and discern exclusively EPC's. (47) Thus, CD34 is considered a first pass surface antigen suitable for capture of a large population of heterogeneous cells, that will include a smaller sub population of stem and progenitor cells, including MSC; CD 34+ is not associated only with hematopoietic cells. 73) Certain sub-populations of CD34+ cells reside in marrow and not blood. (48) Early stage, rare CD 34+ cells, cannot be counted using flow cytometry, but are capable of forming a CFU-f. (48) Lin et al demonstrated that CD34 is not a negative marker of MSC and that freshly isolated CD34+ / BM MSC form greater proportions of CFU-f colonies than their CD34– counterparts. (48) Therefore the CFU-f test is the appropriate analysis to determine how many cells from the heterogeneous population of CD34+ cells from the aspirate are early stage stem cells to include MSC. (73)

Counting cells that reside only in marrow and not blood is a key measure to determine the quality of a marrow sourced the biology. Given the limitations of flow cytometry and the fact that CFU-f reside in marrow and not blood, having a high CFU-f count will correlate with other rare marrow and accessory cells; the full complement of these marrow cells is what drives the transition from inflammation to proliferation and remodelling. (57)

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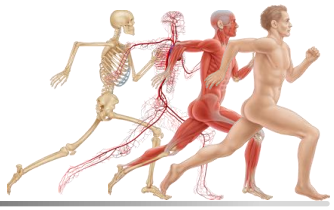


GROWTH FACTORS FROM BLOOD AND MARROW

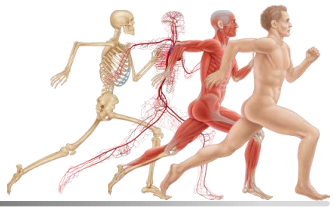
Static in-vitro growth factor analysis does not capture the ongoing cytokine profile of a living cell in-vivo, and the geometric impact it can have by changing the profile of immune cells, those immune cells then impact other cells in a chain reaction that moves the healing cascade forward. In addition, large volume bone marrow aspirates from single locations are predominately comprised of peripheral blood. (1,2) Consequently, the growth factors from the supernatant of such bone marrow aspirates should be comparable to the growth factors from supernatant from peripheral blood samples of matched donors. (36) Despite this significant overlap of peripheral blood cells, in vitro analysis demonstrated that bone marrow supernatants showed greater anti-inflammatory, pro-angiogenic and cyto-protective capability compared to donor controlled supernatants from peripheral blood. (21) Interestingly, in-vivo, the combination of both supernatants in young animals provided the greatest response.(33)

In-vivo, the number of platelets and white blood cells in peripheral blood and their ability to home to sites of tissue damage and form a platelet fibrin clot is an efficient process in a majority of patients. However, the inflammatory profile created by peripheral blood cells increases with age and the ability of one's body to mobilize marrow cells to the site of trauma in response to inflammation to transition from the inflammatory to the proliferation and remodelling phase diminishes greatly over time. (42,44,81,82)

In older patients or healing impaired patients, the vasculogenic and other chemotaxic signals from inflammatory peripheral blood cells and platelets is insufficient to cause adequate marrow cells to migrate into the wound and therefore a chronic condition develops where the wound does not evolve from the inflammatory phase into the proliferation and remodelling phase. (67, 69, 70, 71) Because marrow cells and their related anti-inflammatory, pro-angiogenic and cytoprotective cytokine profile is what is diminished with age, and peripheral blood cells and platelets efficiently infiltrate the site naturally despite age, transplanting marrow only achieves the synergistic effect of both blood and marrow in the clinical setting. (71)

ASPIRATION TECHNIQUE AND IMPLICATIONS OF CENTRIFUGING MARROW

It is well known that the highest quality bone marrow aspirations (greatest quantity of stem/progenitor cells) require aspirating small volumes of bone marrow (1-2ml) from different locations. (1,2,3,4) It is also known that peripheral blood infiltrates bone marrow aspirates when greater than 1-2ml is drawn from any single location. (1,2,3,4) Stem and progenitor cells are enriched in the spongy marrow that is located within the pockets created by the honeycomb of trabecular bone within the medullary space. (1,2,3,4)



Only a finite number of stem cells reside within any given pocket of spongy marrow. (1) Volume over 1ml retrieved from a single site introduces significant peripheral blood into the aspiration. (1,2,3,4) This peripheral blood dilutes further aspiration volume and significantly reduces the stem/progenitor cell quantity of the aspiration per ml. (1,2,3,4) Performing multiple punctures in a clinical setting is often not practical.

To overcome the limitations of lower-quality (reduced cellularity) high volume marrow aspirations from traditional needles, clinicians attempt to enhance the marrow biologic by using a centrifuge-based system. (65)

Centrifuge systems discard 85% of the aspirate by removing lower density plasma and higher density cells composed primarily of red cells while retaining 15% of the starting volume that contains a majority of the platelets, lymphocytes, monocytes, granulocytes and young red cells from both the marrow and the infiltrated peripheral blood components of the aspiration. (65) These systems do not distinguish between nucleated cells from the peripheral blood component of the aspirate compared to the marrow component of the aspirate, (both sets of cells have the same density). (65) In the case of older patients, such systems increase inflammatory peripheral blood macrophages, neutrophils, and related cells within the treating biologic. In addition, within the discarded higher density red cells are a great number of very potent, cycling, high-density, proliferating anti-inflammatory progenitor cells. (6, 7, 8, 65) These cells increase in density as they build up nucleic mass prior to cell division and are always found in the red cell component after centrifugation and consequently, are discarded by all centrifuge protocols. (6,7,8,65)

In the case of a poor aspirate comprised primarily of peripheral blood, the only difference between the biologic that a PRP kit produces compared to what a bone marrow concentrate (BMC) kit produces is that the BMC kit has a higher red cell content and more macrophages and granulocytes. Centrifugation protocols 1) require larger aspiration volumes that are associated with excess peripheral blood and related age dependent inflammatory macrophages and neutrophils 2) have inherent inefficiencies that leaves significant numbers (approximately 40%) of stem cells behind in the discarded red cell portion of the processed marrow 3) require at least 10% dilution by volume for the addition of anti-coagulant to allow the sample to separate 4) and require another 10% dilution in the form of a neutralizing agent such as thrombin and calcium chloride in order for the marrow to clot in the graft. (39,46,65,81,84,85,86)

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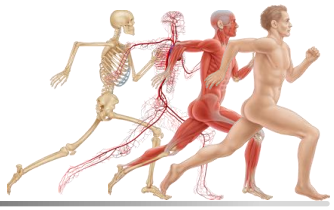


Finally, centrifugation protocols require the marrow to be filtered prior to centrifugation. The cell viability of un-manipulated aspirate after 24 hours is typically between 99% and 100% compared to centrifuged marrow that is typically 93% to 95%. This raises a concern that the stress from the manipulation that led to increased cell apoptosis in the filtered and centrifuged biologic, has potentially damaged the remaining living cells; making them less productive post-transplant. Because marrow based therapies are driven by the stem cell content of the biologic, the sentiment against manipulation, including centrifugation, is best summarized by Muschler et al who concluded "A larger-volume of aspirate (more than 2mL) from a given site is contraindicated with the additional volume contributing little to the overall number of bone marrow cells and results principally in unnecessary blood loss" (p 1707). (1)

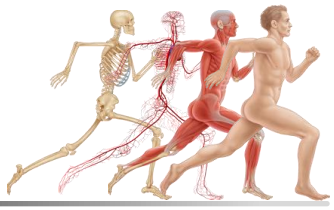
CLINICAL IMPLICATIONS

In older or healing impaired patients a chronic condition results when the cytokine profile from naturally aggregating platelets and white blood cells that home into the clot is not sufficient to stimulate the marrow to cause an adequate vasculogenic response to complete the tissue regeneration process. (9,12)

PRP is often used as an adjunctive therapy for the addition of platelets, white cells, and resulting growth factors beyond what would naturally aggregate at the newly injured site. (74,75) The scientific basis for the intervention is that the enhanced chemotaxic profile from the PRP will create an adequate vasculogenic response to move the healing cascade beyond the inflammatory phase. (74,75) PRP is therefore a growth factor driven mechanism.

When a PRP enhanced therapy is not sufficient, adding additional blood cells and platelets in an attempt to start a new healing cascade is not as reliable as mechanically aspirating and transplanting marrow cells in sufficient quantities to move the cascade beyond the inflammatory phase. (61,99) Moving from the initial inflammatory phase into the proliferation and remodelling phase requires stem cells and complimentary cells to create an anti-inflammatory immune cascade to alter the cell type and growth factor profile in a site-specific manner. (9,10,11,13,14 16,20,28,41,46,83)

Therefore marrow-based strategies are dependent on transplanting adequate numbers of stem cells and complimentary cells from marrow at the site. (5,66,71,78,79) For example, in a tibia non-union setting, the only variable that rose to significance was the number of stem cells in the graft, as measured by CFU-f, not platelets or white blood cells. (66)



MARROW CELLUTION

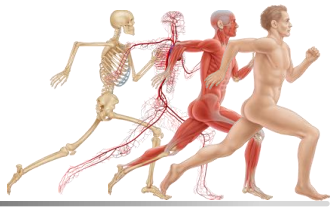
Marrow Cellution is a novel bone marrow access and retrieval device, which incorporate features designed to minimize the limitations of traditional needles. Flow into the aspiration system is collected exclusively laterally because the tip of the aspiration cannula is closed. (72) This design allows for collection of marrow perpendicular to and around the channel created by the tip of the device; traditional needles, even ones with side ports, aspirate primarily through an open-ended cannula which leads to excess peripheral blood in the aspirate. (72) Additionally, Marrow Cellution incorporates technology to precisely reposition the retrieval system to a new location in the marrow after each 1 mL of aspiration. (72) The effects of these two features are that multiple small volumes of high quality bone marrow aspiration are collected from a number of distributed sites within the marrow geography while also retaining clinicians' desire for a single-entry point. (72) The design of Marrow Cellution A) minimizes peripheral blood infiltration, which is potentially inflammatory, and B) significantly increases both the total number of CFU-f and the ratio of CFU-f to total cells when compared to centrifuged marrow. (72) The system enables a total volume of approximately 10 mL to be collected per puncture. In effect, a single puncture with Marrow Cellution appears to be functionally equivalent to repeated small aspirations (1 mL) from a number of puncture sites using traditional needles, but with substantial savings of time, effort, and reduced patient trauma and risk of infection. (72)

CONCLUSION

Vasculogenesis is a key driver of tissue regeneration.

PRP is a growth factor dependent strategy based on the additional growth factors from the platelets and white cells, beyond what would naturally aggregate at the wound site. (32,54,75) These additional growth factors from the PRP causes greater stem cell migration with a resulting enhancement of the proliferation and remodelling phase of the healing cascade. (32,54,75) The heightened inflammatory profile caused by aging on 1) the micro-environment of the wound bed and 2) peripheral blood macrophages and neutrophils, combined with 3) the age dependent diminished vasculogenic capability of marrow, suggests that PRP may be a strategy better suited for younger patients. (64, 67,69,70,71)

Marrow-based interventions are a cell dose driven strategy. (68,78,79) Marrow based treating compositions take advantage of marrow stem cells and marrow complimentary cells to alter the type and function of local cells to create an anti-inflammatory immune driven cascade to transition and amplify the cellular inventory needed to complete the remodelling phase of the healing cascade. (50,68,78,79,80,83)



Consistent with oncology models of marrow stem cell transplantation, the only variable that rose to significance in an orthopaedic clinical setting using marrow as the biologic, was the number of stem cells in the graft, as measured by CFU-f, not platelets or white blood cells. (5,66,78,79) A poor marrow aspirate will be comprised of predominately peripheral blood. (1,2,3,4) Nucleated marrow cells and blood cells have the same density.

Concentrating the cells from a poor aspirate by density centrifugation results in a high proportion of peripheral blood cells in the biologic. In older patients, these cells can lead to excess inflammation. (39,46,81,82) All cells found at the site of surgical trauma can play a beneficial role in the tissue regeneration process. (63,32) The number of platelets and white blood cells in peripheral blood and their ability to home to sites of tissue damage and form a platelet fibrin clot is an efficient process in a majority of patients and does not diminish with age.

Using PRP to further amplify the stem cell homing signals of SDF-1a, ATP, and VEGF provided from naturally aggregating platelets and white cells can have a clinical benefit. (32,54,75) The ability of one's body to mobilize marrow cells to the site of trauma diminishes greatly over time. (42,44) In older patients or healing impaired patients, the vasculogenic signals from PRP is often not sufficient to complete the healing cascade. (43,61) In such cases, marrow rich in CFU-f has been shown to have clinical success. (68,78,79) Central to the coordinated interplay among cells, and the extracellular matrix is the MSC, which coordinates the repair response. (23,24,50,78,79,80) CD34 is not a negative marker of MSC and that freshly isolated CD34+ / BM MSC form greater proportions of CFU-f colonies than their CD34– counterparts. (48) The CFU-f test is the appropriate analysis to determine how many cells from the heterogeneous population of cells to include cd34+ cells, are early stage stem cells, to include MSC. (66,71,78,79) In a clinical setting, CFU-f is the only measured variable that rose to statistical significance. (66,78,79)

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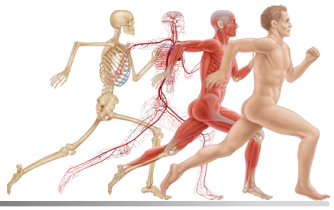


REFERENCES

1 Muschler GF, Boehm C, Easley K. Aspiration to obtain osteoblast progenitor cells from human bone marrow: the influence of aspiration volume. J Bone Joint Surg Am 1997;79:1699-709.

2 Batinic D, Marusic M, Pavletic Z, et al. Relationship between differing volumes of bone marrow aspirates and their cellular composition. Bone marrow transplantation 1990;6:103-7.

3 Bacigalupo A, Tong J, Podesta M, et al. Bone marrow harvest for marrow transplantation: effect of multiple small (2 ml) or large (20 ml) aspirates. Bone marrow transplantation 1992;9:467-70.

4 Hernigou P et al. Benefits of small volume and small syringe for bone marrow aspirations of mesenchymal stem cells. International orthopaedics 2013;37:2279-87.

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.

6 Juopperi T et Isolation of bone marrow-derived stem cells using density-gradient separation. Experimental Hematology 2007;35:335-41.

7 Bhartiya D et al. Very small embryonic-like stem cells with maximum regenerative potential get discarded during cord blood banking and bone marrow processing for autologous stem cell therapy. Stem cells and development 2012;21:1-6.

8 Ahmadbeigi N et al. The aggregate nature of human mesenchymal stromal cells in native bone marrow. Cytotherapy 2012;14:917-24.

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

10 M.E. Bernardo, W.E. Fibbe Mesenchymal stromal cells: sensors and switchers of inflammation Cell Stem Cell, 13 (2013), pp. 392–402

11 El-Jawhari 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 CD8+ regulatory T cells upon mesenchymal stem cell – lymphocyte interaction Haematologica 2007; 92:881-888

14 Einhorn T et al Fracture healing: mechanisms and interventons Nat. Rev. Rheumatol. 2015 Jan 11 (1) 45-54

15 Najar, M et al Mesenchymal stromal cells and immunomodulation: A gathering of regulatory immune cells. Cell Therapy February 2016, Volume 18, issue 2 160-171

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.

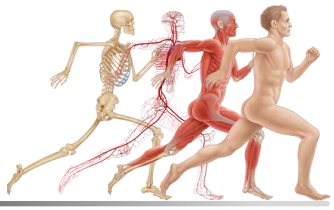
17 Zhang W et al; VEGF and BMP-2 Promote Bone Regeneration by Facilitating Bone Marrow Stem Cell Homing and Differentiation European Cells and Materials Vol 27, 2014 pg 1-12

18 Kusumanto, et al. Platelets and Granulocytes, in Particular the Neutrophils, Form Important Compartments for Circulating Vascular Endothelial Growth Factor ANGIOGENESIS 6(4):283-287 2004

19 Bouletreau et al Hypoxia and VEGF Up-Regulate BMP-2 mRNA and Protein Expression in Microvascular Endothelial Cells: Implications for Fracture Healing Plastic & Reconstructive Surgery: June 2002 - Volume 109 - Issue 7 - pp 2384-2397 38)

20 Hur et al Identification of a Novel Role of T Cells in Postnatal Vasculogenesis: Characterization of Endothelial Progenitor Cell Colonies" Circulation 2007 116; 1671-1682

21 Shoji T et al Comparison of fibrin clots derived from peripheral blood & bone marrow Connective Tissue Research July 2016



22 Stabile et al CD+8 Lymphocytes Regulate the Arteriogentic 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

24 Sackner-Bernstein J et al Abstract 11330: Predictors of Response to Intracoronary Delivery of CD34+CXCR4+ Enriched Bone Marrow Derived stem cells (AMR-001) Early After STEMI Circulation November 20, 2012

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)

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

28 Sudeepta 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- 1 a and ATP Gradient Bone Marrow Res. December 2014

32 Kawazoe T et al Tissue Augmentation by White Blood Cell-Containing Platelet-Rich Plasma Cell Transplantation, Vol. 21, pp. 601–607

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 Sadik 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 perpheral blood Implant Dentistry 2010: Jun: 19(3) : 229-40

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

38 Schmidt-Bleek, 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

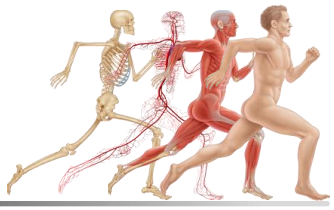
39 Gibon E et al, Aging, inflammation, stem cells, and bone healing Stem Cell Research & Therapy 2016 7:44

40 Linehan E et al. Aging impairs peritoneal but not bone marrow-derived macrophage phagocytosis. Aging Cell. 2014;13:699–708

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 bonemarrow–derived angiogenic cells International Journal of Cardiology Metabolic Endocrine September 2016 Vol 12 pg 19-29

43 Krych A et al Bone Marrow Concentrate Improves Early Cartilage Phase Maturation of a Scaffold Plug in the Knee-A Comparative Magnetic Resonance Imaging Analysis to Platelet-Rich Plasma and Control Am J Sports Med January 2016



vol. 44 no. 1 91-98

44 Scheuble R et al Age-dependent depression in circulating endothelial progenitor cells in patients undergoing coronary artery bypass grafting J AM Coll Cardio. 2003;42(12)2073-2080

45 Ghanaati S et al Advanced Platelet Rich Fibrin: A New Concept for Cell-Based Tissue Engineering by Means of Inflammatory Cells Journal of Oral Implantology December 2014

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 ID2631439

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

49 Peichev M et al Expression of VEGFR-2 and AC133 by circulating CD34+ cells identifies a population of functional endothelial precursors Blood 2000, Feb 1: 95(3): 952-8

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)

52 Ceradini D et al Homing to Hypoxia: HIF-1 as a Mediator of Progenitor Cell ecruitment to Injured Tissue Trends in Cardiovascular Medicine Volume 15, Issue 2, February 2005, Pages 57–63

53 Werner et al Influence of Cardiovascular Risk Factors on Endothelial Progenitor Cells Arteriosclerosis, Thrombosis, and Vascular Biology, Feb. 2006

54 El-Sharkawy et al Platelet Rich Plasma: Growth factors and pro- and anti- inflammatory Properties Journal of Periodontology April 2007, Vol. 78, No. 4, Pages 661-669

55 Laing et al ; Mobilization of Endothelial Precursor Cells: Systemic Vascular Resposne to Musculoskeletal Trauma J Orthop Res 25:44-50 2007

56 Klibansky et al Synergistic targeting with bone marrow- derived cells and PDGF improves diabetic vascular function Am J Physiol Heart Circ290:
H1387-H1392 2006

57 Stellos et al Platelet-Derived Stromal Cell-Derived Factor-1 Regulates Adhesion and Promotes Differentiation of Human CD34 + Cells to Endothelial Progenitor Cells Circulation, 117(2):206-215 2008

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

59 De Boer H et al Fibrin and Activated Platelets Cooperatively Guide Stem Cells to a Vascular Injury and Promote Differentiation Towards an Endothelial Cell Phenotype Arteriscler, Thromb, Vasc Biol. 2006:26;1653-1659

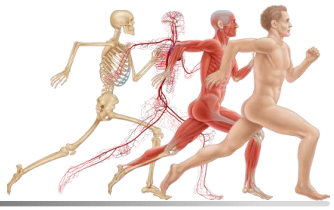
60 Rafii D et al Regulation of Vasculogenesis by Platelet Mediated Recruitment of Bone Marrow Derived Cells" Aterioscler, Thromb. Vasc Biol. 2008; 28; 217-222

61 Nishimoto S et al Impacts of bone marrow aspirate and peripheral blood derived platelet-rich plasma on the wound healing in chronic ischaemic limb Journal of Plastic Surgery and Hand Surgery Vol 47, 2013 issue 3

62 Murry CE et al.: Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 428, 664–668 (2004).

63 Zhang Y et a l PKM2 released by neutrophils at wound site facilitates early wound healing by promoting angiogenesis Wound Repair and Regeneration vol. 24 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

65 Hegde V, Shonuga O, Ellis S, et al. A prospective comparison of 3 approved systems for autologous bone marrow concentration demonstrated non-equivalence in progenitor cell number and concentration. Journal of orthopaedic trauma 2014;28:591-8.

66 Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. J Bone Joint Surg Am 2005;87:1430-7.

67 Zhang L eta l Harmful Effects of Leukocyte-Rich Platelet Rich Plasma on Rabbit Tendon Stem Cells in Vitro Am J Sports Med May 16, 2016

68 Gurkan UA, Akkus O. The mechanical environment of bone marrow: a review. Annals of biomedical engineering 2008;36:1978-91.

69 Dragoo J et al Comparison of the Acute Inflammatory Response of Two Commercial Platelet-Rich Plasma Systems in Healthy Rabbit Tendons Am J Sports Med. June 2012 vol. 40 no 6. 1274-1281

70 Braun H et al The Effect of Platelet-Rich Plasma Formulations and Blood Products on Human Synoviocytes-implicationsfor intra-articularinjuryand therapy AM J Sports Med May 2014 vol. 42 no 5 1204-1210

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, Arthroscopyy 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- Machael A Scarpone MD, Daniel Kuebler

73 Marx RE, Harrell DB. Translational research: The CD34+ cell is crucial for large-volume bone regeneration from the milieu of bone marrow progenitor cells in craniomandibular reconstruction. Oral Craniofac Tissue Eng 2012;2:263-71.

74 Peerbooms J et al Positive Effect of an Autologous Platelet Concentrate in Lateral Epicondylitis in a Double-Blind Randomized Controlled Trial – Platelet Rich Plasma versus Corticosteroid With a 1 year Follow-up Am J Sports Med February 2010, vol 38 no. 2 255-262

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

76 Bratton B et al. Neutrophil Clearance: when the party's over, cleanup begins Trends Immunol. 2011 Aug; 32(8): 350–357

77 Pilling D, Fan T, Huang D, Kaul B, Gomer RH (2009) Identification of Markers that Distinguish Monocyte- Derived Fibrocytes from Monocytes, Macrophages, and Fibroblasts. PLoS ONE 4(10): e7475. doi:10.1371/ journal.pone.000747

78 Hernigou P, Flouzat Lachaniette CH, Delambre J, et al. Biologic augmentation of rotator cuff repair with mesenchymal stem cells during arthroscopy improves healing and prevents further tears: a case-controlled study. International orthopaedics 2014;38:1811-8.

79 Hernigou P, Beaujean F. Treatment of osteonecrosis with autologous bone marrow grafting. Clin Orthop Relat Res 2002:14-23

80 Kim J et al Mesenchymal stem cell-educated macrophages: A novel type of alternatively activated macrophages Experimental Hematology Vol. 37, Issue 12, Dec. 2009 1445-1453

81 Albright J et al Advanced Age Alters Monocyte and Macrophage Responses Antioxidants & Redox Signalling Online ahead of print August 3, 2016

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

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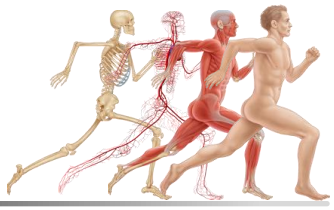

85 Sabine A et al Inflammation in Wound Repair: Molecular and Cellular Mechanisms Journal of Investigative Dermatology 2007; 127, 514-525

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

87 Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC: Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature428, 668–673 (2004).

88 Vasa M et al Increase in Circulating Endothelial Progenitor Cells by Statin Therapy in Patients With Stable Coronary Artery Disease Circulation June 19, 2001, Volume 103, Issue 24

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