

MARROW, BLOOD, INFLAMMATION AND TISSUE REGENERATION

Clinical implications for Application of Marrow and/or PRP

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)

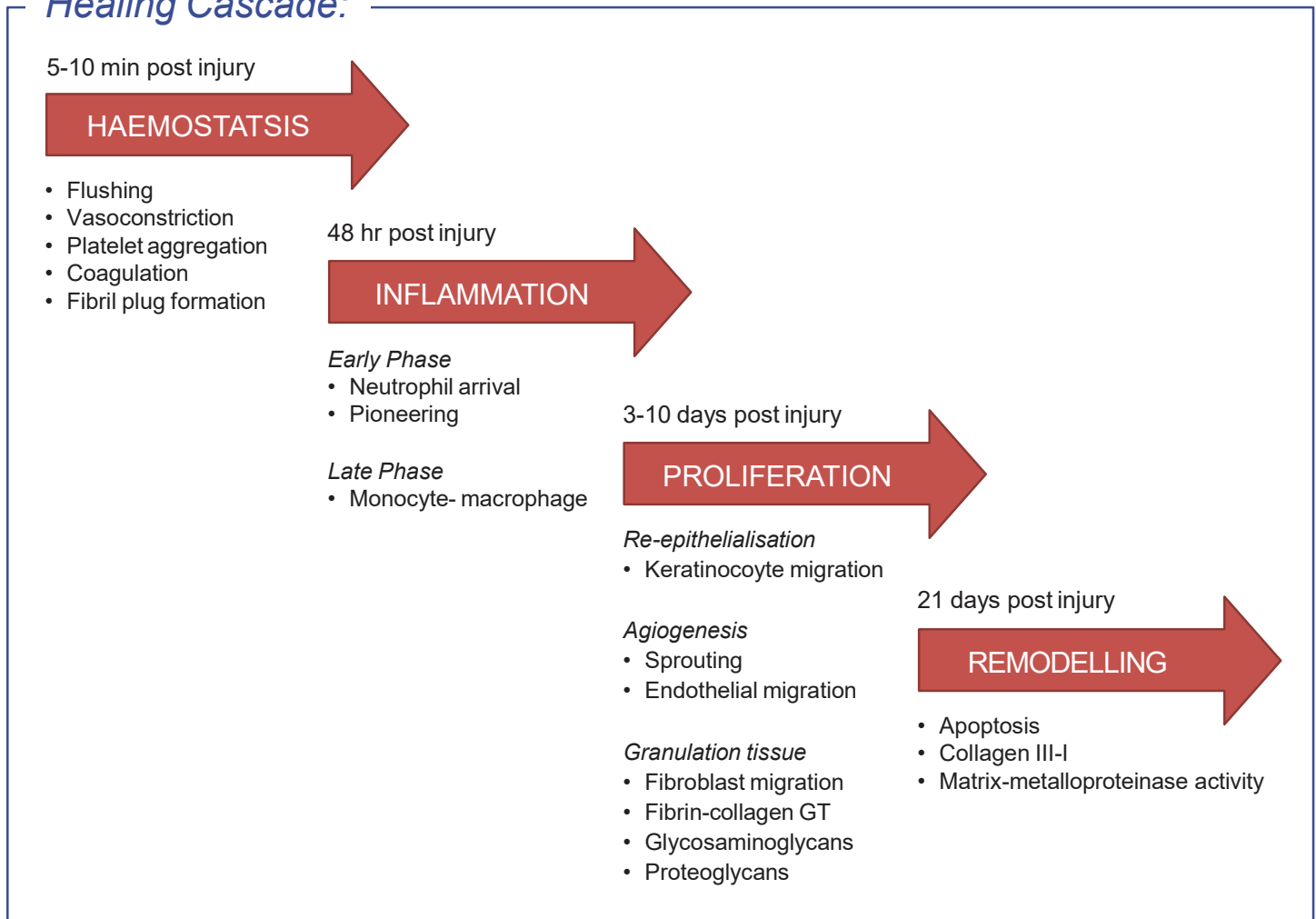
HEALING CASCADE

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)

Healing Cascade:



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) remodeling. 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 remodeling 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 Remodeling 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 remodeling 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 hypoinmunogenic 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 remodeling and provide protection to the developing tissue by up-regulating t-regulatory cells (T-regs) while suppressing inflammation. (20,83) T-regs 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 inflammaging. (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 inflammaging 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 remodeling 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.

PRP—A GROWTH FACTOR DRIVEN THERAPY

PRP is a general term used for a biologic that is made from centrifuging venous whole blood to volume reduce plasma and red blood cells and thereby enriching the treating composition for platelets and white blood cells. The therapy targets the inflammatory phase of the healing cascade. (69) In younger patients with healthy red marrow, or in cases where the injury is minor, adding additional peripheral blood platelets and white blood cells to the clot, (PRP) and the resulting growth factors, beyond what aggregates at the wound bed naturally, can amplify the vasculogenic response. (54,57,74) 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 does not diminish with age. The ability of one's body to mobilize marrow cells to the site of trauma in response to the cytokines released by blood cells in the wound diminishes greatly over time. (42,44) In addition, age impacts the number of stem cells and the function of both peripheral blood mature cells and immature marrow cells. (42,44)

In a stalled partially healed situation, starting a new healing cascade by introducing PRP into micro-wounds created by the PRP delivery needle, is often sufficient to create a corresponding vasculogenic response to complete the healing cascade. (45,69,74) Thus, PRP is a white blood cell and platelet dependent strategy. The additional growth factors from the exogenously added platelets and white cells, beyond what would naturally be present from cells that aggregate at the wound site, causes greater stem cell migration with a resulting transition from the inflammatory phase to the proliferation and remodeling phase of the healing cascade. (54,57,69)

The 1) heightened age dependent inflammatory profile of blood sourced monocytes, macrophages, and neutrophils and their deleterious impact on the micro-environment of the wound bed and 2) the age dependent diminished vasculogenic capability of marrow, suggests that PRP may be a strategy better suited for healthy older patients with minor defects or younger patients. (39,50,56,61,69,71,81,86,99) Also, leukocyte depleted PRP, (often referred to as pure PRP) may be better suited for patients that are older or otherwise have a heightened immune profile associated with such co-morbidities as obesity or diabetes. (32,43,45,54,61,67,70,71)

TREATING COMPOSITIONS SOURCED FROM MARROW ASPIRATE—A CELL DRIVEN THERAPY

Marrow is a Cell Driven Strategy

Properly aspirating and appropriately administering marrow cells is significantly enhancing and exactly mimicking the body's natural healing process. In a hind limb ischemia animal model using aged animals of diminished vasculogenic capacity, mechanically mimicking the natural healing response through autologous transplantation has shown to have a statistically significant clinical benefit. (35,42) Through cytokine release and cell-to-cell contact, bone marrow stem cells orchestrate the transition from inflammation to proliferation and remodeling. (9,10,13,15,16) Marrow based treating compositions are cell dose dependent and take advantage of marrow stem cells and complimentary cells ability to alter the type and function of local cells to create an immune driven cascade to transition and amplify the cellular inventory needed to complete the remodeling phase of the healing cascade. (50,68,78,79,80,83) Mechanically sourcing and placing the cells responsible for transitioning from the inflammatory to the proliferation phase, is often sufficient to complete the healing process. (66,78,79)

Dose Response of Marrow Cells

Critical to successful healing are adequate numbers of immature stem cells and complimentary cells that have migratory capability and whose growth factor profile can influence migrating and resident cells to move into a tissue proliferation and regeneration profile. (23,24,50,78,79) The growth factor profile of a biologic that has a greater proportion of cells from marrow is different from PRP that is made entirely from peripheral blood cells and platelets. (21) Hernigou et al in non-union and osteonecrosis demonstrated that clinical results were linked to the stem cell content of the graft as measured by CFU-f. (66,78,79) This correlation between the CFU-f content of the biologic and outcomes has been repeated by other groups. (5) Interestingly, in the Hernigou work, CFU-f was the only measured variable that rose to statistical significance; not total nucleated cells or platelets. (66,78) This is consistent with bone marrow rescue therapy in oncology where the stem cell content of the graft, not the number of nucleated cells, is the driver of clinical success.

Through cytokine release and cell-to-cell contact, bone marrow stem cells are the quarterbacks of the injury site

The growth factor profile of cells from marrow is different than that produced by blood cells. (21,33,36,80) Paracrine signaling to create synergistic interactions between cells in wound healing requires a coordinated interplay among cells, growth factors, and extracellular matrix proteins. (9,10,13,14,15,16,28,46) MSC's have a substantial involvement in the initial stage of healing by controlling the fate of inflammation. (38,39) By responding to changes in their environment, and using complex growth factor mediated signaling circuitry, MSC's organize site-specific regenerative responses. (9,10,13,14,15,16,28,46,80) Mature resident cells, under the influence of migrating stem cells, demonstrate a plasticity that allows them to make a significant contribution to the healing cascade. (46,80) For example, MSCs modulate the phenotype of macrophages by inducing a shift from inflammatory M1 macrophages to anti-inflammatory M2 macrophages, thereby transitioning the wound healing cascade from inflammatory to proliferation and remodeling.

Complimentary Cells from Marrow

A diverse group of complimentary cells other than stem cells migrate to the source of hypoxia caused by trauma. Removing BMSC's (bone marrow stem cells) from their normal environment of complimentary cells reduces their capacity and that to achieve their maximal potential, BMSC's require direct physical contact with accessory cells. (26,50) In a clinical setting, the bone forming capability of a full compliment of cells was demonstrated to be superior to single cell suspensions of MSC alone. (57)

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 as statins, 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 compliment of these marrow cells is what drives the transition from inflammation to proliferation and remodeling. (57)

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 cytoprotective 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 ones body to mobilize marrow cells to the site of trauma in response to inflammation to transition from the inflammatory to the proliferation and remodeling 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 remodeling 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 (1-2 mL) from different locations. (1,2,3,4) It is also known that peripheral blood infiltrates bone marrow aspirates when greater than 1-2 mL 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 1 mL 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) 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 chemotactic 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 remodeling 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, co-developed by Endocellutions Corp (475 School Street, suite 12, Marshfield MA) and Ranfac Corp, (30 Doherty Dr. Avon MA) which incorporate features designed to minimize the limitations of traditional needles. Flow into the aspiration system is collected mainly 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 remodeling 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 remodeling 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 orthopedic 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.

MARROW, BLOOD, INFLAMMATION AND TISSUE REGENERATION

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 ones 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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