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Operative Techniques in
Orthopaedics

Basic Science: Molecular and Biological Aspects of Platelet-Rich Plasma Therapies

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Knowledge of the basic biological mechanisms involved in tissue response to injury should inform management of healing. Approaches to influence healing may need to integrate multiple cell types and large signaling networks that are necessary for the dynamic communication between cells. Platelet-rich plasma (PRP) therapies deliver a myriad of growth factors and cytokines to the injured tissues. Evolution of our understanding of platelet biology and reinterpretation of some of their more traditional roles in hemostasis and tissue repair have revealed much about the complexity of PRP therapies and provide new insights on PRP therapies' successes and failures. However, many potential molecular mechanisms acting simultaneously in tissue repair present a challenge to the identification of critical mechanisms behind PRP therapies. A vast array of barriers, ranging from deficits in basic research to clinical differences in formulations and administration procedures, undermine current efforts to set effective PRP protocols to manage healing. Identifying which molecular mechanisms are more or less important during the course of healing and clarifying the molecular basis for differences in the healing response across patients will continue to be the priority to tailor PRP therapies for particular sports injuries.

KEYWORDS platelet-rich plasma, sport injuries, tissue healing

Physical activity and sport are fundamental for a healthy lifestyle. However, too much exercise can result in overuse injuries. Many of these occur after repetitive microtrauma or are nagging injuries following an acute event that is not allowed to heal completely. The biological basis appears to be a failed healing response, whether scarce or idle, which seems to be mediated by failure in multiple healing processes. Both acute and overuse musculoskeletal injuries are frequent in sports, but especially the latter are disabling and challenging to manage. They also represent a substantial economic burden to society.[1]

In the past 10 years, the use of platelet-rich plasma (PRP) therapies has become widespread throughout various medical fields and has positively impacted orthopedic sports science with the rationale that such agents have healing properties in addition to an excellent safety profile.[2] Initial studies involving athletes with overuse lesions showed promising results, but more recent level I studies have shown controversial results of PRP injection among patients with tendinopathies.[3-5] Hence, following the originally high expectations by the public, the enthusiasm surrounding PRP therapies may evolve into scepticism if the timeline to optimize PRP therapies will be longer than expected. Evolution of our understanding of platelet biology and reinterpretation of some of their more traditional roles in hemostasis and tissue repair should provide new insights on PRP therapy's successes and failures. This article will highlight some of these recent observations and evolving concepts and paradigms, and will build on and amplify previously published works in the field of PRP therapies.[6-9]

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Understanding Tissue Healing

Knowledge of the basic biological mechanisms involved in tissue response to injury is critical in therapeutic management. Indeed, the most effective way to improve tissue repair is to understand normal healing mechanisms after a perturbation arising from trauma or disease. As healing mecha-

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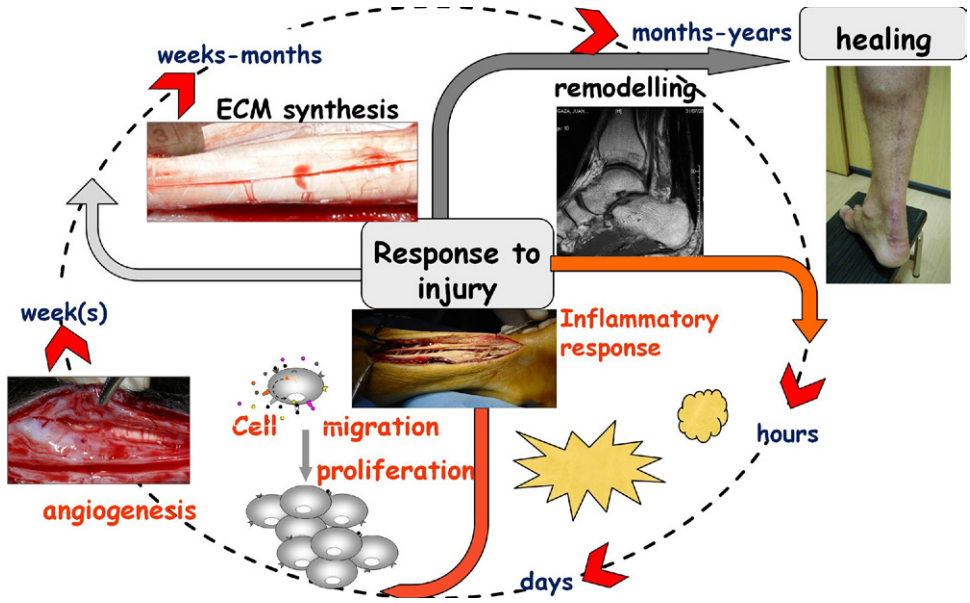

Figure 1 Dynamic phases of tissue healing: The healing response involves 3 broad overlapping phases: the inflammatory response that lasts until a few days after injury, and is characterized by leukocyte extravasation and accumulation in the injured site and monocyte/macrophage activation; the trophic or anabolic phase, including angiogenesis in which endothelial cells are activated to initiate angiogenesis such that new blood vessels are initiated to promote blood flow to support the high metabolic activity of the new tissue. Cells migrate into the site using the fibrin matrix as a scaffold, divide, and differentiate producing collagen, proteoglycans, and other components of the extracellular matrix. Finally, during the remodeling stage, there is a decrease in cell density, and the overall metabolic activity of the injured tissue decreases. In each of the described healing phases, the specific signaling activity is silenced or counterbalanced by other endogenous signals that serve to limit the duration and to promote progression to a new stage.

nisms are capable to restore nearly every tissue in the body, deviations from the normal healing pattern can contribute to various pathologic conditions. For example, disordered or insufficient healing has been hypothesized to lead to osteoarthritis or tendinopathy.[10] Although debated, the repair of overused tendons and joint pathologies can be aided by the correct administration of PRP therapies. However, the correct repair of tissues is extremely complex, and the spatially and temporally dynamic nature of healing mechanisms presents a challenge to the identification of critical mechanisms behind PRP therapies. The healing response involves 3 broad overlapping phases: inflammation, a trophic or anabolic phase (angiogenesis, proliferation, synthesis of extracellular matrix), and remodeling (Fig. 1). These phases can widely overlap, and the synthesis of signaling molecules in 1 phase acts as stimulants for the following phase.

Inflammation

Inflammation and blood coagulation are intimately linked. The coagulation system and innate inflammatory response share common ancestry and are coupled via common activation pathways and feedback regulation systems.[11] The role of platelets is illustrative for this 2-way relationship. Within the blood clot, activated platelets and leukocytes release growth factors and numerous cytokines, establishing the onset of the inflammatory response. Endothelial cells are not only actively involved in hemostasis, limiting clot formation to the sites of injury, but also in localizing inflammatory processes to areas of damage, in part via common pathways.[12] Accordingly, local regulatory mechanisms adjust the magnitude of the innate immune response so that the amount and duration of immune cell infiltration are adequate to phagocyte apoptotic/necrotic cells.[13] In the first few hours after injury, neutrophils are recruited to tissues by chemotactic factors, which are presented in a temporarily and spatially defined manner. Within tissues, neutrophils orchestrate many cell activities and progressively unleash an arsenal of diverse compounds, including radical oxygen species, antimicrobial peptides, and serine proteases, many with a definite biological potential to inflict further tissue injury.[14] Neutrophils are short-lived; their life spans are generally measured in terms of hours. The repertoire of signals engendered by the neutrophils engages the monocyte/macrophage lineage. These, commanded by signals resulting from neutrophil death or activation, largely remove the recruited neutrophils *in situ* and clear the damaged local cells.

The severity of tissue injury—neutrophils' fate and the apoptotic or necrotic condition of resident fibroblasts—may determine the different states of macrophage activation. In fact, macrophages exhibit transition from proinflammatory to prohealing phenotypes. For example, bacterial products (lipopolysaccharide) and proinflammatory cytokines (interferon γ [IFN-γ]) induce the classical proinflammatory phenotype associated with production of high levels of inflammatory cytokines (interleukin 6 [IL-6], interleukin 1β [IL-1β], and tumor necrosis factor α) and reactive oxygen

and nitrogen species. Instead, "alternative" activation through IL-4/IL-23 is associated with the synthesis of healing factors, including transforming growth factors (TGF-β and TGF-α), basic fibroblastic growth factor (bFGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF).[15] These growth factors directly promote healing, contributing to cell proliferation, angiogenesis, and collagen deposition.

Recent research suggests that the switch from proinflammatory to prohealing activities may determine the difference between efficient repair and the failure to repair. Actually, a common characteristic of impaired healing is a persistent inflammatory response, with prolonged accumulation of monocytes/macrophages and elevated levels of proinflammatory cytokines.

Trophic Phase

Cell Migration and Proliferation

In the injured site, platelets and leukocytes release VEGF, also named permeability factor, which increases the permeability of the endothelial cell layer, causing plasma proteins to extravasate and to lay down a provisional matrix scaffold that is initially populated with leukocytes and platelets. In response to the various cytokines secreted by immune cells, stromal cells migrate into this scaffold. Progenitors of differentiated cell types, such as bone, cartilage, muscle, nerve sheath, and connective tissue cells, are thought to contribute to the collection of proliferating cells. The progenitor cells differentiate in response to growth factors and cytokines and become the predominant tissue-specific cell type by the third to fifth day after injury. Moreover, regulated by PDGF, insulin-like growth factor (IGF), and TGF-β, these cells produce collagen, proteoglycans, and other components of the extracellular matrix. Fibroblasts also secrete extracellular zinc-dependent endopeptidases called metalloproteinases (MMPs), which facilitate their movement through the matrix and help with the removal of damaged matrix components.[16]

Angiogenesis

Distinct proteases, such as MMP-2 and MMP-9, modulate angiogenesis-promoting endothelial cell migration and tube formation by proteolytically remodeling the basement membrane. Moreover, proteases liberate angiogenic molecules stored in the extracellular matrix, producing an angiocompetent microenvironment. VEGF-A predominantly regulates angiogenesis in health and disease by signaling through VEGF receptor 2 (VEGFR-2, also known as fetal liver kinase 1). Soluble VEGF isoforms promote vessel enlargement, whereas matrix-bound isoforms stimulate branching. When a quiescent cell senses an angiogenic signal, such as VEGF-A, angiopoietin 2 (ANG-2), FGF, or chemokines released by innate inflammatory or local injured cells, pericytes first detach from the vessel wall in response to ANG-2 and liberate themselves from the basement membrane by proteolytic degradation mediated by MMPs (collagenases, gelatinases, and stromelysins).

To stabilize endothelial cell channels, angiogenic endothelial cells release PDGF-B to chemoattract pericytes. Hence, pericyte deficiency in the absence of PDGF-B causes vessel leakage. Healthy vessels must be equipped with mechanisms to maintain quiescence, while remaining able to respond to angiogenic stimuli. The ANG (ANG-1, -2, -3, and -4 ligands) and tyrosine kinase with immunoglobulin-like and EGF-like domains (TIE) (TIE-1 and -2 receptors) are responsible for this switch.[17]

Tissue Remodeling

Collagen accumulation reaches a maximum at 2-3 weeks after injury, and the transition to remodeling begins. There is a balance between synthesis, deposition, and degradation during this phase. Small capillaries aggregate into larger blood vessels, and there is an overall decrease in the water content of the wound. Similarly, cell density and the overall metabolic activity of the wound decrease. The most dramatic change occurs in the overall type, amount, and organization of the collagen fibers, resulting in an increased tensile strength of the tissue. Initially, there is increased deposition of collagen type III, a reticular collagen, which is gradually replaced by collagen type I. Collagen fibers are cross-linked by the enzyme lysyl oxidase, which is secreted by fibroblasts in the extracellular matrix. The normal adult 4:1 ratio of type I to type III collagen is restored during remodeling. Equilibrium is established as new collagen is formed and collagen type III is degraded.

Most of the clinical differences between chronic and acute healing tissues can be at least partially explained by alterations in the local biochemical environment. Local obstacles to tissue healing include tissue viability, seroma and/or hematoma, infection, insufficient blood supply, and/or mechanical factors. For example, adequate blood supply must exist to provide nourishment and oxygenation to healing tissues. A lack of blood supply may lead to tissue ischemia, an increased risk of infection, and delayed healing.

Therefore, therapeutic approaches to manipulate healing may need to integrate multiple cell types and large signaling networks that are necessary for the dynamic communication between cells. The need to target various signaling pathways simultaneously demands the administration of a balanced combination of mediators. In this context, PRP technologies will draw inspiration for the development of multimolecular therapies (Fig. 2).

PRP Therapies

Platelets are anucleate myeloid cells produced by megakaryocytes in the bone marrow, comprise up to 1.4-4 $\times$ 10^{11} cells/L of blood, and circulate for about 10 days. Platelets adhere avidly at sites of vascular injury—a critical first step in hemostasis. Adhesion and activation, along with fibrin formation, then cause the release of intracellular stores—predominantly α granules (50-80 α granules per platelet), dense granules (3-5 granules per platelet), and lysosomes. The concept that platelets are essential for hemostasis and vascular

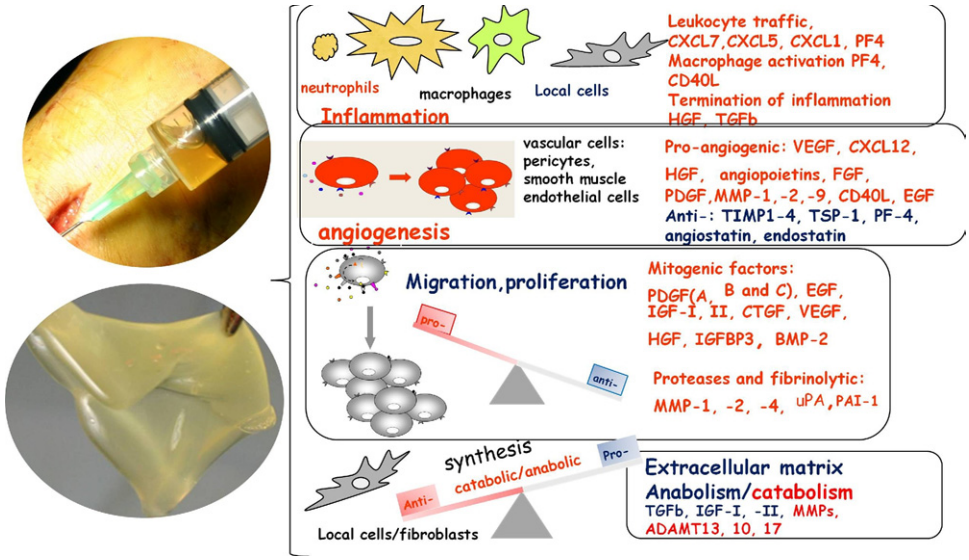

Figure 2 Complexity of PRP therapies: PRP therapies provide a multifunctional microenvironment by releasing a myriad of molecules involved in the healing mechanisms. Therefore, they target multiple cell phenotypes and modulate various biological processes, including inflammation, angiogenesis, cell migration and proliferation, and the anabolism/catabolism (synthesis and remodeling) of extracellular matrix. Globally, the role of platelet secretome at the different stages of the healing response will depend on the relative local balance of pro- and antimolecular activities. Growth factors, including TGF-β 1, PDGF, BDNF, bFGF, and IGF-I, function at various stages during the healing response and produce different outcomes depending on the conditions of the host tissue. For instance, PDGF, a chemotactic and mitotic factor for fibroblasts, also induces the synthesis of collagen type I; IGF-I anabolic and antiapoptotic activities are regulated by IGF BP, IGFBP-2, IGFBP-3, and IGFBP-4, which are also present in the early healing response.

integrity is a fundamental tenet in physiology and medicine that has progressively evolved to the concept that platelets are chief effector cells in the various mechanisms involved in tissue repair with specialized roles in chemotaxis, inflammation, and angiogenesis.[6,8] Substantial progress in understanding the function of platelets has revealed much about the complexity of PRP therapies.

To date, the effectors of the beneficial function of PRP therapies were growth factors, such as PDGF, TGF, FGF, endothelial growth factor (EGF), hepatocyte growth factor (HGF), connective tissue growth factor, and VEGF, among others. However, evolution of our understanding and recent proteomic analyses indicate that α granules contain more than 300 proteins.[18] Thus, reinterpretation of PRP therapies requires us to consider new classes of molecules. As illustrative examples of the different biological mechanisms modulated by PRPs, we quote molecules seldom mentioned in studies of PRPs, such as the chemokine (C-X-C motif) ligand (CXCL)-7 and platelet factor 4 (PF-4 or CXCL-4) in innate immune response, thrombospondin-1 (TSP-1) in angiogenesis, and urokinase plasminogen activator (uPA) in cell migration.

Inflammation

Platelets modulate inflammation largely because of their ability to secrete high levels of chemokines (a subset of small, diffusible cytokines), required to control trafficking and the further accumulation of leukocytes and monocytes in the injured tissue. Supporting this hypothesis, the inhibition of platelet activation with antiplatelet glycoprotein Ib decreased polymorphonuclear leukocyte influx by 50%.[19] Platelets are considered the major source of β-thromboglobulin (CXCL-7 or neutrophil-activating peptide-2 [NAP-2]), a strong chemoattractant and an activator for neutrophils. Actually, platelets secrete 2 CXCL-7 precursors (ie, platelet basic protein) and connective tissue-activating peptide III, but proteolytic processing by neutrophils is essential to make chemotactically active CXCL-7.[20] Hence, leukocyte-PRP fibrin is likely to attract more neutrophils from the bloodstream than PRP fibrin alone. This issue, although needing experimental confirmation, may be clinically relevant when deciding to use pure PRPs or leukocyte-platelet concentrates. *A priori*, we exclude neutrophils because they may exacerbate tissue damage via several different mechanisms (ie, secreting proinflammatory cytokines, such as tumor necrosis factor, interferon α [IFN-γ], and interleukins [IL-6 or IL-1β]) that cause matrix destruction through the production of metalloprotease-1 (MMP-1), -3, and -13. In addition, the interaction of neutrophils with platelets may induce a hyperactive leukotactic response of circulating neutrophils toward the injury site. Thus, a large influx of these cells to the site of injury and subsequent activation of oxidative and enzymatic processes can intensify host tissue damage. Unfortunately, there are few published studies on fixed PRPs, and there is a lack of compelling evidence for the preferential use of either pure PRP or leukocyte PRP.

Another crucial event in inflammation is dying cell removal, which depends on monocyte/macrophage lineage. Platelets are the major source of PF 4, which prevents monocyte apoptosis and promotes macrophage differentiation.

Moreover, recent experiments using microarray technology have shown that PF-4 (representing 25% of the content of α granules) induces a unique macrophage transcriptome distinct from the known macrophage activation patterns (classical and inflammatory) that share diverse molecular similarities with both the pro- and antiinflammatory activation patterns.[21] Minimal research has been conducted to optimize PRP formulation at this critical level, and further basic science knowledge would help in refining PRP therapies. For example, inducing "classical" macrophage activation while avoiding "innate" activation may produce an antiinflammatory environment.

PRPs may terminate inflammation by restoring cells to a noninflammatory phenotype.[22] This effect could be mediated by various GFs, including HGF, VEGF, and TGF-β, which protect the function of the endothelial barrier. For example, HGF treatment induces an antiinflammatory cytokine profile in endothelial cells specifically by suppressing E-selectin. In a model of inflammatory activation (LPS stimulation of macrophages), the presence of HGF induced a decrease in the proinflammatory cytokine IL-6 and an increase in the antiinflammatory cytokine IL-10.[23] Furthermore, these changes were a result of signaling through the mesenchymal epithelial transition factor receptor. Interestingly, the molecular basis of the antiinflammatory action of PRP on human chondrocytes relies on the action of HGF to inhibit NFκB.[24] HGF is primarily found in plasma, and very little is found in platelets. Therefore, it is important to consider the balance between plasma and platelet proteins when formulating PRPs.

These insights on the role of PRP in inflammation may lead to tailored and targeted formulations able to discriminate between the beneficial and harmful effects of this relationship between PRP and inflammation.

Angiogenesis

The influence of platelets on angiogenesis has been well established. However, because platelets contain both stimulators and inhibitors of angiogenesis,[25] the mechanism by which platelets regulate angiogenesis remains unclear.

Platelet α granules contain a variety of angiogenic proteins; paradoxically, α granules also contain established inhibitors of angiogenesis, such as TSP-1, an adhesive protein modulating vascular cell behavior by altering endothelial and vascular smooth muscle cell adhesion, proliferation, motility, and survival. TSP-1 concentration is proportional to the number of platelets. For example, PRP containing a 4-fold concentration of platelets releases 183 $\pm$ 21 μg/mL of TSP-1.[24] By preventing VEGF and bFGF binding, TSP-1 interferes with their mitogenic effects; in addition, it inhibits nitric oxide signaling.[26] Other antiangiogenic proteins in PRPs are angiostatin, endostatin, and fibronectin and the tissue inhibitors of metalloproteinases (TIMPs-1 to -4).[9] Pro- and antiangiogenic proteins may well be stored separately and differentially released because the secretion of pro- versus antiangiogenic stores may be agonist-specific.[27] For example, PAR1-activating peptide, ADP, and the glycoprotein VI-targeting collagen-related peptide induced massive release of VEGF but modest release of PF-4 or endostatin. In contrast, PAR4-AP triggered marked PF-4 and endostatin release.[28] This suggests that different activation mechanisms and environment stimuli evoke distinct secretion patterns of pro- and antiangiogenic factors. Considering these data, further research is required to produce selective pro- or antiangiogenic environment by means of PRP therapies.

Furthermore, vesicles within platelets (ie, dense granules) store and deliver a pool of small molecules, such as histamine, noradrenaline, dopamine, and serotonin, which increase vascular permeability by allowing the extravasation of plasma proteins into the injury.

Cell Migration and Proliferation

Platelets also contain fibrinolytic factors (and their inhibitors) that may regulate precisely the pericellular proteolytic environment required for the control of cell migration and matrix remodeling. For example, several proteases and protease inhibitors released by platelets, most notably uPA and plasminogen activator inhibitor type 1, proceed as both targets and modifiers of pathways that impact proliferative/migratory events and coordinately titrate the overall pericellular proteolytic balance (directly via the generation of plasmin) as well as indirectly by activating several members of the MMP family. Clearly, the binding of plasminogen activator inhibitor type 1 with its several targets, including vitronectin, uPA, and uPA/uPAR, has the potential to affect the motile program on multiple levels, providing the opportunity to therapeutically manipulate this pathway in pathophysiological settings.[29]

Anabolism/Catabolism and Matrix Remodeling

PRP-released anabolic cytokines include connective tissue growth factor (CTGF), TGF-β1 and -β2, IGF I and II, FGF-2, and others.[8] These cytokines have different effects that are tissue-context dependent. For example, TGF-β1 may enhance cartilage repair by triggering chondrocyte differentiation. In other conditions, TGF-β enhances collagen deposition and has been associated with fibrotic healing. Moreover, molecular changes that occur with aging could lead to growth factor dysfunction. Actually, the functionality of both TGF-β1 and IGF-I declines as a result of the aging-related changes in growth factor–receptor interactions. Assuming impaired anabolic growth factor stimulation with increased age, PRP actions would differ when administered to older patients.

The biological paradox is that platelets may be, however, also implicated in matrix destruction (remodeling). Indeed, MMPs are also secreted by platelets, but in an inactive form, and their activity is regulated by the molecular microenvironment. Classically, MMPs are collectively viewed as capable of degrading all components of the extracellular matrix and basement membrane, restricting their functions to tissue remodeling and maintenance. However, extracellular matrix degradation releases noncovalently bound growth factors and cytokines, and thereby increases their bioavailability. Examples include release of VEGF and TGF-β1. VEGF binds

noncovalently to heparan sulfate proteoglycans with release on extracellular matrix proteolysis. TGF-β is maintained in a latent state by binding to the latency-associated peptide (LAP). LAP, in turn, is covalently bound to the fibrillin protein latent TGF-β-binding protein. Extracellular matrix degradation releases the latent complexes, and dissociation of the TGF-β–LAP complexes increases TGF-β availability. Moreover, LAP is a substrate of MMP-2, -9, -13, and -14, and latent TGF-β-binding protein can be cleaved by MMP-7.

The multitude of potential cellular mechanisms acting simultaneously in tissue repair helps to explain why the field can move toward targeting specific pathways by harnessing the effects of particular molecular subsets present in PRP preparations. Moreover, PRP biotechnology can be used for much more than enhancing tissue healing. In the correct hands, PRPs can also be essential tools for discovering the decisive molecules in specific healing pathways.

Critical Parameters in PRP Technologies

Composition of PRP

Depends on the Preparation System

The methods of producing PRPs determine the composition and concentration in terms of leukocytes and platelets in a given plasma volume. Accordingly, PRP preparations have been categorized in pure PRP, in which leukocytes are purposely eliminated from the PRP, and leukocyte- and platelet-rich plasma (L-PRP), containing high concentration of leukocytes.[30] Although leukocytes actually increase the concentration of some growth factors, such as VEGF or PDGF, they also release radical oxygen species, catabolic cytokines, and proteases. According to a recent study,[31] the concentrations of MMP-9 and IL-1β in L-PRP were higher than in pure PRP. Moreover, both MMP-9 and IL-1β correlated with the number of neutrophils in the L-PRP. Moreover, neutrophils release various compounds with the potential for further tissue damage. Hence, the improved homogeneity of pure PRP and its reduced donor-to-donor and intradonor variability would support the view that pure PRPs are more reproducible and predictable than L-PRPs.

Activation Protocol

Activation of the coagulation cascade is a critical step not only for PRP-clot manipulation during surgery but also in stimulating growth factor and cytokine release from PRPs. In fact, the PRP fibrin (gel, clot) that forms on coagulation is a useful delivery system because most growth factors and cytokines are released during fibrin (gel, clot) retraction or during fibrinolysis. Thus, the kinetics of fibrin formation/retraction is crucial in signaling and cellular functions. For instance, PRP activated *ex vivo* with thrombin induces a rapid clot formation/retraction and a sudden burst of signals, compared with $CaCl_2$ or collagen.[32] Alternatively, the clotting cascade can be activated *in situ* by tissue factor, the initiator of the host response to injury. The kinetics of cytokine release is important because most cellular responses are widely influenced not only by cell surface receptors but also by the method by which their cognate ligands (growth factors or cytokines) are secreted or delivered. For example, a receptor may be acutely activated on an immediate increase in ligand concentration (as mimicked by most drugs), but often in physiology, a constitutively secreted factor needs to accumulate over time to reach a threshold set by the affinity of the receptor (as mimicked by slow PRP activators). So far, this aspect is under researched, and no study has addressed the hypothetical differential effects of acute and gradual increases in extracellular factors in PRP-induced signaling events and cellular functions *in vitro*.

Artifacts During Preparation (Unpredictability)

An aspect that has received scant attention, and can greatly affect the biological activity of the PRP, is the length of time between PRP activation and its application. Although all PRP preparations prepared with the same commercial system contain the same molecular pool, the proteases (ie, thrombin, plasmin) ensuring plasma activation may degrade some of the growth factors and activate others, altering the clinical effectiveness for specific applications. Moreover, differences in fibrin stability and in the behavior of platelet-platelet and platelet-leukocyte aggregates should be considered. Leukocyte binding to fibrin profoundly alters leukocyte function, leading to phagocytosis, NFκB-mediated transcription, production of chemokines and cytokines, and degranulation.

The key challenges ahead include identifying present errors and advantages of activation techniques to improve the therapeutic benefits of PRPs.

Barriers to Advancing PRP Therapies

A vast array of barriers, ranging from deficits in basic research to clinical differences in formulations and administration procedures, undermine current efforts to set effective PRP protocols to manage healing. Inconsistencies in clinical findings reported in the literature, even when using the same PRP compound and application protocol, may be the result of donor differences in PRP composition and/or host tissue conditions.

Recent proteomic studies have provided comprehensive catalogs of the molecular content of platelets, which are uniquely valuable to investigate PRP functions. These data sets will be critical in the discovery of PRP functions and expected connections with specific molecular subsets. Hence, elucidating the role of platelet proteome in tissue healing would permit tailoring PRP preparations to the different medical conditions.

Expand Research on Heterogeneity

Other priorities include clarifying the molecular basis for differences in the healing response across patients. So far, thousands of patients have benefited from the administration of platelet-rich compounds, but limited efficacy still remains an outstanding problem. Given the limited response rates, a step forward would be the discovery of predictive biomarkers to identify responders among the large patient group of nonresponders. These biomarkers could be clinical, molecular, or both. Analyses addressing candidate gene biomarker de-

terminants of healing, such as genetic polymorphisms in VEGF and/or its receptors, might improve understanding of the relative impact of some components of these formulations. Moreover, a genetic contribution has been proposed for tendon/ligament vulnerability to injuries, in particular for Achilles tendon and rotator cuff pathologies and the cruciate ligaments in the knee. Specifically, variants within the *TNC* (tenascin), *COL5A1*, and *MMP3* genes co-segregate with chronic Achilles tendinopathy. The variant within the *TNC* gene also appears to co-segregate with Achilles tendon ruptures, while sequence variants within the *COL1A1* and *COL5A1* genes have been shown to be associated with cruciate ligament ruptures and/or shoulder dislocations. Thus, information gained from efficient use of genomics, ie, patient stratification according to functional variants in candidate genes, may be crucial in refining PRP therapies and improving efficacy.

Conclusions

We have focused on a few of the many molecules involved in the various biological pathways that link healing and PRP technologies. As the cellular and molecular contribution to healing is further elucidated, their modulation with PRPs will impact on our understanding, raising the hopes for the development of better therapies. Identifying which molecular mechanism(s) is more or less important during the course of healing will continue to be a challenge, requiring excellent preclinical models for different musculoskeletal conditions and carefully designed clinical trials.

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