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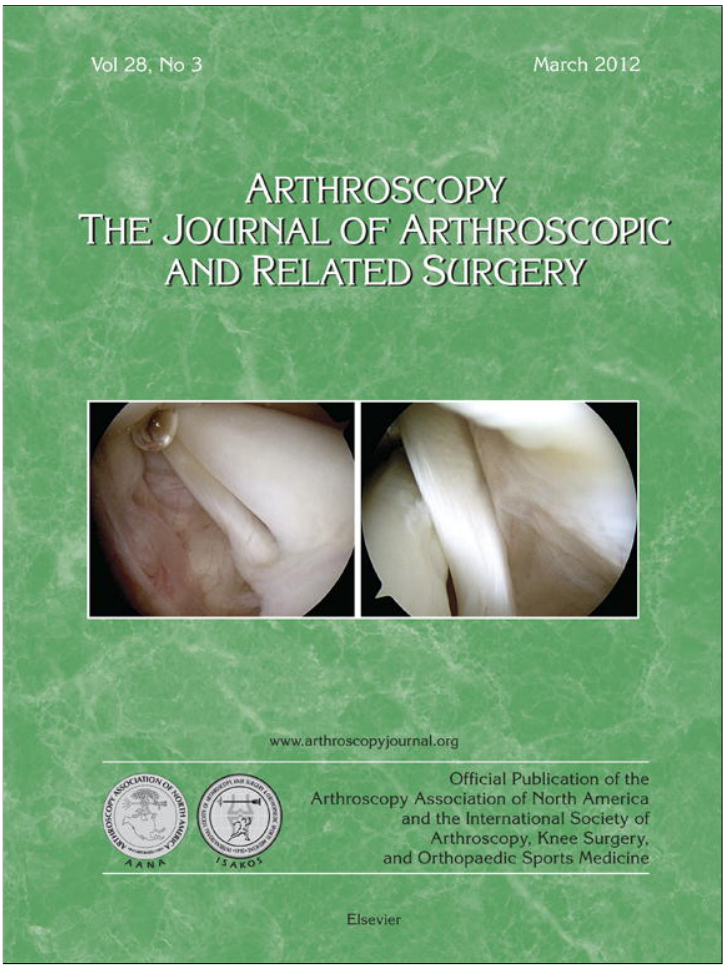

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Current Concepts With Video Illustrations

Platelet-Rich Plasma: A Milieu of Bioactive Factors

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Abstract: Platelet concentrates such as platelet-rich plasma (PRP) have gained popularity in sports medicine and orthopaedics to promote accelerated physiologic healing and return to function. Each PRP product varies depending on patient factors and the system used to generate it. Blood from some patients may fail to make PRP, and most clinicians use PRP without performing cell counts on either the blood or the preparation to confirm that the solution is truly PRP. Components in this milieu have bioactive functions that affect musculoskeletal tissue regeneration and healing. Platelets are activated by collagen or other molecules and release growth factors from alpha granules. Additional substances are released from dense bodies and lysosomes. Soluble proteins also present in PRP function in hemostasis, whereas others serve as biomarkers of musculoskeletal injury. Electrolytes and soluble plasma hormones are required for cellular signaling and regulation. Leukocytes and erythrocytes are present in PRP and function in inflammation, immunity, and additional cellular signaling pathways. This article supports the emerging paradigm that more than just platelets are playing a role in clinical responses to PRP. Depending on the specific constituents of a PRP preparation, the clinical use can theoretically be matched to the pathology being treated in an effort to improve clinical efficacy.

Preparations of platelet concentrates are generically referred to as platelet-rich plasma (PRP) and have gained popularity in fields such as wound healing,[1] dental and maxillofacial surgery,[2] sports medicine,[3] and veterinary medicine.[4] In sports medicine there are numerous clinical objectives motivating the use of PRP, including promotion of tissue regeneration in both bony[5,6] and soft tissues,[7] prevention or treatment of infection,[8,9] and restoration of function.[10] Clinical observation and opinion suggest that pain relief and return to function occur more rapidly than expected for some healing orthopaedic problems after the use of PRP. This has led to investigations of antinociceptive properties of PRP in our laboratory and others.[11] In addition to being evaluated in vivo for efficacy and safety, in vitro investigation of PRP and the growth factors (GFs) contained within it has been performed.

Generation of PRP is accomplished with one of many available commercial systems that are marketed primarily based on their ability to concentrate platelets. Targeted musculoskeletal tissues, such as tendon, ligament, and cartilage, heal slowly because of a limited blood supply, slow cell turnover, and limited extracellular matrix restoration.[12] PRP provides GFs that stimulate neovascularization and increase the blood supply and nutrients needed for cells to regenerate the damaged tissue (Fig 1). Neovascularization also brings new cells and removes debris from damaged tissue. It is hypothesized that the GFs released

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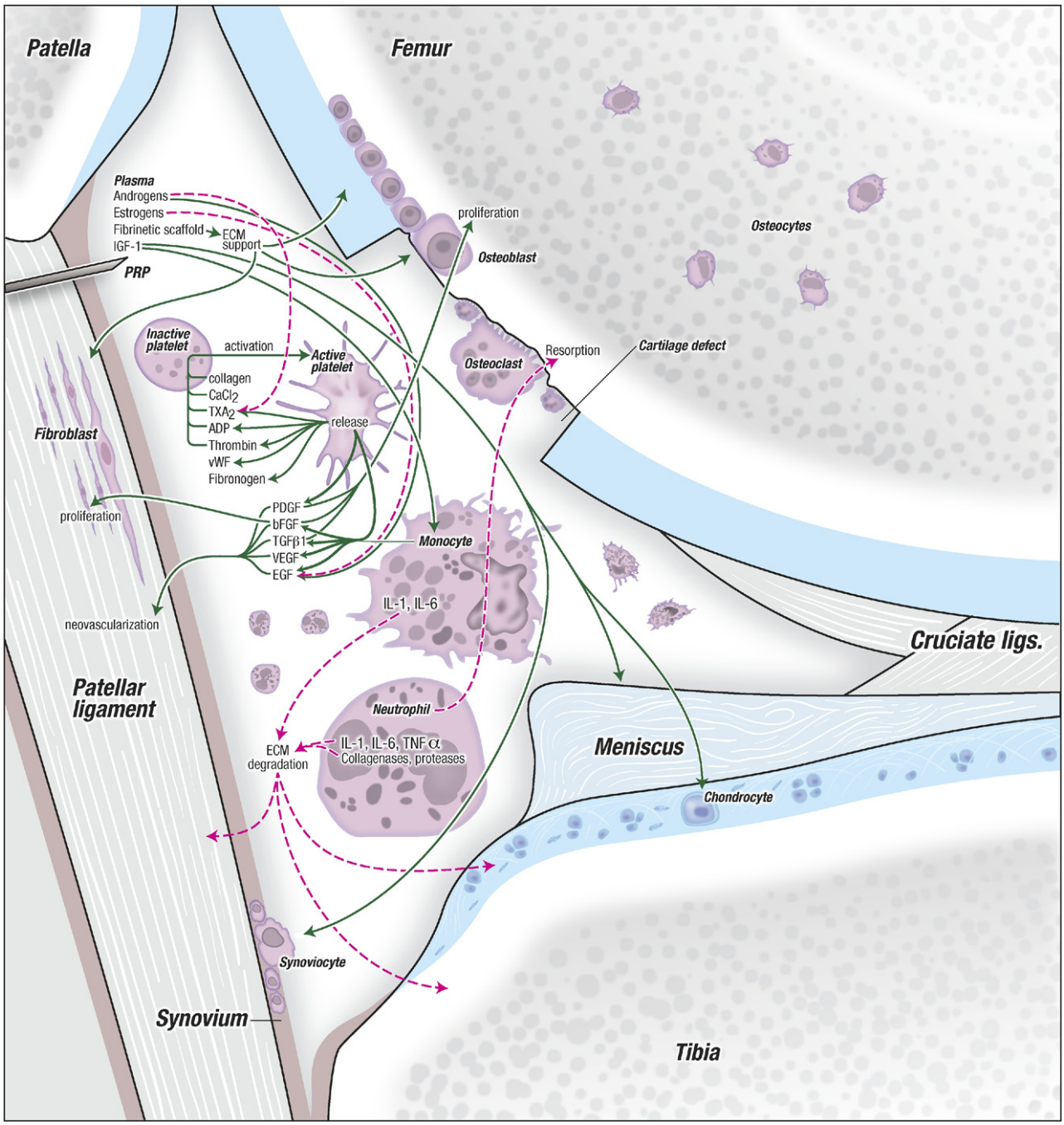

Figure 1. Schema of PRP injection into patellofemoral joint. Cells in PRP include platelets, which are activated to release GFs, and leukocytes such as neutrophils and monocytes that mainly function in phagocytosis, immunity, and inflammation. Soluble hormones (such as IGF-1) are also shown. Cells in the joint that could be exposed to the effects of the bioactive factors in PRP include synovial, meniscal, and ligamentous fibroblasts, chondrocytes, and osteocytes. Green solid arrows indicate positive metabolic paths (such as upregulation of matrix synthesis or cell replication). Red dashed arrows indicate negative metabolic paths (such as increased matrix degradation or inhibition of matrix synthesis). (ADP, adenosine diphosphate; $CaCl_2$, calcium chloride; ECM, extracellular matrix; EGF, epidermal growth factor; IL-1, interleukin 1; IL-6, interleukin 6; ligs, ligaments; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor.)

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from platelets in PRP accelerates integration of biologic grafts and healing so that patients can return more rapidly and functionally to activities.[12,13]

Recently, there has been an increased focus on other components of PRP, particularly leukocytes[14] and fibrinogen.[15] Classification or characterization of PRP based on platelet and white cell counts alone has been attempted.[15] This brings some uniformity to the PRP field and improves the specificity of comparative investigations of PRP. However, PRP is best considered a milieu of bioactive factors, and the resultant blend of these factors will determine the most relevant applications to maximize clinical outcomes.

The purpose of this article is to consider the bioactive cellular and molecular factors in PRP and summarize what is known regarding the effect of each factor on musculoskeletal tissue healing. The clinical application of PRP has been reviewed elsewhere and therefore is not a focus of this report.[13,16]

PRP PRODUCTION

PRP is a plasma suspension that contains all components of whole blood in varying amounts (Videos 1 and 2, available at www.arthroscopyjournal.org). According to the Red Cross, PRP by definition contains a minimum of 200,000 platelets/μL. Preparation processes take advantage of differing density gradients of the components in blood to concentrate platelets. Centrifugation of whole venous blood containing an anticoagulant results in a plasma supernatant with a gradient of cellular concentration. Erythrocytes are the densest and will remain as the packed cell layer at the bottom of the centrifuge container. The buffy coat of white blood cells is at the top of the packed red blood cell layer. The platelets are at the highest concentration in the plasma just above the buffy coat and decrease in concentration toward the top of the plasma layer. Many systems use a 2-spin speed protocol that first reduces the number of erythrocytes and second concentrates platelets. The various systems differ in platelet collection efficiency and repeatability, final leukocyte count, platelet activation, and ease of use.[17]

Differences between PRP preparations can be due to the proprietary system selected or the many other factors that also affect the final product. Peripheral venous blood parameters influence the contents of the final PRP product. For example, platelet count in the final concentrate is dependent on the whole blood platelet count in a linear manner.[18] Hematocrit also influences the final product, especially in fixed-volume separator techniques. These types of systems have a requisite volume of whole blood, which is centrifuged before removal of a predetermined volume of the packed cells and/or plasma layer. Therefore a variable number of erythrocytes remain in the PRP. Storage of whole blood before processing introduces additional variability of the final PRP and platelet characteristics (Table 1).[19,20]

Table 1. *Key Points of PRP Processing*

PRP contains all components of blood in variable quantities.
A final concentration of >200,000 platelets/μL meets the Red Cross definition of PRP.
The final PRP product may differ because of the proprietary system selected for processing.
PRP is affected by the patient's venous blood status (packed cell volume, hydration, medications, circadian rhythms).
A clinician's awareness of exactly what is contained in PRP will allow a better-informed decision to be made regarding its therapeutic application.

There is variability in the number of platelets and leukocytes in PRP between preparations from the same individual that is not system dependent. Both the absolute and relative numbers of each leukocyte type change compared with those found in peripheral blood and are variable with the proprietary system selected. This variation can be partially attributed to a number of factors, including hydration status, inflammation (leukocytosis or leukopenia), lipemia (which increases platelet concentration and is influenced by diet),[21] or circadian rhythms in platelet numbers.[22-24] Although gender does affect hematocrit, it affects the final PRP less if a density-gradient system is used. An investigation using cultured periodontal osteoblasts found that the gender of the blood donor for generation of PRP did not significantly alter either platelet count or GF concentration.[25]

We have observed that some individuals can have a complete failure to concentrate platelets with one system but are successful in concentrating platelets with a system from a different manufacturer (Table 2). This disparate result can occur with one blood draw allocated for use in 2 systems, indicating that failure to generate PRP is possible in any individual and when using any system. These observations suggest that a complete blood count should be performed on each patient's venous blood and PRP so that a clinician can be sure that the patient is truly being treated with PRP. Such complete blood count data would also facilitate determination of which PRP components most affect clinical outcome.

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TABLE 2. *White Blood Cell and Platelet Counts in PRP From Healthy Volunteers, Showing Failed PRP Production*

	Component	Blood (cells × $10^3/\mu L$)	Mfr 1 (cells × $10^3/\mu L$)	Mfr 2 (cells × $10^3/\mu L$)	Mfr 3 (cells × $10^3/\mu L$)
Individual 1	WBC	5.0	0*	14.6	11.7†
	Plt	232	39*	591	873†
Individual 2	WBC	4.3	0.3	20.7*	NA
	Plt	218	442	130*	NA
Individual 3	WBC	5.6	0.2*	47.3	NA
	Plt	215	134*	1,520	NA
Individual 4	WBC	7.5	0.2	28.0*	NA
	Plt	206	328	114*	NA
Individual 5	WBC	4.7	0.4*	NA	14.6†‡
	Plt	106	42*	NA	643†‡
Individual 6	WBC	4.8	0.5*	NA	17.8
	Plt	250	114*	NA	638

Abbreviations: Mfr, manufacturer; NA, not available; Plt, platelet count; WBC, total white blood cell/leukocyte count.

*These 6 individuals had a reduced number of platelets compared with venous blood in at least one of their PRP products, despite an increase in platelet count with another manufacturer's production system. This suggests that clinicians should confirm platelet counts in PRP to ensure that the expected concentration has occurred during processing.

†PRP production on a different day from other values for the same individual.

‡Lipemic sample.

PLATELETS

Platelets (thrombocytes) range from 2 to 3 μm in size while circulating for 7 to 10 days at concentrations of 150 to 400 × $10^3/\mu L$. They are anucleate cytoplasmic fragments of multinucleated megakaryocytes located in bone marrow. Platelets are most often thought of primarily for their hemostatic and coagulation functions; however, proteomic studies have shown that platelets contain over 800 proteins with numerous post-translational modifications, such as phosphorylation, resulting in over 1,500 protein-based bioactive factors.[26,27] Only some of these proteins' physiologic actions have been studied, including GFs, peptide hormones, and chemoattractants for macrophages, neutrophils, and stem cells.

Circulating, inactive platelets have a discoid shape with an open canalicular system. Both native and exogenous molecules can activate platelets, including collagen, platelet-activating factor, serotonin, calcium, magnesium, thromboxane A_2 (TXA_2), adenosine diphosphate (ADP), and thrombin. In a positive-feedback system, activated platelets release TXA_2, ADP, and thrombin and activate other nearby platelets. Facilitated by actin and myosin filaments, the activated platelet undergoes cytoskeleton restructuring to develop multiple filopodia from the location of the canaliculi. Exocytosis and degranulation result in an overall increase of platelet surface area. In vitro observations have shown that when platelets are activated, an initial burst of GF release is followed by further sustained release.[28] Platelet activation results in an increase in anti-inflammatory cytokines because of the presence of hepatocyte GF.[29]

In addition to platelet activation by endogenous chemokines, activation is accelerated by adrenergic activity,[30] oxidative stress,[31] or chemical use, such as smoking.[30] Platelets are heterogeneous in size. Larger platelets from healthy volunteers have been shown to be more active and release more chemokines than smaller platelets.[32] Perhaps most significantly, in vitro experiments have shown that platelets in PRP are activated by bone substitution materials[33] and biphasic osteochondral scaffolds.[28] Platelet aggregation is decreased by a strenuous workload[34] or substances such as caffeine[35] or propofol.[36]

When the concentration of GFs is measured, PRP preparations typically contain a 3- to 5-fold increase compared with baseline.[37] This increase is attributed to both platelet concentration and activation. Several studies have investigated the effects of platelet concentration on musculoskeletal tissue homeostasis.[4,13,14,38] Schnabel et al.[4] showed that a concentrated platelet preparation resulted in an enhanced anabolic gene expression in tendon and ligament. In clinical equine tendonitis, a concentration of 750,000 platelets/μL in PRP was significantly associated with

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a shorter time to recovery, defined as return to race competition.[38] However, a ceiling threshold of benefit from GFs seems to exist. One experiment described an increase in chondrocyte proliferation with addition of recombinant platelet-derived growth factor-BB (PDGF-BB) in culture media. The concentration used varied from 4.7 to 300 ng/mL, but peak proliferation was noted at 75 ng/mL.[39] In a different part of the same study, PDGF-AB was used to stimulate migration of bovine meniscal cells with a maximum effect at 10 ng/mL and inhibition of chemotaxis occurring at higher concentrations.[39] An upper threshold of benefit could occur in vivo if a large volume is injected into a small lesion.

In addition to releasing GFs, some literature supports the concept that PRP regulates local production of these factors. Using PRP in a rabbit model of Achilles tendon healing, Lyras et al.[40] showed an upregulation of intracellular transforming growth factor (TGF) β_1 in the first 2 weeks of healing with downregulation in the third and fourth week of healing compared with controls. Furthermore, an increase of intracellular insulin-like growth factor 1 (IGF-1) expression was shown in tenocytes throughout the 4 weeks of healing.[41]

Platelet alpha granules are 300- to 500-nm microvesicles with a proteome count of approximately 284.[42] These include bioactive molecules such as adhesive proteins (fibrinogen, von Willebrand factor) and receptors, clotting factors (V, XI, XIII, and prothrombin), fibrinolytic factors (antithrombin, plasmin, and plasminogen), other basic proteins, membrane glycoproteins, and GFs (Table 3).[8] Alpha granules give platelets their characteristic purple granular appearance on a typical blood smear. GF peptides released from alpha granules include PDGF, TGF-β_1, vascular endothelial growth factor, basic fibroblast growth factor (bFGF), and epidermal growth factor. Despite reports in earlier literature, IGF-1 is not stored in platelets but it is in plasma. Individual variation in the concentration of GFs released from granules exists, but the platelet number in PRP is positively correlated with GF concentration.[12,14,43] PDGF is integral to cell proliferation, chemotaxis, cell differentiation, and angiogenesis.[39,44] For example, Markopoulou et al.[25] determined that PRP promoted cellular proliferation of human osteoblasts that was due to PDGF, bFGF, and TGF-β_1. Increased cellular proliferation is one way that PRP promotes healing of musculoskeletal tissues. Chung et al.[44] showed that blocking PDGF-BB in a rodent growth plate damage model resulted in reduced chemotaxis of mesenchymal cells.

TABLE 3. *Summary of Cellular and Molecular Components of PRP Relevant to Musculoskeletal Tissue*

	Component
Plasma	
Proteins	Albumin, globulins, fibrinogen, complement, and clotting factors
Electrolytes	Chloride, sodium, potassium, and calcium
Hormones	IGF-1, estrogens, progesterone, androgens, ACTH, and HGH
Biomarkers	COMP, CD11b, protein C, microRNA, osteocalcin, and osteonectin
Platelets	
Alpha granules	Adhesive proteins, clotting factors, and GFs PDGF, TGF-β, VEGF, FGF, EGF, and HGF
Dense bodies	Calcium and neurotransmitters
Lysosomes	Lysosomal enzymes
Leukocytes	
Neutrophils	
Primary granules	Myeloperoxidase, acid hydrolases, defensins, and serine proteases
Secondary granules	Collagenase, lactoferrin, cathelicidin, bactericidal phagocytins, and lysozyme
Tertiary granules	Gelatinase and proteases
Monocytes	Platelet-activating factor, TGF-β, VEGF, FGF, and EGF
Erythrocytes	ATP, S-nitrosothiols, nitric oxide, hydrogen sulfide, hemoglobin, and free radicals

Abbreviations: ACTH, adrenocorticotropic hormone; ATP, adenosine triphosphate; COMP, cartilage oligomeric matrix protein; EGF, epidermal growth factor; FGF, fibroblastic growth factor; HGF, hepatocyte growth factor; HGH, human growth hormone; VEGF, vascular endothelial growth factor.

Modulation of coagulation and vascular repair by GFs in PRP is theorized to result in accelerated and improved wound, tendon, ligament, and bony healing.[12] Extensive reviews on the use and effects of GFs from alpha granules on musculoskeletal tissues are available elsewhere.[37,45,46]

GFs function by binding to cellular transmembrane receptors and regulating cellular signaling pathways.[47] One advantage of PRP over administration of a single exogenous GF is that GFs are released from platelets in native (rather than recombinant) form and presumably in a biologically relevant ratio.[37] In a canine model, use of bFGF alone accelerated the cell-proliferation phase of tendon healing but resulted in peritendinous scar formation and diminished range of motion.[48] This finding

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supports the concept of using a balanced, complementary set of bioactive GFs found in PRP, rather than individual GFs. PRP is also much less expensive and subject to fewer regulatory restrictions than use of a recombinant GF.[49]

Dense bodies (delta granules) are organelles 250 to 300 nm in size. They contain primarily substances that promote clotting, such as calcium (clotting factor IV), magnesium, adenosine, serotonin, and histamine.[47] A deficiency of delta granules results in mild bleeding disorders. Although serotonin is manufactured in the gastrointestinal tract and the brain, it is stored in dense bodies and, when released, promotes hemostasis by constricting vascular tone and permeability. Details are still not fully elucidated, but it is known that serotonin exists in different forms and that it acts as either a hormone or neurotransmitter and can both positively and negatively regulate bone mass.[50]

The lambda granule is the third type of granule whose contents are released during platelet activation. Lambda granules are lysosomal-type organelles. Although literature is scant regarding lambda granules, they do have "clearing" responsibilities to remove infectious agents and cellular debris. As healing progresses, tissue plasminogen activator secreted by the endothelium activates lambda granule enzymes, which convert plasminogen to plasmin and lyse the clot. Plasminogen activators play a role in homeostasis of muscle fibers and adjoining extracellular matrix, including fracture repair.[51,52]

PLASMA

Plasma is defined as the yellow-colored liquid component of blood in which blood cells are suspended. Approximately 200 proteins have been documented in plasma, including albumin, immunoglobulins, complement, and clotting factors.[53] Because plasma transiently contains many proteins released from cells and metabolic processes throughout the body, the presence of as many as 679 proteins has been documented.[54] Biomarkers of bone turnover can be found in plasma as well, including osteocalcin and osteonectin, which are both secreted by osteoblasts. Other musculoskeletal molecules of interest are transiently found in plasma. For example, cartilage oligomeric matrix protein levels are elevated in cases of severe arthritis.[55] Plasma biomarkers can be measured during orthopaedic surgery. Hughes et al.[56] measured markers of ongoing orthopaedic-specific inflammation and leukocyte activation including nonspecific C-reactive protein and orthopaedically related CD11b and protein C and showed that more accurate assessment of intraoperative inflammation was feasible. MicroRNAs present in plasma have been used to document both rheumatoid arthritis and osteoarthritis.[57] A biomarker profile has been used to document high bone turnover in women with osteoporosis.[58] A similar profile could be developed for patients with fractures. Likewise, an equine model showed that a biomarker pattern was present in racehorse plasma before injury.[59]

Plasma proteins involved in hemostasis also affect musculoskeletal healing. After the activated platelet plug provides primary hemostasis at a site of injury and during clot maturation, cell adhesion molecules such as fibronectin, fibrin, and vitronectin move from the plasma into the clot. In vitro, these proteins have been shown to induce chemotaxis of multipotent stromal cells across a membrane.[60] This implies that these molecules may modulate cell migration of fibroblasts, osteoblasts, or other tissue-regenerating cells in musculoskeletal repair.

Electrolytes present in plasma are tightly regulated through transmembrane adenosine triphosphatases to optimize cellular and tissue function. Chloride (reference interval, 340 to 370 mg/dL), sodium (31 to 35 mg/dL), potassium (14 to 20 mg/dL), and total calcium (8.5 to 10.2 mg/dL) are the 4 electrolytes that are most abundant.

About half of the calcium circulating is bound by albumin, and ionized calcium can be measured independently (4.1 to 4.8 mg/dL). In addition to functioning as a platelet activator, intracellular reservoirs maintain calcium homeostasis. It is an important secondary messenger in cells and is a cofactor for several extracellular reactions. It has been well established that within the coagulation cascade, plasma calcium is a cofactor for the formation of both the tenase (factor X) and prothrombinase complexes. In relation to musculoskeletal tissue repair, intracellular calcium signaling is required for the contractile activity of myofibroblasts in vitro.[61]

Hormones such as thyroxine, estradiol, adrenocorticotropic hormone, androgens, estrogens, progesterone, and human growth hormone circulate in plasma. Perhaps the most musculoskeletally relevant hormone found in plasma is IGF-1. IGF-1 has been shown to improve healing in equine tendon and cartilage models.[62,63] The benefits of IGF-1 in enhancing regeneration of injured musculoskeletal tissue has been previously reviewed.[63,64]

How much the other hormones present in PRP affect platelets or musculoskeletal metabolism is not fully known. In general, glucocorticoids provide pro-

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tection to tissues by decreasing the production or activity of inflammatory mediators. Intracellular synthesis of epidermal growth factor is stimulated by testosterone and inhibited by estrogens. Experimentally induced platelet aggregation and release of TXA_2 were reduced in PRP with a physiologic level of dihydrotestosterone.[31] Estrogens alone do not appear to affect platelet aggregation in PRP; however, some estrogens with ADP or adrenaline synergistically increase platelet aggregation.[65]

LEUKOCYTES

Mammalian leukocytes are classified as granulocytes (neutrophils, eosinophils, and basophils) or mononuclear cells (lymphocytes and monocytes or macrophages). Phagocytic cells such macrophages are essential to the in vivo healing process.[66] A murine model was used to show that macrophages were essential for debridement of damaged ligamentous tissue and for cytokine release that mediates the repair process.[66] The presence of leukocytes can result in proinflammatory cellular signaling and local tissue catabolism.[67] For example, McCarrel and Fortier[14] showed that leukocytes in a PRP preparation had a negative correlation with matrix synthesis and a positive correlation with matrix catabolism in tendons. A higher concentration of leukocytes, therefore, is likely undesirable for musculoskeletal applications associated with tendon healing but could be more relevant for other uses, such as healing of large, infected dermal lesions.

The primary function of a neutrophil is to destroy infectious agents. Reservoirs of antimicrobial proteins are contained in primary, secondary, and tertiary granules. Proteases are also found in the cytoplasm of neutrophils. Although these molecules are important for destroying microbes, they can also incite local tissue destruction.

If the goal of PRP is to provide balanced yet augmented healing, adding neutrophils in excess is antagonistic to the goal. To prevent neutrophil-mediated tissue damage, the influx of neutrophils must be controlled.[68] The quantity of neutrophils in the native environment is self-controlled. Secretory leukocyte protease inhibitor is synthesized in myelocytes, stored in neutrophils, and released to limit local tissue damage caused by serine proteases.[67] In addition, neutrophils release reactive oxygen species, which results in a respiratory burst that is intended to damage pathogens. However, the respiratory-burst reaction without a target microbe is likely to cause tissue damage.[13]

Primary (azurophilic) granules are smaller and more numerous than secondary granules and contain at least 10 peptides with antimicrobial properties. When a neutrophil is activated, the primary granules release myeloperoxidase, lysozyme, acid hydrolase, alpha defensins,[68] bactericidal/permeability increasing protein, and serine proteases (including neutrophil elastase [ELA2], azurocidin, cathepsin G, and proteinase 3).[67] These proteases both damage bacteria and degrade the extracellular matrix, allowing cellular migration through the tissue.[69,70] Although these functions are essential for tissue remodeling, they can also result in destruction of normal tissue, as exemplified in an in vitro study using normal equine tendons.[14]

Secondary (specific) granules are less numerous and larger than primary granules. Some molecules carried by primary granules are also carried by secondary granules (lactoferrin, gelatinase, lysozyme).[71] In addition, secondary granules contain matrix metalloproteinase-8 (MMP-8) (neutrophil collagenase) and MMP-9 (type IV collagenase, also known as gelatinase B), which result in extracellular matrix degradation.[13] They also contain the antimicrobial polypeptides lysozymes, lactoferrin, and cathelicidin. Secretory leukocyte protease inhibitor is also present and modulates the neutrophil's proteolytic response in any tissue.[67] Lactoferrin binds iron and is active against bacteria, viruses, fungi, and parasites.[69] Its antiviral action occurs through competitive binding of glycosaminoglycans, preventing viral adsorption and cellular invasion. Thus it could also block glycosaminoglycans found in cartilage, tendon chondroitins, or synovial hyaluronan.

Tertiary granules are both smaller and less numerous than other granule types. They release MMP-9 and MMP-15 along with peptides that sequester iron and other metals, including neutrophil gelatinase–associated lipocalin and natural resistance–associated macrophage protein 1, from phagocytosed bacteria.[70]

Monocytes are found in peripheral blood and differentiate into macrophages when they migrate into connective tissue. Circulating monocytes promote extracellular matrix breakdown by the release of MMP-2, MMP-9, MMP-13, cathepsin, inducible nitric oxide synthase, interleukin-1, and interleukin-6. Concurrently, monocytes suppress inflammation, promote angiogenesis, and support collagen synthesis through TGF-β, vascular endothelial growth factor, and bFGF release, respectively. Monocyte-derived macrophages are necessary for any type of regenerative process, including musculoskeletal tissue repair through active phagocytosis of necrotic tissue and debris. Activated

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macrophages likely play a role in the regeneration of subchondral bone.[72]

Eosinophils function mainly in immunity. They contain major basic protein 1, which is also found in basophils and mast cells and is involved with killing endoparasites. Eosinophil peroxidase is only found in eosinophils and inactivates pathogens through oxidation. Ribonucleases from eosinophils are antiviral and anti-endoparasitic.[69]

ERYTHROCYTES

The centrifugation process used to generate PRP typically reduces or eliminates the presence of erythrocytes (red blood cells). Because some red blood cells are often present, they are worth discussing. The main function of erythrocytes is to carry oxygen, which is essential for tissue repair. As such, red blood cells lack most cellular organelles, including a nucleus, endoplasmic reticulum, and mitochondria. They contain about 750 proteins compared with the estimated 20,000 or more proteins of nucleated cells.[73] Red blood cells also carry some immune complexes.[74] In vivo, erythrocytes release substances that serve to dilate vessels including adenosine triphosphate, S-nitrosothiols, nitric oxide, and hydrogen sulfide. Experiments suggest that nitric oxide mediates insensitivity to IGF-1 in diseased cartilage.[75,76] Hemoglobin carries the oxygen and, technically, is a metalloproteinase. It is made of 4, smaller, protein-bound heme molecules. Under conditions of oxidative stress, heme can become free and cytotoxic. The iron contained within heme molecules catalyzes free radicals and contributes to pathogen destruction. The free radicals, however, can also induce apoptosis of host cells in reaction to proinflammatory signaling. Because of these destructive capabilities, limiting red blood cell contamination in a PRP preparation intended for treatment of musculoskeletal repair seems warranted.

CLINICAL RELEVANCE

Knowing the components of PRP being used will help elicit the important factors in various applications of this regenerative therapy (Table 4). As an example, 5 recent articles in *Arthroscopy* evaluated anterior cruciate ligament reconstruction and compared PRP-augmented procedures with controls.[77-81] Magnetic resonance imaging evaluation times ranged from 3 to 24 months postoperatively, and results of these trials were variable. Several letters to the editor and the authors' replies highlight the controversy and confusion regarding PRP use in these trials and emphasize the need for more Level I trials to determine the efficacy of PRP.[82-85] Of the reports, one included platelet counts in its results and none described leukocyte counts of the PRP. Four of 5 did state which proprietary system was used to produce the PRP used in clinical patients—all of which were different. Understanding what is in PRP is necessary to evaluate why it is or is not efficacious, which patients will receive the most benefit, and when it will provide the maximal benefit.

TABLE 4. *Tips for PRP Use*

Perform a CBC on venous blood and PRP to confirm that the product intended for use is truly PRP.
Always maintain the sterility of the PRP.
When injecting PRP, do not exceed the volume limit of the lesion.
Handle PRP gently to avoid premature platelet activation.
Consider individual, diurnal, and medication-influenced factors when selecting a time to make PRP.

Abbreviation: CBC, complete blood count.

CONCLUSIONS

PRP is a useful regenerative therapy to address many musculoskeletal injuries. It is important to understand that PRP is more than just platelets and that it contains many bioactive factors that act in anabolic, catabolic, proinflammatory, and anti-inflammatory pathways. Some components are also involved in the modulation of the immune response. The precise combination and concentration of platelets, leukocytes, and other plasma components best for musculoskeletal healing are not presently known, and clinicians should be aware that the effects of PRP are not solely based on platelet concentration. A maximal efficacious concentration beyond which the platelet concentration will provide no further clinical benefits likely exists. Although the effects of many of the proteins in PRP on musculoskeletal tissues are still unknown, they likely contribute to the biologic healing process. Finally, it is imperative for individuals involved in clinical study design and all clinicians to take into consideration diurnal variation in platelet count and that, simply, generation of PRP will fail in some patients in some instances. Although PRP is more than just platelets, the clinician should confirm that PRP, by its very definition, has been generated from peripheral blood before application.

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