Platelet-Rich Plasma (PRP) as a Therapeutic Agent: Platelet Biology, Growth Factors and a Review of the Literature

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Abstract The therapeutic basis of platelet-rich plasma use in medicine is derived from the growth factor content and provisional matrix provided by the platelets themselves. This chapter briefly reviews the platelet research which led to the conceptual development of PRP as a treatment and also the early history of its use. An overview of platelet structure and function is provided to enhance the clinician's understanding of the cell biology behind PRP therapy. The 2 major growth factors in PRP (PDGF and TGFβ) are also discussed. Finally, a review of the experimental PRP literature (in vitro and animal studies) is presented, which describes the evidence for use of PRP in tendon/ligament, bone, and joints. Standardization of PRP use remains a challenging prospect due to the number of variables involved in its preparation and administration. It may be that individually-tailored PRP protocols are actually more beneficial for our patients—only time and further research will bear this out.

Origins and Overview of PRP Use in Medicine

As recently as forty years ago, platelets were considered to be exclusively hemostatic cells. Today we know that platelets actually perform myriad diverse functions. The conventional paradigm of limited platelet function began to shift in 1974, as the pathogenesis of atherosclerosis was beginning to be unraveled. Researchers studying the proliferation of smooth muscle cells in the vascular intima knew that 10 % serum was crucial to support cell growth in culture, but did not know which component of serum was responsible for the observed anabolic

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effect. They also knew that “plasma serum”, derived from the addition of calcium to platelet-poor plasma, lacked the stimulatory effect observed in true serum derived from whole blood. In 1974, Ross et al. (1974) determined that the addition of either intact platelets and calcium, or the supernatant derived from thrombin-activated platelets, resulted in significant improvements in the mitogenic capacity of “plasma serum”, such that it equalled that of the serum derived from whole blood. They concluded that platelets must be the major source of the proliferative effect provided by serum. In 1978, Witte et al. (1978) coined the term, “platelet-derived growth factor”, or PDGF, and in the following year Kaplan et al. (1979) used subcellular fractionation to determine that PDGF resided within the platelet’s alpha granules. Over the next 20 years, transforming growth factor beta (TGFβ), (Assoian et al. 1983) insulin-like growth factor (IGF)-1, (Karey and Sirbasku 1989) basic fibroblast growth factor (bFGF) (Brunner et al. 1993) and vascular endothelial growth factor (VEGF) (Banks et al. 1998) were also identified in platelet alpha granules. Platelet suspensions in plasma have been prepared for therapeutic intravenous transfusion (Dimond 1914) and the experimental study of platelet function in the laboratory since the early 1900s, (Eagle 1935) but the notion to use platelet concentrates for non-hemostatic therapy only arose in the late 1990s, after the discovery of these growth factors.

Perhaps not coincidentally, it was also during the late 1990s that the term “Regenerative Medicine” was coined (Haseltine 2011) and a new field was born. The burgeoning fields of stem cell, growth factor and extracellular matrix research converged in a new treatment philosophy, which embraces a more reductionist approach than the concepts of classical Tissue Engineering, but with the common aspiration for restoration of fully functional tissue. Instead of producing completely formed tissues ex vivo and then transplanting them as functional biologic structures, Regenerative Medicine refers to a strategy whereby the injured site is provided with the raw materials necessary for a “scarless repair”, or regeneration, to occur in situ. These therapies provide (at least 1 of) the 3 components considered essential for tissue regeneration—namely, cells, growth factors and scaffold. In Regenerative Medicine the assembly of these resources into new tissue takes place within the lesion site or in proximity to it, and is directed under local influences. The concept is one of augmentation and optimization of the natural healing response, rather than “insertion” of an engineered product. Currently, Regenerative Medicine represents a shift toward more affordable, approachable, and often bed-side strategies to tissue restoration, whereas the construction of entire organs for transplantation remains the purview of true tissue engineering. Nonetheless, the two fields are intimately related and are now often referred to as “Tissue Engineering and Regenerative Medicine”, or “TERM”. Platelet-rich plasma (PRP) is included within the field of Regenerative Medicine, (Torricelli et al. 2011; Okabe et al. 2009; Wu et al. 2011; Sanchez-Gonzalez et al. 2012; Stellos and Gawaz 2007) since it can provide 2 of the 3 components (i.e., growth factors and scaffold) deemed necessary to support true tissue regeneration. Its main advantages include its availability, affordability, and minimally invasive harvest, since it is produced from the patient’s own blood after collection by simple

venipuncture. Because the preparation process is rapid and requires minimal specialized equipment, PRP can be applied to a patient within hours of a treatment decision. These features make PRP extremely attractive for clinical use in a variety of settings, including not only hospitals and outpatient clinics, but also in field applications or other areas with limited medical facilities and resources. Inventory, ordering, and safe storage are not required and shelf-life is not a concern, since the treatment is freshly prepared for each patient. Furthermore, because it is autologous, PRP does not provoke an immune response in the patient and is therefore perceived to have a high margin of therapeutic safety. Interestingly, the disadvantages of PRP therapy also stem from the fact that it is a readily available, autologous blood product. These features mean that, as long as the platelets are "minimally manipulated", PRP is not classified as a drug by the FDA. Since it is therefore not subject to federal regulation, PRP preparation and administration protocols are not specifically defined. As a result, and because of the numerous variables involved in PRP use, clinical and experimental methodologies are extremely inconsistent, making it difficult to draw conclusions about the true efficacy of PRP and best practices for its use. The existing literature is fairly divided on several aspects of PRP use, and authors of recent meta-analyses have concluded that inconsistent clinical methods may be responsible for the inconsistent clinical results also reported (Taylor et al. 2011; Sanchez et al. 2010).

The first clinical report of PRP use to enhance tissue healing was published in 1998, by an oral surgeon who incorporated autologous PRP into cancellous bone graft to reconstruct large mandibular defects in people (Marx et al. 1998). The study was controlled, randomized, blinded, and prospective. The outcome of interest was bone formation within the defect, and the PRP-treated group demonstrated significant improvements in both radiographic and histologic scores of bone density. PRP is now in common use during oral and maxillofacial surgery, as it is believed to enhance the integration of periodontal implants and accelerate the repair process (Del Fabbro et al. 2011; Arora et al. 2010). PRP has also been reported to provide significant improvements in the healing of complex wounds (Mazzucco et al. 2004; Villela and Santos 2010). Most recently, PRP has been used to treat musculoskeletal injuries in both people and horses, where it is applied via an open surgical approach or closed, percutaneous injection (Torricelli et al. 2011; Waselau et al. 2008; Sampson et al. 2008; Taylor et al. 2011; Sanchez et al. 2007; Sanchez et al. 2008; de Vos et al. 2010).

There are a number of variables involved in therapeutic PRP use, which contribute to the reported inconsistency in clinical and experimental methodology and make it difficult to standardize PRP as a product. These factors include preparation method, (Everts et al. 2006; Marx 2004) activation status and methods, (Martineau et al. 2004; Virchenko et al. 2006; Harrison et al. 2011; Kakudo et al. 2008) platelet concentration, (Ogino et al. 2006; Jo et al. 2012; Han et al. 2007; Giusti et al. 2009; Wang et al. 2012; Anitua et al. 2009) leukocyte concentration, (McCarrel et al. 2012; Sundman et al. 2011) effect of the individual, (Mazzocca et al. 2012; Boswell et al. 2012) and physical form of the PRP. Each of these variables has the potential to impact the properties of the resultant PRP.

Interestingly, the very characteristics that make PRP attractive as a therapeutic agent (i.e. autologous in nature, freshly produced at time of need) mean that it will never be a standardized product, by definition. The more realistic and perhaps better goal is instead the development of protocols that can best optimize PRP as it is derived from any individual. Furthermore, in this era of "Personalized Medicine", (Mancinelli et al. 2000) each of these variables may instead be viewed as an opportunity to tailor the PRP according to the specific requirements of a particular individual or a certain tissue, anatomic site, or lesion type.

Basic Concepts of Platelet Biology

Platelets are small, discoid, anucleate cells formed from the fragmentation of long proplatelet extensions of the megakaryocyte. These extensions become interwoven through endothelial pores of the bone marrow sinusoids and are fragmented by shear forces, (Junt et al. 2007) releasing a heterogeneous population (Thon et al. 2012) of nascent platelets into the bloodstream. They have a circulating lifespan of 5–9 days and their predominant mechanism of clearance is via Kuppfer cells and hepatocytes, based upon lectin receptor recognition of altered glycan structures on their surface (Grozovsky et al. 2010). The functional responsiveness of platelets is variable and known to be affected by size (Karpatkin 1978) and age (Hartley 2007) of the cell, with younger and larger platelets demonstrating greater hemostatic function than smaller or older cells.

Physical Properties and Contents

Though they lack a nucleus, platelets possess an extensive cytoskeleton, mitochondria, lysosomes, ribosomes, (Weyrich et al. 2009) and a modified version of smooth endoplasmic reticulum, as well as a number of unique organelles and membrane features (White 2007). There are 3 types of platelet granules: alpha, dense and lysosomes. Alpha granules are the most numerous organelle in the platelet and contain over 300 different proteins, (Coppinger et al. 2004) the majority of which are synthesized or endocytosed by the parent megaryocyte (Rendu and Brohard-Bohn 2001). Recent research has indicated that the distribution of these proteins is not uniform, meaning that distinct subpopulations of alpha granules appear to exist and that they may also have different release kinetics (Sehgal and Storrie 2007; Italiano et al. 2008). Dense granules are relatively few in number and contain only a few small molecules, such as serotonin, ADP, ATP, GDP, GTP, histamine, calcium, magnesium, and polyphosphate (Rendu and Brohard-Bohn 2001). Platelet lysosomes resemble those of other cells and it is unclear whether they play a role specific to platelet function, (White 2007) though

it has been suggested that they may contribute to eventual clot lysis (Rendu and Brohard-Bohn 2001).

The platelet membrane is highly specialized, in that it includes a complex network of invaginations that extend into the center of the cell and are available to increase the surface area of available membrane during the profound shape change that occurs during platelet activation. These invaginations are referred to as the open canalicular system (OCS). Upon activation, the cytoskeleton reorganizes and platelet granules are moved to the center of the cell, where they fuse with the OCS via a vSNARE and tSNARE mechanism, (Blair and Flaumenhaft 2009; Rendu and Brohard-Bohn 2001) releasing their contents into the extracellular environment. A second membranous component within the cytoplasm is the dense tubular system (DTS), which sequesters intracellular calcium in the resting cell and is analogous to the sarcoplasmic reticulum of muscle cells (Rendu and Brohard-Bohn 2001; White 2007).

The platelet cytoskeleton is comprised of a spectrin membrane skeleton, a circumferential microtubular coil, and an abundant network of actin filaments. The platelet is capable of generating remarkable tensile force by virtue of the interactions of the actin network with non-muscle myosin IIA (Ono et al. 2008; Bearer et al. 2002). It was recently estimated that in terms of force generated per unit of cell volume, platelets are capable of generating 100 times the contractile force of a myoblast (Lam et al. 2011). This incredible degree of contractility within the cell means that platelets can be more densely packed within a primary hemostatic plug, conferring stability to the initial platelet thrombus (Ono et al. 2008). When transmitted across a network of fibrin strands as well, the same property leads to clot retraction during secondary hemostasis (Muthard and Diamond 2012).

Platelet Activation in Hemostasis

In the circulation, platelets exist in a resting, discoid state unless specifically activated by stimuli. These stimuli can be physical, chemical, or a combination of both. The main platelet agonists responsible for activation in vivo are subendothelial collagen in combination with exposure to shear and von Willebrand's factor (vWF), thrombin, ADP, or a combination of these. Under experimental conditions, collagen, thrombin, and ADP (as well as their synthetic substitutes and calcium ionophores) are the main agonists used in platelet research. The collagen receptors are the integrin $\alpha2\beta1$, the GPIb-V-IX complex, and GPVI. These receptors engage collagen in a cooperative way: after vWF binds to GPIb, collagen binds to GPV in the same complex, slowing the platelet long enough to allow further collagen binding by $\alpha2\beta1$ and GPVI (Herr and Farndale 2009). These latter steps arrest the platelet and activation ensues. The thrombin receptors are PAR (protease-activated receptor)-1 and PAR-4. These "seven transmembrane" G-protein coupled receptors are unique in that they contain a tethered ligand; namely, the extra-membranous N-terminal portion of the receptor is cleaved by thrombin, revealing a

ligand sequence that itself binds to the active site in the receptor (Brass 2003). ADP is a less potent agonist and is more likely to induce platelet aggregation than complete activation, though it is also important in the final stages of clot retraction; (Muthard and Diamond 2012) it acts via the P2Y1 or P2Y12 receptors (Wang et al. 2003). Each of these receptors ultimately converge in the phospholipase C (PLC) signaling cascade, (Brass 2010) which stimulates the release of the intracellular calcium stores from the dense tubular system (Rink 1988). The resulting spike in cytoplasmic Ca+2 (Feinstein and Fraser 1975) activates gelsolin to begin severing existing actin filaments, which are subsequently reassembled into a new cortical ring (Bearer et al. 2002). Granules are centralized in the process and the release reaction subsequently ensues. As the cytoskeleton reorganizes, the intracellular protein, talin, binds to the cytoplasmic tail of the main platelet integrin, αIIbβ3 (Banno and Ginsberg 2008; Brass 2010). The integrin shifts from a closed (inactive) to open (active) conformation and enables the platelet to bind fibrinogen, in a phenomenon referred to as "inside-out signaling" (Brass 2003). Once it has done so, these integrins cluster together on the platelet surface and transduce an "outside-in" signal back to the interior of the cell: (Zou et al. 2007) focal adhesion plaques are formed around the intracytoplasmic tails of the β3, linking the external fibrin strand to the internal actin cytoskeleton of the platelet (Bearer et al. 2002). The prothrombinase complex is concurrently assembled on the platelet membrane, thrombin is generated as a result, and the platelet is activated via the PAR receptors. Since fibrinogen is the substrate for thrombin, that reaction also proceeds rapidly on the platelet surface, producing fibrin monomers that ultimately assemble into fibers.

Also shortly after the rise in intracellular calcium, the platelet rapidly undergoes reorganization of the actin cytoskeleton, which manifests as 4 phases of dramatic shape change upon activation: rounding into a sphere, extension of pseudopodia, adherence to a surface, and spreading (Bearer et al. 2002). These properties facilitate the sealing of a hole in the vasculature, the formation of a primary platelet thrombus, and subsequently the formation of a fibrin clot for definitive hemostasis. Once flow is arrested, the clot is retracted (Muthard and Diamond 2012) as platelets contract against the fibrin network. In this manner the clot is further stabilized and the absolute wound margin is diminished.

As a further result of platelet activation by thrombin or collagen, phosphatidylserine and specific receptors for the coagulation factors IX, VIII, X, V and II (and their active forms) are exposed on the platelet surface (Ahmad et al. 2003). These changes to the platelet membrane create a procoagulant surface, which provides the platform for the sequence of clotting cascade reactions that ultimately culminate in fibrin formation. The forming thrombus is considered to undergo three main phases, beginning with platelet-collagen binding ("initiation"), followed by the recruitment and activation of other platelets ("extension"), and finally, the formation of a densely packed, platelet-rich fibrin clot ("stabilization") (Brass 2010). This clot is now recognized to be a heterogeneous structure in terms of physical properties, such as porosity, as well as platelet activation state. The central thrombus contains maximally activated platelets, and a gradient of

activation diminishes toward the periphery of the clot (Brass et al. 2011). Platelets on the periphery of the thrombus may even participate in a transient, reversible way. In the center of the clot, however, direct platelet–platelet communication is ongoing, via contact-dependent signaling (Brass et al. 2006). The recent studies cited throughout this section reflect a more nuanced view of platelets as dynamic, living cells within the clot, in striking contrast to previous ideas of platelet activation as a rapid, disintegrating, "kamikaze"-like process (Rodman et al. 1963). Platelets on the periphery of a thrombus may disaggregate and re-enter the circulation, (Weyrich et al. 2003) and those within the center have been documented to synthesize protein for at least 18 h. (Lindemann et al. 2001).

Beyond their physical effects on the vasculature, platelets also possess direct vasoactive effects. Platelets bind directly to endothelial cells by P-selectin-PSGL-1 interactions, respectively. They then chemically influence the endothelial cells by released and surface-expressed substances such as CD40L, leading to increased endothelial surface expression of cell adhesion molecules. In addition, their substantial serotonin content induces vasoconstriction.

Non-Hemostatic Functions

One only need examine the long list of substances in the platelet "secretome" to suspect that they participate in numerous non-hemostatic processes as well as their primary role in hemostasis (Weyrich et al. 2003). This becomes even more apparent when considering the variety of surface receptors they possess, with ligands that include adhesion proteins, cytokines, and lipopolysaccharide (Clemetson and Clemetson 2007). Importantly and perhaps unsurprisingly, platelets are also known to release different substances depending upon the stimulus that activates them and/or the other coincident influences in their environment (Cognasse et al. 2008; Weyrich et al. 2003). This concept makes sense because platelet alpha granules contain many substances with directly opposing activities, (Nurden 2011) and so the existence of a mechanism to selectively release only certain granule contents is logical, though not yet defined (Blair and Flaumenhaft 2009). Most prominent among the non-hemostatic functions of platelets are inflammation, immunity and tissue repair.

Platelets express and release a number of inflammatory chemokines and cytokines, including CD40L, Platelet Factor 4 (PF-4), RANTES, and IL1β (Nurden 2011; Semple et al. 2011). They attract, bind, and activate leukocytes via platelet P-selectin binding to leukocyte PSGL-1, (Weyrich et al. 2003) and circulating platelet-monocyte or platelet-neutrophil aggregates serve as an index of inflammatory insult in several disease states (Brown et al. 1998). Once bound, platelet ligands such as CD40L and CD154 induce direct effects on leukocyte receptors, resulting in activation, migration, immunoglobulin class-switching of B cells, and the generation of more pro-inflammatory cytokines (Semple et al. 2011). As primary immune cells, platelets contain microbicidal proteins that can kill bacteria

within 5 min and possess anti-fungal activity as well (Krijgsveld et al. 2000). Perhaps the most remarkable example of their immune function was recently published in Science, when platelets were reported to kill malarial organisms within infected erythrocytes (McMorran et al. 2009). However, the effects of bacterial-induced platelet activation—or conversely, the cloaking of bacteria which can prevent platelet activation—also play a significant detrimental role in septic processes, (Cox et al. 2011; Leslie 2010; Semple et al. 2008, 2011; Clark et al. 2007b) and platelets directly contribute to many aseptic, pro-inflammatory diseases as well. Platelets are central to the pathogenesis of atherosclerosis, which is now recognized as a primary inflammatory disease, (Ross 1999) and they also contribute to the immune-mediated disorders rheumatoid arthritis, (Pohlers et al. 2006; Boilard et al. 2010) transfusion-related acute lung injury, and multiple sclerosis (Nurden 2011; Semple et al. 2011). Platelets also participate in complement activation, and a small subpopulation can support the formation of membrane-attack complexes on their surface (Martel et al. 2011). Nonetheless, though on the whole platelets must be considered as pro-inflammatory cells, they have the potential to elicit anti-inflammatory effects by inhibiting NF$\kappa\beta$ signaling in target cells (Bendinelli et al. 2010; Van Buul et al. 2011) and by virtue of their tissue inhibitor of matrix metalloproteinase (TIMP) content (Celiker et al. 2002; Villeneuve et al. 2009).

Platelets also directly contribute to the formation of new tissue, from ovulation (Furukawa et al. 2007) to embyogenesis (Finney et al. 2012) to maturity, (Olorundare et al. 2001) in both health and disease (Luttenberger et al. 2000; Dees et al. 2011). Creation and remodeling of the extra-cellular matrix are induced by the combined effects of platelet growth factors, (Montesano and Orci 1988) serotonin, (Dees et al. 2011) matrix metalloproteinases and TIMPs (Nurden 2011). Platelets contain the matrix proteins fibronectin, vitronectin, and laminin, (Nurden 2011) and also bind to these ligands via their integrin receptors (Bennett et al. 2009). In a healing wound, fibroblasts are drawn into the fibrin clot by the chemotactic gradient provided by PDGF and TGFβ. These cells migrate along the necessary physical conduit of fibronectin, (Greiling and Clark 1997) which is also provided and assembled by the platelets. (Olorundare et al. 2001) The fibroblasts begin to synthesize more fibronectin and also collagen, under the influence of platelet-derived serotonin and TGFβ (Dees et al. 2011). In addition to matrix synthesis, platelets induce cell proliferation (Luttenberger et al. 2000; Kakudo et al. 2008; Mishra et al. 2009; Wang et al. 2012; Doucet et al. 2005; Frechette et al. 2005; Jo et al. 2012; Kajikawa et al. 2008; Loppnow et al. 1998; Ogino et al. 2006; Slater et al. 1995) and differentiation (Zhang and Wang 2010; Mishra et al. 2009; Stellos and Gawaz 2007). Platelets directly stimulate the formation of new blood vessels (Kurita et al. 2011; Bosch et al. 2011a) and aid vascular repair at sites of damage, by recruiting and anchoring endothelial progenitor cells at the site (Stellos and Gawaz 2007). In the field of wound healing, the platelet–fibrin clot has been referred to as a “provisional matrix”, (Greiling and Clark 1997) since it provides the anlage for subsequent native tissue formation. Unfortunately, the same properties that facilitate wound healing also implicate platelets as

contributors to neoplastic and fibrotic syndromes. Primary tumor growth is permitted by platelet-driven angiogenesis, and metastasis has been linked directly to the interactions of platelet microparticles with tumor cells (Janowska-Wieczorek et al. 2005; Erpenbeck and Schon 2010). Platelets are also believed to participate in the pathogenesis of alveolar fibrosis, (Piguet and Vesin 1994) pancreatic fibrosis, and systemic sclerosis (Dees et al. 2011; Luttenberger et al. 2000). It should be noted that the anabolic effect of platelets is not only the result of polypeptide growth factors. Bioactive lipids (Langlois et al. 2004; Berg et al. 2003; Nurden 2011; Svensson Holm et al. 2011; Jiang et al. 2008) and reactive oxygen species (Seno et al. 2001; Svensson Holm et al. 2011) have also recently been identified as key components in platelet-directed cell proliferation and tissue repair.

Growth Factors in PRP

The polypeptide growth factors PDGF, (Kaplan et al. 1979) TGFβ, (Assoian et al. 1983) IGF-1, (Karey and Sirbasku 1989) VEGF, (Banks et al. 1998) HGF, (Nakamura et al. 1987) EGF, (Assoian et al. 1984) and bFGF (Brunner et al. 1993) have each been identified within platelet alpha granules. Many of these factors share some common structural features and signaling mechanisms, which will be discussed here in general terms. This section will then focus on the 2 main growth factors of platelets, PDGF and TGFβ.

Growth factors are generally polypeptide dimers, comprised of 2 antiparallel monomers that are arranged in a “cystine knot” configuration. This term refers to the common feature of 8 cysteine residues within each monomer chain, at intervals that are conserved between different growth factors. These cysteines confer the ability for disulfide bonding both between and within the monomer chains, which translates into similar three-dimensional structures among the various growth factors. One intra-chain disulfide bonded loop is nested within another, in a sort of “C-in-a-C” arrangement, referred to as the “cystine knot” (Heldin and Westermark 1999; Reigstad et al. 2005). Most of these growth factors (all but EGF) have several isoforms, which produce overlapping but slightly different outcomes on target cells and tissues. The receptors for most of these growth factors (all but TGFβ) are tyrosine kinase receptors (“RTK”s), and the PDGF, EGF, IGF, and VEGF receptors dimerize themselves upon ligand binding, (Reigstad et al. 2005; Andrae et al. 2008) and then autophosphorylate by virtue of the tyrosine kinase activity between the paired intracellular tails. Once phosphorylated, the tyrosine kinase itself has enhanced catalytic efficiency to phosphorylate (and thereby activate) other intracellular proteins. In addition, phosphorylation of the non-kinase domains provides a binding site for proteins that contain Src-homology 2 (SH2) domains. These latter proteins induce signaling via several pathways including the PI3-kinase and PLC cascades (Heldin and Westermark 1999). These 2 pathways induce myriad downstream effects including transcription, translation,

cell division, and/or migration. Examples of these specific signaling effects include the release of intracellular calcium, or activation of the Ras G-proteins Rac and/or Rho, causing cytoskeletal reorganization and cell migration (Wozniak et al. 2005). Alternatively, the SH2 domains may belong to adaptor proteins that ultimately lead to the MAPK signaling cascade, which drives the cell cycle past its restriction point and causes cell proliferation (Heldin and Westermark 1999; Alberts et al. 2004; Andrae et al. 2008).

PDGF

Platelet-derived growth factor was the original growth factor discovered in alpha granules, (Kaplan et al. 1979) after observation of its potent mitogenic effect on cultured cells (Ross et al. 1974; Kohler and Lipton 1974). PDGF has since been identified as a product of many other cell types, but platelets remain its primary source. There are 5 isoforms of PDGF (AA, AB, BB, CC, DD), each of which are approximately 30 kDa in molecular weight and are derived from the combination of 4 different monomers (Reigstad et al. 2005). There are 3 PDGF receptors, based upon the combination of α and β chains into homo- or heterodimer configurations; PDGF-BB has been called the "universal" isoform of PDGF (Caplan and Correa 2011) because it binds to all 3 receptor configurations. PDGF is crucial for the development of the heart, lungs, kidneys, and central nervous system, and PDGF knock-out generally results in an embryonic or perinatal lethal phenotype (Heldin and Westermark 1999; Andrae et al. 2008; Reigstad et al. 2005). PDGF-AA, -AB, and -BB are secreted as active molecules, whereas PDGF-CC and -DD are secreted as inactive proteins and are cleaved by plasmin, tissue plasminogen activator, or urokinase plasminogen activator (Reigstad et al. 2005). Active isoforms may subsequently be sequestered by binding to matrix and plasma proteins (Heldin and Westermark 1999; Clark et al. 2007a; Caplan and Correa 2011).

After tyrosine kinase-induced phosphorylation begins at the receptor, PDGF signaling occurs by 4 different pathways: Src, PI3 K, PLC and Ras. Phosphatases are active concurrently, and the balance between these competing forces ultimately determines the degree and type of PDGF effect on the cell (Heldin and Westermark 1999; Andrae et al. 2008). When fibroblasts are exposed to platelets, signaling is rapid and sustained: Akt phosphorylation was observed within 15 min and lasted for 48 h in normal dermal fibroblasts (Giacco et al. 2006). These signaling cascades collectively result in a triad of cellular effects: migration, proliferation, and matrix synthesis. Specifically, PDGF is released by platelets in the wound bed and creates a chemotactic concentration gradient for fibroblasts, neutrophils and macrophages. It then activates macrophages to produce more growth factors and to aid debridement of damaged tissue (Heldin and Westermark 1999; Uutela et al. 2004). PDGF induces mitosis in fibroblasts and smooth muscle cells, and it stimulates these cells to produce proteoglycans, hyaluronic acid, fibronectin, (Pierce et al. 1991) and, to a lesser extent, collagen (Heldin and Westermark

1999). The diversity of PDGF effects (and that of other growth factors) is regulated according to the integrin phenotype of the target cell, which varies over time according to the extra-cellular matrix composition (Xu and Clark 1996).

Far more is known about the contribution of PDGF in pathologic states than in normal physiologic (and therefore potentially therapeutic) states. However, PDGF-BB has recently been proposed as a cornerstone growth factor, linking the processes of angiogenesis and mesengenesis, (Caplan and Correa 2011) and it is also recognized to help orchestrate the production of and response to other growth factors, such as TGFβ (Donnelly et al. 2006).

As an agent of disease, dysregulated PDGF signaling is specifically implicated in atherosclerosis, neoplasia and fibrotic diseases (Heldin and Westermark 1999; Reigstad et al. 2005; Barrientos et al. 2008). For this reason, most PDGF research has centered on methods for its inhibition (Andrae et al. 2008; Heldin and Westermark 1999) rather than its therapeutic provision. However, PDGF is constitutively expressed in many tissues (Reigstad et al. 2005; Andrae et al. 2008; Donnelly et al. 2006) and each PDGF isoform has been confirmed to play a role in wound healing, inducing angiogenesis and matrix synthesis (Reigstad et al. 2005) in addition to cell proliferation and migration. Since its discovery, it has therefore been investigated for therapeutic use in a variety of tissues, either singly or in concert with other growth factors (Barrientos et al. 2008; Haupt et al. 2006). The results of studies on single growth factors for therapeutic use have been somewhat disappointing, and on that basis many investigators have suggested a shift in approach toward a more physiologic "cocktail" of multiple factors (Haupt et al. 2006; Lynch et al. 1987; Costa et al. 2006). PDGF, however, has proven successful as a single agent in some clinical applications. Becaplermin is an FDA-approved recombinant PDGF product, licensed for topical use on refractory wounds such as diabetic ulcers. Its margin of improvement in wound healing is estimated to be only about 25 %, and it is expensive and requires daily application and therefore dressing changes (Clark et al. 2007a). Regardless, any improvement in the healing of these complex wounds is clinically significant, and a positive result has been documented in large clinical trials (Steed 2006). In the experimental setting, PDGF has been applied to the cells of non-cutaneous tissues as well, such as tendon, bone, cartilage, and meniscus (Haupt et al. 2006; Kaigler et al. 2011; Schmidt et al. 2006). Overall, studies of PDGF effects on these tissues indicate only mild to moderate anabolic impact in tendon, (Haupt et al. 2006; Thomopoulos et al. 2009; Costa et al. 2006) matrix synthesis and proliferation but not differentiation of chondrocytes, (Kieswetter et al. 1997) improved matrix synthesis by chondrocytes of meniscal fibrocartilage, (Bhargava et al. 1999; Imler et al. 2004) and osteoplastic, osteoclastic and regulatory effects on bone formation (Chang et al. 2010; Choo et al. 2011; Kaipel et al. 2012; Marden et al. 1993; Vordemvenne et al. 2011; Ranly et al. 2005). Recently, there is renewed interest in PDGF as an adjunct therapy for fracture healing and periodontal alveolar reconstruction (Caplan and Correa 2011; Kaigler et al. 2011). It has been suggested that by mobilizing pericytes (which are believed to mesenchymal stem cells) from the vasculature

(Ribatti et al. 2011) surrounding a fracture, PDGF not only aids the development of new vessels within the site, but also directly recruits a progenitor cell with osteogenic potential into the fracture bed (Caplan and Correa 2011).

TGFβ

Whereas PDGF is considered to be the predominant mitogen among growth factors, the main activity of TGFβ is synthesis and preservation of the extracellular matrix (Luttenberger et al. 2000). There are 3 isoforms (TGFβ1-3) of this 25kD homodimer, all of which play an important role in wound healing. TGFβ3 in particular is recognized as the main determinant of scarless healing in fetal wounds (Ferguson and O'Kane 2004; Larson et al. 2010) and the shift from TGFβ1 to TGFβ3 expression is recognized as an important step in adult wound healing as well (Theoret et al. 2002).

Most cells secrete TGFβ as a Large Latent Complex, which then binds to the ECM to provide a "controlled release" of the growth factor to its target cells. This process requires release from the ECM and then cleavage for activation of the growth factor, which normally occurs by proteolytic or mechanical means (Albro et al. 2012; Doyle et al. 2012). The interaction of TGFβ with its tetramer receptor involves a series of steps and begins with TGFβ binding the homodimer Type II receptor on the target cell surface. This process recruits the homodimer Type I receptor component into the complex and activates Smad proteins, which translocate to the nucleus to serve as transcription factors to induce TGFβ effects on the cell (Doyle et al. 2012; Hinck 2012).

As was the case for PDGF, much of our knowledge about TGFβ has been elucidated by its role in pathologic states, particularly those that involve the ECM. The hallmark example of this is Marfan syndrome, (Doyle et al. 2012) which is a primary fibrillin defect that results in abnormalities in the great vessels, heart, chest wall and skin. It was determined that the morphogenetic abnormalities could not be based on abnormal fibrillin-1 structure, but were instead the manifestation of increased TGFβ availability from the abnormal ECM. This disease illustrates that normal physiology as well as potential therapeutic uses of TGFβ depend not only on the presence of the growth factor, but also on the nature and degree of its delivery to tissues. Interestingly and in contrast to other cellular sources of this growth factor, the TGFβ contained by platelets is secreted in active form upon release from the alpha granules, (Blakytny et al. 2004) and this characteristic may have implications for TGFβ as delivered by PRP treatment. TGFβ1 is strongly associated with pathologic fibrosis because of its strong induction of collagen synthesis in both health and disease (Barrientos et al. 2008; Plaas et al. 2011). It is specifically anti-proliferative for many immune cells and tumor cells, by inducing the synthesis of the 2 main cyclin-dependent kinase inhibitors (p15 and p21). In this way, TGFβ is considered to be a tumor suppressor early in neoplastic processes, though it can facilitate metastasis and invasion in the advanced stages of malignancy. In normal

physiologic states, TGFβ is generally considered to exert anti-inflammatory and immunosuppressive effects, and to promote mesenchymal tissue development while inhibiting epithelial cells (Moustakas et al. 2002). It is commonly described as "pleiotropic", however, and it exerts almost opposite effects in wounds, where it is a chemoattractant for neutrophils and macrophages, and stimulates the migration of keratinocytes once epithelialization begins. It strongly induces granulation tissue formation by attracting fibroblasts and stimulating collagen production and angiogenesis, and then promotes wound contraction by inducing their phenotypic shift to myofibroblasts (Barrientos et al. 2008; Montesano and Orci 1988; Pierce et al. 1991; Theoret et al. 2002). In orthopedic tissues, TGFβ is required for cartilage matrix homeostasis and intrinsic repair (Blaney Davidson et al. 2005; Grimaud et al. 2002; Scharstuhl et al. 2002; Plaas et al. 2011) and also for the chondrogenic induction of MSCs, (Freyria and Mallein-Gerin 2012) but its fibrogenic effects pose concerns for its use as an intra-articular therapeutic agent (Fortier et al. 2011). TGFβ effects on bone are contradictory as well. Acting in concert with bone morphogenetic proteins (BMPs), which are themselves part of the TGFβ superfamily, TGFβ induces matrix production and proliferation in osteoblasts, and serves as a negative regulator of osteoclasia by inhibiting the release of receptor-activator of nuclear factor kappa beta ligand (RANKL) from osteoblasts (Chen et al. 2012a). TGFβ is a key regulator of embryonic skeletal development, but recent studies in adult knock-out mice, as well as follow-up studies using TGFβ inhibitors, have demonstrated an inverse relationship between TGFβ signaling and the stiffness, hardness, and ultimately, resistance to fracture in intact bones (Balooch et al. 2005; Mohammad et al. 2009). This data may be more relevant for the constitutive influence of TGFβ on fracture prophylaxis in osteoporotic bones than in the process of fracture healing, where TGFβ supplementation of demineralized bone matrix has been shown to accelerate the repair process (Servin-Trujillo et al. 2011). In tendon repair, the opposing effects of TGFβ are again illustrated by a study in Smad3 -/- mice: although these tendons healed with less adhesion formation and scarring, they were weaker overall by virtue of lower collagen expression (Katzel et al. 2011). TGFβ signaling is reduced in chronic, degenerative tendinosis lesions (Fenwick et al. 2001) and TGFβ blockade in tendon explants results in reduced tensile strength, (Azuma et al. 2007) suggesting that TGFβ is important for the maintenance of normal tendon integrity and repair. Successful therapeutic use of TGFβ, either as a lone agent or as a component of PRP, will require the ability to select for desired TGFβ effects on matrix production and quality without incurring pathologic fibrosis.

Review of the Literature on Platelet-Rich Plasma

There are many published reports that compare the various proprietary PRP preparation systems, but the consideration of these numerous devices and methods (Everts et al. 2006; Sutter et al. 2004; Weibrich et al. 2012; Zimmermann et al. 2001; Arguelles et al. 2006) is beyond the scope of this review. This discussion will instead

focus on studies that have applied PRP to cells or tissues of musculoskeletal origin, and which have therefore provided insight into its potential therapeutic use. With regard to tissue type, PRP has been most heavily investigated in tendon and bone, with studies on articular tissues being performed more recently. It should be pointed out that these studies employ a variety of platelet concentrations, activation methods, and PRP products (i.e. whole PRP which includes platelets versus platelet-rich clot releasate which does not). Platelet concentrations less than 300×10^3 platelets/μL are referred to as "low", $300–800 \times 10^3$ platelets/μL are considered "moderate", and $> 800 \times 10^3$ platelets/μL are referred to as "high". These factors are included in the description of each study so that they may be considered in addition to the results. Lastly, it is important to note that randomized, controlled clinical trials are still rare in the PRP literature.

Tendon and Ligament

With regard to tendon, Anitua et al. (2005) were among the first investigators of the effects of PRP on normal tenocytes in culture. Their work utilizes a platelet-rich clot releasate (PRCR), which is the acellular serum product extruded from PRP of low-moderate platelet concentration (i.e. $200–500 \times 10^3$ platelets/μL) after activation with 23 mM $CaCl_2$. In a 6 day experiment, they observed significant increases in proliferation and synthesis of VEGF and HGF in human tenocytes after treatment with PRCR. Subsequent studies again demonstrated increased proliferation and also hyaluronic acid synthesis—but not increased collagen synthesis—in response to PRCR treatment, (Anitua et al. 2007) as well as improved migration of tenocytes exposed to a combination of PRCR and HA in culture. (Anitua et al. 2011) De Mos et al. (2008) replicated these results in a 14 day experiment with varying concentrations of a similar PRCR, and also reported increased proliferation and also collagen production in human tenocytes. They also observed an increase in MMP1, MMP3, VEGFA, and TGFβ1 gene expression after PRCR treatment. Anabolic effects of PRCR on tenocytes have also been reported by other groups, (Tohidnezhad et al. 2011; Wang et al. 2012) including after exposure to insult: PRCR-conditioned media reversed the tenocyte senescence and death caused by ciprofloxacin or dexamethasone (Zargar Baboldashti et al. 2011). More recently, the effects of PRCR on tendon stem cells have also been evaluated. Zhang et al. reported a significant influence of PRCR on the differentiation of these cells toward a tenocyte lineage and also increased collagen production; this effect was dose-dependent and was compared to controls in 10 % FBS (Zhang and Wang 2010). PRP is also frequently evaluated in conjunction with various scaffolds, with a view toward PRP-enhanced, engineered constructs. Over a 14-day culture experiment, platelet lysate (prepared from repeated freeze–thaw cycles of PRP) induced significantly more collagen production and cell proliferation than controls in a study of canine patellar tenocytes seeded onto a poly-L-lactic scaffold (Visser et al. 2010). A recent study by Jo et al. (2012). was

particularly informative, in that it investigated the effect of varying platelet concentrations and activation methods, and did so on abnormal tenocytes derived from damaged human rotator cuffs. The study examined several outcomes and provides a comprehensive view of PRP effects on this cell type. Cell proliferation over 7 days increased in a dose-dependent manner relative to the platelet concentration of PRP, over a range of 0–16,000 $\times$ 10^3 platelets/μL. Gene expression of collagen Types I and III and tenascin C was greatest in PRP activated by a combination of calcium gluconate and thrombin (approximately 10 mg/mL and 17 U/mL, respectively), in comparison to activation by calcium gluconate alone. However, total collagen and GAG synthesis were not different between the 2 activated PRP groups, which were both significantly greater than a 2 % FBS control. Interestingly, collagen synthesis was greatest in a platelet-poor plasma (PPP) control. A few studies have examined the effects of PRP on equine tendon and ligament explants. McCarrel et al. (2009) examined the effects of resting PRP and also a freeze-dried platelet product on gene expression in superficial digital flexor tendon (SDFT) and suspensory ligament (SL): in both tissues, the ratio of Type I: Type III collagen expression was significantly increased after exposure to both platelet products in comparison to controls. Another study from the same laboratory also found increased Type I collagen expression in SDFT after treatment with PRP lysed by 1 freeze–thaw cycle, (Schnabel et al. 2007) but in SL there were no significant differences observed between PRP and plasma or whole blood controls (Schnabel et al. 2008). Unfortunately, studies that examine only gene expression provide little insight into the ultimate cellular effect induced by PRP. A study on canine deep digital flexor tendon explants (Morizaki et al. 2010) reported significantly increased breaking strength and stiffness in explants treated with a collagen graft containing PRP + MSCs as compared to no graft or graft with MSCs alone. This study employed a PRCR generated from activation of high concentration PRP with 143 U/mL of bovine thrombin and 14.3 mg/mL $CaCl_2$, and the MSCs were harvested from canine bone marrow. With regard to cruciate ligament repair, one in vitro study on cells cultured from damaged human ACLs reported significant increases in cell proliferation but no increase in collagen synthesis when corrected for cell number (Fallouh et al. 2010). In summary, there is consistent in vitro evidence for a mitogenic effect of PRP on both normal and diseased tenocytes, but results are less conclusive with regard to collagen production.

Animal models of tendon injury have most often been performed on the rat and rabbit, with one study on sheep and one study on horses. Several tendon studies have reported the effect of PRP in concert with stem cells of tendon, bone marrow, or peripheral blood origin. One relatively early example in 2007 (Kajikawa et al. 2008) was conducted using chimeric rats that expressed GFP on their bone marrow derived cells. High concentration, lysed PRP (1 freeze–thaw cycle) was injected at the time of injury into patellar tendons that had been partially transected. In comparison to controls, a higher number of GFP-positive cells were present in tendons treated with PRP at 3 and 7 days, suggesting greater recruitment of bone-marrow derived cells to the injured site. A recent study in sheep (Martinello et al. 2012) compared the effects of resting (high concentration) PRP, PRP + MSCs, or MSCs alone to a saline

control in collagenase lesions in the DDFT. Treatment was applied once at 7 days after injury, and histologic outcomes were assessed at 30 and 120 days. There were no significant differences between treatment groups in terms of collagen or COMP staining; surprisingly, cell number was greatest in the control group. Greater vascularity was reported in the PRP-treated tendons. A similar study was conducted in rats which underwent Achilles transaction (Chen et al. 2012b) and were treated with resting, high concentration PRP alone, PRP + tendon stem cells (TSC), TSC alone or saline controls. Treatment was applied at the time of injury in a collagen sponge. There were no significant differences in collagen content between treatments and controls; the PRP + TSC group trended toward the highest collagen content at 3 days, but differences were not statistically significant and all groups appeared equivalent by 14 days. Studies which employ biomechanical testing of treated tissues are especially useful: in a rat Achilles transection model, (Aspenberg and Virchenko 2004) high concentration, thrombin-activated PRP was injected 6 h post-injury. Tendon harvested 1-3 weeks later had significantly greater force to failure, strength and stiffness, by a margin of approximately 30 % over control values. A subsequent study from these investigators used similar methods but examined the effects of thrombin, thrombin-activated PRP, resting PRP, and saline in comparison to untreated controls. The activated PRP gel produced a 44 % increase in force-to-failure at 14 days, as compared to 22 % for resting PRP, 24 % for thrombin alone, and 10 % for saline. Because thrombin is itself a known a mitogen, these results were important to clarify the results of the previous study, and also demonstrated a significant difference between activated and resting PRP in terms of tendon strength. The sole in vivo experimental study on equine tendon also employed mechanical testing outcomes: Bosch et al. (2010) created surgical lesions in the SDFT of both forelimbs and, at 7 days post-injury, treated 1 limb with resting, moderate concentration PRP and the other limb with saline. At 6 months, significant increases in cell number were observed in the PRP group, which translated into significant differences in collagen and GAG content as well. Most importantly, PRP-treated tendons were stronger by a margin of approximately 30 %, as indicated by both force to failure and elastic modulus. Ultrasonographic examination revealed significantly greater fiber alignment and neovascularization in the PRP-treated tendons (Bosch et al. 2011a; Bosch et al. 2011b). A study of Achilles transection in rabbits also found significantly increased vessel density after treatment with a PRP gel. Other findings included significantly increased immunohistochemical staining for IGF-1 expression within the tendon and also significantly increased force-to-failure for 4 weeks after injury (Lyras et al. 2010; Lyras et al. 2009a; Lyras et al. 2009b).

Bone

There are numerous, somewhat conflicting in vitro studies on the effects of PRP on osteogenic cells in culture. As for other cell types, a proliferative response to PRP

is commonly reported in osteoblast-like cells, (Mooren et al. 2010; Graziani et al. 2006; Celotti et al. 2006; Ferreira et al. 2005; Kanno et al. 2005) but in other studies PRP (resting or activated) has significantly inhibited proliferation relative to a 10 % FBS control (Slapnicka et al. 2008). Thrombin-activated PRP releasates have been shown to stimulate osteoclastic development by increasing RANKL expression, (Gruber et al. 2002; Weicht et al. 2007) but other authors have reported PRP inhibition of osteoclasia (Cenni et al. 2010). Recently, muscle satellite cells have been investigated as an alternative for bone formation. One study examined the osteoinductive effect of PRP (lysed by 1 freeze–thaw cycle) on these cells, in comparison to treatment with autologous serum or 10 % FBS. The authors observed significantly more cell proliferation, ALP production, and Alizarin red staining after in vivo implantation in the cells treated with PRP. Gene expression for Type I collagen, osteocalcin, and osteopontin was also enhanced by PRP treatment (Huang and Wang 2010).

The results of in vivo studies of PRP in bone formation are also contentious. The original clinical PRP study by Marx et al. (1998) preceded most of the experimental reports in the literature. This study demonstrated significantly improved bone formation in clinically-occurring, critical-sized mandibular defects in human patients, and therefore largely supersedes many of the studies with negative results of PRP in experimental models. Platelet concentration appears to be particularly important for bone formation, with no bone produced at low-intermediate concentrations or at very high platelet concentrations (Weibrich et al. 2004; Graziani et al. 2006). These findings may explain some of the inconsistency in experimental results, and there may also be significant species differences that account for the bone formation that occurs in people but is sometimes lacking in experimental animals (Plachokova et al. 2009). Activation method also seems to play an important role in whether bone formation occurs or not: one study demonstrated a negative impact of thrombin-activated human PRP on ectopic bone formation in athymic rats, whereas resting PRP performed significantly better than controls (Han et al. 2009). Another study (Kim et al. 2010) demonstrated a better osteogenic effect with low-dose thrombin and calcium activation of human PRP in calvarial defects of athymic rats, in comparison to high-dose thrombin activation as originally described by Marx (143U/mL + 14.3 mg/mL of $CaCl_2$) (Marx et al. 1998).

It is important to note that most of the in vivo studies on PRP effects on bone formation utilize xenogeneic (human) PRP to treat a critical-sized calvarial defect model in athymic rats. This model is probably useful to predict bone formation in the mandible and maxilla, but may or may not be relevant to osteogenesis in weight-bearing long bones. Many of these studies do not report specifics on the platelet concentration or activation status of the PRP, and most bone formation studies use PRP in combination with a variety of osteoconductive scaffold materials. (Please note: although activated PRP is considered to provide a scaffold for the formation of soft extracellular matrix, the term "scaffold" here refers to materials that contain the rigid, mineral components necessary for bone formation.) For these reasons, it is somewhat difficult to determine the true effect of PRP alone on bone healing. Because the focus of this discussion is on the potential

orthopedic applications of PRP, preference will be given here for any studies that are more pertinent to the load-bearing skeleton.

With regard to long bones, PRP has been tested in a few experimental long-bone fracture models. One study created similar defects in goats and treated them with scaffold + PRP (autologous, high concentration, activated) or scaffold alone: the inclusion of PRP resulted in a significant increase in new bone formation at 4, 8 and 16 weeks (Bi et al. 2010). Another study used high concentration, activated PRP in combination with cancellous bone graft to treat critical sized, unicortical defects in the tibiae of mini-pigs. The treatment group was compared to bone graft alone, with outcomes at 6 weeks. The area of new bone formation in the defect was significantly greater for PRP treated animals (i.e. new bone filled approximately 54 % of the original defect vs. 38 % in the control group) (Hakimi et al. 2010). These results are impressive and uncommon because autologous cancellous bone graft is considered the "gold standard" in terms of bone repair, as it provides all 3 properties necessary for new bone formation (osteoconduction, osteoinduction, and osteogenesis). In rabbits with a distal radial ostectomy, (Kasten et al. 2008) allogeneic PRP (pooled from 6 donors, high concentration, lysed by 1 freeze–thaw cycle) + scaffold increased new bone formation as compared to the scaffold alone. However, these PRP results were significantly inferior to those obtained with cancellous bone graft alone (i.e., the positive control) and mechanical stiffness was not improved by the addition of PRP into the repair. Nonetheless, the authors concluded that allogeneic PRP would be of benefit as an "off the shelf" adjunct to improve bone formation in conjunction with osteoconductive scaffold, thereby preventing the need for cancellous bone harvest from the patient. A study in rats (Gumieiro et al. 2010) used PRP (high concentration, $CaCl_2$-activated, allogeneic) alone to treat unicortical tibial defects created after irradiation of the bone. Fourteen to 84 days later, new bone formation was significantly greater in PRP-treated defects than in empty control defects. In another study in rabbits with unicortical defects in the femoral condyle, (Dallari et al. 2006) PRP (autologous, thrombin/$CaCl_2$-activated, high concentration) was used alone or in combination with BMSCs and freeze-dried allogeneic bone. The combination induced significantly greater filling of the defect: at 2 weeks, the PRP-alone group had 35–40 % healing, whereas the combination group was 95 % healed. At 12 weeks, the PRP-alone group had not progressed further, whereas BMSCs alone or freeze-dried bone alone had progressed significantly from 2 weeks but were also inferior to the combination treatment. With regard to osseointegration of implants used in either fracture repair or as periodontal prostheses, PRP has not shown a demonstrable advantage in terms of bone-implant contact (Garcia et al. 2010; Weibrich et al. 2004; Jensen et al. 2005, 2004).

In summary, the answer to the question, "How useful is PRP in osseous restoration?" depends on the control group to which it is being compared. By virtue of the osteoinductive properties of its growth factors, the addition of PRP improves new bone formation in comparison to either no treatment or a synthetic scaffold alone. If osteogenic cells are also added to a combination of PRP+scaffold, the triad of osteoconduction (scaffold), osteoinduction (PRP) and osteogenesis (cells)

is theoretically provided. This is reflected in some studies that show superior effects of combination therapy, but even these results are not uniformly obtained. In short, there may be a role for PRP as an adjunct to bone repair, but it does not appear to confer any advantage as a single agent. It may be of particular use in cases where cancellous bone harvest is not possible or is of insufficient volume for treatment of a large defect.

Articular Cartilage and Synovial Tissues

Research examining the effects of PRP on articular tissues has commenced only recently. In vitro studies thus far have reported only positive effects of PRP on chondrocytes or chondrocyte precursors, in terms of proliferation and increased matrix synthesis. In a study by Kruger et al. (2012) human cortico-spongious progenitor cells were cultured under the influence of very high concentration PRP that had been lysed by 1 freeze–thaw cycle. PRP induced a dramatic chemotactic effect on these cells (approximately 14x that of 10 % serum controls), as well as significant increases in immunohistochemical staining for Type II collagen and GAG. In another study on human chondrocytes, PRP lysate (high concentration, 2 freeze–thaw cycles) led to increased SOX9 and aggrecan gene expression as well as increases in Toluidine blue staining for GAG content (Spreafico et al. 2009) Van Buul et al. (2011) reported that diminished aggrecan and Type II collagen gene expression by IL-1β-conditioned human chondrocytes could be restored to normal levels by PRCR (high concentration, $CaCl_2$-activated). The mechanism for this effect was determined to be PRP-mediated inhibition of NF$\kappa\beta$ signaling. Another study also confirmed this mechanism of action in high concentration, thrombin/$CaCl_2$-activated PRP on human chondrocytes, and determined that NF$\kappa\beta$ inhibition was specifically mediated by hepatocyte growth factor (HGF)-induction of the protein, IκBα. Notably, this group evaluated resting PRP as well as activated and did not observe the anti-NF$\kappa\beta$ effect after exposure to resting PRP (Bendinelli et al. 2010). A third group also reported that PRP restored collagen and proteoglycan synthesis by chondrocytes after IL-1β/TNFα insult (Wu et al. 2011). In porcine chondrocytes, PRP (high concentration, thrombin/Ca $CaCl_2$-activated) stimulated significant increases in DNA content, proteoglycan synthesis and total collagen synthesis (Akeda et al. 2006).

The effects of PRP on synovial fibroblasts and meniscal chondrocytes have also been examined. Anitua et al. determined that normal synovial fibroblasts produced significantly more HA after exposure to PRCR (moderate concentration, $CaCl_2$-activated), even in the face of IL-1β insult. In a subsequent study using synovial fibroblasts from osteoarthritic patients, HA and HGF production increased after PRCR treatment, but only HA synthesis was restored after IL-1β exposure and PRCR did not diminish the accompanying increases in MMPs (Anitua et al. 2007). HA production and cell proliferation were dose-dependent in terms of increasing platelet concentration (Anitua et al. 2009). Synovial fibroblasts migrated best when

exposed to a combination of PRCR and HA (Anitua et al. 2011). Regarding PRP effects on the meniscus, meniscal cells of rabbits were cultured and exposed to PRP (high concentration, 1 freeze–thaw) in combination with a hydrogel Ishida et al. (2007). After 8 days of culture, the cells treated with PRP had significant increases in proliferation, GAG production and small proteoglycan expression (which is characteristic of meniscal chondrocytes) in comparison to hydrogel+PPP or hydrogel alone. When these constructs were implanted in vivo, proteoglycan staining and chondrocyte number were greatest in the PRP group.

A few experimental animal studies have been reported on the impact of PRP in osteoarthritis or repair of osteochondral lesions. In a cruciate-transection osteoarthritis model in rabbits, very high concentration, activated PRP was mixed with gelatin microspheres and injected intra-articularly 4 and 7 weeks after injury. At 10 weeks post-injury, gross and histologic scores were significantly improved in the PRP-microsphere group in comparison to untreated controls or PRP alone. The authors concluded that PRP dramatically attenuated the progression of early OA when used with a vehicle such as gelatin microspheres (Saito et al. 2009). Another rabbit study used PRP in combination with a polyglycolic acid scaffold to treat large (5 mm diameter), full-thickness osteochondral defects in the stifle. The PRP was activated and of high platelet concentration. At 4 and 12 weeks, the results indicated significantly better gross and histologic scores and significantly more subchondral bone formation in the PRP treated group, as compared to untreated controls or those treated with scaffold alone (Sun et al. 2010). In another study in sheep, Kon et al. (2010) created 7 mm defects in the femoral condyles and treated them with a collagen-hydroxyapatite scaffold with or without PRP (high concentration, $CaCl_2$-activated). Significant improvements were reported in the scaffold-only group, whereas the inclusion of PRP had a detrimental effect on gross and histologic scores at 6 months. However, another study which created very similar lesions in sheep reported significant improvements in gross appearance, histologic scores, and also cartilage stiffness after microfracture followed by activated PRP + additional fibrin gel in the defect. The other treatment groups underwent microfracture alone or microfracture plus liquid PRP injection; lesions treated with microfracture + liquid PRP had a better histologic appearance than those that underwent microfracture alone (Milano et al. 2010). In a follow-up study, the same authors evaluated whether intra-articular injections of PRP could augment the healing of the same lesions treated with microfracture alone. After surgery, they performed 5 weekly injections of PRP (high concentration) into the stifle. Gross and histologic scores and cartilage stiffness were significantly better in the PRP treatment group at 3, 6, and 12 months (Milano et al. 2012).

Literature Review: Conclusions

With regard to PRP use for tendons, there is good experimental and clinical evidence to support the use of PRP in the healing of acute lesions. In vitro studies suggest a definite increase in tenocyte number and vascularity after PRP treatment,

but evidence for improved matrix synthesis or correct collagen alignment is lacking. Despite this, the few studies that have performed mechanical testing have demonstrated increased tendon strength after PRP treatment. There are no experimental studies examining the effects of PRP on chronic or degenerative models in tendon. This gap in the literature is important because degenerative tendinopathy is encountered at least as often as acute tendon injury.

The evidence for PRP use as an adjunct to bone formation is not as clear. With regard to long-bone healing in particular, PRP improves the performance of osteoconductive scaffolds and may therefore be useful for large bone defects or when cancellous graft is not available. PRP in the absence of a scaffold is probably of minimal use in acute bone defects, including fracture repair or fusion procedures. However, percutaneously applied PRP may be useful in cases where fibrous callus (i.e. native scaffold) is already present, such as in cases undergoing distraction osteogenesis or possibly in the treatment of delayed or non-union fractures. Platelet concentration and activation methods appear to be of greater significance in bone than in other tissues, since very high concentrations of platelets and/or thrombin are reported to inhibit osteogenesis.

PRP use in joints is in its infancy, but the literature thus far is quite favorable. Chondrocytes appear to respond well to PRP exposure in terms of proliferation and most importantly, matrix production. In vivo, PRP is likely to be of benefit in early osteoarthritis but may require a vehicle for sustained release, or could be administered as repeated injections. PRP appears to augment osteochondral repair but results may be influenced by unfavorable interactions with certain types of implanted scaffolds. PRP also shows promise for meniscal repair.

In summary, there is good experimental evidence to support PRP use in orthopedic applications, particularly in tendon/ligament injuries and in arthropathies. The current trend toward prospective, randomized, controlled clinical studies will likely continue to substantiate the use of PRP as a therapeutic agent in orthopedic and sports medicine. However, because of the autologous nature of the product, standardized results may not be obtained in all patients. Experimental studies are still necessary to optimize each of the variables involved in PRP preparation and use, so that the best PRP product possible can be produced from and delivered to each individual patient.

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