

# ADVANCES IN CONDENSED MATTER AND MATERIALS RESEARCH

VOLUME

8

*Hans Geelvinck  
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# CONTENTS

<b>Preface</b>		<b>vii</b>
<b>Chapter 1</b>	Development, Properties, Applications and Machinability of Superalloy Inconel <i>Chandra Nath, Kapil Krishna Baidya and Mustafizur Rahman</i>	<b>1</b>
<b>Chapter 2</b>	Zirconia and Allumina Bioceramic Biocompatibility <i>G. Maccauro, P. Rossi Iommetti, P.F. Manicone and L. Raffaelli</i>	<b>33</b>
<b>Chapter 3</b>	Platelet-Rich Plasma: Its Biological Properties and Applications <i>Hongshuai Li and Bingyun Li</i>	<b>53</b>
<b>Chapter 4</b>	Biocompatibility Assessment of Gelatin Carriers Used in Corneal Endothelial Cell Delivery <i>Jui-Yang Lai</i>	<b>75</b>
<b>Chapter 5</b>	The Thermoelectric Power, an Alternative Non-Destructive Method for the Characterization of Inconel 718 <i>E. López Cuéllar , L. López Pavón , U. Ortiz Méndez , E. Reyes Melo, A. Martínez de la Cruz, M. Morin and H. Guajardo Martínez</i>	<b>97</b>
<b>Chapter 6</b>	Electron Backscatter Diffraction (EBSD) Analysis of the Microstructure of Ni <sub>2</sub> MnGa Shape Memory Alloys <i>A. Koblishka-Veneva and M.R. Koblishka</i>	<b>115</b>
<b>Chapter 7</b>	Capacitor Discharge Welded Bars of Inconel 718 Superalloy Under Fatigue <i>S. Chiozzi, V. Dattoma, and F. Panella</i>	<b>133</b>
<b>Chapter 8</b>	Biointegration of Novel Enabling Technologies <i>W. John Kao</i>	<b>147</b>

<b>Chapter 9</b>	Aeronautical Superalloys Joined by Projection CDW Process <i>S. Chiozzi, V. Dattoma and F. Panella</i>	<b>153</b>
<b>Index</b>		<b>163</b>

*Chapter 3*

## **PLATELET-RICH PLASMA: ITS BIOLOGICAL PROPERTIES AND APPLICATIONS**

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### **ABSTRACT**

Platelet-rich plasma (PRP) is an emerging biomaterial used to improve healing of soft and hard tissues; its development has led to breakthroughs in stimulation and acceleration of tissue healing. PRP is a concentration of platelets, plasma, and leukocytes. Upon activation, PRP releases more than 30 growth factors and other biologically active proteins. The released growth factors, in turn, set the stage for tissue healing which includes cellular chemotaxis, proliferation, and differentiation; removal of tissue debris; angiogenesis; laying down of extracellular matrix; and regeneration of appropriate types of tissues. PRP has also been combined with synthetic biomaterials to enhance their integration into surrounding tissues. This chapter provides an overview of PRP properties, preparation, and potential applications and limitations. The first part of this chapter presents the fundamental biological properties of PRP, such as physiology, structure and biocompatibility. The second part describes the methods for preparation of PRP. The third part reviews the broad applications of PRP. The last part discusses possible limitations of the use of PRP and its future development. The potential application of nanotechnology to encapsulate PRP to obtain sustained release and to protect its biological activity will be briefly discussed.

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## 1. INTRODUCTION

Platelet-rich plasma (PRP) is also known as platelet-enriched plasma, platelet-rich concentrate, autogenous platelet gel, platelet leukocyte-rich plasma, or platelet releasate. In general, PRP is a volume of autologous plasma that has a platelet concentration well above that of normal blood (e.g. 1,000,000 platelets/ $\mu$ l vs. 150,000-350,000 platelets/ $\mu$ l), and it also contains clotting factors and secretory proteins. PRP with 1,000,000 platelets/ $\mu$ l has been shown to be effective in enhancing bone and soft tissue healing. This platelet concentration in a 5-ml volume is currently the working definition of PRP.

Recently, PRP has attracted attention as an emerging biomaterial for improving healing of soft and hard tissues. The use of PRP has led to breakthroughs in stimulation and acceleration of tissue healing[1-9]. More studies testing new applications, for instance, preventing bone infection and treating osteomyelitis, are under investigation[10, 11]. The following sections will provide readers a review on the physiology, preparation, and general applications, as well as potential limitations of PRP.

## 2. PHYSIOLOGY

PRP is a mixture of platelets, leukocytes, and plasma; platelets play a key role in a variety of PRP applications. In this section, we will focus on the biocompatibility and structure of PRP, the biology of platelets, and the platelet-expressed growth factors and cytokines.

### 2.1. Biocompatibility and Structure of Platelet-Rich Plasma (PRP)

Autogenous PRP is inherently biocompatible, safe and free from risk of transmissible diseases such as HIV, hepatitis, West Nile fever, and Creutzfeldt-Jakob disease. PRP, therefore, is biologically compatible in clinical scenarios.

The structure and contents of PRP were observed using Transmission Electron Microscopy (TEM) imaging[3]. Figure 1 shows images taken from PRP-gel samples that were prepared within one minute after mixing PRP with thrombin (platelets were not activated). Concentrated and aggregated cells as well as fibrin strands were visualized, and there were plenty of platelets, surrounded by granulocytic neutrophils, monocytes, and lymphocytes. After mixing PRP with thrombin for a relatively longer time, platelets are activated. Cell structures revealed that, upon platelet activation, more than 80% of alpha ( $\alpha$ ) granules were empty, indicating the release of platelet growth factors to the extracellular milieu (Figure 2). The leukocytic cellular structures were retained, the cell membranes were intact, and minor signs of leukocytic pseudopodium development were observed.

### 2.2. Platelet Origin, Morphology, and Content

Platelets are one of the key functional components of PRP. Platelets are cytoplasmic fragments of megakaryocytes, a type of white blood cells, and are formed in bone marrow.



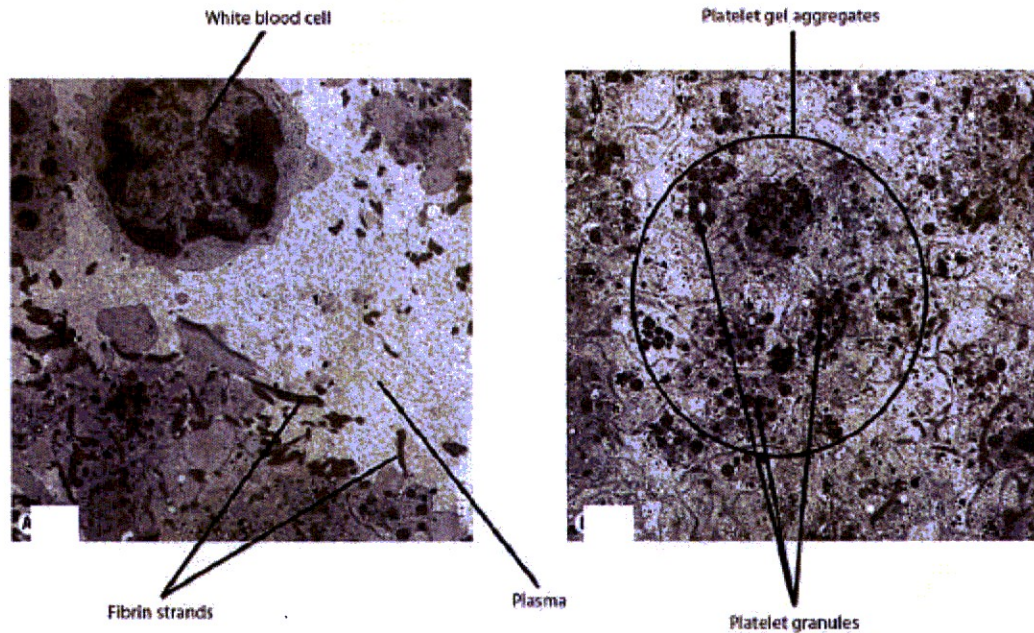


Figure 1. (A) TEM image of a mixture of platelets, neutrophilic granulocytes and fibrin strands present in PRP-Gel. (B) Close-up of platelet aggregates with platelet granules inside the platelet structure. Magnification  $\times 7,000$ . Reprinted from Ref. 3, Everts, P.A., et al. Reviewing the structural features of autologous platelet-leukocyte gel and suggestions for use in surgery. *Eur Surg Res*, 39(4), 199-207, Copyright(2007), with permission from S. Karger AG, Basel.

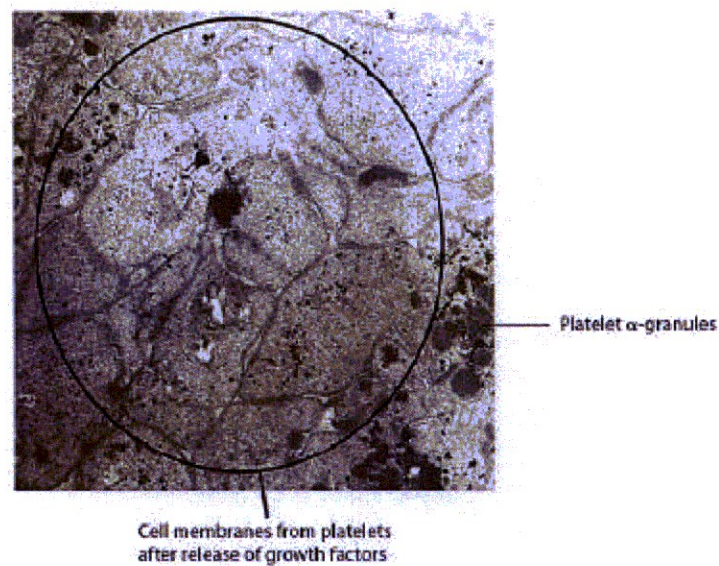


Figure 2. TEM image showing platelet cell aggregates in PRP-Gel after clot retraction has occurred and the platelet granules are emptied in the plasma. Only a few platelets have filled granules. Reprinted from Ref. 3, Everts, P.A., et al. Reviewing the structural features of autologous platelet-leukocyte gel and suggestions for use in surgery. *Eur Surg Res*, 39(4), 199-207, Copyright(2007), with permission from S. Karger AG, Basel.



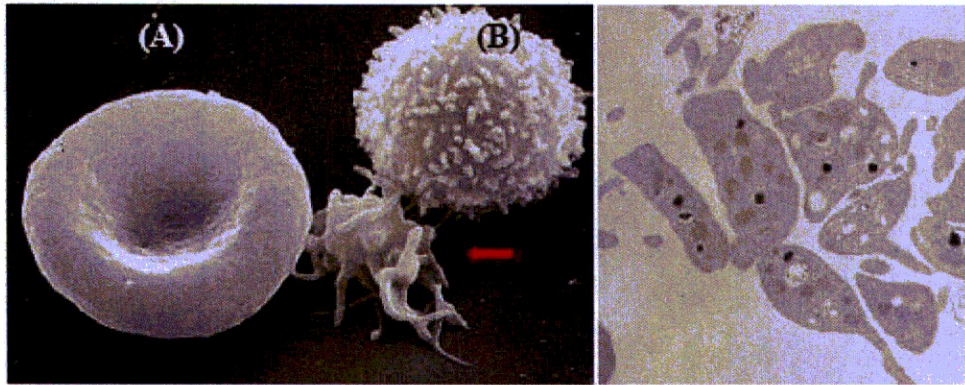


Figure 3. (A) SEM and (B) TEM pictures of platelets. ( ) refers to platelet.

They are the smallest of blood cells, round or oval in shape, and approximately 2-4  $\mu\text{m}$  in diameter (Figure 3)[12-15]. Their cell membrane is tri-laminar with a glycoprotein receptor surface and partially interspersed with a penetrating bi-layer of phospholipids and cholesterol. Platelets lack nuclei and, as a result, young platelets can perform only limited translations using residual megakaryocyte mRNA templates[16-18]. Platelets contain organelles and structures such as mitochondria and microtubules, and they also have three major types of cytoplasmic granules (alpha, delta, and lambda, Figure 4). The  $\alpha$  granules are about 200-500 nm in diameter, and there are approximately 50-80 of them in each platelet. The contents and corresponding biological activities of  $\alpha$ -granules are described in Table 1. The  $\alpha$ -granules contain more than 30 bioactive proteins, and these proteins may be involved in hemostatic functions such as adhesion (e.g., fibrinogen, thrombospondin, vitronectin, laminin, and Von Willebrand factor), modulation of coagulation (e.g., plasminogen,  $\alpha$ 2-plasmin inhibitor, and thrombasthenin), and endothelial cell repair (e.g., platelet-derived growth factor or PDGF, permeability factor, and transforming growth factors  $\alpha$  and  $\beta$  or TGF- $\alpha$  and TGF- $\beta$ )[15, 19]. The  $\alpha$ -granules in mammalian animals also likely contain an arsenal of microbicidal proteins[19-23]. The delta ( $\delta$ ) granules store mediators of vascular tone including serotonin, adenosine nucleotide diphosphate (ADP), eicosanoids, thromboxane A2 (TXA2), calcium, and phosphate[15, 19]. The lambda ( $\lambda$ ) granules contain enzymes that may play a key role in mediating thrombus dissolution[15, 19]. The distinct platelet granules are subject to discrete or synchronous release, depending on agonist specificity and potency. For example, low levels of thrombin or ADP may induce  $\alpha$  and  $\delta$  degranulation, while  $\lambda$  granules may not be secreted until the agonists are present in relatively high concentrations. From this perspective, platelets may be viewed as vehicles that respond to agonists and ligands which are expressed at sites of endovascular damage or microbial colonization; they can release a variety of bioactive molecules.

### 2.3. Platelet-Expressed Growth Factors and Cytokines

A variety of growth factors and cytokines have been identified in platelets; those that have been quantified in the use of PRP for tissue regeneration are discussed below. A full description of the growth factors involved in the regulation of bone remodeling is not within the scope of this chapter.



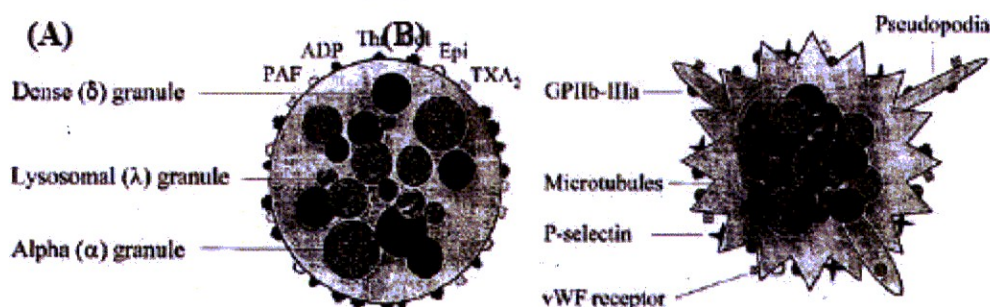


Figure 4. Ultra-structure of platelets. (A) Unactivated platelet, illustrating granule variety and composition. Also shown are predominant constitutive receptors for platelet agonists, including platelet activating factor (PAF); ADP; thrombin (Thr); collagen (Col); epinephrine (Epi); and TXA<sub>2</sub>. (B) Activated platelet, illustrating changes in platelet shape, pseudopod formation, granule centralization, and expression of inducible ligands such as P-selectin (GMP-140; ligand for neutrophil adhesion) and receptors such as GPIIb-IIIa (fibrinogen receptor) and von Willebrand factor (vWF) receptor. Reprinted from Ref. 19, Yeaman, M.R. The role of platelets in antimicrobial host defense. Clin Infect Dis, 25(5), 951-70, Copyright(1997), with permission from The University of Chicago Press.

### 2.3.1. Platelet-Derived Growth Factors (Pdgfs)

PDGF is a dimeric disulfide linked polypeptide composed of two subunits, A and B. It exists in three different combinations: PDGF-AA, PDGF-BB, and PDGF-AB. The A and B chains share approximately 56% sequence homology[24]. PDGF has been isolated from blood cells and is considered a potent mitogen for most of the healing cells (including osteoblasts and fibroblasts) of mesenchymal origin; it is important in the early phases of wound and bone repair[25, 26]. PDGF stimulates bone cell replication and bone collagen synthesis; PDGF may stimulate the proliferation of progenitor cells, such as mesenchymal stem cells and osteo-progenitor cells, which are part of the connective tissue-bone healing cellular composite; PDGF may stimulate bone resorption by increasing the number of osteoclasts, which can lead to faster bone remodeling; PDGF may also promote the replication of endothelial cells, causing budding of new capillaries into the wound[27]. Furthermore, macrophages can be activated by PDGF, resulting in debridement of damaged tissue. The activated macrophage may then trigger a second source of growth factors released from host tissues that continue the process of repair and bone regeneration.

*In vivo*, PDGF, in concentrations of 20-100 ng, has been shown to increase ectopic bone formation and alkaline phosphatase activity[28]. PDGF, applied locally, has also stimulated bone healing in rabbit osteotomies[29] as well as in rabbit calvaria defects in combination with barrier membranes. More than 48 PDGFs have been expressed by different cell types during different stages of normal fracture healing[30], and the production of PDGF may be regulated by other growth factors such as TGF- $\beta$ [27, 31, 32].

### 2.3.2. Transforming Growth Factor-B (TGF-B)

TGF- $\beta$  is synthesized in many tissues; bone and platelets are the primary sources for this cytokine[33]. TGF- $\beta$  is a polypeptide that stimulates the proliferation of osteoblast precursor cells and it has a direct stimulatory effect on bone collagen synthesis. TGF- $\beta$  modulates bone matrix synthesis via various mechanisms, including (1) increasing the number of cells that are



capable of expressing osteoblast genotypes, (2) directly acting on the differentiated osteoblasts by up-regulating their function, (3) decreasing bone resorption by inducing apoptosis of osteoclasts, and (4) activating fibroblasts to form collagen, endothelial cells for angiogenesis, chondroprogenitor cells for cartilage, and mesenchymal cells to increase the population of wound healing cells[34-36].

**Table 1. Platelet  $\alpha$ -granule contents and their functional categories. Modified and reprinted from Eduardo A, et al. Autologous platelets as a source of proteins for healing and tissue regulations. *Thromb Haemost*, 91, 4-15, Copyright(2004), with permission from Schattauer GmbH, Germany**

Categories	Terms	Biological functions
Clotting factors	Factor $\delta/\delta_a$ , Factor $\phi$ , multimerin, gas6, protein S, high-molecular weight kininogen, antithrombin, tissue factor pathway inhibitor (TFPI)	Released by thrombin activation, regulate homeostasis, angiogenesis
Adhesive proteins	VWF, Fg, Fn, Vn, TSP-1, Laminin-8	Cell contact interactions, clotting, extracellular matrix composition
Fibrinolytic factors and associated proteins	Plasminogen, PAI-I, u-PA, Osteonectin, $\alpha_2$ -antiplasmin, histidine-rich glycoprotein, TAFI, $\alpha_2$ -macroglobulin	Plasmin production and vascular modeling
Proteases and anti-proteases	Tissue inhibitor of metalloprotease-4 (TIMP-4), Metalloprotease-4, platelet inhibitor of FIX, protease nexin-2, C1 inhibitor, $\alpha_1$ -antitrypsin	Angiogenesis, vascular modeling, regulation of coagulation, regulation of cellular behavior
Growth factors, cytokines and chemokines	PDGF, TGF- $\beta$ , EGF, IGF-1, VEGF, bFGF and FGF-2, hepatocyte growth factor, CTGF, BMP-2, -4 and -6 RANTES, IL-8, MIP-1 $\alpha$ , growth-regulated oncogene- $\alpha$ , ENA-78, MCP-3, angiopoietin-1, IL-1 $\beta$ , IGF BP-3, neutrophil chemotactive protein	Chemotaxis, cell proliferation and differentiation, angiogenesis
Anti-microbial proteins	Microbicidal peptides (e.g., PMPs and tPMPs), thrombocidins, phospholipase A2, Fc $\gamma$ R $\alpha$ (for antibody Fc), Fc $\epsilon$ (for IgE antidody Fc), Factor D and H (for CR3)	Direct antimicrobial activity, antibody-dependent cell cytotoxicity, modulation of complement activation
Membrane glycoproteins	$\alpha\alpha\beta_3$ , $\alpha\nu\beta_3$ , GPIb, PECAM-1, most plasma membrane constituents, receptors for primary agonists, CD40L, tissue factor, P-selectin	Platelet aggregation and adhesion, endocytosis of proteins, inflammation, thrombin generation, platelet-leukocyte interactions
Basic proteins and others	PF4, $\beta$ -thromboglobulin, platelet basic protein, connective-tissue-activating peptide $\beta$ , neutrophil-activating-peptide-2, endostatins.	Regulation of angiogenesis, vascular modeling, cellular interactions



*In vivo*, TGF- $\beta$  has led to bone formation in a rabbit cranofical onlay model[37], and a single application of rhTGF- $\beta$  has resulted in dose-dependent bone formation in a 12-mm diameter calvarial defect[38, 39]. Exogenous administration of TGF- $\beta$  in experimental fractures has also led to an increase in callus size and some improvements in biomechanical properties[40]. The bone-forming properties of TGF- $\beta$  are maximized when combined with demineralized bone matrix[41].

### **2.3.3. Other Associated Platelet Proteins**

Among platelet proteins, fibronectin and vitronectin are cell adhesion molecules that help cells move around during the proliferation and migration phases in bone and cartilage healing. Fibrin, another platelet protein, contributes to cell mobility in the wound by serving as a scaffold for cell migration and platelet entrapment. In PRP, the concentration of these proteins is high and may accelerate bone and soft tissue healing.

## **2.4. Platelet functions**

The two major functional roles of platelets are hemostasis and initiation of wound healing, a somewhat arbitrary division because hemostasis can be considered to be the first stage of healing. Nevertheless, for convenience, the physiological roles of platelets are described as two separate parts.

### **2.4.1. Hemostasis**

When tissue damage occurs, platelets aggregate at the injury site and rapidly change from a rounded shape to one that includes large sticky protuberances or pseudopodia; this process is called platelet activation. The platelets adhere to elements (such as collagen, basement membranes of capillaries, and subendothelial microfibrils) that have been exposed due to blood vessel damage[42]. Platelet adhesion further leads to ADP release which causes more platelet aggregation. Other factors, such as thrombin and adrenalin, may also mediate platelet aggregation[42]. When the vascular defect is small, platelet aggregates are sufficient to stop blood loss caused by injury. When the vascular defect is large, however, formation of blood clot is necessary in order to stop the bleeding. In general, blood clot formation is initiated by two pathways: intrinsic and extrinsic[42]. In both mechanisms, there is a cascaded reaction sequence in which inactive factors become activated and catalyze the formation of products from their precursors, which in turn activate more factors until the final products are formed. The two pathways share many steps of the sequence, and the intrinsic pathway includes some additional initial steps. The intrinsic pathway is initiated by damage, or alteration, to blood and is independent of contact with damaged tissue, whereas the extrinsic pathway is initiated by exposure to factors derived from damaged tissues[42].

Figure 5 shows a schematic diagram that illustrates the pathways by which platelets affect clot formation. Factor V secreted by granules of activated platelets binds to activated factor X to produce prothrombin activator which, in the presence of calcium, catalyzes the formation of thrombin from prothrombin[42]. Thrombin then catalyzes fibrinogen into fibrin monomers which, in the presence of calcium and fibrin stabilizing factor (factor XIII), form fibrin threads. Thrombin can also bind to platelet surface receptors and activate serum factor



VIII, which complexes with factor IX on the platelet surface. Activated factors VIII and IX participate in the activation of factor X via the intrinsic pathway.

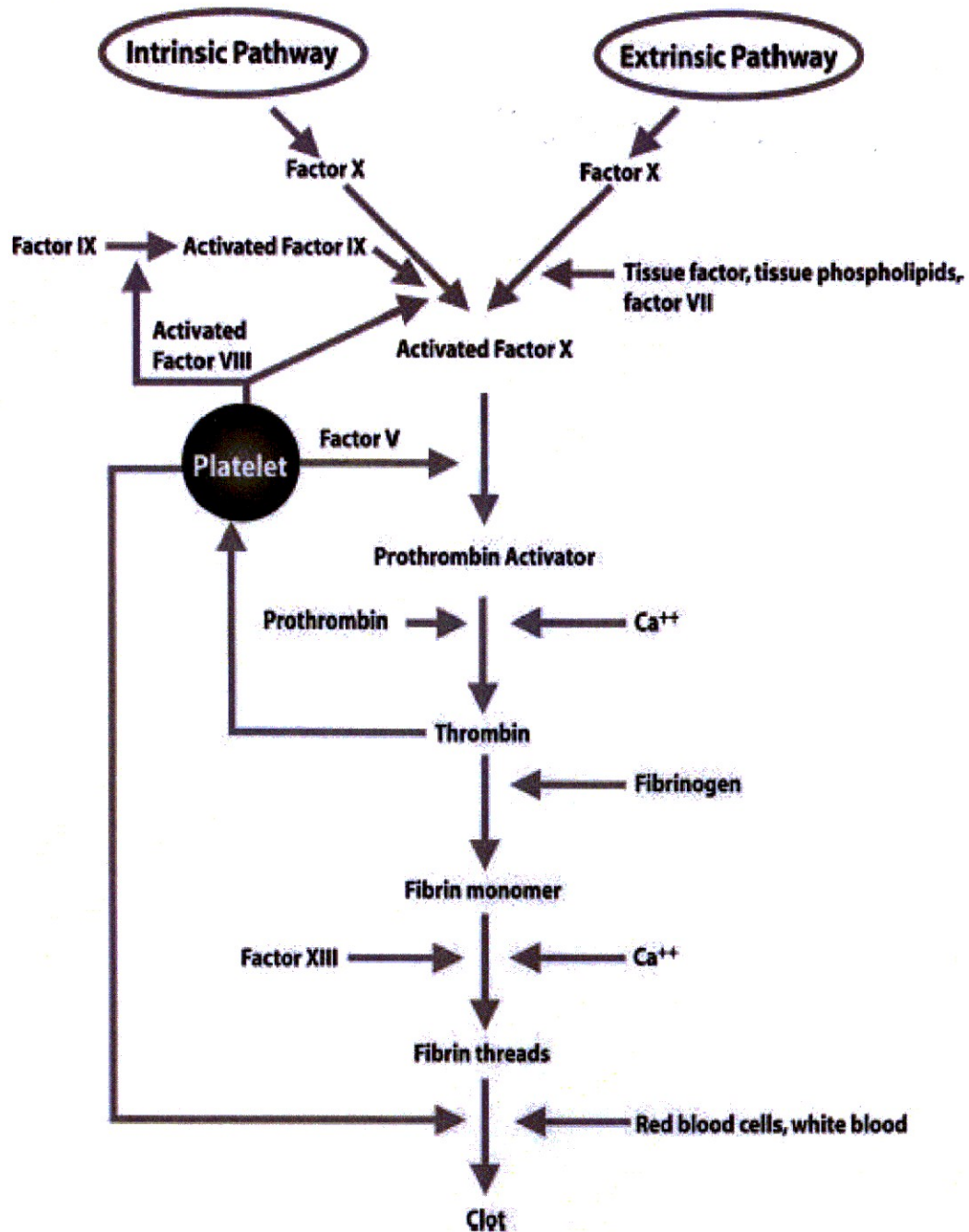


Figure 5. Schematic diagram describing the roles of platelets in clot formation. Reprinted from Ref. 6, Pietrzak, W.S. and Eppley, B.L. Platelet rich plasma: biology and new technology. J Craniofac Surg, 16(6), 1043-54, Copyright(2005), with permission from Wolters Kluwer Health.

The blood clot consists of a fibrin mesh containing platelet aggregates, as well as entrapped red and white blood cells. Contraction of the platelet actin myosin fibers is responsible for retraction of the clot, and this further closes the vessel[42]. During clot retraction, platelet releasates are expressed. Thromboxane and serotonin, released from platelet aggregates, cause vasoconstriction, which also aids in hemostasis.

#### **2.4.2. Wound Healing**

In PRP, the  $\alpha$  granules of platelets contain numerous proteins that provide a powerful influence on wound healing, including PDGF, TGF- $\beta$ , platelet factor 4 (PF4), interleukin-1 (IL-1), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet derived endothelial growth factor (PDEGF), epithelial cell growth factor (ECGF), IGF, osteocalcin (Oc), osteonectin (On), fibrinogen (Fg), vitronectin (Vn), fibronectin (Fn), and thrombospondin-1 (TSP-1)[42-46]. These proteins are growth factors, cytokines, or chemokines. In this chapter, these proteins are broadly referred to as secretory proteins.

Upon platelet activation, the  $\alpha$  granules start to fuse to the platelet cell membrane where at least some of the secretory proteins (e.g., PDGF and TGF- $\beta$ ) are transformed to a bioactive state by the addition of histones and carbohydrate side chains[44, 45]. The proteins are then secreted and bind to the transmembrane receptors of cells like mesenchymal stem cells, osteoblasts, fibroblasts, endothelial cells, and epidermal cells. These agonist-bound transmembrane receptors activate intracellular signal proteins and lead to the expression of gene sequences that direct cellular proliferation, matrix formation, osteoid production, and collagen synthesis, among other things[44].

Upon clot formation, the platelets secrete a variety of secretory proteins within 10 minutes, and more than 95% of the pre-synthesized growth factors are secreted within one hour[44]. After this initial burst release of proteins, the platelets synthesize and secrete additional proteins for the balance of their life span (5-10 days)[44, 47, 48]; macrophages subsequently assume the responsibility for wound healing regulation. Macrophages arrive at the wound site through vascular ingrowth stimulated by platelets and secrete a variety of cytokines. Therefore, it is believed that the platelets at the wound repair site set the pace for wound repair[44, 48].

The numerous proteins secreted by activated platelets influence many aspects of healing; some of the proteins are absent in chronic and nonhealing wounds. Table 1 provides a brief introduction to the proteins and substances that are secreted by platelets and indicate which may participate in wound healing. For example, PDGF is chemotactic for macrophages. A combination of PDGF, TGF- $\beta$ , and IGF are chemotaxis and mitogenesis of stem cells and osteoblasts, angiogenesis for capillary ingrowth, bone matrix formation, and collagen synthesis. TGF- $\beta$  and PDGF may also assist in bone mineralization. The adhesive proteins Fg, Fn, Vn, and TSP-1, as a group, participate in thrombus formation, and some also have mitogenic characteristics. It was previously believed that platelets did not contain bone morphogenetic proteins (BMPs); however, BMP-2 and BMP-4 were recently identified within platelet lysates and may contribute to bone formation and repair[49].



### 3. PRP PREPARATION

PRP with 1,000,000 platelets/ $\mu$ l in a 5ml volume of plasma is the current working definition of PRP. However, platelet concentrations from 2-fold to 8.5-fold compared to normal blood have been reported[44, 50-53], and the relationship of clinical benefit to platelet concentration vs. total number of platelets in PRP await further investigation[54]. To prepare PRP, many protocols have been developed, and, in general, can be divided into two categories: (i) complex techniques using hematology cell separators, such as CATS (Fresenius, Wilmington, DE), Sequestra (Medtronic, Minneapolis, MN), Haemonetics Cell Saver 5 (Haemonetics Corp., Braintree, MA), and others, generally operate on a full unit of blood[52, 54], and (ii) simplified techniques utilizing ready-to-use commercially available separation kits or office setting 2-step centrifugation. Such systems include the GPS (Cell Factor Technologies, Inc., Warsaw, IN), the PCCS (Implant Innovations, Inc., Palm Beach Gardens, FL), the Symphony  $\alpha$  (Depuy, Warsaw, IN), the SmartPRP (Harvest Technologies Corp., Norwell, MA), and the Magellan (Medtronic, Minneapolis, MN)[52, 55-57]. The common procedures could be described as follows:

- 1) Draw venous blood and add anticoagulant(s) to avoid platelet activation and degranulation.
- 2) Carry out a first centrifugation to separate blood into three distinct layers (Figure 6):  
(i) the bottom layer, ~55% of the total blood volume, constitutes mainly corpuscles;  
(ii) the top layer (sometimes called platelet-poor plasma or PPP), ~40% of the total blood volume, is mainly made up of circulating plasmatic molecules (in particular, fibrinogen) and is low in platelets; and  
(iii) the intermediate layer, ~5% of the total blood volume, is the concentrated platelets, which presents a characteristic buffy aspect called the "buffy coat."
- 3) Using a sterile syringe, transfer the top and intermediate layers, along with some blood corpuscles, to a second centrifugation tube.
- 4) Conduct a second centrifugation, longer and/or faster than the first centrifugation, to concentrate platelets at the bottom of the tube and to once again obtain three distinct layers (Figure 6): some residual red blood corpuscles at the bottom; acellular plasma (PPP), ~80% of total volume, at the top; and a buffy layer in the middle.
- 5) Aspirate PPP using a sterile syringe. The majority of the PPP is discarded; only a small volume is kept to suspend the concentrated platelets. The tube is then gently shaken to obtain ready-to-use PRP which can be stored at room temperature for a few hours[58]. PRP can be obtained in gel or membrane form, depending on the syringe nozzle used. The small number of red blood corpuscles trapped at the bottom of the tube is part of the PRP; this gives the final PRP a rosy color.
- 6) At the time of application, mix PRP with thrombin and calcium chloride using a mixing syringe; gelling of platelet concentrates occurs very quickly. The quick gelling is related to the concentrated fibrinogen in PRP since the polymerization of fibrinogen forms a fibrin matrix with hemostatic and adhesive properties.

Variables including the type of anticoagulants used during blood collection, centrifugation forces applied, duration of centrifugation, and gel preparation, may affect



platelet yields and growth factor levels in the final PRP. The following centrifugation parameters may be used as a reference: a first spin of 300 g for 10 minutes at a constant temperature of 22°C and on a rotor brake off; followed by a second spin of 5,000 g for 5 minutes within the same parameters of temperature and braking curve[59].

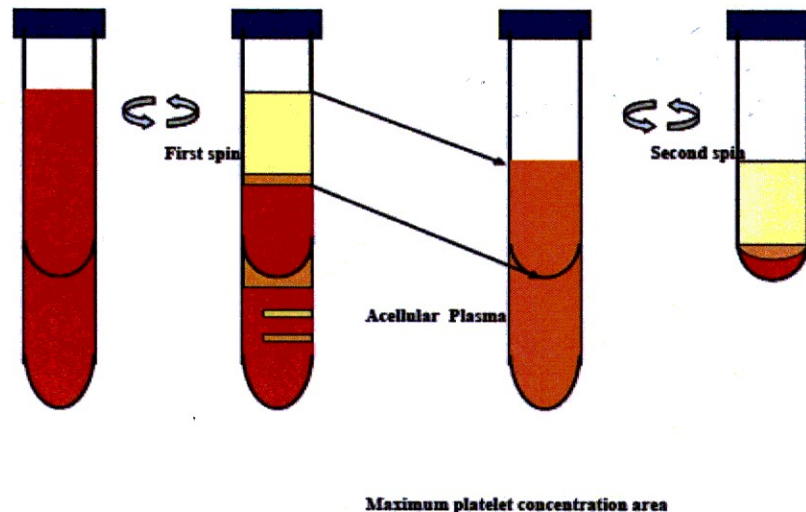


Figure 6. Technological concept of PRP processing.

## 4. APPLICATIONS OF PRP

One of the first clinical applications for PRP was in oral and maxillofacial surgical literature, where autologous fibrin adhesive was added to cancellous bone during mandibular continuity reconstruction. The study, published in 1994, revealed earlier radiographic bone consolidation (4 weeks vs. 8 weeks) which was attributed to enhanced osteoconduction by concentrated platelet formulation[60]. Another early application of PRP was in the realm of hemostatic control, which is important for any surgical procedure. Autologous PRP was sprayed on a partial thickness skin wound model and, compared to placebo controls, reduced bleeding by 70% after 5 minutes[61].

Today, PRP is widely used for clinical applications in a variety of orthopaedic surgeries, periodontal and oral surgeries, maxillofacial surgeries, plastic surgeries, heart bypass surgeries, and treatment of chronic skin and soft-tissue ulcers[5, 60, 62-65]. PRP has shown beneficial effects on bone and soft-tissue restoration and wound healing and can decrease postoperative pain and blood loss[5]. PRP has been increasingly used in treating pathologic conditions of bone, ligament, tendon, and cartilage. Hence, we will review its application in orthopaedics in detail.

### 4.1. Bone

There are several basic science and clinical studies examining the role of PRP in bone healing[65-67]. The bone healing process can be accelerated by initiating the cascade of events early in the cycle with the use of PRP[44, 50]. Treatment of human mesenchymal stem



cells in an osteo-conductive environment with PRP enhanced bone formation by modulating cellular pathways. Initiation of bone regeneration starts with the release of PDGF and TGF- $\beta$  from the degranulation of platelets in PRP. PDGF stimulates mitogenesis of marrow cells and endosteal osteoblasts. PDGF also initiates angiogenesis by inducing capillary budding into the surgical site through endothelial cell mitosis. TGF- $\beta$  activates fibroblasts, induces pre-osteoblasts to divide and increase in number, and triggers differentiation of pre-osteoblasts to mature osteoblasts. TGF- $\beta$  also induces osteoblasts to produce bone matrix. As these cellular activities occur, growth factors including PDGF and TGF- $\beta$  from PRP can rapidly increase the numbers of bone cells and promote their activities during the time of injury and/or surgery. When the platelets come to the end of their life cycle, the growth factors have already activated related chemotaxis, and macrophage-derived growth and angiogenic factors subsequently become the primary cellular drivers of bone healing. Once the site is revascularized (about 4 weeks), the secretion of growth factors is self-sustained, and maturation of the bone then comes from BMPs produced by the bone matrix. In fact, previous studies have confirmed that PRP mediates the early aspects of bone repair through an osteopromotive mechanism[66].

Currently, it is common to combine PRP with autograft, allograft, demineralized bone matrix, or other graft materials for bone applications. It was found that when PRP was applied in conjunction with autogenous bone grafts, the rate of bone formation doubled and bone density increased by 25% compared to controls[50]. More recent data also showed improved efficacy of PRP and bone graft materials on human bone marrow stromal cell activities, accompanied by significantly improved bone formation. However, caution should be taken since PRP may have limited or even negative efficacy in certain delivery vehicles; PRP may reduce the osteoinductivity of active demineralized bone matrix[62,63].

## 4.2. Nonunion

Nonunion occurs most commonly after a scaphoid, tibial or femoral fracture, and it may also accompany fractures of the humerus, radius, ulna and clavicle. Researchers were able to measure the levels of PDGF and TGF- $\beta$  in the fracture hematoma of 24 patients who had fresh fractures of the foot and ankle; however, these investigators were unable to detect the same proteins in the nonunion tissues of seven patients presenting with similar fractures. High levels of the missing growth factors were detected in the autologous PRP prepared from the seven nonunion patients. After applying PRP to the nonunions during revision surgeries, radiographic union was observed in an average of 8.5 weeks[51]. Such studies, although not randomized and prospective, may provide evidence of the utility of PRP for treating nonunion patients.

It should be noted that PRP can help augment fusions, but it does not eliminate the need for meticulous techniques or the use of structural grafts when required. Surgeons should not expect PRP to promote adequate bone growth in bone defects or voids, but they can use PRP to augment the healing of two well-opposed adjacent surfaces, comprised mainly of cancellous or cortico-cancellous bone; currently, there is no evidence showing that cortical bone alone can benefit from PRP treatments[68].



### 4.3. Total Joint Arthroplasty

Recently, attention has turned to using biological materials to assist in hemostasis after total knee arthroplasty. In a retrospective review of 98 total knee arthroplasty patients, 61 of them had PRP applied intraoperatively to exposed tissues, synovium, and the lining of the wound at closure. The patients that received PRP during surgery had less postoperative blood loss, less oral and intravenous narcotics, greater range of motion at discharge, and a shorter hospital stay than their counterparts who did not receive PRP. PRP applied directly to the operative site after knee replacement may have sealed the tissues and delivered platelets directly to the wound[69]. This observational study indicates that PRP may lead to improved outcomes after total knee arthroplasty; further prospective trials with a placebo control group and comparable treatment modalities would be needed.

Another potential application of PRP is in trauma or total joint arthroplasty and involves using PRP at the interface between the implant and bone. With the decline in cement use and the increase in use of press-fit implants, PRP at the implant/bone interface may promote earlier and more complete osteointegration of implants into host bone. Siebrecht and colleagues demonstrated that PRP prepared from human blood significantly increased bone and total tissue ingrowth distance compared with controls in an athymic rat bone chamber model[70]. The same technology is currently being applied in oral surgery where implants are placed into extraction sites augmented with PRP[65].

### 4.4. Tendon

A recent review of commonly used growth factors suggested that PRP may be useful for tendon and ligament healing *in vivo*[9]. In one study, PRP was used to treat chronic severe elbow tendinosis in patients who failed non-operative treatments. Fifteen patients with recalcitrant lateral epicondylitis were treated with PRP and demonstrated significant improvement in healing with no reported complications[71]. A six-month prospective study also showed significant improvement in healing of chronic tennis elbow with the use of autologous PRP[71]. In addition, PRP enhanced Achilles tendon callus strength as well as stiffness in a rat model[72].

### 4.5. Diabetic Fracture

The association between diabetes mellitus and impaired osseous healing has been documented in clinical and experimental settings. Diabetes impairs the fracture healing process beginning with a reduction in early cellular proliferation, followed by a delay in chondrogenesis, and ending with a decrease in the biomechanical properties of the fracture callus. Several clinical studies have noted that the healing time for diabetic patients is approximately twice as long as that of nondiabetic patients[73]. In addition, diabetic patients undergoing elective arthrodesis had a significantly increased incidence of delayed union, nonunion, and pseudoarthrosis[74]. Moreover, in a diabetic fracture model study, significant reduction in PDGF, TGF- $\beta$ , IGF, and VEGF expression was observed in diabetic fracture callus compared to non-diabetic fracture callus[66]. Applications of PRP were found to



restore early cell proliferation during healing to levels comparable to nondiabetic controls and biomechanical testing revealed improved fracture healing in PRP treated diabetic fractures compared to those in non-treated diabetic controls[66]. Percutaneous injection of PRP also normalized early diabetic fracture callus, but the biomechanical properties were only partially restored in late diabetic fracture callus. In addition, PRP improved healing and decreased complications in diabetic patients after ankle fusion[75].

#### **4.6. Wound Healing**

Application of autologous PRP can enhance wound healing, as demonstrated in controlled animal studies for both osseous and soft tissues[65, 67] and in numerous clinical trials. One study showed that 17 out of 21 chronic lower extremity wounds re-epithelialized during a nine-week course of wound treatment with PRP (twice daily). In contrast, only two out of 13 similar wounds had comparable healing after treatment with placebo. After crossover of the placebo group, the remaining 11 non-healed wounds achieved epithelization in an average of seven weeks[62]. In another study, 171 patients with 355 wounds were initially recommended for amputation but instead, a 78% limb salvage rate was reported after treatment with PRP[76].

Another emerging trend in wound care is to apply and retain the PRP using an occlusive dressing at graft donor sites. Added as a clot in a split thickness skin graft donor model, PRP induced early cellular proliferation without leading to cell overgrowth or inhibition of cellular differentiation. Results showed that when the dressing was removed at seven days, the donor site had a similar appearance to that of a 21-day placebo control and the site treated with PRP also showed better epithelialization than the control.

#### **4.7. PRP as a Bone Healing Enhancer for Bone Graft Materials**

Over 1.3 million medical and dental procedures related to bone loss occur annually in the U.S. Current clinical methods of treating skeletal defects involve bone transplantation or the use of bone graft materials[77]. There are four types of bone graft materials: autografts, allografts, alloplasts, and xenografts. PRP has been studied as a bone healing enhancer for such bone graft materials; to date, its findings are contradictory and further investigation is needed. On one hand, PRP was found to improve bone healing in conjunction with bone grafts. PRP led to an increase in TGF- $\beta$ 1 as well as PDGF and enhanced new bone formation when PRP was used in allografts[39, 78]. More volume fractions of bone in the maxillary sinus of rabbits were also found when PRP was used with  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) compared with the use of  $\beta$ -TCP alone. In addition, a higher percentage of bone contact with particulate dentin-plaster of Paris was observed when PRP was used compared to controls without PRP[79].

However, in some cases, PRP did not appear to enhance bone healing in conjunction with bone graft applications. No evidence of osteoinductivity enhancement was observed when PRP was used with a mixture of ground mineralized bone matrix, demineralized bone matrix gelatin powder, and reconstituent fluid in the recti abdomini muscles of actymic nude rats[8]. A critical-size defect pig model with allograft showed no enhanced osteoinductivity[8]. PRP,



as maxillary sinus augmentation in minipigs, also failed to appear superior to the control animals regarding new bone formation and bone-implant contact[8]. PRP, in a series of clinical cases, did not enhance the quantity or quality of new bone formation in alveolar ridge defects, although histological evaluation revealed the presence of residual allograft particles surrounded by connective tissue as well as newly formed bone within the grafted areas[80].

#### **4.8. Infection Prevention**

In addition to platelets, PRP also contains high concentrations of several differentiated and non-activated leukocytes including lymphocytes, neutrophilic granulocytes, and monocytes. These white blood cells were reported to be two to four times greater in PRP than in normal blood[81]. The presence of concentrated neutrophils, lymphocytes, monocytes, and platelets indicates that PRP could be used to prevent osteomyelitis and infection, because

1. Neutrophils are known for their host-defense mechanism actions against bacteria and fungi through the actions of myeloperoxidase, present in neutrophilic granulocytes. At injury sites, myeloperoxidase catalyzes the oxidation of chloride to produce hypochlorous acid and other reactive oxygen derivatives. These substances act as potent bactericidal oxidants and are toxic to microorganisms and fungi[82]. As a result, neutrophils may play an important role in immune defense against infections.
2. Lymphocytes produce immunocompetent cells that play a role in immunologic defense.
3. Monocytes, precursors of macrophages, may produce cytokines and chemotactic factors that participate in inflammation.
4. Platelets also contain multiple antimicrobial peptides[19], which are released upon platelet activation.

Therefore, it is speculated that PRP, an engineered biological blood product, may have the potential to prevent osteomyelitis or infection. This was confirmed in a recent study of a large cohort of cardiac surgery patients. The intraoperative use of PRP during wound closure was found to significantly reduce the incidence of superficial and deep sternum infections[83]. Unfortunately, limited data related to infection prevention using PRP are available, and the roles of leukocytes and platelets in the use of PRP for infection prevention are still unknown.

### **5. LIMITATIONS OF PRP USE**

PRP has been used in a variety of applications; however, its application in some cases is still controversial and special attention should be paid to the timing of PRP preparation and safety issues related to PRP processing and use of PRP devices.



### 5.1. Time for Usage

After the addition of thrombin to PRP, the platelets start to change structurally, and growth factors are excreted to the platelet extracellular milieu where they are capable of binding to tissue receptors. As a result, it is very important to understand that PRP-gel should be prepared and applied in a timely fashion. Once the PRP has been activated, the PRP-gel, alone or in combination with other materials, should be applied to tissues within a short time (e.g., one minute). Late application of PRP after activation will most likely lead to significant losses of platelet growth factors thereby resulting in no positive effects of PRP application. This might be a reason for inconsistencies in some PRP studies.

### 5.2. Safety Issues Related to Processing PRP and PRP Devices

Autogenous PRP is inherently safe and free from transmissible diseases. However, the practitioner should keep in mind that liability, consent, and licensing must be discussed because both patient and auxiliary staff safety issues are pertinent. Although the patient is protected from transmissible diseases because of the autologous nature of PRP, the practitioner and the auxiliary staff are not. Devices that leak blood or have the potential to malfunction from centrifuge imbalance are a real health, medical, and legal risk. Practitioners are encouraged to use devices that have FDA clearance to process PRP from autologous whole blood. In addition, appropriate caution should be used if allogeneic PRP will be used in clinic.

Also note that no dental or medical practitioners are licensed to infuse or re-infuse blood or blood products systemically in an operating room setting. However, it is within the licensure to apply blood products, e.g. PRP, topically in the operating room. It takes approximately 45-60 ml of blood, a relatively insignificant amount compared to a normal 4-5 L of blood volume in humans, to produce the PRP needed.

## 6. FUTURE DEVELOPMENT OF PRP

PRP has been successfully used in orthopaedics, maxillofacial surgery, cosmetic surgery and dental implant surgery. However, the procedures to prepare PRP are likely to differ greatly among researchers and clinicians, and may result in inconsistent outcomes. There is also no consensus in the literature on the terminology of platelet products, as indicated by Bielecki and colleagues[84]. Therefore, standardization of PRP methodology is warranted. Moreover, randomized controlled clinical trials are needed to study the effects of PRP in wound rehabilitation as well as tissue engineering, and further studies are needed to clarify the bactericidal effect of PRP. Once activated, the platelets will release their growth factors and cytokines within a very short time, and multiple blood draws are required in many PRP applications. Obtaining sustained release of growth factors and cytokines from activated platelets could be advantageous and could avoid multiple blood draws. Nanotechnology-enabled drug delivery systems are expected to generate over \$4.8 billion in 2012; nanotechnologies, e.g. electrostatic layer-by-layer self-assembly[85,86], could be applied to



form a nanoshell on cells within PRP thereby achieving sustained release of growth factors and cytokines. The nanoshell may also protect the growth factors and cytokines from losing their biological activity[87]. Therefore, nanotechnology-enabled delivery of PRP is likely to be of interest to the medical community, in view of the increasing number of PRP applications.

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