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SPECIAL FOCUS

Platelet-Rich Plasma and Its Uses in Foot and Ankle Surgery

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ABSTRACT

With advances in basic science, the use of biological adjuncts is currently being explored to supplement orthopedic surgery. Platelet-rich plasma is a clinical concept of autologous intraoperative concentration of serum platelets to provide high levels of early critical growth factors for implantation into fracture, arthrodesis, or nonunion sites. The theoretical benefit of a biological adjunct like platelet-rich plasma has been to significantly enhance fusion rates. *Platelet-rich plasma* has been defined as a volume of plasma that has a platelet concentration above physiological levels, typically a 5-fold increase. Application of autologous platelet concentrate is a simple procedure with minimal morbidity, which is also inexpensive. Although an abundance of literature exists pertaining to dental applications, few data exist on orthopedic applications.

Keywords: platelet-rich plasma, bone healing, growth factor, nonunion, fracture

HISTORICAL PERSPECTIVE

At the site of any trauma involving bone, a clot forms consisting of red blood cells, white blood cells, and platelets entrapped within a fibrin matrix. In the bone repair process, platelet α-granules act as a reservoir of exogenous growth factors. The degranulation of the α-granules results in the release of platelet-derived growth factor (PDGF), insulinlike growth factor (IGF), vascular endothelial growth factor (VEGF), and transforming growth factor (TGF) β among a host of other growth factors providing an ideal delivery system localized to the site of injury. Each of these factors plays a critical role in bone healing.[1–3]

Platelet-derived growth factor enhances DNA synthesis, increases collagen deposition, and stimulates synthesis of extracellular matrix.[4] In vitro, PDGF has been shown to stimulate type I collagen production and messenger RNA expression in osteoblasts and chondrocytes.[5] Platelet-derived growth factor has enhanced chemotactic and proliferative effects and the ability to initiate differentiation of osteoprogenitor cells toward an osteoblastic lineage.[6] Platelet-derived growth factor functions in a macrophage autocrine feedback loop stimulating production and release of growth factors or cytokines.[7] Joyce and colleagues[5] analyzed the effect of daily injection of PDGF into uninjured newborn rat femurs. Their findings suggest that PDGF initiated osteogenesis and chondrogenesis processes. Daily injections of PDGF resulted in a dose-dependent increase in mesenchymal cell proliferation with a mass of new bone formation. In a unilateral rabbit tibial osteotomy model, the application of exogenous PDGF-BB exhibited a stimulatory effect on bone healing.[8] Fujii and colleagues[9] analyzed the expression of PDGF along with α- and β-receptor messenger RNA to further elucidate its role in the inflammatory phase (days 2–4) after fracture. These investigators theorized that the function mediated by the β-receptor, including cell migration, might be a prerequisite to the recruitment of mesenchymal cells in the initial step and to the interaction between osteoclasts and osteoblasts in the bone remodeling phase.

Platelet-derived growth factor has been identified at fracture sites in humans throughout the stages of healing.

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Andrew and colleagues[10] demonstrated PDGF expression from many cell types throughout a normal human healing fracture process including endothelial and mesenchymal cells in granulation tissue and osteoblasts, chondrocytes, and osteoclasts during later stages of fracture healing.

Insulinlike growth factor is another growth factor that is critical in bone formation. Insulinlike growth factor 2 has been found to be the most abundant growth factor in the bone; however, IGF-1 has been found to be more potent and has been localized in healing and fractures in rats and humans.[11] Insulinlike growth factor 1 stimulates proliferation of osteoblast precursors and early-stage osteoblasts and promotes bone matrix formation by fully differentiated osteoblasts.[12–15] In vivo, endogenous and exogenous IGF has been associated with indices of active bone matrix production.[16–18] Insulinlike growth factor expression was also greatest in osteoblasts that were involved in active bone remodeling. The data suggest that IGF is involved in cell proliferation or differentiation of mesenchymal cells, periosteal cells, osteoblasts, and chondrocytes by way of an autocrine or paracrine fashion.

Transforming growth factor β is part of a family of related proteins called the TGF-β superfamily. It influences a broad range of cellular activities, including growth, differentiation, and extracellular matrix synthesis. It is found in many tissues but particularly bone, platelets, and cartilage.[11] Bone and platelets contain almost 100 times more TGF-β than any other tissue, and osteoblasts have the highest number of TGF-β receptors.[19,20] Transforming growth factor β has the ability to stimulate differentiation of mesenchymal cells into cells with a chondrocytic phenotype[21] and the synthesis of type II collagen and proteoglycans by mesenchymal cells[22]; these suggest a role for TGF-β in regulating chondrocyte differentiation and cartilage matrix production.

These aforementioned studies suggest that the growth factors (PDGF, IGF, and TGF-β) that are contained within platelets present at the site of injury play a critical role in the healing process. The delivery of increased levels of these critical growth factors is accomplished by producing platelet-rich plasma (PRP).

Platelet-rich plasma is defined as a volume of plasma with an increased platelet concentration of 4 to 6 times above normal values (approximately 1,000,000/mL). Multiple commercially available systems exist that can create PRP from a patient's own blood in the operating room for intraoperative application to a surgical site (Table 1). One of the first available commercial orthopedic PRP systems was Symphony PCS (DePuy, Warsaw, Ind). This system centrifuges 55 mL of autologous blood and combines the platelet-rich layer with thrombin to produce a concentrate gel. This gel can be sprayed directly onto the prepared bone surfaces and/or mixed with the autologous or allogeneic bone graft.

One key concept is the high variability of growth factor levels to platelet levels. Neither whole blood nor PRP platelet counts are predictive for the resulting growth factor levels in PRP. Although these systems are able to create a highly predictable platelet count, currently, no simple procedure exists for obtaining preoperative estimates of individual growth factor levels in a PRP sample.[23] Weibrich et al[23] reported considerable variability in growth factor concentrations between PRP samples. Determining preoperative growth factor levels is crucial for future clinical trials of the effectiveness and reliability of PRP.

Platelet-rich plasma contains increased levels of growth factors, which are proteins secreted by cells that act on an appropriate target cell or cells to carry out a specific action. Once the growth factor binds to the target cell receptor, it induces an intracellular signal transduction system and produces a biological response critical for chemotaxis, cell proliferation, and osteoblast differentiation.[11]

Clinically, PRP has been demonstrated to enhance early maturation of fusion masses in the lumbar spine and after maxillofacial surgery. One of the first reports of the use of PRP in patients who had foot and ankle injuries was by Gandhi and colleagues.[24] This preliminary study centered on the use of PRP in 9 patients that had sustained a foot and ankle nonunion. All patients had undergone initial surgical intervention that was performed within 20 days of their foot and ankle fracture and were diagnosed with a nonunion for a minimum duration of 4 months after the index procedure. Their revision treatment operation was the focus of this study. The study population possessed a nonunion of 4 to 10 months' duration after diagnosis; the mean patient age was 42 years. The investigators applied PRP with autogenous bone graft to the nonunion site along with standard fixation techniques. Their findings indicated that,

TABLE 1. *Commercially Available Platelet Concentration Systems*

Symphony PCS (DePuy, Warsaw, Ind)
Vivostat PRF Preparation Kit (Vivolution A/S, Birkeroed, Denmark)
PCCS Platelet Concentrate Collection System (3i Implant Innovations, Palm Beach Gardens, Fla)
Harvest SmartPrep 2 APC 60 Process (Harvest Technologies Corporation, Munich, Germany)
Fibrinet Autologous Fibrin & Platelet System (Cascade Medical Enterprises, Wayne, NJ)
Curasan (Curasan, Kleinostheim, Germany)
Haemonetics Cell Saver 5 (Haemonetics Corp, Braintree, MA)
Haemonetics MCS (Haemonetics Corp, Braintree, MA)

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with the addition of PRP and bone graft, resolution of a nonunion was achieved at a mean time of 60 days. Within this study, Gandhi and colleagues[24] compared growth factor concentrations at the surgical site within the fracture hematoma in patients who had a nonunion versus with patients who did not have a nonunion. In examining growth factor levels, plasma levels of PDGF and TGF-β were consistent between patients who had a nonunion versus those who had fresh fracture; however, a significant reduction was noted in the growth factor levels at the nonunion site compared with the hematoma at the site of a fresh fracture. This study clearly implicates that the local milieu in fresh fractures versus those that have gone on to a nonunion exhibits a significant difference in these important healing factors.

Bibbo and colleagues[3] also demonstrated the use of autologous platelet concentrate (APC) in high-risk foot and ankle surgery patients. The criteria for a high-risk patient included those patients who were active smokers, patients with diabetes (with neuropathy), patients who were immunologically or nutritionally impaired, patients who demonstrated a clinical history of poor bone healing (nonunion or delayed union at proposed surgical site), patients who had previously undergone multiple surgeries at the proposed operative site (>2 open surgeries at the proposed surgical site), and patients with a history of open treatment after high-energy trauma.[3] The study followed 62 high-risk patients with a mean age of 51 years and with at least 1 identifiable risk factor for poor bone healing and followed them for 6 months with checks for radiographic union every 2 weeks. Overall, a 94% union rate was achieved at a mean of 41 days. For patients treated with APC alone, mean time to union was 40 days, and when APC was used with autograft, the mean time to union was 45 days. These data suggest that adjuvant APC results in an acceptable time to union and may be a useful adjunct to promote osseous healing in high-risk patients undergoing elective foot and ankle surgery.

Reconstructive surgery of the foot and ankle remains a challenging endeavor in many regards. In response to tissue injury, the lower extremity, particularly the foot and ankle, exhibits a different course and time frame for the healing than do many other areas of the musculoskeletal system. The innate biological factors that influence the response of foot and ankle region to injury include the pattern of arterial networks with a reliance on small vessels to provide inflow to distal parts, a paucity of muscle coverage over most of the region, a high level of back pressure within the venous system, and the high mechanical stresses acting across the foot and ankle. Thus, when performing foot and ankle surgery, surgeons are faced with overcoming a number of factors that act as obstacles to achieving successful healing. These obstacles are magnified in compromised patients and are truly a challenge to overcome.

In a preliminary study by Coetzee and colleagues,[1] PRP was used to promote syndesmosis fusion, which is critical for proper functioning of the Agility Total Ankle Replacement. The prepared Symphony platelet concentrate was sprayed directly onto the prepared bone surfaces and mixed with autologous bone graft. Bone graft was placed in the syndesmosis and over the anterior fins of the tibial and talar components. They found a statistically significant improvement in 8- and 12-week fusion rates and a statistically significant reduction in delayed unions and nonunions at 6 months with the addition of PRP. A high-risk subset of smokers achieved a 50% union rate at 6 months in the control group, whereas 80% of patients who were treated with PRP and bone graft achieved union at 6 months. The data demonstrated that PRP, in this clinical setting, improves the time to union in ankle syndesmotic fusions, which is a critical "next step" required before patients can proceed with advanced weight-bearing status and the overall success of the Agility Total Ankle Replacement.

Other case reports demonstrated the use of PRP in orthopedic surgery; however, most are either case studies or extended case series. In a case report by Sanchez and colleagues,[25] PRP was given during an arthroscopic repair of a large nontraumatic avulsion of articular cartilage in the knee of an adolescent soccer player. The author found an improvement in articular cartilage healing and accelerated functionality with treatment of PRP. Given the poor blood supply of articular cartilage of the knee, regeneration can be a long process, despite the age and health of the patient.[25] A correlation can be made with the ankle and its limited blood supply because of its pattern of arterial networks with a reliance on small vessels to provide inflow to distal parts.

Platelet-rich plasma has also been used to enhance human Achilles tendon repair. In a study of 12 athletes undergoing Achilles tendon repair, Sanchez and colleagues[26] demonstrated that the group receiving PRP application recovered their range of motion earlier, had no wound complications, and took less time to take up gentle running and resume training activities. Although the group studied was relatively small and composed of athletes in generally good health, correlations can be made to poorly healing foot and ankle fractures. Achilles tendons have a poor blood supply, and repair after a tear can be prolonged for many weeks. These conditions are similar to poorly healing ankle fractures that possibly have a decreased blood supply compared with normal ankles, where application of PRP may prove beneficial.

When combined with early physiotherapy, Virchenko and Aspenberg[27] demonstrated that a single PRP treatment after tendon injury can improve healing. This

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TABLE 2. *Risk Factors for Impaired Bone Healing*

Medically/pharmacologically immunosuppressed
Nonunion/pseudoarthrosis at the proposed surgical site
Smoking
Multiple surgeries (more than 2 at the same proposed surgical site)
History of trauma (open, high-energy)
Diabetes (neuropathy, Charcot arthropathy)
Avascular necrosis at proposed surgical site
History/active infection at proposed surgical site
Suboptimal arterial inflow from peripheral vascular disease (not requiring revascularization)

improvement was lost in the treatment group given botulinum toxin type A (Botox; Allergan, Inc, Irvine, Calif), which simulated a group without mechanical stimulation. This study demonstrates that other important factors play a role in tendon healing when treating with PRP and provides critical area for future study.

■ INDICATIONS AND CONTRAINDICATIONS

The use of PRP in orthopedic surgery seems to be beneficial in many clinical cases of fractures, arthrodesis, and nonunions especially in high-risk patients (Table 2). Currently, experimental basic science data and initial clinical evidence suggest that the use of PRP as an adjuvant modality to augment bony healing (namely fusions) holds significant potential and seems to be a safe agent to use in foot and ankle surgery. Recent studies of the effectiveness of PRP have only included high-risk patients, and further investigations need to focus on non–high-risk patients. According to DePuy Symphony II Platelet Concentrate System, its use may be contraindicated when there is clinical or laboratory evidence of septicemia and for patients who have taken aspirin, or other medications that alter platelet function within 3 days

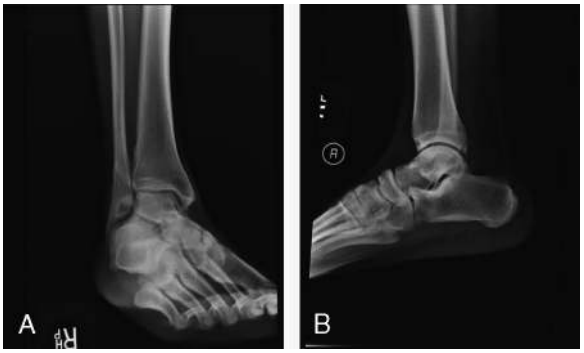

FIGURE 1. Preoperative radiographs of right ankle demonstrating fracture of fibula and posterior malleolus; mortise view (A) and lateral view (B).

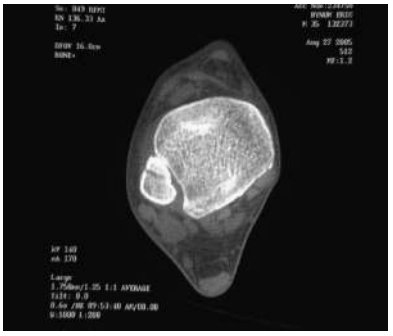

FIGURE 2. Preoperative computed tomography of the right ankle demonstrating persistent fracture of fibula despite an additional 4 weeks of casting.

before surgery, or patients with disorders associated with platelet dysfunction.[28]

The long-term effects of the use of PRP have not been studied in depth in human applications. However, Schmidmaier and colleagues[29] studied the long-term effects of IGF-1 and TGF-β1 application in a rat model. At the last time point studied at 84 days, there were no differences measured in the biomechanical testing and callus composition between groups treated with and without growth factors. No side effects such as neoplasia or deficient bone production were found. There are no current case studies demonstrating long-term complications of PRP application during orthopedic surgery (Figs. 1–5).

■ TECHNIQUE

After induction of sedation, peripheral blood is collected via upper extremity venous cannulation or radial artery catheterization (when arterial blood pressure monitoring

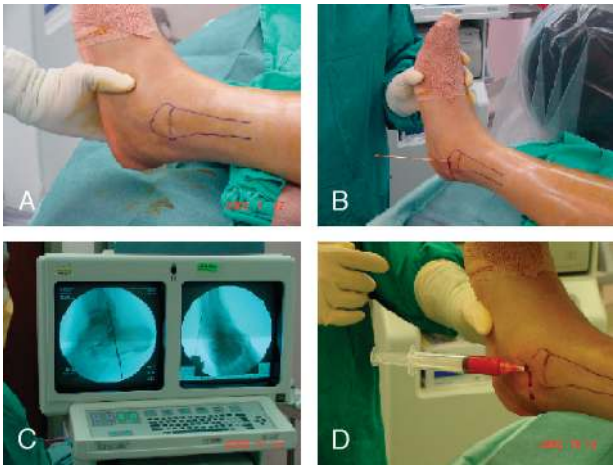

FIGURE 3. Intraoperative photographs with percutaneous injection, ink drawings demarcating fracture site (A), guidewire placement at the fracture site (B), fluoroscopic confirmation (C), and injection of PRP (D).

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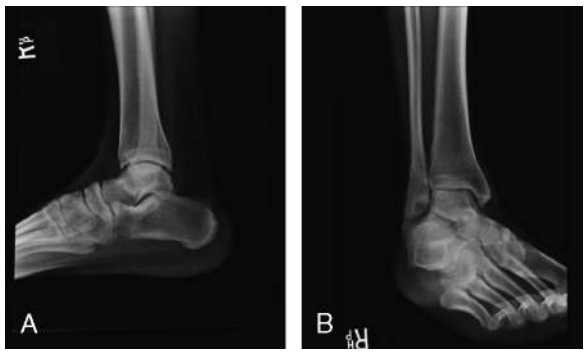

FIGURE 4. Postoperative radiographs 6 weeks after PRP injection demonstrating union of fibula and posterior malleolar fracture; mortise view (A) and lateral view (B).

is medically required). The amount of peripheral blood collected is determined via a nomogram, adjusted based on the patient's hematocrit, the details of which are published elsewhere. The amount of platelet-rich concentrate prepared is predetermined based on an estimate of the surgical surface area (eg, ankle joint accommodates more APC than a single midfoot joint). Blood specimens are immediately processed for platelet concentration. The platelet processing technique results in "platelet-rich" and "platelet-poor" products, with the final PRP applied as a final product in combination with calcium chloride and thrombin. The platelet-poor product is discarded. Autologous platelet concentrate product is applied intraoperatively after preparation of bony surfaces and final operative site irrigation.

Currently, when compared with implantable direct current stimulators and with recombinant growth factors, PRP seems to be a cost-effective adjuvant modality. This observation is based upon actual device cost and the low associated complications from its use.

■ POSSIBLE CONCERNS, FUTURE OF THE TECHNIQUE

Many future areas of study are needed for the use of PRP in orthopedic surgery in high-risk, and in non–high-risk, patients. In a current study by Lin and Pinzur, growth factor levels (PDGF, VEGF, IGF-1, and TGF-β) of 12 high-risk patients undergoing ankle fusion were measured using enzyme-linked immunosorbent assay technique in PRP and bone samples (Tables 3 and 4). These patients were all diabetic for at least 10 years. Procedures performed included ankle fusion, hindfoot fusion, and pantalar fusion. Platelet-rich plasma was applied during each surgery, and the patients were followed for either union or nonunions and any other complications. Four of 12 patients went onto nonunion after the procedure. When normalized to bicinchoninic acid assay analysis, in the 4 nonunions, there was a 65% decrease in PDGF-AB (0.052 pg/μg nonunion to 0.144 pg/μg union; $P = 0.036$) and a 48% decrease in VEGF (0.485 pg/μg nonunion to 0.940 pg/μg; $P = 0.029$) growth factor levels compared with the values

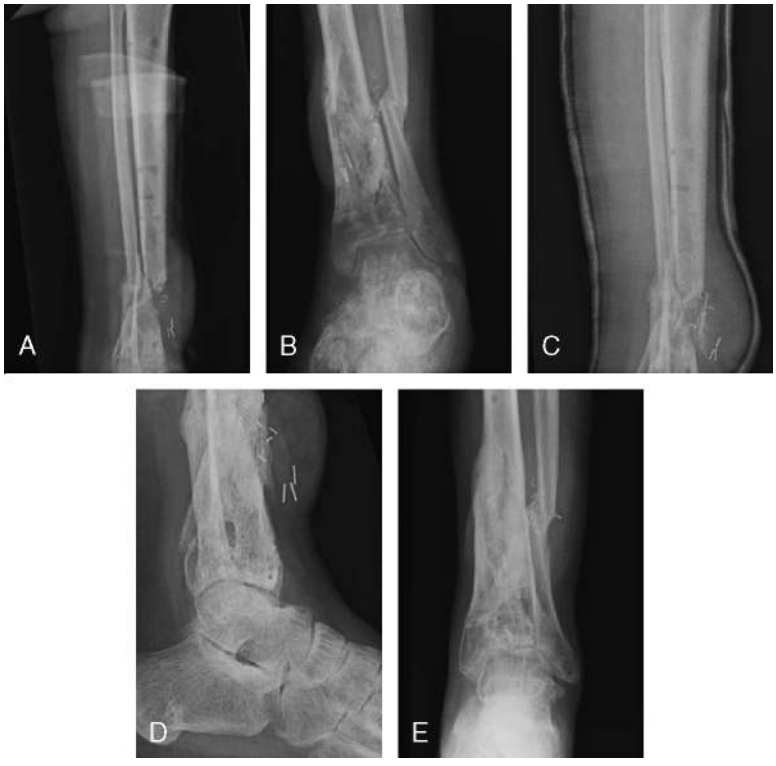

FIGURE 5. Preoperative anteroposterior and lateral view of the left ankle shows distal tibial nonunion (A and B). Fourteen-day postoperative bone grafting with Symphony PCS shows early callus formation (C). Twenty-eight–day postoperative bone grafting with Symphony PCS shows more callus formation (D). Two-year follow-up anteroposterior view of the left ankle shows healed nonunion (E). (Reprinted with permission from Ankur Gandhi, PhD, Frank Liporace, MD, Vikrant Azad, MD, James Mattie, BS, and Sheldon S. Lin, MD. Diabetic Fracture Healing Foot Ankle Clinics, 2006.)

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TABLE 3. *Growth Factor Quantification/Union Status in PRP Samples*

	PDGF, pg/mL	VEGF, pg/mL	IGF-1, ng/mL	TGF-β, ng/mL	Result
Patient No.					
1	210.986	12.074	9.145	5.873	Union
2	196.106	8.737	6.442	4.955	Fusion
3	318.583	10.482	6.002	6.699	Union
4	316.052	13.71	8.22	7.085	Nonunion
5	322.704	14.503	5.263	9.53	Fusion
6	326.969	15.087	9.85	9.61	Nonunion (infection)
7	330.465	12.649	5.952	5.941	Union
8	214.865	13.041	8.907	8.883	Foot union/ankle nonunion
9	322.321	14.221	19.425	9.85	Union
10	282.607	8.694	9.696	7.439	Union
11	132.68	14.95	8.48	9.594	Nonunion
12	153.394	9.768	9.789	6.737	Fusion
Mean growth factor levels					
Union	267.146	11.391	8.964	7.128	
Nonunion	247.642	14.197	8.864	8.793	

seen in group that achieved union. No difference exists in the levels of IGF-1 between the union and nonunion groups. An increased level of TGF-β1 in the nonunion group compared with the union group was detected (208.940 pg/μg nonunion to 127.495 pg/μg union; $P = 0.257$) (Table 5). Clearly, differences exist among local growth factor levels in patient's bone. Potentially, growth factor levels (PDGF-AB and VEGF) in the bone may affect the outcome of successful arthrodesis in diabetic patients. This study supports the concept that growth factor levels within the fusion site of a diabetic patient may affect the outcome of a successful hindfoot fusion. This study only included 12 patients, and a larger scale study needs to be done.

TABLE 4. *Growth Factor Quantification/Union Status in Bone Samples*

	PDGF, pg/mL	VEGF, pg/mL	IGF-1, ng/mL	TGF-β, pg/mL	Result
Patient No.					
1	5.901	29.153	9.106	1.992	Union
2	4.645	30.786	6.271	0.729	Fusion
3	8.341	61.416	7.035	1.021	Union
4	2.808	30.457	8.431	15.162	Nonunion
5	11.354	42.773	5.624	8.182	Fusion
6	6.694	32.015	18.597	20.497	Nonunion (infection)
7	5.733	43.259	3.936	0.925	Union
8	3.725	25.558	7.268	17.283	Foot union/ankle nonunion
9	7.727	31.058	6.335	18.367	Union
10	9.113	67.564	9.032	10.239	Union
11	1.803	48.076	6.139	4.196	Nonunion
12	0.606	34.027	3.774	10.909	Fusion
Mean growth factor levels					
Union	6.678	42.505	6.389	6.546	
Nonunion	3.758	34.027	10.109	14.285	

TABLE 5. *Growth Factor Levels Normalized to Total Protein Levels*

	Ratios of GF Levels to BCA Protein	
Growth Factor	Union	Nonunion
PDGF	1.444375	0.524
VEGF	9.396875	4.8475
IGF-1	0.000141387	0.00014044
TGF-β	1.274874125	2.0893975

The exact indications for PRP have not been delineated. However, it seems to be a promising adjunct to present autograft, allograft, and synthetic graft techniques. Platelet-rich plasma alone carries no potential risk of disease transmission. In addition, the "ideal" amount of PRP has not been firmly established.

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