

# The Utilization of Autologous Growth Factors for the Facilitation of Fusion in Complex Neuropathic Fractures in the Diabetic Population

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Type 2 diabetes mellitus is an enormous and growing public health problem affecting an estimated 18.2 million Americans [1]. Diabetic neuropathy is the most frequent complication of diabetes and, although the etiology is unknown, metabolic and ischemic nerve injury are likely factors [2]. It is believed that approximately 25% of adults who have diabetes have appreciable peripheral neuropathy within 10 years of diagnosis [3]. This complex set of clinical syndromes affects distinct regions of the nervous system and, in developed countries, is responsible for 50% to 75% of nontraumatic amputations [4].

## Neuropathic osteoarthropathy

Neuropathic osteoarthropathy, also known as Charcot's arthropathy, is a common complication in patients who have diabetes and severe neuropathy [1]. Pathologic fractures, joint dislocations, and deformity characterize Charcot's foot

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and commonly affect the midfoot but also occur in the hindfoot, forefoot, and ankle [3,5,6]. Its prevalence ranges from 0.16% in the general population to 13% of patients presenting to high-risk diabetic foot clinics [7–9].

The pathogenesis of Charcot's arthropathy has been the subject of many long debates and various theories that are not mutually exclusive. Volkman and Virchow proposed the so-called "neurotraumatic theory," which suggests that insensate joints undergo repetitive damage, resulting in fractures. Alternatively, Mitchell and Charcot favored the so-called "neurovascular theory," which suggests that the increased blood flow due to autonomic neuropathy causes bone resorption and weakness, resulting in pathologic fracture.

Histologic and biochemical studies in recent years found that the tissues of patients who have Charcot's arthropathy are abnormal. In evaluation of the bone structure, it was found that these patients have increased incidence of osteoporosis, reduced bone density, and increased osteoclastic activity [10–12]. These findings create a bone structure that is at high-risk for fracture and that has an impaired healing potential. In addition to changes noted in the skeletal system, the soft tissues in a person who has diabetes are adversely affected by the hyperglycemic state, especially through nonenzymatic glycosylation of various proteins including collagen. Grant and colleagues [13] found that secondary to nonenzymatic glycosylation, the collagen fibers in the Achilles tendon become abnormal. Electron microscopy revealed increased packing density, decreased fibrillar diameter, and overall abnormal fibril morphology. These changes in soft tissue morphology may lead to shortening of tendinous structures and abnormalities in the ligamentous structures, changing the anatomic configuration of the lower extremity [13–15]. Myerson [16] proposed that this hyperglycemic state might adversely act on the ligamentous structures of the foot, increasing the potential for structural failure and the creation of the Charcot's deformity [14,15]. Grant and coworkers [13,14] determined that the alterations in the collagen at the histologic level alter the morphologic characteristics of the Achilles tendon, with reduced elasticity and decreased tensile strength. It is believed that the abnormal stress applied to the foot in a person who has diabetes by a short, tight Achilles tendon creates changes to the anatomic structures within the foot. Charcot's foot is associated with equinus, which contributes to the collapse of the midfoot, exacerbates collapse of the longitudinal arch [14,17,18], and in turn, alters the gait mechanics. The senior author has altered the outcome of patients who have acute Charcot's arthropathy by way of surgical intervention with Achilles tendon lengthening and has arrested the disease process by simply eliminating the deforming force of the Achilles tendon.

Charcot's arthropathy often presents with swelling, warmth, and erythema [6,19]. As such, it may be difficult to differentiate Charcot's changes from infection, cellulitis, or deep venous thrombosis [6,19–21]. Often, Charcot's foot goes unrecognized until severe complications occur [20]. These complications include bone fragmentation, fracture, and dislocation that progress to foot deformity, bony prominence, and instability, which can lead to ulceration and deep infection and may require amputation [19,22]. If caught sufficiently early,

Charcot's foot can be treated conservatively with immediate non-weight bearing immobilization to stabilize the foot and protect the soft tissues, followed by a lifelong program of preventive care [16,19,20,23]. Closed management of neuroarthropathy with total-contact casting or walking boots have been found to be approximately 75% effective in patients who have early stages of the disease [16].

The role of early surgical stabilization is among the list of current treatment controversies [5,19,21]. Reconstructive surgery is challenging, but operative correction and salvage can result in stability in patients who have severe deformity. Reconstruction is an alternative to amputation. Surgery may be indicated for (1) unbraceable deformity; (2) recurrent ulceration secondary to deformity, instability, or both; and (3) pain unresponsive to conservative measures [22]. For example, over a 6-year period, Pinzur [3] treated 198 patients (201 feet) for Charcot's foot arthropathy, including 147 feet with midfoot, 50 with ankle, and 4 with forefoot disease. After at least 1-year follow-up, 87 of the 147 feet with midfoot disease (59.2%) achieved the desired endpoint with only conservative treatment, with the balance requiring surgery: corrective osteotomies with or without arthrodesis in 42 feet and debridement or exostectomy in 18 feet. Three patients had initial amputation and 5 patients had amputation performed after attempted salvage failed. In another study, Pakarinen and colleagues [23] reviewed 29 patients (36 feet) presenting with Charcot's foot in various stages. Treatment with cast immobilization ranged from 4 to 37 weeks. Fourteen surgical procedures were performed on 10 patients, including 6 exostectomies, 4 midfoot arthrodeses, 1 triple arthrodesis, 1 tibiocalcaneal arthrodesis, and 2 below-knee amputations. Radiologic fusion was achieved in 6 of the 9 attempted arthrodeses. Foot/ankle patients who have diabetes are at higher risk for treatment complications than those who do not have diabetes. For example, McCormack and Leith [24] and Flynn and coworkers [25] treated ankle fractures of patients who had diabetes and those who did not, using surgical and conservative methods. Incidence of infection, malunion, and wound necrosis was higher in the diabetic cohorts, indicating the difficulty in treating this population. The high incidence of Charcot's foot, the high complication rate, and the devastating consequences of improper treatment make it highly desirable to explore additional treatment strategies. One potential strategy is to provide a mechanism to accelerate the healing process.

### **The healing process**

Normal wound healing comprises three overlapping phases: (1) inflammation, which includes the initial hemostatic response; (2) proliferation; and (3) remodeling [26–29]. Three critical components of the wound “healing cascade”—appropriate cells, extracellular matrix, and signaling proteins—must be present at the correct levels and at the right place and time for normal wound healing

to occur. Platelets play a key role in the inflammatory phase in hemostasis and clot formation [29] and in the release of key signaling proteins that influence the chemotaxis, differentiation, and proliferation of a variety of cells that provide extracellular matrix, stimulate angiogenesis, and generate repair tissue [30]. Such signaling proteins include platelet-derived growth factor (PDGF), transforming growth factor  $\beta$  (TGF- $\beta$ ), platelet-derived angiogenesis factor, platelet-derived endothelial cell growth factor, insulin-like growth factor, and many others that initiate proliferation and differentiation of mesenchymal progenitor cells [30–34].

Platelets are cytoplasmic fragments of megakaryocytes, which are a type of white blood cell and are formed in the marrow [29]. Normal blood contains approximately 140,000 to 400,000 platelets per microliter [29]. They have a circulation lifetime of approximately 10 days, after which they are removed by macrophages of the reticuloendothelial system [29]. Although they have no nucleus, platelets contain a variety of organelles, including alpha granules. The alpha granules contain numerous protein signaling molecules from the family of growth factors, cytokines, and chemokines, which are secreted after platelet activation [30]. For convenience, these proteins are collectively referred to as secretory proteins.

When tissue damage occurs and there is a rent in the vascular system, the platelets become activated through contact with collagen, the basement membranes of capillaries, and subendothelial microfibrils [29]. During activation, the platelets change shape, developing long thin projections, and become sticky. This morphological change enables the platelets to aggregate at the site of the vascular defect and provide hemostasis. As the platelets aggregate, they secrete ADP, causing further platelet activation and aggregation. Thrombin, produced from prothrombin, is also a potent platelet activator. There are several stages in the coagulation cascade at which platelets interact and exert their influence on clot formation. The reader is referred to Conley [29] for the details of this interaction.

During platelet activation, the alpha granules fuse to the platelet plasma membrane, releasing their contained secretory proteins [35–37]. During activation, at least some of the secretory proteins (eg, PDGF and TGF- $\beta$ ) are transformed to a bioactive state by the addition of histones and carbohydrate side chains [35,36]. These secreted proteins bind to transmembrane receptors of target cells (eg, mesenchymal stem cells, osteoblasts, fibroblasts, endothelial cells, and epidermal cells). The agonist-bound transmembrane receptors then activate intracellular signal proteins that direct the expression of a gene sequence that codes for activities such as cell proliferation, matrix formation, osteoid production, collagen synthesis, and so forth [35]. Anitua and colleagues [30] have provided a recent, detailed review of the manner by which platelets can influence many of the aspects of wound healing.

Protein secretion begins about 10 minutes after clot formation, with more than 95% of the presynthesized, secreted proteins released within 1 hour [35]. After this initial burst of secretory activity, the platelets continue to synthesize and release additional proteins for the balance of their life (on the order of days)

[35,38]. By the end of the platelet life span, macrophages, which have arrived by way of platelet-stimulated vascular ingrowth, assume responsibility for wound healing by secreting their own factors. Thus, as wound healing progresses, there is an evolution in the quantities and types of cells present, with the earlier cell types giving way to the later cell types, and so on, in a cascaded sequence. Because platelets are the first inflammatory cell-type to invade the wound site, it can be said that platelets set the pace for wound healing [35,38]. There are many similarities between soft- and hard-tissue healing, with platelets playing a direct fundamental role in the inflammatory phase of both. During the proliferative phase, the damaged, necrotic tissue is removed and replaced with living tissue that is specific to the local tissue environment (eg, bone, cartilage, fibrous tissue, and so forth). Local tissue factors, including the growth factor and cytokine profile, hormones, nutrients, pH, oxygen tension, and the electrical and mechanical environment, mediate the differentiation of mesenchymal stem cells into osteoblasts, fibroblasts, chondrocytes, and other types of cells as required to generate the appropriate tissue type [26]. Finally, during the remodeling phase, which takes place over a period of years, cell density and vascularity decrease, excess repair matrix is removed, and the collagen fibers of the repair matrix become oriented along lines of stress to maximize strength [26,27]. A major difference between soft- and hard-tissue healing is that the former heals by scar formation, whereas the latter typically does not [26,39].

Bone tissue is capable of true cellular, morphologic, and functional restoration. The initial phase in fracture healing is characterized by an inflammatory response and consolidation of hematoma within the fracture site. This consolidation of hematoma is followed by proliferation of periosteal, endosteal, and marrow cells adjacent to the fracture site and by recruitment of undifferentiated cells from nearby soft tissue. These cells, under the influence of various growth factors and environmental signals, differentiate to become chondrocytes and osteoblasts. The second phase is intramembraneous ossification, whereby new bone matrix is synthesized by osteocytes in the region of the fracture. The third phase is chondrogenesis, when chondrocytes appear and replace the soft callus with fibrous tissue. The fourth phase—endochondral ossification—begins with revascularization and leads to increased oxygen tension and subsequent osseous proliferation. The cartilage formed in the third phase will eventually be replaced by woven bone and undergo remodeling to form mature lamellar bone.

The factors that have been found to play an essential role in bone healing are TGF- $\beta$ , bone morphogenic proteins (BMP), fibroblast growth factors (FGFs), insulin-like growth factor (IGF), and PDGF. Because of the multiple growth factors present in the osseous microenvironment, synergistic and additive interactions may play an important role in regulating and mediating bone metabolism. These mediators have endocrine, paracrine, and autocrine functions.

PDGF has been studied intensively and found in platelets and osteoblasts. PDGF is released from platelets and macrophages at the initiation of bone healing and stimulates mesenchymal cell proliferation for the generation of new osteocytes. PDGF acts locally to promote protein and collagen synthesis. It causes

endothelial migration or angiogenesis to create new stroma to receive nutrients and promote fibroblastic proliferation and migration, formation of granular tissue, and re-epithelization and is chemotactic for mesenchymal cells [31,40]. In addition, it activates the release of TGF- $\beta$  from macrophages [31].

TGF- $\beta$  is released from platelets and osteoblasts; it has activities in bone tissue, connective tissue, and the immune system; and it belongs to the same superfamily as bone morphogenic protein. The broad range of cellular activities regulated by TGF- $\beta$ s includes the proliferation and expression of the chondrocytes and osteocytes and the promotion of angiogenesis. During endochondral ossification, the cells produce and secrete TGF- $\beta$  into the surrounding extracellular matrix. The concentration of TGF- $\beta$  in osteoblastic tissue and platelets has been found to be 100-fold greater than in other tissues. TGF- $\beta$  was found to increase osteogenesis and enhance bone ingrowth into mechanical fixation of implants inserted into trabecular bone in mature dogs [37,38].

Gandhi and colleagues [40] were able to measure 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 these proteins in the nonunion tissue of 7 patients presenting with nonunion of similar fractures. They prepared autologous platelet-rich plasma (PRP) and were able to measure high concentrations of these growth factors in the concentrate. After application of the PRP to the nonunions during revision surgery, radiographic union was observed by an average of 8.5 weeks. Such studies, although not randomized and prospective, provide evidence of the utility of platelet therapy applied to the foot and ankle in high-risk patients.

Fracture healing is largely controlled by local regulatory interactions among the cells and tissues near the site of injury; however, many systemic hormones, including insulin, glucocorticoids, and gonadal steroids, also influence the course of tissue repair, particularly in the case of pathologic hormone excess or deficiency. Patients who have diabetes demonstrate significant incidence of delayed union, nonunion, and pseudoarthrosis. There have been limited investigations evaluating the difference between the osseous healing processes in patients who have diabetes, but the studies that appear in literature have significant findings. Follak and colleagues [41] evaluated the histomorphology of bone healing in diabetic rats and found severe mineralization disorders associated with poor metabolic control.

Gooch and coworkers [42] found that in experimentally induced diabetic animals there was a significant difference in collagen formation and chondrocytes maturation in comparison to the controls. In addition, there was impaired healing of fractures that may be associated with changes in collagen expression and chondrocyte maturation. Gandhi and Berberian [31] measured the amount of PDGF and TGF- $\beta$  in the fracture hematoma of patients who had diabetes and those who did not. There were significantly lower amounts of these growth factors in the patients who had diabetes, suggesting at least a partial mechanism for the poorer healing response typical of these patients. These growth factors, which are locally produced within the fracture environment, play a critical role in

cellular chemotaxis, proliferation, extracellular matrix formation, and angiogenesis [43–45]. In addition, Baumhauer and colleagues [46] found an increase in the number of osteoclasts and cell mediators involved in bone resorption present in Charcot's bone specimens, which may have a detrimental effect on the healing in this patient population. The ideology behind the addition of autologous growth factors in these patients is to overcome the deficiencies and stimulate the healing pathways in an autologous manner. The healing mechanisms in a person who has diabetes appear to be impaired not only systemically but also locally.

PRP is produced using a single or dual centrifuge cycle to separate the PRP fraction from the balance, based on differential density [35,36,47–53]. Typically, a volume of PRP approximately equal to 10% of the volume of drawn blood is prepared [36]. The use of acid citrate dextrose–A anticoagulant and the use of low G forces during centrifugation help to preserve the integrity of the platelet membrane during processing [53,54]. Preservation of platelet viability is important so that the platelets can maintain function in situ and provide the mechanism by which the tertiary structure of at least some of the secretory proteins are completed on activation. Platelet fragmentation during processing could result in the release of high levels of proteins with compromised bioactivity [54]. Furthermore, platelet activation during processing should be kept to a minimum because although biologically active proteins would be secreted, they would be contained in the releasate and may not be transferred to the surgical bed when the clot is implanted. Lack of transference, however, may be a function of the mode of delivery (see later discussion) [50]. Platelet activation during processing can be quantified by measuring the level of the protein P-selectin residing on the platelet plasma membrane. This protein is normally contained on the inner surface of the alpha granule membrane [37]. On platelet activation, as the alpha granule membrane fuses with the platelet plasma membrane, P-selectin becomes expressed on the platelet surface, where it can be measured [37,50,54]. Thus, measurement of P-selectin in PRP is an important method of determining the quality of the product.

After the PRP is produced, it is stable in the anticoagulated state for 8 hours or longer [35,53,55]. Activation is typically performed by mixing the PRP with an activation solution formed by combining 1000 units of topical bovine thrombin per milliliter of 10% calcium chloride. Thrombin directly activates the platelets. Calcium ion is a necessary component to several of the steps of the coagulation cascade. Addition of calcium replaces that which was bound by citrate in the acid citrate dextrose–A anticoagulant to facilitate clot formation. In practice, 1 mL of activation solution is often mixed with 10 mL of PRP [27,50], which can be applied using a dual-spray system [56]. The PRP is placed in a 10-mL syringe and the activation solution is placed in a 1-mL syringe. The two syringes are connected to a dual-spray applicator tip. The two syringe plungers are advanced in tandem to create two overlapping sprays that mix the two fractions in the correct ratio as the sprays combine. In this manner, the PRP is activated as it reaches the surgical bed. Alternatively, Marx [57] described a technique in which 6 mL of PRP and 1 mL of activation solution are placed in a 10-mL syringe,

along with 1 mL of air to be used as a mixing bubble. After 6 to 10 seconds of agitation, the clot is formed and can be expressed from the syringe. Activation of the PRP, by way of the introduction of bovine thrombin and calcium, initiates the clotting cascade with the formation of fibrin and degranulation of the alpha granules. The alpha granules release serotonin, catecholamines, ADP, ATP, thromboxane A<sub>2</sub>, calcium, fibrinogen, fibronectin, factor V, von Willebrand factor, osteocalcin, PDGF, TGF- $\beta$ , insulin-like growth factor, platelet-derived angiogenesis factor, and platelet-derived endothelial cell growth factor [31,32,58–60]. These secretory proteins have autocrine and paracrine functions that stimulate the healing cascade.

In general, the degree of hematoma that forms in response to an injury is, at least on first approximation, proportional to the degree of trauma. The delivery of PRP to the wound site can be thought of as responding to the wound with a greater number of platelets than would otherwise have been physiologically produced. It is likely, however, that the effect of PRP on healing is a function of many variables, including platelet concentration, volume of PRP delivered, the extent and type of injury, and perhaps, the overall medical condition of the patient. For instance, if it is the number of platelets delivered that is important, it may be possible to partially offset a low concentration of platelets in the PRP with delivery of a large volume. Another issue is whether the fold-increase of platelets in the PRP over baseline is important versus the actual concentration in the PRP. Several investigators have suggested that the PRP should achieve a threefold to fivefold increase in platelet concentration over baseline [33,36,54]; however, following this recommendation, a patient who has a baseline platelet value of 150,000/mm<sup>3</sup> would require a PRP concentration in the range of 450,000 to 750,000/mm<sup>3</sup>, whereas a patient with a baseline value of 300,000/mm<sup>3</sup> would require 900,000 to 1,500,000/mm<sup>3</sup>. According to Marx [35,53], a platelet concentration of 1,000,000/mm<sup>3</sup> provides a “working definition” of PRP. Of course, to achieve this level, the fold-increase requirement will vary depending on the baseline level for a given patient. Finally, because it is the release of secretory proteins from platelets that accounts for much of their contribution to healing, it is possible that the desired concentration of platelets in PRP will vary from patient to patient and be a function of the secretory protein content present in the platelets. Several studies have demonstrated wide variability in platelet secretory protein levels in the general population [50,61–63]. Weibrich and colleagues [62] suggested that different individuals may require different concentration ratios to achieve comparable biologic effect. For reference, among several of the PRP concentrations systems available, platelet concentration ratios of less than 2 to 8.5 fold have been reported [33,35,50,54,57]. PRP instrumentations on the market include Sequestra 1000 (Medtronic, Parker, Colorado); Cell Saver 5 (Haemonetics, Braintree, Massachusetts); CATS (Fresenius USA, Walnut Creek, California); Compact Advanced and BRAT II (Sobe, Arvada, Colorado); SMART PreP (Harvest Technologies, Plymouth, Massachusetts); Symphony (DePuy, Warsaw, Indiana); AGF (Interpore Cross, Irvine, California) systems and GPS II (Cell Factor Technologies, Inc., Warsaw, Indiana).

Ultraconcentrator devices that also increase the concentration of the plasma proteins of the clotting cascade include Ultra Concentrator (Interpore Cross, Irvine, California) and the Access System (Interpore Cross, Irvine, California).

Despite the intuitive appeal of PRP, clinical application remains controversial due to the paucity of controlled clinical studies to conclusively support its use. Both soft- and hard-tissue controlled animal studies indicate the efficacy of PRP. For instance, Carter and colleagues [64] found that equine-derived PRP accelerated healing in a full-thickness cutaneous equine wound model compared with untreated controls. Fennis and coworkers [65] found an enhancement of bone healing compared with controls in a mandibular goat model. Most but not all published clinical studies are on the use of PRP in periodontal and oral surgery [36,57,66–72], and most of these show excellent clinical outcome. Perhaps the most compelling controlled clinical study supporting the use of PRP was reported by Marx [36]. Core biopsies were taken for placement of dental implants in 88 patients. Half received grafts containing PRP, whereas the other half received grafts without PRP. Graft sites in patients receiving PRP matured faster and had significantly greater trabecular bone area at 6 months.

During normal fracture healing, the hematoma provides an osteoconductive matrix and scaffold for the bone healing response. Growth factors act synergistically to stimulate bone healing and remodeling. PRP provides a supplementary source of fibrinogen and glycoproteins, such as fibronectin (an important regulator of osteoblast morphogenesis and differentiation), stem cell factor, and other factors that are critical to cell adhesion and growth. PRP, when mixed with graft material (allogenic or autogenic), not only acts to augment the maturation and incorporation of the graft but also acts as an extender to reduce the amount of graft material required. In addition, the fibrin gel provides an autogenous biodegradable substratum that is an ideal environment to enhance cell migration and attachment [34]. Fibrin provides an environment conducive to vascular invasion and promotes wound closure. PRP provides a relatively inexpensive and autologous source of numerous growth factors without antigenicity compared with genetically engineered growth factors.

PRP use is contraindicated in patients who have pre-existing coagulation defects (thrombocytopenia, hypofibrinogenemia, or on anticoagulant therapy) or have a hypersensitivity to bovine products.

In summary, the following statements can be made regarding PRP technology:

1. The local accumulation of activated platelets represents the initial phase of the natural wound healing response of the body.
2. Controlled animal studies and a limited number of controlled human studies indicate that increasing the local activated platelet level at the wound site by application of PRP can accelerate both soft- and hard-tissue healing.
3. There are many commercially available systems for creating PRP from autologous blood, with the systems differing in terms of the user interaction with the system, the efficiency with which platelet levels are in-

creased above baseline, and the impact of processing on platelet integrity and viability.

4. Much additional clinical work needs to be performed to substantiate the effects of the use of PRP on wound healing in general and to document differences in response to the use of PRP produced from different systems.

Advocates of the clinical use of PRP technology believe that the benefits include an increase in bone and wound healing and a decrease in postoperative infection, pain, and blood loss [48].

### **A retrospective comparison of the Symphony and the Interpore Cross AGF systems**

A retrospective study was performed on the comparison of the fusion rates of Charcot's foot reconstruction surgeries in 50 cases in which the Interpore Cross AGF system (Interpore Cross, Irvine, California) or the Symphony PRP concentration system (DePuy, Warsaw, Indiana) was used to facilitate fusion between June 1, 2000 and May 31, 2003. The procedures included Achilles tendon lengthening, reconstruction of the pathology with arthrodesis and realignment maintained by way of internal large-diameter screw fixation, the use of autologous growth factors (Interpore Cross AGF or Symphony) across the arthrodesis site or sites randomly, and the use of an external fixator (Ilizarov hybrid frame) to compress the fusion site or sites. The senior author performed all the surgical procedures with the use of Interpore Cross AGF system (24 cases) or the Symphony system (26 cases). The cases consisted of 14 ankle/tibiocalcaneal fusions, 14 triple arthrodeses, 12 midtarsal arthrodeses, and 10 Lisfranc's joint arthrodeses through the use of a combination of allogenic and autogenic bone graft, autogenic growth factors, and internal fixation (Table 1).

The use of autologous growth factors enhances bone fusion through osteo-inductive properties. Growth factors are morphogens that activate an endogenous cascade of events to stimulate bone growth. In the past, autologous growth factors have been used to increase the rates of fusion through the stimulation of biochemical pathways in the healing process.

Table 1  
Distribution of diabetic salvage procedures

Distribution of procedures	Symphony	AGF	Solid fusion (%)
14 Ankle/tibiocalcaneal fusion	8	6	43
14 Triple arthrodesis	3	11	85
12 Midfoot (midtarsal arthrodeses)	9	3	72
10 Lisfranc's joint arthrodeses	6	4	83

When considering the patient population presenting with this deformity, the investigator must be alert to the multiplicity of disease states in these individuals. These disease states include but are not limited to diabetes, obesity, renal disease, dysvascularity, malnutrition, peripheral neuropathy, and the presence or absence of osteomyelitis. These entities, individually and in combination, impair healing of the operative procedure. Risk factors that are associated with reduction in fusion rates are smoking, diabetes mellitus, steroid use, prior pseudoarthrosis, prior infection, and poor nutrition.

The Interpore Cross AGF system is a hollow-fiber hemoconcentrator that uses 60 mL of the product obtained from an intraoperative salvage device or 450 to 475 mL of whole blood. A specific system must be used to provide housing for the syringes, hemoconcentrator, and air flow. This process increases the platelet concentration and the concentration of the growth factors by 7 to 10 times the hemodynamic baseline. The entire process takes 25 minutes for the initial 20 mL of the concentrated platelet product.

The Symphony system uses 50 to 60 mL of whole blood that is added to the first chamber and centrifuged. The red blood cells and the PRP are separated, with the PRP automatically being decanted into a second chamber, whereby the platelet concentration is further concentrated by centrifuge and the use of a floating shelf. The platelet-poor plasma is discarded and the remaining platelets are resuspended. This process produces 20 to 35 mL of platelet concentrate at 5 to 8 times the hemodynamic baseline. The entire process takes 12.5 minutes for the initial 20 mL of the concentrated platelet product and can produce multiple products at a given time.

In the retrospective study, the incidence of solid fusion of the joints were as follows: ankle/tibiocalcaneal, 43% triple arthrodesis, 85%; midtarsal, 72%; and Lisfranc's, 83% (see Table 1). Evaluation of the results showed an 83% fusion rate with the use of Interpore Cross AGF and a 62% fusion rate with Symphony (Table 2). This patient population showed an increased rate of fusion with the use of autologous growth factor compared with the results found in literature. Papa and colleagues [73] found nine pseudoarthroses in 29 patients for arthrodesis of Charcot's joints, with a 31% nonunion rate. In a study by Stuart and Morey [74], 38% of the patients who had neuropathic arthropathy received a satisfactory ankle fusion. Only 40% of patients in a study by Samilson and coworkers [75] went on to ankle fusion. In the authors' retrospective study, there was a success rate of 43% for the ankle/tibiocalcaneal fusion group, which in this population is

Table 2  
Comparison of fusion rates with the utilization of Interpore Cross AGF and Symphony

System	Complete fusion	Incomplete fusion	Total	Percentage fused
Interpore Cross AGF	20 cases	4 cases	24 cases	83
Symphony	16 cases	10 cases	26 cases	62

Chi-Square Test,  $p = 0.162$ .



Fig 1. Blood draw of 55 mL. (Symphony, DePuy, Warsaw, IN.)

the most difficult salvage procedure (the realignment of the foot on the leg to create a plantigrade platform for propulsion).

Experimental studies comparing Symphony with Interpore Cross AGF show a difference in the quality of the product produced. Kevy and Jacobson [33] compared the various instrumentations available for the preparation of autologous growth factors, and Kevy and colleagues [60] evaluated Interpore Cross AFG and Symphony in another study. These studies found that Symphony demonstrated the greatest percentage yield of intact platelets (72%), with a fourfold increase over baseline. In addition, Symphony had the least number of procedural steps and the most reproducible results. Interpore Cross AGF was found to have the lowest yield of intact platelets, with a 0.6-fold increase over baseline and 15 procedural steps. The transfer of the platelet through the hollow-fiber hemoconcentrator resulted in platelets that were fragmented, clumped, or lost to



Fig 2. Blood centrifuged for PRP. (Symphony, DePuy, Warsaw, IN.)



Fig 3. PRP coating the bone graft material. (Symphony, DePuy, Warsaw, IN.)

binding to the vessel walls, creating a product of platelet releasat rather than viable platelet concentration. In a direct comparison of these two systems, Symphony produced a higher yield of PDGF and TGF- $\beta$  when challenged with ADP than did Interpore Cross AGF. Kevy and colleagues [61] stated that for benefits of the autologous growth factors, a high concentration of normally functioning platelets with direct release of platelet-associated growth factors into the defect site is optimal.

The authors' comparison of clinical outcomes, however, found that this might not be the case. In their diabetic patient population, there exists an intrinsic loss of healing potential, with a noted decrease in local mediators and response to injury [31,46,76–79]. Overall, treatment using platelets produced from the Interpore



Fig 4. PRP bone graft amalgam for insertion into defect site. (Symphony, DePuy, Warsaw, IN.)

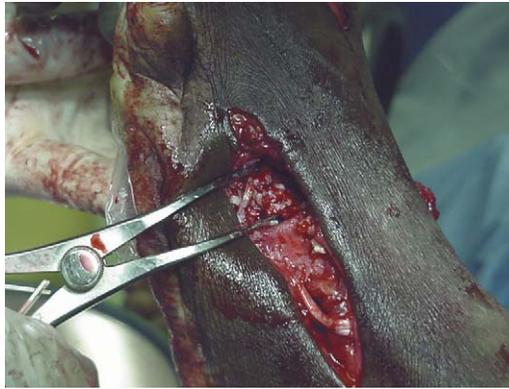


Fig 5. Placement of PRP bone graft amalgam into defect site. (Symphony, DePuy, Warsaw, IN.)

Cross AGF system resulted in a greater average rate of fusion than did corresponding treatment using the Symphony system, although this difference was not statistically significant. The earlier studies cited that the Symphony system tended to produce a greater percentage of intact platelets than did the Interpore Cross AGF system [33,60]. Despite this, the latter treatment was associated with a fusion rate that was comparable, or better, than that using Symphony-derived PRP or historical controls [73–75]. It is important to note that in the compromised patients of this population, i.e., a long duration of diabetes, there are marked changes in the morphology and the biochemistry of the osseous environment [31,46,76–79]. It is possible that such changes may favor treatment with a PRP releasate whose growth factors are immediately available to induce the healing process. While the nature of tissue healing is understood in basic form, much detail remains to be elucidated. As understanding of this process becomes further refined, improved interventions will become apparent and integrated into clinical practice. Currently, platelet technology, both theoretically and prac-



Fig 6. Preoperative radiograph of patient MB showing complex Charcot's fracture dislocation of the midtarsal and Lisfranc's joints.



Fig 7. Anteroposterior view of patient MB.

tically, appears to have clinical merit as an augmentation of the body's natural healing response.

### Case presentations

#### *Case 1*

DB, a 29-year-old man who had type 1 diabetes mellitus since the age of 8 years and a history of diabetic neuropathy, three prior ulcerations, hyperlipidemia, and anemia, developed an ulceration on the plantar lateral aspect of the right foot with cellulitis and subsequent osteomyelitis of the fifth ray. DB underwent a fifth-ray resection for osteomyelitis and was treated with parenteral



Fig 8. Patient MB at 12 weeks postoperative status after frame removal. Note ensuing fusion and bone consolidation. (AGF, Interpore Cross, Irvine, CA.)



Fig 9. Patient MB at 12 weeks postoperative, an anteroposterior view. (AGF, Interpore Cross, Irvine, CA.)

antibiotics for 6 weeks. He was later evaluated with a Ceretec Scan and found negative for osteomyelitis. DB subsequently developed a severe Charcot's foot deformity, which limited ambulation. The left Charcot's foot was stabilized surgically with a reconstructive procedure that used Tendo Achilles lengthening, midfoot and Lisfranc's joint fusion by way of internal fixation and application of autologous growth factors (Symphony) with bone graft (Figs. 1–5), and application of an external fixator (Ace DePuy, DePuy, Warsaw, Indiana) applied with bent-wire Ilizarov principles in February 2002. Postoperative films reveal the graft material saturating areas of bone defects. Twelve-week postoperative radiographs showed incorporation of bone graft and healing across the osteotomy site. Within 6 months of the surgical reconstruction of the deformed left Charcot's foot, DB had a stable plantigrade platform for propulsion that fit into standard shoe gear.



Fig 10. Intraoperative radiograph of patient AA demonstrating lack of viable bone medially secondary to severe Charcot's fracture and dislocation.



Fig 11. Intraoperative lateral view of patient AA demonstrating defect engendered by severe Charcot's fracture and dislocation.

### Case 2

MB, a 48-year-old man who had type 2 diabetes mellitus for 19 years and a history of hypertension, developed edema, erythema, calor, and deep pain to his left foot in April 2004 and was placed on antibiotic treatment for cellulitis. In June 2004, MB was referred to the senior author's office for treatment of Charcot's neuroarthropathy of the left foot. In September 2004, the left Charcot's foot was stabilized surgically with a reconstructive procedure that used Tendo Achilles lengthening and Lisfranc's joint and midtarsal arthrodesis with internal fixation and application of autologous growth factors (Interpore Cross AGF) with bone graft. A subtalar arthrodesis procedure was performed to realign his medial column, and this was compressed with application of an external fixator (Stryker



Fig 12. Intraoperative radiograph of patient AA subsequent to large-diameter screws bridging the defect and autologous growth factor bone graft amalgam filling the void. (AGF, Interpore Cross, Irvine, CA.)



Fig 13. Postoperative radiograph of patient AA. Note ensuing fusion and production of bone from autologous growth factor bone graft amalgam (Interpore Cross AGF).

Orthopaedics, MahWah, New Jersey) (Figs. 6–9). Intraoperative films showed grafting materials bridging the void and anatomic alignment of the medial and lateral arches of the foot. MB underwent frame removal in December 2004 and was placed in a posterior splint with continued non-weight bearing to the left lower extremity. Radiographic and clinical evaluation in January 2005 revealed a stable triple arthrodesis, with an anatomically aligned reconstructed foot for propulsion. MB was placed in a Charcot restraint orthotic walker (CROW) boot and permitted to weight bear. After being evaluated in April 2005, he was returned to normal footwear.

### Case 3

AA, a 45-year-old male pediatrician who had type 2 diabetes mellitus for 25 years and a history of diabetic retinopathy, nephropathy, and neuropathy,



Fig 14. Patient AA at 12 weeks postoperative. Note ensuing solid fusion from autologous growth factor bone graft amalgam (Interpore Cross AGF).



Fig 15. GPS II centrifuge. (Biomet, Warsaw, IN.)

hypertension, and cardiovascular disease, developed edema, erythema, calor, and deep pain to his right leg in October 2000. AA was initially treated with parenteral antibiotics to no avail. In November 2000, AA presented to the senior author's office and a diagnosis of Charcot's arthropathy of the right foot was determined. In November 2000, the right Charcot's foot was stabilized surgically with a reconstructive procedure that used Tendo Achilles lengthening, triple arthrodesis with internal fixation and application of autologous growth factors (Interpore Cross AGF) with bone graft, and the application of an external fixator (Ace DePuy) (Figs. 10–14). Intraoperative films showed grafting materials bridging the void. AA underwent frame removal in February 2001. Radiographic and clinical evaluation in April 2001 revealed a stable triple arthrodesis with a planigrade platform for propulsion. Follow-up in February 2005 revealed a solid stable foot with no collapse of the medial longitudinal arch.



Fig 16. Note central buffy coat of PRP for autologous growth factor. (Biomet, Warsaw, IN.)



Fig 17. PRP bone graft amalgam inserted across Lisfranc's joint for fusion in Charcot's subluxation and collapse in patient DR. (Biomet, Warsaw, IN.)

#### Case 4

Recently, a new system for platelet concentration has become available [50]. This system, used in the treatment of this patient, was not included in the statistical analysis cited above. DR, a 58-year-old man who had type 2 diabetes mellitus for 25 years and a history of diabetic neuropathy, hypertension, chronic renal failure, hyperlipidemia, and asthma presented with severe pain and swelling of his left lower extremity in April 2005. A diagnosis Charcot's arthropathy of Lisfranc's joint of the left foot was made. In April 2005, the left Charcot's foot was stabilized surgically with a reconstructive procedure that used Tendo Achilles lengthening, Lisfranc's joint arthrodesis with internal fixation and



Fig 18. Application of hybrid Ilizarov-type bent-wire foot plate and modified CAM boot (EZ-Frame) for compression of the fusion site in patient DR. (EZ-Frame, Signal Medical Corp., Port Huron, Michigan.)

application of autologous growth factors (GPS-II, Biomet Co., Warsaw, Indiana) with bone graft, and the application of an external fixator (EZ-Frame, Signal Medical Corp., Port Huron, Michigan). Intraoperative films showed grafting materials bridging the joint and realignment of the Lisfranc's joint (Figs. 15–18).

The GPS-II system uses 50 to 60 mL of whole blood that is added to the first chamber and centrifuged. This technology uses a modified centrifugation tube that contains a buoy to separate the red blood cell plasma interface and to increase the concentration of decanted platelets. The entire process takes 12 minutes and produces a concentration of 6.0 mL per process.

## References

- [1] Segal KR. Type 2 diabetes and disease management: exploring the connections. *Dis Manage* 2004;7(Suppl 1):S11–22.
- [2] Ross MA. Neuropathies associated with diabetes. *Med Clin N Am* 1993;77(1):111–24.
- [3] Pinzur M. Surgical vs. accommodative treatment for Charcot arthropathy of the midfoot. *Foot Ankle Int* 2004;25(8):545–9.
- [4] Vinik AI. Management of neuropathy and foot problems in diabetic patients. *Clin Cornerstone* 2003;5(2):38–55.
- [5] Sinacore DR. Acute Charcot arthropathy in patients with diabetes mellitus: healing times by foot location. *J Diabetes Complications* 1998;12(5):287–93.
- [6] Heckman JD. Fractures and dislocations of the foot. In: Rockwood Jr CA, Bucholz RW, Green DP, et al, editors. *Fracture in adults*. New York: Lippincott-Raven; 1996. p. 2267–405.
- [7] Armstrong DG, Todd WF, Lavery LA, et al. The natural history of acute Charcot's arthropathy in diabetic foot specialty clinic. *Diabet Med* 1997;14:357–63.
- [8] Sander LJ, Frykberg RG. The Charcot foot. In: Frykberg RG, editor. *The high risk foot in diabetes mellitus*. New York: Churchill Livingstone; 1991. p. 525–35.
- [9] Fabrin J, Larsen K, Holsein PE. Long term follow-up in diabetic Charcot feet with spontaneous onset. *Diabetes Care* 2000;23(6):796–800.
- [10] Childs M, Armstrong DG, Edelson GW. Is Charcot arthropathy a late sequela of osteoporosis in patients with diabetes mellitus? *J Foot Ankle Surg* 1998;37:437–9.
- [11] Young MJ, Marshall A, Adams JE, et al. Osteopenia, neurological dysfunction, and the development of Charcot neuropathy. *Diabetes Care* 1995;18:34–8.
- [12] Gough A, Abraha H, Li F, et al. Measurement of markers of osteoclast and osteoblast activity in patients with acute and chronic diabetic Charcot neuroarthropathy. *Diabet Med* 1997;14: 527–31.
- [13] Grant WP, Sullivan R, Sonenshine DE, et al. Electron microscopic investigation on the effects of diabetes mellitus on Achilles tendon. *J Foot Ankle Surg* 1997;36(4):272–8.
- [14] Grant WP, Foreman EJ, Wilson AS, et al. Evaluation of Young's modulus in Achilles tendon with diabetic neuroarthropathy. *J Am Podiatr Med Assoc* 2005;95(3):242–6.
- [15] Rajbhandari SM, Jenkins RC, Davies C, et al. Charcot neuropathy in diabetes mellitus. *Diabetologia* 2002;45:1085–96.
- [16] Myerson MS. Evaluation of the diabetic neuroarthropathy guides to treatment. *Biomechanics* 1999;9:37–46.
- [17] Wang JC, Le AW, Tsukuda RK. A new technique for Charcot's foot reconstruction. *J Am Podiatr Med Assoc* 2002;92(8):429–36.
- [18] Catanzanti AR, Blich EL, Karlock CG. Elective foot and ankle surgery in the diabetic patient. *J Foot Ankle Surg* 1995;34:22–41.
- [19] Trepman E, Nihal A, Pinzur MS. Current topics review: Charcot neuroarthropathy of the foot and ankle. *Foot Ankle Int* 2005;26(1):46–63.

- [20] Caputo GM, Ulbrecht J, Cavanagh PR, et al. The Charcot foot in diabetes: six key points. *Am Fam Physician* 1998;57(11):2705–10.
- [21] Pinzur MS. Charcot's foot. *Foot Ankle Clin* 2000;5(4):897–912.
- [22] Herbst SA. External fixation of Charcot arthropathy. *Foot Ankle Clin* 2004;9(3):595–609.
- [23] Pakarinen TK, Laine HJ, Honkonen SE, et al. Charcot arthropathy of the diabetic foot. Current concepts and review of 36 cases. *Scand J Surg* 2002;91(2):195–201.
- [24] McCormack RG, Leith JM. Ankle fractures in diabetics. Complications of surgical management. *J Bone Joint Surg Br* 1998;80(4):689–92.
- [25] Flynn JM, Rodriguez-del Rio F, Piza PA. Closed ankle fractures in the diabetic patient. *Foot Ankle Int* 2000;21(4):311–9.
- [26] Buckwalter JA, Einhorn TA, Bolander ME, et al. Healing of musculoskeletal tissues. In: Rockwood Jr CA, Buchholz RW, Green DP, et al, editors. *Fractures in adults*. Philadelphia: Lippincott-Raven; 1996. p. 261–304.
- [27] Bhanot S, Alex JC. Current applications of platelet gels in facial plastic surgery. *Facial Plast Surg* 2002;18(1):27–33.
- [28] Anderson JM. The cellular cascades of wound healing. In: Davies JE, editor. *Bone engineering*. Toronto, Canada: em squared; 2000. p. 81–93.
- [29] Conley CL. Hemostasis. In: Mountcastle VB, editor. *Medical physiology*. St. Louis, Missouri: C.V. Mosby Co.; 2004. p. 1137–46.
- [30] Anitua E, Andia I, Ardanza B, et al. Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost* 2004;91(1):4–15.
- [31] Gandhi A, Berberian WS. Reductions in growth factors in the fracture hematoma of diabetic patients. Presented at the 49th Annual Meeting of the Orthopaedic Research Society. New Orleans, LA, February 2–5, 2003.
- [32] Pietrzak WS, Eppley BL. Platelet rich plasma: Biology and new technology. *J Craniofac Surg* 2006, In press.
- [33] Kevy SV, Jacobson MS. Comparison of methods for point of care preparation of autologous platelet gel. *J Extra Corpor Technol* 2004;36:28–35.
- [34] Lettice JJ, Kula TA, Kelly J, et al. Autologous growth factors (AGF). Presented at the 34th Annual Meeting of the Scoliosis Research Society. San Diego, CA, September 23–25, 1999.
- [35] Marx RE. Platelet-rich plasma: evidence to support its use. *J Oral Maxillofac Surg* 2004;62:489–96.
- [36] Marx RE. Platelet concentrate: a strategy for accelerating and improving bone regeneration. In: Davies JE, editor. *Bone engineering*. Toronto, Canada: University of Toronto; 2000. p. 447–53.
- [37] Harrison P, Cramer EM. Platelet alpha-granules. *Blood Rev* 1993;7:52–62.
- [38] Tischler M. Platelet rich plasma. The use of autologous growth factors to enhance bone and soft tissue grafts. *N Y State Dent J* 2002;68:22–4.
- [39] Roseborough IE, Grevious MA, Lee RC. Prevention and treatment of excessive dermal scarring. *J Natl Med Assoc* 2004;96:108–16.
- [40] Gandhi A, Van Gelderen J, Berberian WS, et al. Platelet releasate enhances healing in patients with a non-union. Presented at the 49th Annual Meeting of the Orthopaedic Research Society. New Orleans, LA, February 2–5, 2003.
- [41] Follak N, Kloting L, Wolf E, et al. Delayed remodeling in early period of fracture healing in spontaneous diabetic BB/OK rats depending on the diabetic state. *Histol Histopathol* 2004;19(2):473–86.
- [42] Gooch HL, Hale JE, Fujioka H, et al. Alterations of cartilage and collagen expression during fracture healing in experimental diabetes. *Connect Tissue Res* 2000;41(2):81–91.
- [43] Trippel SB, Coutts RD, Einjom TA, et al. Growth factors as therapeutic agents. *J Bone Joint Surg Am* 1996;78(8):1272–82.
- [44] Joyce ME, Jingushi S, Scully SP, et al. Role of growth factors in fracture healing. *Prog Clin Biol Res* 1991;365:391–416.
- [45] Lind M. Growth factors: possible new clinical tools. *Acta Orthop Scand* 1996;67(4):407–17.
- [46] Baumhauer JF, O'Keefe R, Schon LC, et al. Free cytokine induced osteoclastic bone resorption

- in Charcot arthropathy: an immunohistochemical study. *American Orthopaedic Foot and Ankle Society*; 2004.
- [47] Wilson RM. Clinical use of autologous platelet gel: hemostasis and wound sealing. Presented at the Proactive hemostasis management: The emerging role of platelets symposium. Aspen, CO, Jan 23–24, 1997.
- [48] Waters JH, Roberts KC. Database review of possible factors influencing point-of-care platelet gel manufacture. *J Extra Corpor Technol* 2004;36:250–4.
- [49] Marlovits S, Mousavi M, Gabler C, et al. A new simplified technique for producing platelet-rich plasma: a short technical note. *Eur Spine J* 2004;13(S1):S102–6.
- [50] Eppley BL, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. *Plast Reconstr Surg* 2004;114:1502–8.
- [51] Kevy SV, Jacobson MS, Blasetti L, et al. Preparation of growth factor enriched autologous platelet gel. *Society for Biomaterials 27<sup>th</sup> Annual Meeting Transactions*, St. Paul, MN, April 24–29, 2002, paper 262.
- [52] Welsh W. Autologous platelet gel-clinical function and usage in plastic surgery. *Cosmet Dermatol* 2000;13:13–8.
- [53] Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? *Implant Dent* 2001;10:225–8.
- [54] Gonshor A. Technique for producing platelet-rich plasma and platelet concentrate: background and process. *Int J Periodontics Restorative Dent* 2002;22:547–57.
- [55] Anderson NA, Pamphilon DH, Tandy NJ, et al. Comparison of platelet-rich plasma collection using the Haemonetics PCS and Baxter Autopheresis C. *Vox Sang* 1991;60:155–8.
- [56] Man D, Plosker H, Winland-Brown JE. The use of autologous platelet-rich plasma (platelet gel) and autologous platelet-poor plasma (fibrin glue) in cosmetic surgery. *Plast Reconstr Surg* 2001;107(1):229–37.
- [57] Marx RE, Carlson ER, Eichstaedt RM, et al. Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998;85:638–46.
- [58] Lowery GL, Kulkarni S, Pennisi AE. Use of autologous growth factors in lumbar spinal fusions. *Bone* 1999;25(2):47S–50S.
- [59] Slater M, Patava J, Kingham K, et al. Involvement of platelets in stimulating osteogenic activity. *J Ortho Res* 1995;13:655–63.
- [60] Kevy SV, Jacobson MS, Lazar R. Quantitative and qualitative analysis of autologous platelet products prepared using Symphony™ and Interpore Cross AGF™ system: a comparative study. 2002.
- [61] Logan JB, Dietz DD, Giuffrè JM. Autologous bone augmented with autologous growth factors and resorbable porous ceramic for posterolateral lumbar fusion: a preliminary report. *International Meeting on Advanced Spine Technologies*. Paradise Island, Bahamas, July 12–14, 2001.
- [62] Weibrich G, Kleis WKG, Kunz-Kostomanolakis M, et al. Correlation of platelet concentration in platelet-rich plasma to the extraction method, age, sex, and platelet count of the donor. *Int J Oral Maxillofac Implant* 2001;16(5):693–9.
- [63] Zimmermann R, Arnold D, Strasser E, et al. Sample preparation technique and white cell content influence the detectable levels of growth factors in platelet concentrates. *Vox Sang* 2003; 85:283–9.
- [64] Carter CA, Jolly DG, Worden Sr CE, et al. Platelet-rich plasma gel promotes differentiation and regeneration during equine wound healing. *Exp Mol Pathol* 2003;74(3):244–55.
- [65] Fennis JP, Stoelinga PJ, Jansen JA. Mandibular reconstruction: a clinical and radiographic animal study on the use of autogeneous scaffolds and platelet-rich plasma. *Int J Oral Maxillofac Surg* 2002;31(3):281–6.
- [66] Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. *J Oral Implantol* 1999;14:529–35.
- [67] Della VA, Sammartino G, Marenzi G, et al. Prevention of postoperative bleeding in anticoagulated patients undergoing oral surgery: use of platelet-rich plasma gel. *J Oral Maxillofac Surg* 2003;61:1275–8.

- [68] Froum SJ, Wallace SS, Tarnow DP, et al. Effect of platelet-rich plasma on bone growth and osseointegration in human maxillary sinus grafts: three bilateral case reports. *J Periodontol* 2002;22:45–53.
- [69] Kassolis JD, Rosen PS, Reynolds MA. Alveolar ridge and sinus augmentation utilizing platelet-rich plasma in combination with freeze-dried bone allograft: case series. *J Periodontol* 2000; 71:1654–61.
- [70] Petrunaro PS. Using platelet-rich plasma to accelerate soft tissue maturation in esthetic periodontal surgery. *Compend Contin Educ Dent* 2001;22(9):729–46.
- [71] Robiony M, Polini F, Costa F, et al. Osteogenesis distraction and platelet-rich plasma for bone restoration of the severely atrophic mandible: preliminary results. *J Oral Maxillofac Surg* 2002;60:630–5.
- [72] Soffer E, Ouhayoun JP, Anagnostou F. Fibrin sealants and platelet preparations in bone and periodontal healing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003;95:521–8.
- [73] Papa J, Meyerson M, Girard P. Salvage with arthrodesis in intractable neuropathic arthropathy of the foot and ankle. *J Bone Joint Surg Am* 1993;75(7):1056–66.
- [74] Stuart MJ, Morey BF. Arthrodesis of diabetic neuropathic foot. *Clin Orthop* 1990;253:209–11.
- [75] Samilson RL, Sankaran B, Bersani FA, et al. Orthopedic management of neuropathic joints. *AMA Arch Surg* 1959;78:115–21.
- [76] Bouillon R, Bex M, Van Hereck E, et al. Influence of age, sex, and insulin on osteoblasts function: osteoblasts dysfunction in diabetes mellitus. *J Clin Endocrinol Metab* 1995;80(4): 1194–202.
- [77] Reddy GK, Stehno-Bittel L, Hamade S, et al. The biomechanical integrity of bone in experimental diabetes. *Diabetes Res Clin Pract* 2001;54(1):1–8.
- [78] Lin SS. Impaired bone healing in patients with diabetes mellitus. Available at: <http://www.theuniversityhospital.com/trauma/impaired.htm>. Accessed April 14, 2005.
- [79] Margolis DJ, Kantor J, Santanna J, et al. Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. *Diabetes Care* 2001;24:483–8.