JECT. 2010;42:20–29
The Journal of ExtraCorporeal Technology

Application of Autologous Derived-Platelet Rich Plasma Gel in the Treatment of Chronic Wound Ulcer: Diabetic Foot Ulcer

Akinfemi Ayobami Akingboye, MRCS;*§ Stephen Giddins, ACP;† Philip Gamston, FCCP;† Arthur Tucker, PhD;‡ Harshad Navsaria, PhD;* Constantions Kyriakides, FRCS, MD§

**Centre for Cutaneous Research, Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom; †Department of Clinical Perfusion Science, St. Bartholomew Hospital, West Smith Field, London, United Kingdom; ‡Microvascular Unit, Ernest Cooke Laboratory, St. Bartholomew Hospital, West Smith Field, London, United Kingdom; and §Vascular Surgery Unit, Department of General Surgery, Barts and The London Hospitals, NHS Trust, Whitechapel, London, United Kingdom*

Abstract: The treatment of chronic wounds remains problematic, despite new insight into the cellular and molecular basis of wound healing. Although the aetio-pathogenesis of chronic wounds is said to be multi-factorial, it is evident from literature that effective and adequate wound debridement has produced the most consistent effect in chronic wound treatment. There is a growing body of evidence that suggests that wound healing in chronic diabetic foot ulcers is growth factor dependent and that the therapeutic delivery of these growth factors to wounds topically, has the potential ability to accelerate wound healing in conjunction with conventional wound care. Autologous derived platelet concentrate is activated to release growth factors that are stored in the platelet granules. These secretory proteins include cytokines and growth factors such as transforming growth factor–beta, vascular endothelia growth factor, platelet derived growth factor, and so on. The enhancement of soft tissue healing by the application of autologous derived platelet rich plasma gel (APG) is supported by basic science and some clinical studies. This review article will attempt to provide a concise report of current concepts on the use of APG in treating chronic ulcers.
Keywords: autologous derived platelet rich plasma, diabetic foot ulcer, growth factors, wound healing. *JECT. 2010;42:20–29*

Chronic wounds pose a major management problem in the health sector often requiring a multi-disciplinary approach. The majority (90%) of chronic wounds result as a progression from diabetic wounds, venous ulcers (chronic venous insufficiency ulcers), and pressure sores. Diabetic foot ulcers (DFU) seem to account the most for the global burden of the disease, especially with the attending complications of recurrence, chronicity, and the resultant lower limb amputation (1).

There is a global increase in the prevalence of diabetes mellitus, particularly type II diabetes mellitus, which is directly linked with obesity and a sedentary life style. It is believed that 20% or more of the United Kingdom adult population over the age of 65 years suffer with type II diabetes mellitus (2). Two studies from North European countries reported the annual incidence of foot ulcers in the general population to be just more than 2% (3). The annual incidence rates in diabetic neuropathic individuals vary from 5–7% (4). The lifetime risk of a person with diabetes having a foot ulcer could be as high as 25% (5). Of this population, 15% will either suffer foot or limb amputation (5). It is likely that the cumulative lifetime incidence of foot ulcers may be as high as 25% (5). Shearer et al. confirmed that diabetic patients with neuropathic risk factor incur five times more direct medical costs for ulcers and amputations, and live for 2 months less than individuals without neuropathy (6). Lower limb amputation is performed 15 times more frequently among diabetics, as compared with non-diabetic patients (7).

The American Diabetic Association did a cost analysis of medical expenditure for the United States population with and without diabetes in 2002, which was based on national health care survey data. The direct and indirect expenditure attributable to diabetes was estimated at 132 billion U.S. dollars. The direct medical expenditure alone totaled 91.8 billion U.S. dollars (23.2 billion U.S. dollars for diabetic care, 24.5 billion for chronic complications, and 44.1 billion U.S. dollars for excess prevalence of general medical conditions). The attributable indirect expenditure

Received for publication October 23, 2008; accepted September 27, 2009.
Address correspondence to: Akinfemi Ayobami Akingboye, Vascular Surgery Unit, Department of General Surgery, Barts and The London School of Medicine and Dentistry, White Chapel, London, E1 1BB. E-mail: akinfemi.akingboye@bartsandthelondon.nhs.uk
The senior author has stated that authors have reported no material, financial, or other relationship with any healthcare-related business or other entity whose products or services are discussed in this paper.

resulting from lost workdays, restricted activity days, mortality, and permanent disability due to diabetes totaled 39.8 billion U.S. dollars (8,9).

Fundamentally, the development of diabetic foot ulcers and the resultant chronicity could be said to have resulted from peripheral vasculopathy, peripheral neuropathy, and immunopathy. There are other contributory factors which stem from these three major risk factors. These include repetitive trauma to areas of the foot exposed to moderate to high pressure, reduced resistance to infection, immunologic disturbances, and microcirculation alterations.

Different authors and researchers have attempted to classify diabetic wounds from the view point of aetiopathogenesis and failure to demonstrate significant improvement following good clinical care as a guide to determine poor outcome. Presently, there are no early predictive factors to guide clinicians to differentiate patients who will heal readily from those who will have a protracted course of treatment. The American Diabetic Association have attempted to offer a lasting solution by clarifying the definition of a chronic wound, which they defined as "failure to show continuous progress towards healing. These are wounds that remained unhealed after 4 weeks, were a source of concern and were associated with worse outcomes, including amputation" (10). The decision to change to advanced therapeutic intervention should not be based solely on timing, but also in conjunction with careful monitoring of the percentage wound closure. A reduction in wound area of 10–15% per week represents a normal healing process and should not necessitate a change in wound management strategy or re-evaluation (11).

Other authors have demonstrated that chronic diabetic wounds lack significant growth factors which are stimulatory agents for wound healing. These growth factors play important roles in the tissue-remodeling phase through mesenchymal cell recruitment and extra matrix synthesis. This understanding has led to the development of recombinant platelet derived growth factor-BB (becaplermin) that has been approved for use and has shown a degree of success in treating DFU (12). Some authors have shown improved rate of wound healing in acute and chronic wounds following the use of autologous platelet rich plasma gel (Table 1). This review article aims at examining the role of the delivery system of multiple growth factors in healing chronic diabetic foot ulcers.

Aetio-Pathogenesis of Diabetic Foot Ulcers

The physiological alteration in diabetic foot ulcers could partly explain the chronicity and/or the recurrence of the ulcers. The triad of neuropathy, vasculopathy, and immunopathy forms the cardinal point on which the chronicity of diabetic wounds rest (1,13). The neuropathic effect on the foot produces a biomechanical abnormality, which could predispose areas of concentrated pressure to significant risk of ulceration, repetitive trauma, vasodilatation, and decreased sweating (14). Atherosclerotic plaque deposit contributes to the development of diabetic peripheral vasculopathy resulting in either occlusion or stenosis of the vessels. This causes decreased blood flow and ultimately ischemia will ensue. Infection has more deleterious effect in this group of patients, whose impaired immunity increase their risk for local and systemic infections. The wound infections may either be superficial or deep and are commonly poly-microbial in etiology. Superficial wound infections may be treated with antibiotic therapy only whilst deep-seated wound infections might require surgical debridement which may be followed by antibiotic and or other wound care adjunct.

Physiological Changes in Diabetic Foot Ulcers

Aggressive sharp wound debridement is believed to convert chronic wounds to acute ones and allows growth factors to function more effectively. This allows the wound to progress through the normal phases of wound healing. Regular and frequent wound debridement removes collagenase, matrix metalloproteinase, and elastase which are inhibitors of wound healing (15,16).

The cellular and molecular alteration has a direct link with the major physiological changes mentioned earlier and there is evidence to support hyperglycaemia—related

Table 1. Summary of clinical studies investigating the use of PRP and PPP in treating chronic non-healing wounds.

Authors	Study Design	Results
Knighton DR, et al.	32 patients, prospective randomized, controlled, blinded, crossover study	By 8 weeks, 81% of treated group had 100% epithelialization, 15% of the controls did ($p < .0001$); All of the remaining 75% in the placebo group achieved complete healing after crossover (57).
Ganio C, et al.	Conventional treatment of patients, 171 patients presented with 355 wounds of average duration of 75 weeks	100% epithelization achieved at 10 weeks, with 78% limb salvage rate for at-risk patients (58).
Driver, et al.	RCT, blinded, multicentre study involving 72 patients treated over 12 weeks	13 of 19 patients treated with PRP had total re-epithelization; 9 of 21 of the control (42%) also re-epithelized (65)
Yuan, et al.	A plot study of 13 refractory diabetic dermal ulcers were treated with PRP	69.2% were cured; significant reduction in ulcer size after 3 weeks of initial treatment

deleterious molecular and cellular alteration in the cellular environment. Hyperglycemic non-enzymatic glycosylation has an inhibitory effect on structural and enzymatic proteins. In addition, glycosylated collagen is resistant to enzymatic degradation and less soluble than the normal protein products, which makes connective tissue inelastic (17). High deposition of sorbitol has been implicated in diabetic vasculopathy. This is associated with increase in dermal vascular permeability and results in peri-capillary albumin deposition (18), which impairs oxygenation and nutrient distribution (19). Bennett et al. demonstrated increased destruction of growth factors by elevated levels of proinflammatory cytokines and metallo-matrix proteins following repeated trauma and infection (19,20).

Most chronic wounds are characterized by increased protease levels, particularly matrix metalloproteinase's (MMPs) and neutrophil elastases. Furthermore, tumor necrosis factor–alpha (TNF-alpha) has been shown to increase the production of MMPS, whilst inhibiting the production of tissue inhibitory metalloproteinase (21,22). The response mounted from the host immune system following critical wound colonization by bacteria is followed by polymorph nuclear leukocyte reaction that releases protease and oxidant species. This is followed by degradation of cytokines and extra-cellular matrix which distort the internal cellular milieu, thereby contributing to the non-healing of wounds. The cellular response to injury is poorly coordinated in diabetic wounds, most of which are complex and inter-related biochemical activities.

Molecular Mechanisms Involved in Chronic Wounds

The understanding of the molecular mechanisms involved in chronic wounds holds the key to solving the huge problem posed by this disease entity. Several efforts made at understanding this process have been undermined by the absence of an easily reproducible animal model that mimics the human chronic wound. Keratinocyte biology attempts to explain the mechanism involved in chronic wound healing through cellular injury. Keratinocytes are the first cells to respond to injury; they are programmed to maintain skin integrity based on their specific attachment to extracellular matrix and vice versa. The absence of normal migration and proliferation of cells caused by failure of keratinocyte activation during wound healing results in non-healing (23). The activation of C-myc affects epidermal biology directly and its relevance to wound healing. C-myc is required for transition from the G1 to S1 phase of the cell cycle and it also promotes proliferation of transit amplifying cells. Deregulation of c-myc results in depletion of epidermal stem cells thereby making the tissue non-responsive to injury (24–26).

Stojadinovic and colleagues discovered that the stabilization of β-catenin (a subtype of keratinocyte adhesion molecule) inhibits keratinocyte migration and wound healing of human skin in organ culture (26). This is done through different mechanisms: (a) activation of c-myc, (b) blocking of epidermal growth factor (EGF) effects and by synergizing with glucocorticoids. β-catenin also participates in glucocorticoid signaling and repression of keratin genes, which are involved in cytoskeletal network and keratinocyte migration. The main features of chronic wound such as persistent inflammation, decreased activity of growth factors, and angiogenesis may be affected by the activities of β-catenin and c-myc. Other authors suggested that c-myc was found to inhibit the expression of platelet derived growth factor (PDGF)-BB and its receptors (28), whereas fibroblast growth factor (FGF)-Basic and EGF were shown to induce c-myc expression (29,30). In contrast to their stimulatory effects, these growth factors may also stimulate negative feedback in chronic wounds by sustaining c-myc expression.

Physiology of Platelets in Wound Healing

Platelets are formed from megakaryocytes and are synthesized in bone marrow by pinching off pieces of the cytoplasma. Platelets are small discoid blood cells, with the average platelet count ranging from $1.5–3.0 \times 10^{-5}$/ML of the circulating blood, and with an average half-life of about 7 days. They have a trilaminar cell membrane with a glycoprotein receptor surface overlying and are partially interspersed with a penetrating bilayer of phospholipids and cholesterol. Platelets lack nuclei but contain organelles and structures such as mitochondria, microtubules, lysosomes, and granules (α, δ, λ). The cytoplasma contains an open canalicular system that increases the effective surface area for intake of stimulatory agonists and the discharge of effectors' secretions. The dense granules contain adenosine diphosphate adenosine triphosphate, serotonin, and calcium. The α-granule contains clotting factors, growth factors, and other proteins. Platelets are normo-thrombogenic and require a trigger, before they become potent and active in hemostasis and wound healing. They are found intravascularly and are concentrated in the spleen.

Hemostasis is the balanced interaction of platelets, endothelia cells, and plasma clotting proteins. Platelets are responsible for initiating the immediate and most important step in coagulation. Platelet adhesion occurs through the interaction of the damaged vessel wall and exposes the subendothetial collagen, which binds with von Willebrand factor, with a resultant change in the platelet structure, thus, making it possible for its adherence to blood vessels. This effect is accomplished through the activities of glycoprotein 1b, and 11b/111a receptors on the platelet membrane. After aggregation, the granular contents are then released; serotonin probably assists with vasoconstriction. Adenosine diphosphate (ADP) promotes release of granules, which further encourages platelets aggregation.

Phospholipase A_2, when activated, results in release of arachidonic acid, which is converted into thromboxane A_2, resulting in further platelet aggregation and growth factor release. The process described attempts to produce a platelet plug—the primary hemostasis. The platelet plug acts as a barrier to micro-organism invasion of wounds, with the help of highly concentrated leukocyte buffer. The clotting cascade is also activated along with the complement system, producing the secondary hemostasis with a resultant fibrin network formation. Degranulation of α-granules results in the fusion of platelet cell membrane during which some of the secretory proteins (e.g., PDGF) are transformed to a bioactive state by the addition of histones and carbohydrate side chain (52). These active proteins then bind to the transmembrane receptors of target cells such as mesenchymal stem cells, osetoblast, and fibroblast, endothelial and epidermal cells. Once bound to the transmembrane receptors, intracellular signal proteins are activated, resulting in expression of gene sequence that directs cellular proliferation, matrix formation, and initiation of collagen synthesis (52). These proteins are secreted within 10 minutes of clotting, with more than 95% of the pre-synthesized growth factors secreted within 1 hour.

Platelet Derived Growth Factors in Wound Healing

Wound healing is a well-orchestrated and complex series of events involving cell-cell and cell-matrix interactions, with growth factors acting as messengers to regulate the various processes. The role of growth factors in wound healing has been well described in acute wound healing; growth factors are stored in the form of α-granules in platelets and when these platelets are activated, they in turn release a multiplicity of growth factors. After the formation of platelet coagulum, the activated platelets are interspersed among the fibrin strands forming a matrix within the clot, which helps to keep the growth factors within the mesh. They eventually diffuse out into the surrounding tissue. Growth factors act locally to recruit undifferentiated cells to the injury site by chemoattraction and also stimulate mitosis in the undifferentiated cells. Stem cells are attracted to areas of high concentration of growth factors and cellular movement occurs by forming attachments to the matrix/scaffold. Growth factors attach to receptors on the stem cell membrane, thereby activating genes controlling cell division. They also attach to cell receptors and control the genetic expression of stem cells via the modulation of signal transduction pathways of secondary proteins, resulting in cellular division and differentiation. Mitosis occurs via a signal transduction pathway through the tryosinase kinase located on the cell membrane. Receptor activation leads to the activation of secondary messenger proteins, which enter the cell nucleus and influence the expression of the genes responsible for triggering mitosis, angiogenesis, and macrophage activation (31).

The growth factor that was first identified was the platelet derived growth factor, which has other isomers (αα, ββ, αβ). PDGF has been synthesized through recombinant DNA technology and has been used topically for treating chronic diabetic foot ulcers. The first efficacy study documented showed complete healing of non-ischemic foot ulcers in 25% of the placebo-treated group and 48% in the PDGF-treated group ($p < .01$) (32). The second efficacy study found complete healing in 35% of the placebo-treated group, and 50% of the PDGF-treated group, which represented a 43% improved healing ($p < .007$). The PDGF-treated group also showed a reduced healing time by 6 weeks ($p < .013$) (33). Other identifiable growth factors include transforming growth factor (TGF-β), which has β1 and β2 as its isomers; platelet derived angiogenesis factor; platelet derived epidermal growth factor; fibroblast growth factor; keratinocyte growth factor; insulin like growth factor; interleukin-1; vascular endothelial growth factor; epidermal growth factor; osteocalcin; osteonectin; fibrinogen; and fibronectin. The summary of the roles of the super-family growth factors in wound healing cascade is shown in Table 2. Growth factors have special roles they play at different phases of the wound healing, and they also appear to have synergistic effects on one another. The other groups of growth factors not discussed in the table are collectively called the adhesive proteins. These include fibrinogen, fibronectin, and thrombospondin. They are known to participate in thrombus formation and some mitogenic action (55). More recently, Kubota et al. (49) described a new platelet growth factor known as connective tissue growth factor (CTGF). They showed in their experiment that CTGF is released after the activation of platelet rich plasma. They are said to be expressed along with the platelet coagulation process. The expression of CTGF following autologous derived platelet rich plasma gel (APG) activation is said to be more than a 20-fold increase when compared with any other platelet growth factors.

Rationale for the Use of Autologous Platelet Gel

In the late 1970s the importance of growth factors within the wound-healing cascade was first identified. These began with platelet-derived growth factor and the subsequent identification of many other growth factors now known to be important within the different stages of the wound healing cascade. These growth factors aid the three phases of wound healing; inflammatory, proliferative, and remodeling phases. These phases involve complex paracrine mediated growth factors which influence mitogenic and cellular differentiation activity.

Chronic wounds are thought to have increased proteases, increased proinflammatory cytokines, decreased protease inhibitors, and decreased growth factor activities. Cooper et al. showed that a number of growth factors were markedly reduced in wound fluid from chronic wound as compared

Table 2. Growth factor super-families and their role in wound healing.

Super-family	Member	Discussion
Platelet-Derived Growth Factor	PDGF	PDGF has three isoforms; PDGF-AA, AB, BB. PDGF affects cells of mesodermal origin. PDGF has its primary effect on these cells and could also be secreted from other polymorph nuclear cell cells, fibroblast, and smooth muscle cells. Its roles include chemotaxis, proliferation, and new gene expression in these cells (34).
	Vascular endothelia growth factor (VEGF)	Its receptors are exclusive to the endothelial cells and it acts as an effective mitogen during angiogenesis. FGF-4, PDGF, Tumor necrosis factor (TNF)-α, Insulin-like growth factor (IGF), and some interleukins stimulate VEGF production and others inhibit it (35).
Epidermal Growth Factor	EGF	EGF is released after degranulation of platelets. Most cells have receptors for EGF but epithelial cells have the largest number of receptors. Other sites for the receptors include endothelial cells, fibroblasts, and smooth muscle cells. EGF is chemotactic in nature, stimulates angiogenesis and collagenase activity and acts as a potent mitogenic stimulant for epithelial cells (36,37).
	TGF-α	TGF-α has 30% structural homology as EGF. It is produced by activation of macrophages, platelets, and keratinocytes. It stimulates mesenchymal, epithelial, and endothelial cells via chemotactic effect (36,37).
Fibroblast Growth Factor	aFGF, bFGF	FGF has 2 different forms; the acidic and basic, both have 50% homology. Both forms stimulate endothelial cell proliferation and motility, thereby contributing to wound angiogenesis. bFGF is 10 times more potent as an angiogenic stimulator. Other stimulatory effects include collagen synthesis, wound contraction, epithelialization, and fibronectin and proteoglycan synthesis (36,38).
	Keratinocyte growth factor (KGF)-1, KGF-2	High quantity of KGF is produced after tissue damage, which is mainly produced by fibroblast. KGF-1 is the most potent mediator of keratinocyte proliferation and motility. KGF-2 has been shown to increase granulation tissue formation by directly stimulating the migration of fibroblasts into wounds. Glutathione peroxidase, a DNA repair enzyme, protects the damaging effect of reactive oxygen species, which are released into the wound by neutrophils (39,40).
Transforming Growth Factor	TGF-β1, β2, β3	TGF-β has been isolated from platelets, macrophages, lymph-cytes, bone, and kidneys. It is also released by platelet after degranulation. It stimulate monocytes to secrete other growth factors (FGF, PDGF, TNF- α, and IL-1). TGF is chemotactic for macrophages and regulates its own production within the macrophages in an autocrine fashion. It also stimulates fibroblast chemotaxis and proliferation. The presence of other growth factors and the variability in concentration of TGF-b could modulate the role of TGF-b. This is either an inhibitory or stimulatory effect on cellular proliferation. TGF-b stimulates fibronectin, proteoglycan synthesis by fibroblast. It organizes extracellular matrix and may be involved in scar remodeling and contracture. It also stimulates epithelial cell proliferation and inhibits endothelial cell proliferation, but with a co-factor it will stimulate angiogenesis (36,41–46).
Insulin Growth Factor	IGF-I, IGF-II	IGF-II is most prominent during fetal development, where-as IGF-I persists throughout life and is synthesized in the liver, heart, lung, kidney, pancreas, brain, and muscle. IGF is stimulated by human growth hormone (especially in the liver) and the two together stimulate skeletal cartilage and bone growth. Degranulation of platelets will also produce IGF-I and the stimulation of fibroblasts. IGF-I is a potent chemotactic agent for endothelial cells, and is also involved in neo-vascularization. It may also act with PDGF to enhance epidermal and dermal growth. The effect of IGF-I on wound healing depends on the amount of available free IGF-I (47,48).

with acute wounds (50). Also, FGF and TGF-β concentrations are down regulated in chronic wounds and are significantly lower when compared with acute wounds (50). It has also been shown that the use of a synthesized single growth factor in treating chronic diabetic wounds has only produced a limited degree of success. With the recent understanding of bio-tissue engineering, autologous derived platelet rich plasma gel can effectively and safely deliver all the needed growth factors to stimulate tissue reparation. The rationale for employing this technique is to mimic the normal physiological wound healing and reparative tissue process.

Autologous derived platelet gel (APG) has a wide and safe application within the post operative surgical field both as a wound sealant and a tissue repair agent. Its application has extended to patients that are prone to higher surgical complications, and this is also true of diabetic patients. The modification of cell saver technology has made it possible to synthesize AGP with structural and functional properties, as close as possible, to those of natural soft tissue and epidermis. Besides, the ability of AGP to deliver multiple growth factors with a synergistic effect to wound sites, the clot formed from AGP serves as a scaffold and a protein reservoir, thereby locally concentrating and magnifying their effect. The platelet plug acts as a barrier to micro-organism invasion of wounds, achieved with the help of highly concentrated leukocyte buffer, present within APG. The availability of concentrated leukocyte with about 5-fold the baseline value makes the graft matrix infection free. It also promotes mitogenesis of mesenchymal stem cells at the wound site. As a three dimensional volumetric soft connective tissue replacement, it provides a matrix medium for cell migration, granulation tissue formation, and epithelia wound contracture in addition to serving as a non-disturbed wound patch. The overall efficacy of AGP in treating wounds is likely to be a function of many variables such as the platelets concentration, the volume of AGP delivered to the wound, the extent and the type of injury and perhaps, the overall medical condition of the patient.

AGP contains autologous-derived living cells, which are able to deliver a program of healing to the wound that may not be achieved with a single growth factor. Another added advantage of autologous preparation technique is the inherent safety and therefore freedom from concerns of transmissible disease, such as, human immunodeficiency virus, hepatitis, and Creutzfeldt-Jakob disease.

Preparation of Platelet Rich Plasma Gel

Platelet rich plasma gel (PRP) can be prepared either through standard blood banking techniques or through a point of care device. The blood bank techniques are more costly, highly controlled logistic systems, to prevent mismatch. However, the point of care device does not require large predonated blood volumes. The tabletop centrifuge system has been used to manufacture smaller volumes of PRP from lesser amounts of whole blood (50–150 mL). With the advent of small compact office devices, 6 mL of platelet-rich plasma could be prepared from 45–60 mL of blood (54). This in a way obviates the need for re-transfusion of the remaining blood products (54). All the different devices available, such as Smart PReP (Harvest Technologies Corp., Norwell, MA), angel (Sorin Group, Arvada, CO), and the Magellan (Medtronic, Minneapolis, MN.), operate on a small volume drawn and on the principle of centrifugation. They all differ widely in their ability to collect and concentrate platelets, but they are able to increase the PRP concentration to about 2-fold to 8-fold of their original platelet concentration (51,54). It is expected that the concentration of the released growth factors would be linearly proportional to the platelet concentration ratio. However, some authors have experienced a little variation in this relationship. Marx (52,54) states that a "working definition" of PRP should be 1,000,000 platelets/μL. Marx states that lesser concentrations of platelets were unable to demonstrate healing properties. However, greater concentrations have not yet been shown to be advantageous. In another study by Anitua et al. (55), they state that the aim is to prepare PRP with a platelet count in the excess of 300,000 platelets/μL. Eppley et al. and Weibrich et al. realized little value in using platelet concentration ratio to predict the resultant platelet-rich plasma secretory protein levels (51,53). Selecting the type of device to be used will depend on the type of surgical procedure, the technical expertise available, and the amount of platelet-rich plasma required.

Preparation Methodology

In the clinical setting, the tabletop device or the cell saver may be used. But for the purposes of this review, we shall concentrate on the tabletop device. Blood is drawn from the anticubital vein carefully and gently into a syringe, using a large bore cannula (>17 gauge). It is important to avoid negative pressure whilst filling the syringe, to avoid damaging the platelets and to avoid activating them before processing. The autologous blood is collected in a sufficient amount of anticoagulation citrate dextrose-A solution (ACD-A). A ratio of 1 mL of ACD-A to 7 mL of whole blood should be maintained. The acid citrate dextrose serves to preserve the integrity of the platelet membrane. The aspirated blood is gently agitated by mixing the anticoagulant with the blood. The whole blood is sequestered into a semiautomatic controlled operation mode by centrifugation, separating the platelet-poor plasma (PPP) from the Buffy coat layer and erythrocytes. The PPP volume is separately collected into another bag. The centrifugation continues to obtain the Buffy coat layer consisting of PRP and leukocytes, which is collected into another separate bag. After this procedure, the erythrocytes are also collected into a separate bag. The PPP and erythrocyte are not re-transfused when a table top processing unit is used but could be re-infused in the case of cell saver. Part of the PPP is used in processing the thrombin.

Platelet Rich Plasma Gel Activation

To initiate the release of the growth factors from APG, the platelets have to be activated. The regenerative potential of platelet-rich plasma depends on the level of secretory proteins that are released during the activation process (51). The protein levels will depend on several factors including: (1) patient factor, the concentration of the protein in the platelet, (2) the platelet handling and processing technique, which determines the quality and quantity produced, and (3) the completeness of platelet activation before measurement (51–53). Thrombin, a potent activator will induce immediate platelet growth factor release when added to the PRP. Human thrombin is generated from a commercial kit Activa (Mirandola, Modena, Italy). It is generated after PPP is mixed with the (Activa) beaded material in a pressurized glass syringe for about 20–25 minutes and the thrombin is squeezed out of the glass syringe following application of pressure on the syringe plunger. Autologous thrombin is preferred in the United Kingdom, so as to avoid the potential complication of pre-prepared bovine thrombin, which has been implicated in the development of antibodies to clotting factor Va, XI, and thrombin. Life-threatening coagulopathies have been previously documented following these reactions. However, it is believed that the antibodies developed against factor Va in bovine thrombin has been essentially eliminated in the manufacturing and processing of bovine thrombin (52).

The harvested cellular Buffy coat is combined with thrombin and 10% calcium chloride ($CaCl_2$) for platelet activation to produce the gelatinous material. The $CaCl_2$ antagonises the effect of anticoagulant in the citrate solution present in the pre-donated blood bag. Once PRP is prepared, it remains stable in the non-coagulated state for about 6–8 hours. The PRP produced can be used to the

desired need of the physician; either to fill three dimensional volumetric wounds or as a spray-on graft for superficial wounds (Figure 1). The tissue graft constructed in this manner has a semisolid physical integrity much like bilaminant skin graft. The graft also serves as an occlusive dressing, which could remain on the wound for 5–10 days (Figure 2). Another additional advantage of PRP is that it has been applied on bone and fracture sites, particularly following amputation or removal of dead bone in osteomyelitis. Mixing autologous platelet gel with sequestered autologuos bone graft materials might create a bioengineered graft. This has the potential to support, promote, and accelerate bone healing (51). The quality and integrity of the formed gel appears central to the delivery of its role. The thrombelastography hemostasis analyzer (TEG) and Sonoclot measures the elasticity of a clot as its forms and subsequently degrades naturally. Cassidy et al., in their attempt to determine the structural integrity of formed gel, concluded that TEG is valid for analyzing platelet gel and Sonoclot appears to be an unreliable tool (66).

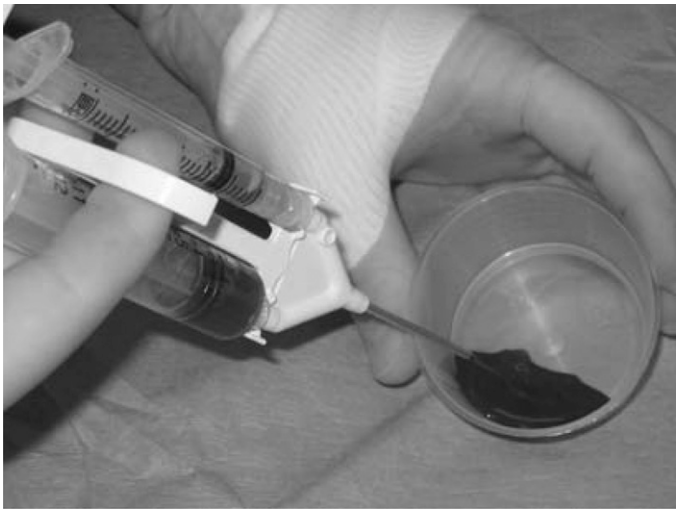

Figure 1. Making of platelet rich plasma gel, a consistent gel that can be used to fill up a three dimensional volumetric wound.

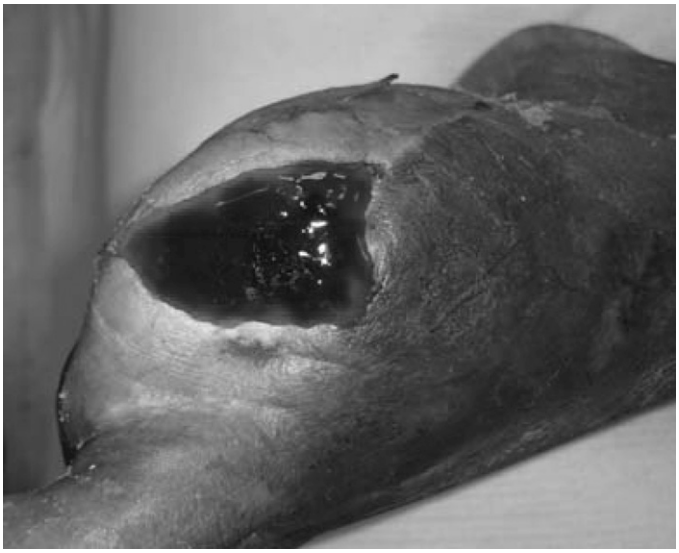

Figure 2. Application of the platelet gel on chronic diabetic foot ulcer after a sharp surgical debridement of a chronic wound.

Pitfalls in Autologous Derived Platelet Rich Plasma Gel Production

Centrifugation forms the basis of the current methods for producing platelet-rich plasma. From the drawn whole blood, platelet fragmentation should be avoided during this process, which could result in the untimely release of high levels of proteins with compromised bioactivity.

A large bore cannula (17G) is recommended for drawing the blood and possibly from a large anticubital vein under no tension. Veno-puncture done under tension could activate the platelet before the centrifugation, which may negate the yield. Low gravity force during centrifugation and minimal platelet activation should be used. Despite all the efforts to prevent activation, bioactive secretory proteins could still be produced properly, but lost during transferring to the surgical bed. This failure is largely due to the delivery technique used. It is important to achieve a good quality gel; the gel serves as a vehicle that delivers the secreted proteins to the wound site. It is imperative to safely transfer the gel to the surgical site and use it within 10 minutes of its production. Otherwise, the gel will retract and lose the originally expressed growth factors. It has been reported that the quality and quantity of the PRP produced is dependent on the quality of the platelet. However, there are other conditions that are relatively contraindicated to the use of PRP, such as pre-existing coagulation defects (thrombocytopenia, hypofibrinogenemia) or potential hypersensitivity to bovine products (64).

Evidence to Support the Clinical Application of Platelet Rich Plasma Gel

Different authors have proposed that platelet-rich plasma technology has relevant application in promoting hard and soft tissue wound healing; and potentially decreasing postoperative wound infections, blood loss, and pain. Platelet-rich plasma has found clinical application in most fields of surgical practice. There are scientific literatures to support its efficacy in periodontal and oral surgery, maxillofacial surgery, aesthetic plastic surgery, heart bypass surgery, orthopedic and spinal fusion surgery, and in the treatment of chronic skin and soft tissue ulcers (51,52,54). Although the various published data appears promising on its efficacy in wound healing, most are case studies or series; hence, most of the evidence is anecdotal. There are very few randomized controlled prospective clinical trials to support the acclaimed potentials. Knighton et al. reported that 17 of 21 patients achieved complete re-epithelization of the

treated chronic lower limb ulcers over an average period of 8.6 weeks, with a course of twice-daily wound treatment with platelet releasate suspended on a collagen base, compared to two of 13 similar wounds treated with placebo (56). After crossover of the placebo group, all the remaining 11 chronic ulcers were treated with the same bio-active agent as the active group. All treated placebo patients achieved 100% re-epithelization in an average of 7.1 weeks (55). Other studies published on the role of platelet-rich plasma in treating diabetic foot ulcers are highlighted in Table 2.

Multidisciplinary Approach to Wound Management and its Future

The complexity and the poor outcome associated with healing chronic diabetic foot ulcers necessitate the need for a multi-disciplinary approach to treating these groups of patients. Most physicians involved with the care of these patients are increasingly advocating for an integrated wound care center. This is aimed at improving the communication amongst the different care givers involved with treating these patients. This center will provide the requisite collaborations for optimal healing, which include in-patient and out-patient care, general medicine, podiatrists, vascular and orthopedic surgeons, diabetologists, nutritionists, radiologists, and neurologists. This center has the potential of galvanizing already available resources, streamlining protocol implementation, reducing cost, and ultimately accelerating wound healing.

Advances in Wound Care

The search for an ideal wound dressing material still remains pivotal to improving the clinical outcome of complex wounds. The ideal dressing needs to ensure that the wound remains moist with exudates but not macerated, and free of infection, excessive slough, toxic chemical, particles, and fibers. It also has to ensure that the wound is at an optimum temperature and pH for healing, and undisturbed by the need for frequent changes (56). The choice of an appropriate local wound dressing material depends on identification of the type of wound that is in question, the phase of the wound healing, the clinical behavior of the wound, such as granulating or epithelializing, the presence of infection, and the conformability of the dressing. The advancement in wound-dressing technology has been directed at solving the problems associated with the non-availability of the ideal dressing material. In the search for the ideal dressing material, many researchers have shown that simulation of the living skin equivalent may hold the key to efficient tissue healing and remodeling (59).

Biological Therapy

Tissue engineering is a biological science that specializes in the development of biological substitutes to restore, maintain, or improve the function of the skin. It seeks to create a readily available tissue replacement with the biologic and pharmacologic properties of the human skin (59). The principle of implementing this method in treating chronic wounds is called biological or cellular therapy. Implementation of cellular therapy is recommended when wound size cannot be decreased by more than 10% within 3 weeks. There are a number of commercially available dermal matrices that have been approved by the United States Food and Drug Administration. This includes Alloderm™ (Life Cell Corp., The Woodlands, TX), altered allograft; Integra™ (Integra Life Science Corp., Plainsboro, NJ), dermal regeneration template; Dermagraft™ (Smith and Nephew, London, UK), synthetic dermal replacement/dermal regeneration template; Apligraf™ (Organogenesis, Inc., Canton, MA), composite skin replacement, and Hyalograft-3D™ (Fidia Advanced Biopolymers, Abano T., Italy).

Biological therapy may be an ideal treatment for diabetic foot ulcers because it adds cells that release growth factors to a growth factor dependent environment, increases cytokines and matrix proteins, and promotes angiogenesis (60). It is also believed that they accelerate healing time and decrease the risk of wound infection. Of all the available cellular therapy techniques, Apligraf™ (organogenesis, previously Graft skin) and Hyalograft-3D™ (esterified hyaluronic acid beneath silicone) have shown efficacy in the treatment of chronic diabetic foot ulcers (61). Falanga et al. in a prospective randomized multicentre clinical trial showed a 55% increased healing rate of chronic neuropathic diabetic foot ulcers.

CONCLUSION

Successful treatment of chronic non-ischemic diabetic ulcers remains challenging for clinicians. Because of the huge financial burden on the health sector and the psychosocial impact on the patients' well being, more clinical trials will be needed to define a definitive treatment protocol and standardization. Sharp wound debridement and a combination of many other discussed treatment modalities hold a promising future. The enhancement of wound healing through the application of PRP is supported by basic scientific and clinical studies. Research has shown that PRP are responsible for actively extruding growth factors, which initiate soft tissue healing and recruitment of stem cell. Although, autologous platelet derived growth factors appear promising, more properly structured clinical randomized controlled trials will be required to confirm these results and to establish under which conditions the application of platelet-rich plasma has merit.

REFERENCES

1. Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, Apelqvist, J. The global burden of diabetic foot disease. Lancet. 2005;366:1719–24.
2. Ragnarson Tennvall G, Apelqvist J. Health-economic consequence of diabetic foot lesion. Clin Infect Dis. 2004:39:S132–9.

3. Abbott CA, Carrington AL, Ashe H, et al. The Northwest Diabetes Foot Care Study: Incidence of, and risk factors for, new diabetic foot ulceration in a community-based patient cohort. Diabet Med. 2002; 19:377–84.
4. Abbott CA, Vileikyte L, Williamson S, Carrington AL, Boulton AJM. Multicentre study of the incidence of and predictive factors for diabetic neuropathic foot ulcers. Diabetes Care. 1998;21:1071–5.
5. Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. JAMA. 2005;293:217–28.
6. Shearer A, Schuffham P, Gordiois A, Ogleshy A. Predicted costs and outcomes from reduced vibration detection in people with diabetes in the US. Diabetes Care. 2003;26:2305–10.
7. Most RS, Sinnock P. The epidemiology of lower extremity amputations in diabetic individuals. Diabetes Care. 1983;6:87–91.
8. Hogan P, Dall T, Nikolov P; American Diabetes Association. Economic costs of diabetes in the US in 2002. Diabetes Care. 2003;26:917–32.
9. Saar WE, Lee TH, Berlet GC. Economic burden of diabetic foot and ankle disorder. Foot Ankle Int. 2005;26:27–31.
10. Consensus development of conference in diabetic foot wound care. 7–8 April 1999, Boston, Massachusetts, American Diabetes Association. J Am Podiatr Med Assoc. 1999;89:475–83.
11. Sheehan P, Jones P, Caseli A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12 week prospective trial. Diabetes Care. 2003;26: 1879–82.
12. Robson MC, Thomason A, Pierce GF, Philips LG, Robson LE. Platelet-derived growth factor BB for the treatment of chronic pressure ulcers. Lancet. 1992;339:23–5.
13. Reiber GE, Vileikyte L, Boyko EJ, et al. Causal pathway for incident lower extremity ulcers in patients with diabetes from two settings. Diabetes Care. 1999;22:157–62.
14. Shaw JE, van Schie CH, Carrington AL, Abbott CA, Boulton AJ. An analysis of dynamic forces transmitted through the foot in diabetic neuropathy. Diabetes Care. 1998;2:1955–9.
15. Steel DL, Donohoe D, Webster MW, Lindsley L; Diabetic Ulcer Study Group. Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. J Am Coll Surg. 1996;183:61–4.
16. Nwomeh BC, Liang HX, Cohen IK, Yager DR. MMP-8 is the predominant collagenase in healing wounds and nonhealing ulcers. J Surg Res. 1999;81:189–95.
17. He Z, King GL. Micro vascular complication of diabetes. Endocrinol Metab Clin North Am. 2004;33:215–58.
18. Bucalo B, Eaglstein WH, Falanga V. Inhibition of cell proliferation by chronic wound fluid. Wound Repair Regen. 1993;1:181–6.
19. Bennett NF, Schultz GS. Growth factors and wound healing. Part 11: Role in normal and chronic wound healing. Am J Surg. 1993;166: 74–81.
20. Yager DR, Nwomeh BC. The proteolytic environment of chronic wounds. Wound Repair Regen. 1999;7:433–41.
21. Palolahti M, Lauharanta J, Vaheri A. et al. Proteolytic activity of leg ulcer exudates. Exp Dermatol. 1993;2:29–37.
22. Chen SM, Ward SI, Olutoye OO, Diegelmann RF, Kelman CI. Ability of chronic wound fluids to degrade peptide growth factors is associated with increased levels of elastase activity and diminished levels of proteinase inhibitors. Wound Rep Regen. 1997;5:23–32.
23. Morasso MI, Tomic-Canic M. Epidermal stem cells, the cradle of epidermal determination and wound healing. Biol Cell. 2005;97:173–83.
24. Waikel RL, Kawachi Y, Waikel PA, Wana XJ, Roop DR. Deregulation expression of c-myc depletes epidermal stem cells. Nat Genet. 2001;28:165–8.
25. Arnold I, Watt FM. c-myc activation in transgenic mouse epidermis results in mobilization of stem cells and differentiation of their progeny. Curr Biol. 2001;11:558–68.
26. Stojadinovic O, Brem H, Tomi-Canic M, et al. Molecular pathogenesis of chronic wounds: The role of β catenin and c-myc in the inhibition of epithelialization and wound healing. Am J Pathol. 2005;167: 59–69.
27. Gandarillas A, Watt FM. c-Myc promotes differentiation of human epidermal stem cells. Genes Dev. 1997;11:2869–82.
28. Oster SK, Marhin WW, Asker C, et al. Myc is an essential negative regulator of platelet-derived growth factor beta receptor expression. Mol Cell Biol. 2000;20:6768–78.
29. Wang Y, He H, Zigler JS Jr, et al. bFGF suppresses serum-deprivation-induced apoptosis in a human lens epithelia cell line. Exp Cell Res. 1999;249:123–30.
30. Izadnegahdar MF, Rathanasiwami P, Shah RM. Effects of EGF and TGF beta-1 myc gene expression and DNA synthesis in embryonic hamster palate mesenchyme cells. Anat Rec. 1999;254:453–64.
31. Alder SC, Kent KJ. Enhancing wound healing with growth factors. Facial Plast Surg Clin North Am. 2002;10:129–46.
32. Steel DL. The Diabetic Ulcer Study Group: Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity diabetics ulcers. J Vasc Surg. 1995;21:71–81.
33. Wieman TJ, Smiell JM, Su Y. Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. A phase III randomised placebo-controlled study. Diabetic Care. 1998;21:822–7.
34. Everts PA, Knape JT, Weibrich, et al. Platelet-rich plasma and platelet gel: A review. J Extra Corpor Technol. 2006;38:174–87.
35. Neufeld G, Cohen T, Gengrinovitch S, et al. Vascular endothelial growth (VEGF) and its receptors. FASEB J.1999;13:9.
36. Lawrence W, Diegelmann R. Growth factors in wound healing. Clin Dermatol. 1994;12:157–69.
37. Grotendorst G, Soma Y, Takehara K, et al. EGF and TGF-alpha are potent chemo attractants for endothelial cells and EGF-like peptides are present at sites of tissue regeneration. J Cell Physiol. 1989;139:617–23.
38. McGee G, Davidson JM, Buckley A, et al. Recombinant basic fibroblast growth factor accelerates wound healing. J Surg Res. 1998;45: 145–53.
39. Cross K, Mustoe T. Growth factors in wound healing. Surg Clin North Am. 2003;83:531–45.
40. Beer H, Gassmann M, Munz B, et al. Expression and function of keratinocyte growth and activin in skin morphogenesis and cutaneous wound repair. J Investig Dermatol Symp Proc. 2000;5:34–9.
41. Massague J. The TGF-beta family of growth and differentiation factors. Cell. 1987;49:437–8.
42. Wahl S, Hunt D, Wakefield L, et al. Transforming growth factor type beta induces monocyte chemo taxis and growth factor production. Proc Natl Acad Sci USA. 1987;84:5788.
43. Sporn MB, Roberts AB, Wakefield L, et al. Transforming growth factors-beta: Biological function and chemical structure. Science. 1986; 233:532–4.
44. Edwards DR, Murphy G, Reynolds JJ, et al. Transforming growth factor beta modulates the expression of collagenase and metalloproteinase inhibitor. EMBO J. 1987;6:1899–904.
45. Bassols A, Massague J. Transforming growth factor beta regulates the expression and structure of extracellular matrix chondroitin/dermatan sulphate proteoglycans. J Biol Chem. 1988;263:3039–45.
46. Fukamizu J, Grinnell F. Spatial organization of extracellular matrix and fibroblast activity: Effects of serum, TGF-beta and fibronectin. Exp Cell Res. 1990;190:276–82.
47. Guler HP, Zapf J, Scheiwiller E, Friesch ER. Recombinant human insulin-like growth I stimulate growth and has distinct effects on organ size in hypophysectomized rats. Proc Natl Acad Sci USA. 1988;85:4889–93.
48. Grant M, Jerdan J, Merimere T. Insulin-like growth factor-I modulate endothelial cell chemotaxis. J Clin Endocrinol Metab. 1987;65:370–1.
49. Kubota S, Kawata K, Yanagita T, Doi H, Kitoh T, Takigawa M. Abundant retention and release of connective tissue growth factor (CTGF/CCN2) by platelets. J. Biochem. 2004;136:279–82.
50. Cooper DM, Yu EZ, Hennessey P, Ko F, Robson MC. Determination of endogenous cytokines in chronic wounds. Ann Surg. 1994;219: 688–91.
51. Eppley BL, Woodell JE, Higgins J. Platelet quantification and growth factors analysis from platelet-rich plasma: Implications for wound healing. Plast Reconstr Surg. 2004;114:1502–8.
52. Marx RE. Platelet-rich plasma (PRP): What is PRP and what is not PRP? Implant Dent. 2001;10:225–8.
53. Weibrich G, Kleis WK, Hafner G, et al. Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count. J Craniomaxillofac Surg. 2002;30:97–102.
54. Marx RE. Platelet-rich platelet plasma: Evidence to support its use. J Oral Maxillofac Surg. 2004;62:489–96.

55. Anitua E, Andia I, Ardanza B, et al. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost. 2004;91:4–15.
56. Knighton DR, Ciresi KF, Fiegel VD, Austin LL, Butler ER. Classification and treatment of chronic nonhealing wounds. Successful treatment with autologous platelet-derived wound healing factors (PDWHF). Ann Surg. 1986;204:322–30.
57. Knighton DR, Ciresi K, Fiegel VD, Schumerth S, Butler EM, Ceira F. Stimulation of repair in chronic, non-healing cutaneous ulcers using platelet-derived wound healing formula. Sur Gynecol Obstet. 1990;170:56–60.
58. Ganio C, Tenewitz FE, Wilson RC, Moyles BG. The treatment of chronic non-healing wounds using autologous platelet-derived growth factors. J Foot Ankle Surg. 1993;32:263–8.
59. Fohn M, Bannasch H. Artificial skin. Methods Mol Med. 2007;140: 167–82.
60. Trent JF, Kirsner RS. Tissue engineered skin; Apligraf, a bilayered living skin equivalent. Int J Clin Pract. 1998;52:408–13.
61. Stark HJ, Willhank MJ, Mirancea N, et al. Authentic fibroblast matrix in dermal matrix in dermal matrix equivalents normalises epidermal histogenesis and dermoepidermal junction in organotypic co-culture. Eur J Cell Biol. 2004;83:631–45.
62. Veves A, Falanga V, Armstrong DG, Sabolinski ML. Apligraf Diabetic Foot Ulcer Study. Graft skin, a human skin equivalent, is effective in the management of non-infected neuropathic diabetic foot ulcers. A prospective randomised multicentre clinical trial. Diabetic Care. 2001;24:290–5.
63. Christie RJ, Carrington L, Alving B. Post operative bleeding induced by topical bovine thrombin: Report of cases. Surgery. 1997;121:708–10.
64. Grant WP, Jerlin EA, Pietrzak WS, et al. The utilization of autologous growth factors for the facilitation of fusion in complex neuropathic fractures in diabetic population. Clin Podiatr Med Surg. 2005;22: 561–84.
65. Driver VR, Hanft J, Fylling CP, Beriou JM. Autologous Diabetic Foot Ulcer Study Group. A prospective randomised, controlled trial of autologous platelet-rich plasma gel for the treatment of diabetic. Ostomy Wound Manage. 2006;52:68–74.
66. Cassidy LK, Finney AS, Ellis WC, Spiwak AJ, Riley JB. Quantifying platelet gel coagulation using Sonoclot and Thrombelastograph hemostasis analyser. J Extra Corpor Technol. 2005;37:48–51.