

## REVIEW

# Platelet-Rich Plasma: Support for Its Use in Wound Healing

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Previous topical growth factor studies have shown that recombinant human platelet-derived growth factor-BB isomer (rhPDGF-BB<sup>†</sup>) is an efficacious treatment of chronic diabetic foot ulceration. A newer treatment, autologous platelet-rich plasma (PRP), represents a greater similarity to the natural healing process as a composite of multiple growth factors, is safe due to its autologous nature, and is produced as needed from patient blood. A review of the literature shows few studies performed with scientific rigor, although the safety of PRP appears to be validated. As the use of PRP increases, additional studies may establish PRP as an efficacious treatment modality and guide future treatment of chronic diabetic foot ulceration.

Platelet-rich plasma (PRP) is defined as a portion of the plasma fraction of autologous blood having a platelet concentration above baseline [1,2]. PRP also has been referred to as platelet-enriched plasma, platelet-rich concentrate, autologous platelet gel, and platelet releasate [1]. Platelet releasates have been used to treat wounds since 1985 [3]. PRP serves as a growth factor ag-

onist [4] and has both mitogenic and chemotactic properties [2,5,6,7]. It contains a high level of platelets and a full complement of clotting and growth factors [1].

In addition to use in the treatment of chronic skin and soft tissue ulcerations [8-10], publications regarding the use of PRP include periodontal and oral surgery [8,11-14], maxillofacial surgery [8,9,11,13], or-

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<sup>†</sup>Abbreviations: rhPDGF-BB, recombinant human platelet-derived growth factor-BB isomer; PRP, platelet-rich plasma; PF4, platelet factor 4; IL-1, interleukin-1; PDAF, platelet-derived angiogenesis factor; EGF, epidermal growth factor; IGF, insulin-like growth factor; Oc, osteocalcin; On, osteonectin; Ff, fibrinogen; Vn, vitronectin; Fn, fibronectin; TSP-1, thrombospondin-1; PRFM, platelet-rich fibrin matrix membrane; PDWHF, platelet-derived wound healing formula; PF-4, platelet factor 4; TGF- $\beta$ , transforming growth factor- $\beta$ ; PDEGF, platelet-derived endothelial growth factor; PG, platelet gel; PR, platelet releasate; VEGF, vascular endothelial growth factor; ECGF, epithelial cell growth factor.

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thopedic and trauma surgery [12,15-17], cosmetic and plastic surgery [17,18], spinal surgery [8,9], heart bypass surgery [8], and burns [19].

### MECHANISM OF ACTION OF PLATELET-RICH PLASMA

PRP functions as a tissue sealant and drug delivery system [20], with the platelets initiating wound repair by releasing locally acting growth factors [7,21,22] via  $\alpha$ -granules degranulation [22]. The secretory proteins contained in the  $\alpha$ -granules of platelets include platelet-derived growth factor (PDGF-AA, BB, and AB isomers) [6,13,19,22-27], transforming growth factor- $\beta$  (TGF- $\beta$ ) [6,13,18,19,22-27], platelet factor 4 (PF4) [13,19,22], interleukin-1 (IL-1), platelet-derived angiogenesis factor (PDAF) [6,13,19,22], vascular endothelial growth factor (VEGF) [12,23,24], epidermal growth factor (EGF) [6,12,13,19,26,27], platelet-derived endothelial growth factor (PDEGF) [6], epithelial cell growth factor (ECGF), insulin-like growth factor (IGF) [27], osteocalcin (Oc), osteonectin (On), fibrinogen (Ff), vitronectin (Vn), fibronectin (Fn), and thrombospondin-1 (TSP-1) [28]. These growth factors aid healing by attracting un-differentiated cells in the newly formed matrix and triggering cell division [18]. PRP may suppress cytokine release and limit inflammation, interacting with macrophages to improve tissue healing and regeneration [16], promote new capillary growth [5,29], and accelerate epithelialization [21] in chronic wounds.

Platelets in PRP also play a role in host defense mechanism at the wound site by producing signaling proteins that attract macrophages [11]; PRP also may contain a small number of leukocytes [15,18] that synthesize interleukins as part of a non-specific immune response. Previous studies of PRP have demonstrated antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* [30,31], including methicillin-resistant *Staphylococcus aureus* [30], *Candida albicans* [31], and *Cryptococcus neoformans* [31].

### THE PRODUCTION AND ACTIVATION OF PLATELET-RICH PLASMA

PRP is easy to produce with minimal effort [14,18] and can be prepared as needed at the point of care [16]. In a two-step process, whole blood from the patient is first centrifuged to separate the plasma from packed red blood cells and then further centrifuged to separate PRP from platelet-poor plasma [32]. This concentrate is then activated with the addition of thrombin or calcium [16,33], resulting in a gelatinous platelet gel [33]. Clinically valuable PRP contains at least one million platelets per microliter [2,16]. Lesser concentrations cannot be relied on to enhance wound healing, and greater concentrations have not been shown to increase wound healing [2].

Predictable and efficient [25] compact systems to develop PRP can be used in both office and hospital settings [18,23,29]. While medical practitioners are able to apply blood products in the office, as is done with PRP [2], they are not licensed to infuse or re-infuse blood or blood products in an office setting. Because PRP producing systems only require a small amount of blood to produce, there is no need for reinfusion [23], and studies have shown that these frequent but small blood draws do not have an effect on hemoglobin, hematocrit, or platelet count [3].

Not all currently marketed PRP devices are equivalent because not all concentrate viable platelets in sufficient numbers to enhance healing, with these differences accounting for many of the criticisms regarding the efficacy of PRP [23]. Although previous PRP studies have used a wide range of devices for the preparation of PRP [34], not all have been approved for use in humans. The only autologous PRP separation system currently indicated for use in diabetic ulcers is the AutoloGel™ System (Cytomedix, Inc., Rockville, MD), which contains all materials, including bovine thrombin, necessary to activate the PRP gel [35] and can be used by health care providers without specialized technicians [3].

## ROLE OF PRP IN PATIENT CARE IN ADDITION TO STANDARD MODALITIES

Topical growth factor products are typically used as adjuvant treatments along with the standard of care for treatment of diabetic foot ulceration, including debridement, off-loading, frequent dressing changes, and compression for wounds with an origin of vascular insufficiency. The efficacy of these therapies has been shown in previous studies, and their importance cannot be stressed enough.

Debridement is the most important step to promote healing in diabetic foot ulceration, with a goal of removing all devitalized tissue, including callus, necrotic, and infected tissue, and leaving only healthy tissue [5,36-38], effectively converting a chronic wound into an acute wound [36,39]. Debridement reduces the bacterial load of an ulcer even in the absence of overt infection [4] and can be used on both neuropathic and venous ulcers [38]. Previous studies have suggested that foot ulcers that are debrided are more likely to heal than those that are not, independent of treatment group [40], supporting frequent debridement as an important adjuvant treatment in these wounds.

Pressure, shear, and friction have been shown to impede wound healing [5,41], and the off-loading of high pressure areas is important in managing diabetic foot ulcers [42,43]. Although the gold standard for off-loading is plaster casting or total contact casts [4,5,36,37,41], they are indicated only if ulcers are clean and free from infection; therefore, the wounds must be inspected and dressings must be changed daily, which is impossible with these devices [5], making them impractical for everyday use. Many physicians advocate the use of a removable contact walker with custom orthotic, which has the same amount of pressure reduction as total contact casts and can be removed daily for wound inspection and dressing changes. Although compliance may be an issue with these easily removable devices [5], they are more practical for everyday use.

The ideal dressing removes excess exudate, maintains a moist environment, pro-

tects against contamination, does not cause trauma when removed, and leaves no debris on wound bed [38]. Dressings that retain moisture are less likely to be associated with infections than conventional dressings [43]. There is no evidence to support one type of dressing over another [44,45], and no single dressing is appropriate for every type and location of ulcer [37]. Moist saline or hydrocolloid dressings create a moist wound bed that enhances the healing process and prevents desiccation of the wound [5].

Compression is considered first-line therapy for venous ulcers [38,46] and is more effective in healing these ulcers than no compression [45,46]. Methods of compression vary and may include stockings, multilayer bandages, high-pressure compression boots, intermittent pneumatic compression, and Unna boots [46]. Compression relieves edema and stasis by reducing distention in superficial veins and assisting the calf muscle pump action [38].

## METHODS

A thorough review of the literature was performed in multiple sessions between July 2008 and March 2009. PubMed was searched for literature on PRP and rhPDGF-BB using the keywords platelet-rich plasma/PRP/platelet releasate/platelet gel/platelet concentrate, Platelet-derived growth factor/PDGF/rhPDGF-BB/becaplermin, and diabetic foot ulcers/neuropathic foot ulcers/chronic diabetic foot ulcers. Only sources available in English were used. The reference lists of appropriate studies and review articles were reviewed for additional sources regarding the use of PRP and rhPDGF-BB.

## STUDIES SUPPORTING THE USE OF PLATELET-RICH PLASMA

Due to the novelty of PRP and its more recent discovery, few studies have been conducted on its efficacy in human subjects. PRP has been found to be effective in several case control studies in addition to several non-controlled clinical trials. Only one

prospective, randomized, controlled clinical trial has been reported regarding the use of autologous PRP for the treatment of diabetic foot ulcers.

McAleer et al. (2006) found that the use of autologous PRP was successful in healing a chronic lower extremity wound in a case study of a 57-year-old man with type 2 diabetes and a wound of six months duration. The treatment of this man's wound with PRP followed a failed attempt at a living skin graft application accompanied by off-loading and daily wet-to-dry dressing changes. The autologous PRP was synthesized in the treating clinician's office, and wound inspection, debridement, and reapplication of platelet gel were performed weekly. Complete closure of the ulcer was achieved by the fourth week of treatment with PRP. Although this study is limited as a case study involving a single patient, it suggested that PRP can be successful in healing wounds that have failed to heal by other treatment techniques [29].

Salemi et al. (2008) was a more recent case study evaluating the effectiveness of a combination of autologous adipose tissue and PRP in a lower extremity ulcer of three years duration in a non-diabetic 65-year-old male patient. This study lasted for four weeks with follow-up at one, three, six, and 15 months. Although no statistical analysis was done in this study, the researchers noted that the graft appeared to take well and the patient suffered no local infection or any other complications. At a 15-month follow-up, the wound had healed completely with regained functioning of the limb and a greatly enhanced quality of life reported by the patient. This study, although also limited as a case study involving a single patient, further supported previous findings suggesting that PRP may be successful in the treatment of wounds that have failed to heal using other treatment modalities. This study differed from previous studies due to the investigators' use of autologous adipose tissue in addition to PRP for the treatment of a chronic lower extremity ulcer [10].

Margolis et al. (2001) was a retrospective cohort study devised to estimate the ef-

fectiveness of platelet releasate (PR) in the treatment of diabetic neuropathic foot ulcers. Of the 26,599 patients included in the study, 21 percent were treated with PR by the end of the 12-week run-in period before the 20-week study period began. The investigators used logistic regression-derived propensity scores to account for selection bias. The relative risk for a wound to heal after treatment with PR, when compared to the standard of care, was 1.14 (95 percent CI 1.03-1.27) to 1.59 (1.49-1.70). Overall, 43.1 percent of patients healed within 32 weeks, including 50 percent of patients treated with PR and 41 percent of patients not treated with PR treatment. The investigators concluded that PR was more likely to be used in more severe wounds and was also more effective in treating these wounds than the standard of care. One limitation of this study was the varying timing of commencement of PRP treatment, such that patients did not always receive 20 full weeks of PRP therapy. However, this study was one of the first to incorporate the most severe wounds, which were excluded from the majority of previous studies, and suggested that PR would be appropriate for use in these wounds [47]. In addition, the authors of this study found that the percentage of healed diabetic foot ulcers leveled off after 20 weeks, indicating little incremental continued healing after this time [47] and setting a precedent for the use of a 20-week study period in future growth factor studies.

Crovetti et al. (2004) published a prospective non-blinded study regarding the efficacy of platelet gel (PG) in healing cutaneous chronic wounds. The wounds of the 24 patients enrolled in this study varied in origin, and etiologies included diabetes-related, vascular insufficiency, infectious disease, post-traumatic, neuropathic, and vasculitis-related. The protocol for this study consisted of once-weekly PG applications of either autologous or homologous origin. At the time of the study publication, nine patients had healed completely, two went on to receive cutaneous grafts, four had stopped treatment, and nine had responded partially and were still receiving treatment. Although

pain was reported as reduced with the application of PG, neither patients nor clinicians were blinded to the treatment, possibly introducing bias to the self-report of pain. In addition to being a non-blinded study, it is limited in that only three of the 24 subjects were able to perform autologous blood donations, meaning that the majority of patients received PG of homologous origin [48].

O'Connell et al. (2008) presented promising findings from a pilot study involving the treatment of chronic lower-extremity ulcers with autologous platelet-rich fibrin matrix membrane (PRFM). This prospective trial ( $n = 21$ ) of eligible patients aged 18 to 85 included 12 patients with 17 venous lower-extremity ulcers and nine patients with 13 nonvenous lower-extremity ulcers, all who had failed to respond to at least four weeks of conventional treatment. The primary endpoints were the incidence and time to complete closure, and the secondary endpoints were the incidence and time to 75 percent closure. The study duration was 12 weeks with follow-up at one month. Complete healing was achieved in 66.7 percent of the patients with venous lower-extremity ulcers in 7.1 weeks (median six weeks) following an average of two applications of PRFM per patient. Of the nonvenous lower-extremity ulcer group, 44 percent of patients treated with PRFM healed completely during the study period. Although this study is limited as a small-scale pilot, it suggests that platelet-rich substances such as PRFM have the potential to heal chronic lower-extremity ulcers that have failed to heal by conventional methods [49].

Driver et al. (2006) carried out the first reported prospective, randomized, controlled multicenter trial in the United States regarding the use of autologous PRP for the treatment of diabetic foot ulcers. Participants included 72 patients with type 1 and type 2 diabetes between the ages of 18 and 95 from 14 investigation sites suffering from an ulcer of at least four weeks duration. In this study, investigators compared the effectiveness of autologous PRP gel to that of normal saline gel for 12 weeks. The primary objective of

this study was to evaluate the safety of PRP and the incidence of complete wound closure, defined as 100 percent re-epithelialization, when compared to the control treatment, and a secondary objective was rate of wound closure. Patients were randomized into two groups — standard of care with PRP gel or control (saline gel) — and were evaluated biweekly for 12 weeks. After excluding 32 patients from the final per-protocol analysis because of failure to complete treatment and protocol violations, the authors found that 68.4 percent (13/19) of patients in the PRP group and 42.9 percent (9/21) in the control group had wounds that healed. Wounds in the PRP group healed after a mean of 42.9 days (SD 18.3) vs. 47.4 days (SD 22.0) in the control group. This study was limited somewhat because it excluded ulcers with “challenging presentations” such as mild to moderate vascular disease and exposed tendon or bone, in addition to patients with hyperglycemia and/or inadequate nutritional status; however, this study is the first published to use the AutoloGel™ System [3].

## STUDIES NOT SUPPORTING THE USE OF GROWTH FACTOR THERAPY

Margolis et al. (2002) carried out a retrospective cohort study of 31,106 individuals in order to evaluate risk factors in patients with diabetic neuropathic foot ulcers that may help predict who will and will not heal among patients receiving standard therapy for their wounds. The patients receiving an adjuvant therapy such as PR, PDGF, or graft skins were found to have wounds that were less likely to heal, although including these therapies in a multivariate model did not change the point estimate of any risk factor by more than 10 percent, suggesting that these therapies had little effect on the study results. The authors of this study attributed this effect to selection bias, with physicians likely selecting those patients doing poorly to receive adjuvant care. It also was noted that many patients did not receive a full 20 weeks of any adjuvant

therapy. For further support of this attribution, the authors cited a previous study by Margolis et al. (2001), which showed that PR was successful in treating even the most severe diabetic neuropathic foot ulcers. In addition to selection bias, information bias may have led to systematic differences in reporting of presence or absence of certain risk factors or differences in the way risk factors were measured [50].

One randomized prospective double-blind placebo-controlled study by Krupski et al. (1991) investigated the use of autologous platelet-derived wound healing formula (PDWHF), a mixture of growth factors including PDGF, platelet factor 4 (PF-4), TGF- $\beta$ , platelet-derived epidermal growth factor (PDEGF), and platelet-derived angiogenesis factor (PDAF) [51]. PDWHF was investigated in 18 patients with 26 lower extremity wounds of at least eight weeks duration (mean of  $5.5 \pm 4.3$  months duration). Only 78 percent of the patients were diabetic and all were men ranging from 57 to 75 years old. Over the 12-week study period, the investigators did not find any improvement in wound healing with the use of PDWHF. Three (33 percent) wounds healed in two patients in the control group, and four (24 percent) wounds healed in three patients in the PDWHF group ( $p > 0.05$ ). The wounds in the PDWHF group increased in size during treatment, averaging  $-4.3 \pm 12.2$  cm<sup>2</sup>/week, while healing in the control group was  $1.9 \pm 2.7$  cm<sup>2</sup>/week. While this study was limited by a small sample size, its results suggested that treatment of chronic wounds with PDWHF is no better than traditional therapy [52].

### **POSSIBLE CONFOUNDING VARIABLES IN THESE STUDIES**

Several possible confounding variables exist in these studies, including the variation in patient characteristics between patients with type 1 and type 2 diabetes mellitus [5,53,54], duration of diabetes diagnosis [5,55], patient age [21,27,47,53,56-59], patient gender [27,47,53,56,58,60], patient race [53], initial wound area [6,21,53,56-

58,60], wound depth [57], wound duration [6,21,53,57,58,60], and wound location [21]. Other possible confounding variables include variation in PRP characteristics between studies, as growth factor content of autologous PRP can vary from patient to patient [2,17,20,61], even in patients with similar platelet counts [17]. The extent of platelet activation before application of gel may vary as well [2,20]. It is unclear if these factors are relevant in studies regarding the use of PRP in diabetic foot ulceration.

### **RATIONALE TO USE AUTOLOGOUS PLATELET-RICH PLASMA RATHER THAN RECOMBINANT HUMAN PLATELET-DERIVED GROWTH FACTOR-BB ISOMER**

The use of PRP represents a greater similarity to the natural healing process, with the application of multiple growth factors in their biologically determined ratios, more closely than the addition of a single growth factor such as rhPDGF-BB [62]. The autologous nature of PRP distinguishes it from recombinant human growth factors such as rhPDGF-BB, which are purely human but are foreign growth factors to that individual, synthesized by a culture of Chinese hamster ovarian cells with a human gene inserted into the nucleus through a bacterial plasmid vector [2]. In addition, growth factors may be more effective when directly delivered via a “depot” platelet plug, allowing a slow release of these factors than when administered in a bolus dose [24,63] such as commonly performed with rhPDGF-BB treatment.

The short shelf life of recombinant human growth factors such as rhPDGF-BB is not a concern with the use of PRP, which can be made as needed from an autologous donation [13]. More recent studies linked the expression of PDGF-BB to malignant transformation in human cells [64], and a black box warning was added to Regranex (becaplermin) in June 2008 following a post-market epidemiologic study linking the use of more than three tubes of becaplermin to a five times increased risk of mortality in

patients with a history of malignancy. Although the risk of developing a new malignancy during treatment with becaplermin was not increased [65], these recent findings remain cause for concern.

Finally, PRP may be a more cost-effective and economical use of resources for the treatment of diabetic foot ulcers. Dougherty (2008) carried out an evidence-based model of 200,000 hypothetical patients comparing the cost-effectiveness of PRP to a wide range of current therapies for diabetic foot ulceration and found that the use of PRP resulted in an improved quality of life and was less expensive over an estimated five-year period when compared to other treatment modalities, including rhPDGF-BB. While this economic model is hypothetical and used peer-reviewed data to simulate expected costs and outcomes of treatment with PRP, this study further suggests the promise of PRP in treatment of these ulcers in the future.

### **RATIONALE TO USE AUTOLOGOUS PLATELET-RICH PLASMA RATHER THAN ALLOGENIC OR HOMOLOGOUS PLASMA**

As an autologous preparation, PRP is safer to use than allogenic or homologous preparations and is free from concerns over transmissible diseases [1,2,13,14,24] such as HIV, hepatitis, West Nile fever, and Creutzfeldt-Jakob disease. PRP requires no special considerations regarding antibody formation [66], effectively preventing the risk of graft vs. host disease [29] and leading to better acceptance by patients [1].

### **CONCLUSIONS AND OUTLOOK**

Despite the many advances in the treatment of diabetic foot ulceration, this common complication continues to devastate the community of patients suffering from diabetes. It is important to motivate both patients and clinicians to attempt these more advanced treatment modalities, as treatment with growth factors may result in faster healing times and regained limb function in ad-

dition to a decreased rate of amputation, thereby improving the quality of life for diabetic patients suffering from diabetic foot ulceration. If PRP proves to be a more effective treatment for diabetic foot ulceration than rhPDGF-BB, there is potential for its use in other chronic wounds of varying etiology and of greater surface area.

Many unanswered questions remain regarding the use of topical growth factors in the treatment of chronic ulceration. Future studies should focus on the use of these growth factors in wounds of varying etiology and greater surface area. In addition, prospective studies with greater follow-up periods are necessary to verify the long-term safety of PRP, as the use of the growth factor preparation rhPDGF-BB has been associated with an increased risk of mortality in patients with a history of malignancy and is not recommended for use in these patients. In spite of these remaining questions, PRP shows promise as an effective treatment modality in the setting of diabetic foot ulceration.

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