Original Article

Proficiency of Topical Platelet-Rich Plasma with Vacuum-Assisted Closure over Platelet-Rich Plasma Alone in Diabetic Foot Ulcers – A Clinical, Prospective, Comparative study

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Abstract

Background and Objectives: Type 2 diabetes mellitus is usually associated with peripheral neuropathy, peripheral vascular disease with consequential limb ischemia, and eventually diabetic foot ulcers (DFUs). The healing process is slow due to microangiopathy and wound is easily infected with microbials leading to superficial infection, progressing to deep infection, and eventually landing in amputation most of the times. Platelet-rich plasma (PRP) is very cost effective, readily available blood derivative and has the capability to stimulate cell proliferation and differentiation. It improves tissue healing and regeneration and exhibit potent activities against a number of pathogens. Vacuum-assisted closure (VAC), on the other hand, is a new novel way to treat DFUs by having negative pressure wound healing. The present study focused on the advantage of (PRP + VAC) dressing over (topical PRP application with its peripheral injection) alone for aiding and enhancing the process of wound healing in DFU. **Materials and Methods:** This was a prospective comparative study of 100 cases to compare the outcomes of wound healing by topical PRP application with its peripheral injection. **Results:** Mean time taken for the appearance of granulation tissue, 100% granulation tissue, average reduction in wound surface area, showed significant ($P \geq 0.005$) differences between the (PRP + VAC) and the (topical PRP application with its peripheral injection) dressing groups. **Conclusions:** (PRP + VAC) dressings are more effective than conventional (topical PRP application with its peripheral injection) dressings in wound healing of DFUs.

Keywords: Platelet-rich plasma, type 2 diabetes mellitus, vacuum-assisted dressing

Introduction

The healing process of diabetic foot ulcer (DFU) is a complex mechanism involving the integrated interface between molecular signals and different cells together with platelets. These platelets in the presence of a diabetic wound get activated with thrombin and this activated platelet rich plasma (aPRP) releases growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor, insulin-like growth factor, and transforming growth factor beta (TGF-β). These growth factors trigger angiogenesis, extracellular matrix production and cytokine release, and help in wound healing.[1]

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Activated platelet-rich plasma, aPRP with × 4 of normal concentration platelets (1,000,000 platelets/microliter) is very cost-effective, readily available blood derivative, with a capacity to stimulate cell proliferation and differentiation. Cellular mitogenesis and angiogenesis are both upregulated by activated platelets.[2] Its high leukocyte concentration, autologous property helps in local debridement and antibacterial activity without an immune reaction.[3] Autologous aPRP not only enhances wound healing but also helps to regenerate skin tissue.[4] It is much advantageous in fracture wound healing is remedy for skin defects or dental mucosal wounds.[5,6]

Proper preparation and centrifuge technique are decisive to obtaining high quality active platelet-rich plasma (PRP).[7,8] Lack of biological effect may be due to poor PRP processing or inadequate standard laboratory centrifuges that cannot properly prepare PRP rather than the specialized FDA cleared equipment with validated processes.[9]

As aPRP is autologous, immune rejections are a nonissue.[3] It contains the same materials present in the blood that induce clotting, except in higher concentration.[10] It secretes growth factors which function by activating a cytoplasmic signal that further promotes normal gene expression.

Platelets are composed of a cytoskeleton and intracellular structures such as glycogen, lysosomes, and two granules, the *dense granule* and the *alpha-granule*.

The *alpha-granule* contains clotting factors, growth factors, proteins, and works through degranulation process.[9] PRP is collected in an anticoagulated form in a tube containing sodium citrate for the these growth factors to remain inactive.[11,12] These alpha granules bind to the transmembrane receptor of target cells such as mesenchymal stem cells, fibroblasts, endothelial cells, and epidermal cells, activating these.[13] This further activates intracellular signal proteins that express a gene sequence directing cellular proliferation, collagen synthesis, extracellular matrix formation, and numerous other pathways to promote healing and repair processes.[14]

Damaged platelets with degraded/nonviable cellular components are incapable of inducing this response.[15]

Negative pressure wound therapy (NPWT), on the other hand, is a novel way to treat DFUs by creating an intermittent negative pressure across these ulcers (NPWTs) is effective in managing wound infections, soft tissue loss, vascular insufficiency, and traumatic wounds.[16,17] It is a noninvasive method based on well defined, controlled negative pressure application through medical grade reticulated polyurethane ether or polyvinyl foam dressing to wound surfaces. The technique characteristically removes exudates from wounds and hence reduces extravascular, interstitial fluid; subsequently leading to enhanced microcirculation.[18] Taking away of wound fluid removes factors that suppress fibroblasts, vascular endothelial cells and keratinocytes, all of which promote wound healing.

Experimental studies have revealed a positive influence on both local microcirculation and granulation tissue formation. Local mechanical physical factors, yet not completely understood, similar to tissue expansion, and seem to promote cell growth. These studies have revealed that cells, allowed to stretch, tend to divide and proliferate in the presence of soluble mitogens.[19]

One explanation for the high acceptance on part of the therapists and the widespread use of the vacuum-assisted closure (VAC) method in these are the excellent clinical results.[20]

The present was a clinical, prospective comparative study to check the proficiency of topical platelet-rich plasma (PRP) with VAC over only topical PRP application in DFUs.

Materials and Methods

This was a clinical, prospective comparative study, done on 100 patients of either sex, in their middle years of life, from the demographic profile, having type 2 diabetes mellitus and on oral hypoglycemic drugs (biguanide/thiazolidinediones/alpha-glucosidase inhibitors) with DFU admitted in the department of orthopedics at a tertiary care hospital in Punjab, India, to compare the outcomes of wound healing with topical aPRP application, to topical aPRP combined with VAC from January 2019 to December 2020. An informed consent was obtained from each participant. Thereafter, ethical clearance was taken from IEC of the institution to conduct the study.

Inclusion criteria

- Patients within the age group of 30–60 years
- Patients with a DFU of >4-week duration, not healed with conventional conservative methods of treatment.

Exclusion criteria

- Patient with systemic disorder (chronic obstructive pulmonary disease and tuberculosis) etc.
- Psychological disorders, unable to maintain personal hygiene
- Patient with coagulopathies
- Advanced peripheral vascular disease secondary to type 2 diabetes mellitus
- Other confounding factors (old age, compliance, arteriosclerosis, varicose ulcers, alcoholism, and smoking).

Patients allocated to one of the treatment arms through sampling without replacement method for their random allocation. Patients included in the study underwent routine investigations and initial debridement. Wound size (cm^2) measured on day "0" (zero) with two largest perpendicular diameters and calibrated on a graph paper, depth of the wounds was not a consideration in the present series as the included patients had sloughing of epidermis and dermis only. On the same day, deep tissue swab was taken and appropriate antibiotics initiated as per culture and sensitivity report. After achieving adequate hemostasis after debridement, a foam band dressing applied over the wounds under aseptic condition with

the application of intermittent negative pressure after 4 h of application of dressing; removed and replaced with a new one every 4 days. All the patients had punch biopsies taken as a part of protocol during follow up at 3, 6, and 12 weeks and at regular intervals thereafter. This was done to confirm the appearance, presence and progression of the granulation tissue, revascularization and to crosscheck any adverse change/malignant transformation of wound, if any. Decrease in size of wound documented at each visit until permanent healing of the wound was there.

The following steps followed in the study.

Preparation

Autologous PRP (1000000 platelets per microliter of blood) obtained from freshly drawn blood (30 ml of venous blood) of the patient with an added anticoagulant (sodium citrate). It drawn blood experienced two centrifugation (spin) steps.[7]

The first spin, known as HARD SPIN, (more than 3000 rpm for 15 min) separated the red blood cells (RBCs) from the plasma containing the platelets, white blood cells (WBCs), and clotting factors. Three layers resulted from the hard spin: An upper layer-containing platelets and WBCs, a middle layer known as the buffy coat containing maximum number of platelets, and a bottom layer containing RBCs. The RBC layer removed and discarded.

The second spin (also called SOFT SPIN [more than 2000 rpm for 5 min]), separated the PRP in the bottom of the tube of the platelet poor plasma (PPP) in the top of the tube by removal of more RBC. This created a bottom layer rich in platelets and leukocytes used for aPRP dressing [Figure 1].[8]

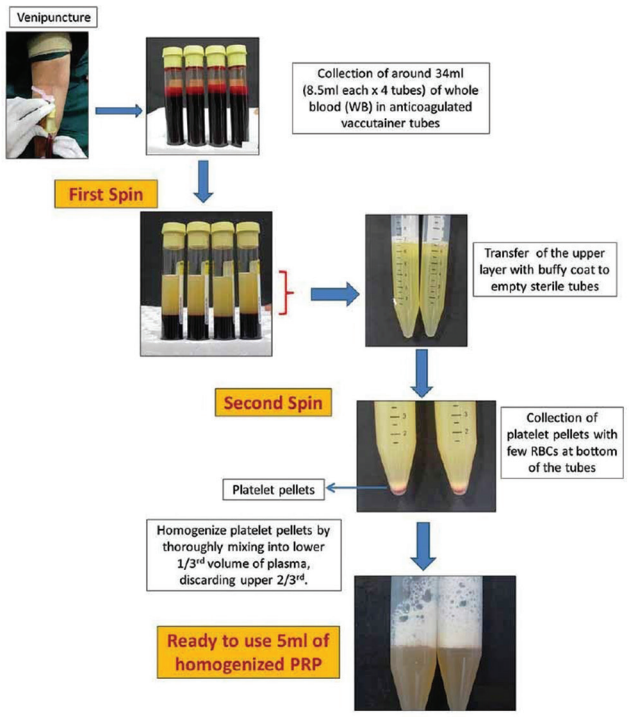

Figure 1: Preparation of platelet rich plasma

In (aPRP + VAC), the dressing sealed at periphery of the wound and connected it to VAC unit through a drainage tube to produce a desired pressure of 125 mm of hg, intermittently for two minutes every five minutes for half an hour, six times a day, after four hours of PRP dressing to allow the maximum imbibition of topically placed aPRP over the ulcer bed. Once the vacuum was on, it sucked air out of the dressing causing its collapse and drawing the edges of the wound inwards. It also took away exudates from the wound in tandem, through evacuation tube embedded in the dressing on one side and connected to a fluid collection canister contained within the vacuum/suction machine on other side, to collect these.

Method of application of dressings

1. In the first group, the sterile foam dressing cut to the approximate size of the wound placed gently into position after the application of freshly prepared aPRP.
2. In the second group, as per VAC application guidelines described by Lee *et al*.,[12] a drainage tube was placed on the top of the foam and a second piece of foam was placed over it.(For shallower wounds, a single piece of foam used and the drainage tube inserted inside it). The foam, together with first few inches of the drainage tube and the surrounding area of healthy skin, checked to ensure that the dressing formed an airtight seal both with the skin and drainage tube [Figure 2]. The distal end of the drain connected to a vacuum unit, programmed to produce the desired negative pressure of 125 mm of Hg, intermittently for two minutes every 5 min for half an hour, six times a day, after four hours of PRP dressing. It sucked air out of the dressing causing its collapse and drawing the edges of the wound inwards. It also took away exudates from the wound in tandem through

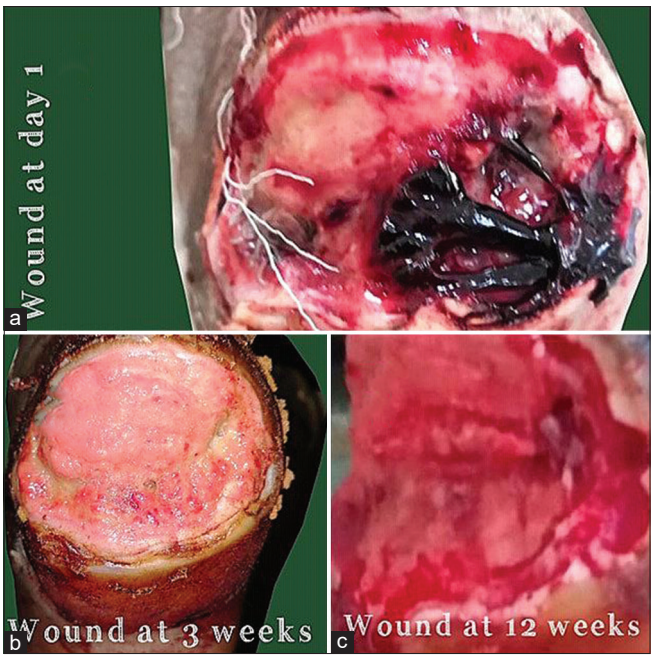

Figure 2: Progression of healing with the topical application of a platelet-rich plasma (clockwise). (a) Infected wound at presentation, (b) wound status at 3 weeks, (c) Healing at 12 weeks

evacuation tube connected to a fluid collection canister contained within the vacuum/suction machine.

Change of dressing

Performed every 4 days in both the groups with wound inspection at that time. The resident doctor conducted manual measurement of size and granulated area in cm^2. Findings calibrated on a graph paper.

Parameters for evaluation

Patients evaluated clinically for appearance of granulation tissue, 100% appearance of granulation tissue, full coverage of the ulcer, reduction in wound surface area and duration of hospital stay.

Statistical analysis

The results of observations of individual patients pooled for each intervention group. Data analysis performed using SPSS Statistics 20 software; SPSS Inc., Chicago, IL, USA. Numerical data expressed as mean, ± standard deviation (SD) or percent as proportionate to the sample size. The significance of difference between two groups was determined using "P" value.

A $P < 0.05$ considered significant.

Period of follow up

The follow up done at 3, 6 and 12 weeks [Figures 3 and 4] and thereafter at regular intervals until definitive wound coverage was there.

Results

All the patients were having type 2 diabetes mellitus (80% were on oral hypoglycemic agents and rest 20% were on insulin therapy) with an ulcer of >4 weeks duration ulcer on sole/dorsum of foot. The size of the wound ranged from 5.1 cms to 8.7 cms (mean of 7.6 ± 0.8 cms). Foot angiopathy (assessed by color Doppler) was the most associated co-morbidity. The mean time taken for appearance of granulation tissue, 100% granulation tissue, permanent wound coverage, reduction in wound surface area and hospital stay, was lesser in the (aPRP + VAC) dressing group, with a significant *P* value ($P \leq 0.005$), than in the (topical aPRP application) dressing group. Successive punch biopsies at regular follow up visits showed no adverse cellular change/malignant change in the present series. Complications such as infection, presence of exudates and a persistant pain were there in both modalities of treatment but were less in the (aPRP + VAC) dressing group. Only 86% of the patients needed the split skin grafting in (aPRP + VAC) dressing group when compared to topical aPRP application dressing group [Table 1].

Discussion

We all know that that despite many advances in the treatment of DFU, patients are yet living with amputation as destructive complication.[21] Topical application of aPRP and NPWT are two familiar modalities in the management of chronic DFUs. Although the former could significantly increase the rate of healing of DFUs, latter leads to a faster granulation tissue formation and reduction in wound size in comparison to the former.[22,23]

Autologous aPRP is economical and affordable as it is prepared with a small volume (7cc of aPRP prepared from 30cc of freshly drawn venous blood) of patient's blood and the risk

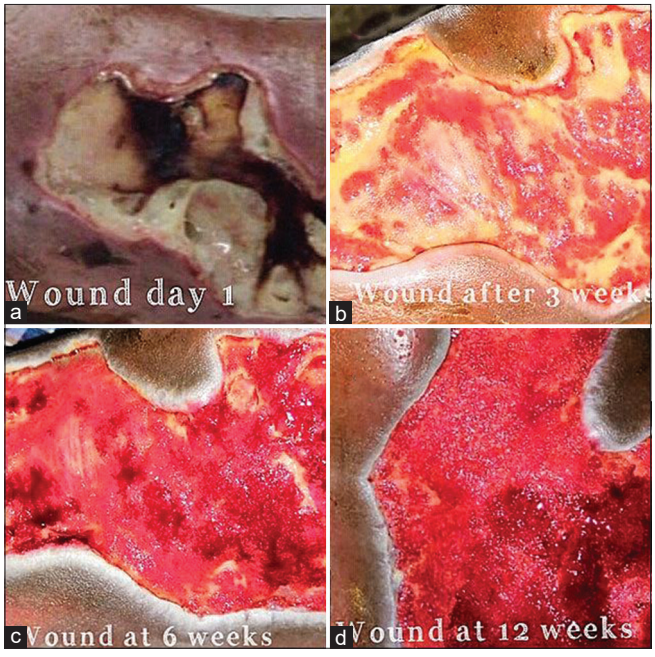

Figure 3: Progression of healing with topical application of a platelet-rich plasma (clockwise). (a) Infected wound at presentation, (b) wound status at 3 weeks, (c) Healing at 6 weeks, (d) Healing at 12 weeks

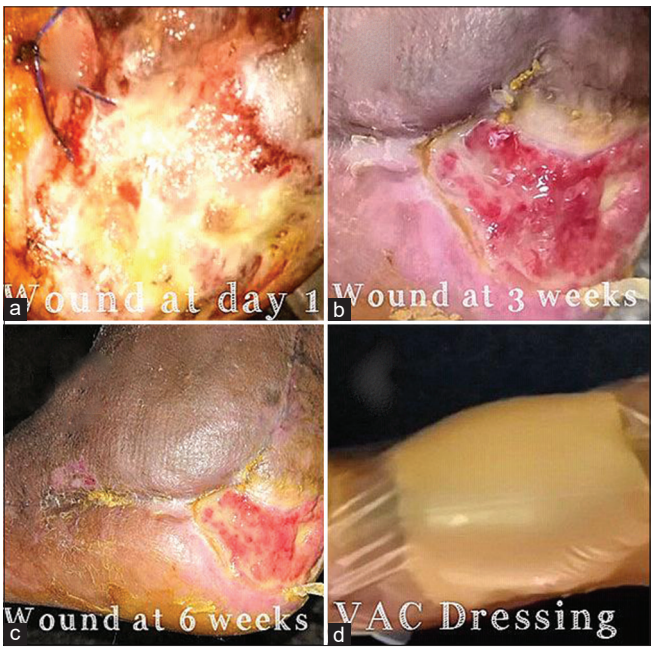

Figure 4: Progression of healing with topical application of a platelet-rich plasma (clockwise) and vacuum-assisted closure dressing (a) infected wound at presentation, (b) wound status at 3 weeks, (c) healing at 6 weeks, (d) vaccum assisted closure dressing

Table 1: Comparison of results of topical PRP application with its peripheral injection dressing and (PRP+VAC) dressing

Parameters	Group I (topical PRP application with its peripheral injection) dressing	Group II (PRP + VAC) dressing
Mean age (years) and sex (male: female)	33.28 (48:3)	35.46 (47:2)
Duration of diabetes	7-10 years (mean of 10.5±2.5 years)	
Size of the wound	5.1-8.7 cm (mean of 7.6±0.8 cm)	
Associated co-morbidities (%)		
Foot angiopathy	45	39
Controlled hypertension	62	
Nephropathy	30	
Smoking	60	
P=0.718, in significant		
Appearance of granulation tissue, *P*<0.005, significant (days)	9.12	4
Total appearance of granulation tissue, *P*<0.005 significant (days)	22.04	13
Total wound coverage (days)	25	14.88
Hospital stay (days)	36	22.04
Reduction in wound surface area/day, *P*≤0.005 significant (%)	1.6	3.9
Split skin grafting	90	86
Complications (%)		
Infection	22	14
Exudates	24	10
Pain	14	8

PRP: Plate rich plasma, VAC: Vacuum-assisted dressing

of transmission of blood borne diseases or immunological reaction is not an apprehension.

Autologous aPRP provides the growth factor needed for natural healing process in diabetic patients.[24] Seven fundamental protein growth factors, actively secreted by platelet initiate wound healing process. aPRP also has three proteins known to act as cell adhesion molecules i.e., fibrin, fibronectin and vitronectin.[7] Platelets also secrete TGF-beta and monocyte chemo attractant protein-1 that would attract monocytes and neutrophils to the wound site.

Present study found that PRP can facilitate healing of DFUs and therefore can reduce the risk of amputation[25] but more so when it used in alliance with NPWT.

Previous studies in support of "plate rich plasma" dressing

Driver *et al*.[23] conducted a study on 72 patients type 2 diabetes, aged between 18 and 95 years suffering from an ulcer of at least 4 weeks duration and compared the effectiveness of autologous aPRP gel to that of normal saline gel for 12 weeks. The primary objective of the study was to evaluate the safety of aPRP and 100 percent re-epithelialization, when compared to the control treatment, and a secondary objective was rate of re-epithelialization. Patients were randomized into two groups: Standard of care with aPRP gel or control (saline gel) and were evaluated biweekly for 12 weeks. They found that 68.4% (13/19) of patients in the aPRP group and 42.9% (9/21) in the control group had wounds that healed. Wounds in the aPRP group healed after a mean of 42.9 days (SD 18.3) versus 47.4 days (SD 22.0) in the control group. They thus confirmed the positive effect of autologous aPRP gel in the treatment of DFUs.

Lacci and Dardik[26] used aPRP as an adjuvant therapy and showed the effectiveness of it on healing of chronic wounds. The most important steps in their study were debridement, offloading and frequent dressing changes, procedures that transformed a chronic wound into an acute wound. They eliminated other factors that impeded healing like pressure, friction, and sheer. After preparing the wound bed, aPRP applied as a gel or injected in the wound. The healing time in their study was shorter as compared to saline dressings.

Mehrannia *et al*.[27] reported a single case of nonhealing DFU, successfully treated by injection of aPRP inside and around the peripheral skin.

Tran *et al*.[28] conducted a study aimed to evaluate the effects of aPRP on DFU healing on 6 patients. aPRP was isolated from peripheral blood and activated with calcium chloride. Patients were injected with aPRP two times with 14-day interval. All patients were monitored during 12 weeks. The results showed that 100% (6/6) ulcers completely closed after about 7 weeks. This result initially suggested that aPRP injection was efficient method to treat the nonhealing foot ulcers.

Suthar *et al*.[29] demonstrated the potential safety and efficacy of autologous aPRP for the treatment of chronic nonhealing ulcers. The mean age of the treated patients was 62.5 ± 13.53 years and they were followed-up for a period of 24 weeks. All the patients showed reduction in wound size, and the mean time duration to ulcer healing was 8.2 weeks.

Horn *et al*.[30] reported that aPRP gel could effectively decrease the width and depth of chronic wounds.

So most of the researchers stated above used PRP effectively in nonhealing DFU. Several of the researchers used it effectively

in dental and oral surgeries[31] and also in the treatment of musculoskeletal injuries,[32] but still studies about its usage in DFU are to be explored.

Previous studies supporting usage of "vacuum-assisted closure"

Ali M Lone *et al*.[33] conducted a prospective case control study on usage of VAC versus conventional dressings in management of DFUs. He reported that granulation tissue appeared in 26 (92.85%) patients by the end of week 2 in-group A (VAC), while it appeared in 15 (53.57%) patients by that time in-group B (conventional dressing). They achieved 100% granulation in 21 (77.78%) patients by the end of week 5 in Group A compared to only 10 (40%) patients in Group B. Patients in Group A had fewer number of positive blood cultures, secondary amputations. They concluded that VAC was more effective, safe and patient satisfactory compared to conventional dressings for the treatment of DFUs.

Jeffrey D. Lehrman[34] combined the benefits of Collagen and NPWT to heal a chronic DFU in a case and suggested combining the advanced wound healing properties of collagen with NPWT has a beneficial effect on wound healing.

Swaminathan GA *et al*.[35] conducted a study on 30 patients with DFUs to evaluate the efficacy of VAC wound therapy for the treatment of DFUs. They showed that mean initial wound surface area before VAC therapy was 103.07 cms and after VAC therapy there was significant reduction in wound size to 94.53 cms over a mean duration of 31.9 days. That significantly increased the wound bed granulation tissue and good percentage of graft and flap take up. The daily requirement of antibiotic and analgesic was reduced. Duration of hospital stay was reduced due to faster wound healing. There was overall reduction in pain and further complications like amputations were avoided thereby increasing the patients compliance. They Concluded that VAC therapy is newer and a safe method of treatment for DFUs.

Ahmed *et al*.[36] documented that PRP and NPWT were effective in neovascularization and stimulation of healing process but the latter was more effective. They showed expression of VEGF in PRP patients after the 3rd week was mild 10%, moderate 20%, strong 70% and was mild 10%, moderate 20%, strong 70% in peripheral and central tissue biopsy respectively. While in NPWT patient was mild 20%, moderate 30%, strong 50% in both peripheral and central tissue biopsy. The average of blood vessels formation in CD-31 was 9.95 ± 3.64 after 3 weeks in peripheral tissue biopsy and was 8.58 ± 3.51 in central tissue biopsy in PRP patients. While in NPWT patients was 8.35 ± 3.25 in peripheral tissue biopsy and was 8.38 ± 3.12 in central tissue biopsy.

Therefore, despite all controversies, the previous studies inveterate the effectiveness of aPRP in the treatment of DFUs, but also at the same time, studies are there to establish that VAC is more effective, safe, and patient satisfactory for the treatment of DFUs.

Previous studies-supporting synergism in using "negative pressure wound therapy" with "vacuum-assisted closure"

Leon G.[37] reported synergism in using NPWT with alternated applications of autologous PDGFs. He concluded that there was a significant change in wound volume (20%–50%), 2–6 weeks after beginning one or both treatment modalities. They further demonstrated that using NPWT and PRP treatments alternately resulted in successful wound healing.

The present study focused to have a synergistic effect of both methods proved that mean time taken for appearance of granulation tissue, 100% granulation tissue and average reduction in wound surface area showed statistically significant ($P \leq 0.005$) differences between two above stated dressing groups. This study showed that (aPRP + VAC) dressing, which was an attempt to amalgamate the advantages of both methods, is a superior alternative for achieving a better healing, in shorter duration, with lesser complications, thus establishing it, a better method of management of DFUs.

Conclusions

We conclude that (aPRP + VAC) dressings are superior to topical aPRP application dressings in the wound healing of DFUs.

Limitations of the study

Lack of control over some confounding factors such as patients' nutrition, activities, and their level of adherence to their medical treatments are some of the limitations of the present study. Moreover, a very small sample is another limitation of the study. Therefore, replication of the study with larger sample sizes is recommended.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Eppley BL, Pietrzak WS, Blanton M. Platelet-rich plasma: A review of biology and applications in plastic surgery. Plast Reconstr Surg 2006;118:147e-59e.
2. Sclafani AP, Azzi J. Platelet preparations for use in facial rejuvenation and wound healing: A critical review of current literature. Aesthetic Plast Surg 2015;39:495-505.
3. Kim DH, Je YJ, Kim CD, Lee YH, Seo YJ, Lee JH, *et al.* Can platelet-rich plasma be used for skin rejuvenation? Evaluation of effects of platelet-rich plasma on human dermal fibroblast. Ann Dermatol 2011;23:424-31.
4. Carter CA, Jolly DG, Worden CE Sr., Hendren DG, Kane CJ. Platelet-rich plasma gel promotes differentiation and regeneration during equine wound healing. Exp Mol Pathol 2003;74:244-55.
5. Lindeboom JA, Mathura KR, Aartman IH, Kroon FH, Milstein DM, Ince C. Influence of the application of platelet-enriched plasma in oral mucosal wound healing. Clin Oral Implants Res 2007;18:133-9.
6. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost 2004;91:4-15.
7. Dhurat R, Sukesh M. Principles and methods of preparation of

platelet-rich plasma: A review and author's perspective. J Cutan Aesthet Surg 2014;7:189-97.
8. Boswell SG, Cole BJ, Sundman EA, Karas V, Fortier LA. Platelet-rich plasma: A milieu of bioactive factors. Arthroscopy 2012;28:429-39.
9. Everts PA, Knape JT, Weibrich G, Schönberger JP, Hoffmann J, Overdevest EP, *et al.* Platelet-rich plasma and platelet gel: A review. J Extra Corpor Technol 2006;38:174-87.
10. Marx RE. Platelet-rich plasma: Evidence to support its use. J Oral Maxillofac Surg 2004;62:489-96.
11. Alves R, Grimalt R. A review of platelet-rich plasma: History, biology, mechanism of action, and classification. Skin Appendage Disord 2018;4:18-24.
12. Chan RK, Liu P, Lew DH, Ibrahim SI, Srey R, Valeri CR, *et al.* Expired liquid preserved platelet releasates retain proliferative activity. J Surg Res 2005;126:55-8.
13. Sclafani AP, McCormick SA. Induction of dermal collagenesis, angiogenesis, and adipogenesis in human skin by injection of platelet-rich fibrin matrix. Arch Facial Plast Surg 2012;14:132-6.
14. Eppley BL, Pietrzak WS, Blanton M. Platelet-rich plasma: A review of biology and applications in plastic surgery. Plast Reconstr Surg 2006;118:147e-159e.
15. Andia I, Abate M. Platelet-rich plasma: Underlying biology and clinical correlates. Regen Med 2013;8:645-58.
16. Venturi ML, Attinger CE, Mesbahi AN, Hess CL, Graw KS. Mechanisms and clinical applications of the vacuum-assisted closure (VAC) Device: A review. Am J Clin Dermatol 2005;6:185-94.
17. Morykwas MJ, Argenta LC, Shelton-Brown EI, McGuirt W. Vacuum-assisted closure: A new method for wound control and treatment: Animal studies and basic foundation. Ann Plast Surg 1997;38:553-62.
18. Horch RE. Basics foundation and results of the vacuum therapy in the reconstructive surgery. Zentralbl Chir 2004;129 Suppl 1:S2-5.
19. Saxena V, Hwang CW, Huang S, Eichbaum Q, Ingber D, Orgill DP. Vacuum-assisted closure: Microdeformations of wounds and cell proliferation. Plast Reconstr Surg 2004;114:1086-96.
20. Renner R, Rogalski C, Friedlein H, Simon JC. Vacuum therapy in dermatology: A review J Dtsch Dermatol Ges 2006;4:468-76.
21. Driver VR, Hanft J, Fylling CP, Beriou JM; Autologel Diabetic Foot Ulcer Study Group. A prospective, randomized, controlled trial of autologous platelet-rich plasma gel for the treatment of diabetic foot ulcers. Ostomy Wound Manage 2006;52:68-70, 72, 74.
22. Lee HJ, Kim JW, Oh CW, Min WK, Shon OJ, Oh JK, *et al.* Negative pressure wound therapy for soft tissue injuries around the foot and ankle. J Orthop Surg Res 2009;4:14.
23. Arcand V, Born C, Bliss J, Ray J, Patel A. Negative pressure wound therapy and its application in orthopaedics Part II: Clinical application. Osteosynth Trauma Care 2006;14:254-8.
24. Moura LI, Dias AM, Carvalho E, de Sousa HC. Recent advances on the development of wound dressings for diabetic foot ulcer treatment – A review. Acta Biomater 2013;9:7093-114.
25. Kargar S, Javadzadeh Shahshahani H, Tabkhi N. The effect of platelet gel on the treatment of diabetic foot ulcer. Sci J Iran Blood Transfus Org 2010;6:283-91.
26. Lacci KM, Dardik A. Platelet-rich plasma: Support for its use in wound healing. Yale J Biol Med 2010;83:1-9.
27. Mehrannia M, Vaezi M, Yousefshahi F, Rouhipour N. Platelet rich plasma for treatment of nonhealing diabetic foot ulcers: A case report. Can J Diabetes 2014;38:5-8.
28. Tran TD, Le PT, Van Pham P. Diabetic foot ulcer treatment by activated platelet rich plasma: A clinical study. Biomed Res Ther 2014;1:1-6.
29. Suthar M, Gupta S, Bukhari S, Ponemone V. Treatment of chronic non-healing ulcers using autologous platelet rich plasma: A case series. J Biomed Sci 2017;24:16.
30. Horn SD, Fife CE, Smout RJ, Barrett RS, Thomson B. Development of a wound healing index for patients with chronic wounds. Wound Repair Regen 2013;21:823-32.
31. Albanese A, Licata ME, Polizzi B, Campisi G. Platelet-rich plasma (PRP) in dental and oral surgery: From the wound healing to bone regeneration. Immun Ageing 2013;10:23.
32. Lone AM, Zaroo MI, Laway BA, Pala NA, Bashir SA, Rasool A. Vacuum-assisted closure versus conventional dressings in the management of diabetic foot ulcers: A prospective case-control study. Diabet Foot Ankle 2014;5:1-5. [doi: 10.3402/dfa.v5.23345].
33. Lehrman JD. Combining the benefits of collagen and negative pressure wound therapy to heal a chronic diabetic foot ulcer: A case report. Wounds 2020;32:E11-3.
34. Cervelli V, Gentile P, Scioli MG, Grimaldi M, Casciani CU, Spagnoli LG, *et al.* Application of platelet-rich plasma in plastic surgery: Clinical and *in vitro* evaluation. Tissue Eng Part C Methods 2009;15:625-34.
35. Swaminathan AG, Subramanian CS, Sundaram S. Novel effective treatment for diabetic foot ulcers [DFU] by vacuum assisted wound closure therapy. Int J Surg Sci 2020;4:458-61.
36. Ahmed G, Khalil AA, Hawary AK, Samy A, Eldin SS, Ahmed MB. Comparative study between negative pressure wound therapy and platelet rich plasma in neovascularization of chronic wound healing. Egypt J Plast Reconstr Surg 2018;42:35-43.
37. Gurvich L. Synergism in using negative pressure wound therapy with alternated applications of autologous platelet-derived growth factors in treating post-acute surgical wounds. Wounds 2009;21:134-40.