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Original Research Article

Leucocyte Platelet-Rich Fibrin (L-PRF) in non-healing ulcers

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ABSTRACT

Introduction: The therapeutic use of autologous platelet concentrates represents a newer regenerative avenue to stimulate and accelerate complex wound healing. These non-healing ulcers have repercussions in terms of decrease functional outcome and productivity of the patients. Here, we intend to wider the horizon of an alternative approach for treating small-to-moderate-sized complex wounds of lower extremities by using novel Leucocyte Platelet-Rich Fibrin (L-PRF) which embraces healing potentiality over that of bare soft tissue, including bone, tendon, and ligaments respectively.

Materials and Methods: A total of 23 cases of non-healing ulcers were receiving L-PRF gel application. The cases with small and large ulcers received L-PRF gel weekly once for 3 and 6 weeks respectively. All the cases were followed up in regular intervals at the end of every week and the end of 3rd month. To document the progress of ulcer healing, we calculated the area and volume of the ulcer at the beginning of the procedure and every week till the size of the ulcer gets contracted. Photographic documentation was performed for all the cases.

Results: The duration of non-healing ulcers ranged from 6 months to 24 months with a mean duration of 15.05 ± 2.37 months. According to Wagner's ulcer classification scale, 15 ulcers (65.21%) belong to grade 1 and 8 ulcers (34.78%) belong to grade 2. In grade 1 ulcer cases, the mean area and volume improvement observed was 100% at the end of 3 months whereas, in grade 2 ulcer cases, the mean area and volume improvement observed was 95.67% and 97.31% at the end of 3 months. The volume of 15 ml of venous blood was sufficient to cover an ulcer with a maximum area of 23.12 cm² with 1 mm thickness of L-PRF.

Conclusion: Our study adds to the string of positive evidence for treating complex wounds using L-PRF wherein this autologous preparation is available at greater ease to facilitate the process of granulation tissue formation and epithelization. This serves as a good alternative to manage wounds of small to medium size and minimizes the need to plan for relevant soft tissue surgeries.

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1. Introduction

Complex wounds have been described as well-known wounds posing a challenge for medical and nursing teams irrespective of their acute or chronic onset.¹ The entity

not only represents a considerable financial burden on the health care set-up of the country but also undermines the functional and productive outcome of the patients. Notably, ulcers located in the lower extremities constitute the most commonly encountered chronic wounds and on average usually last for about 12-13 months, warranting extra wound care.²

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Over the past decades, there has been tremendous development in the management of complex wounds. The intricate interest in the aspect of wound care has resulted in the therapeutic advancement with the employing of newer regenerative modalities being directed to stimulate and accelerate complex wound healing. In this alignment, autologous platelet based concentrates represent one such biological avenue being underutilization at present for the healing of complex wounds. The use of platelet based derivatives for treating wounds of skin dates back to the 20th century, albeit, the notable terminology “PRP” in the field of regenerative medicine was introduced by Marx et al in the year 1998 wherein their study reported a positive effect of this platelet based product- PRP on the healing of bone in maxillofacial surgery.³ Following the ascription of the term, it attained popularity and turned into an area of keen interest of many researchers including oral and maxillofacial surgery, cosmetic surgery, dermatology, and dentistry. Despite multiple advantages of PRP, factors like utilization of anticoagulants, the requirement of apt equipment, and the high cost of available pre-kits have critically made it difficult to use it in routine clinical practice.⁴ The aforementioned notion paved the way to develop low-cost platelet concentrates with more beneficial biological characterizations. Subsequently, in 2001, second-generation PRP emerged which lacked coagulation factors and was characterized by a dense matrix of fibrin, later named platelet-rich fibrin (PRF).⁴

The contents of platelet-rich fibrin (PRF) include platelets, leukocytes, cytokines, and adhesive proteins including fibrinogen, fibronectin, vitronectin, and thrombospondin-1 respectively.^{5,6} The second-generation platelet concentrates include products such as leukocyte-platelet-rich-fibrin (L-PRF) and advance-platelet-rich-fibrin (A-PRF) wherein offer an edge over the first generation in terms of easier, cost-effective, and rapid protocol resulting in a solid formulation of higher density with the characteristic tridimensional organization. To reiterate, Choukroun et al. had developed this open technique of L-PRF preparation which involves subsection of peripheral blood to one-step based centrifugation either at 3000 rpm for 10 minutes or 2700 rpm for 12 minutes without application of anticoagulants and blood activating substances. Briefly, venous blood collected in a glass tube without anticoagulants is centrifuged at low speed and the resultant yield is a three-layered solution. The middle fraction of this product has a fibrin clot which is the biological product of interest and can be readily applied at the wound site in its membranous formulation.⁷

The salient facet of L-PRF is the presence of white blood cells which indeed secrete growth factors in abundant amounts and facilitate the healing process. Notably, there is a slow release of 273.4 ± 15.3 ng transforming growth factor- β 1 (TGF- β 1), 6071 ± 773 pg vascular endothelial

growth factor (VEGF), and 50.3 ± 6.3 ng platelet-derived growth factor-AB (PDGF-AB) by an intact PRF membrane for 7 days, which represent large amounts of these growth factors.⁷ The unique organization in the form of the 3-dimensional fibrin matrix provides a binding site for platelets as well as growth factors. This flexible mesh serves as a scaffold to promote cellular migration in the micro-environment and perquisite in repairing and regenerating tissue. Overall, in L-PRF preparation, leukocyte and fibrin act as mutual stimulatory actors by imitating the physiological process of wound healing and boosting angiogenic, osteogenic, and antimicrobial activities.⁸

Herein, we used L-PRF on non-healing ulcers of small to medium-sized located in the lower extremities as an alternative, cost-effective and simple treatment modality with the background rationale of neoangiogenesis and healing of wound under physiological dimensions. This method is comparatively fast and applicable without any requirement of hospitalization with the benefit of reduction in hours lost for work.

2. Materials and Methods

After obtaining institute ethical clearance, a total of 23 cases with chronic non-healing ulcers were recruited. All cases were evaluated for the underlying pathology of the non-healing ulcers. An informed and written consent was obtained for treating non-healing ulcers with L-PRF. All the cases were subjected to routine hematological and radiological investigations. The cases of non-healing ulcers secondary to diabetes, venous pathology, and leprosy, wounds secondary to open injury, and trophic ulcers were included in the study. The cases of non-healing ulcer with malignant etiology, peripheral artery disease (distal pulses absent or ankle-brachial index <0.8 and/or >1.2), connective tissue disorders, cutaneous granulomatous diseases, mycobacterial or fungal infection, chronic steroidal, and/or immunosuppressive drugs were excluded from the study.

A total of 15 ml venous blood was withdrawn and were subjected to centrifugation of 1300 rpm for 8 minutes in plain glass vacutainer tubes under sterile conditions. The resultant column contains a whitish-yellow gel-like substance which represents L-PRF and reddish gel which represents RBC gel as shown in Figure 1. L-PRF gel has to be separated and applied over the chronic non-healing ulcer.

The patients with small and large ulcers received L-PRF gel weekly once for 3 and 6 weeks respectively. All the cases received non-absorbable dressings. All the cases were followed up in regular intervals at the end of every week and the end of 3rd month. At every visit, the ulcer was gently cleansed with normal saline to get rid of the exudates without performing any mechanical debridement. Subsequently, L-PRF gel was placed in the ulcer area and covered the ulcer with non-absorbable

dressings. Such procedure was repeated till the ulcer get contracted. To document the progress of ulcer healing, we calculated the area and volume of the ulcer at the beginning of the procedure and every week till the size of the ulcer gets contracted. Photographic documentation was performed for all the cases. The descriptive statistics were reported as mean (SD) for continuous variables, frequencies (percentage) for categorical variables. Data were statistically evaluated with IBM SPSS Statistics for Windows, Version 24.0, IBM Corp, Chicago, IL.

3. Results

In our study, a total of 23 cases with non-healing ulcers of various etiology were included to receive L-PRF gel management. Out of 23 cases, 16 cases (69.56%) were males and 7 cases (30.43%) were females with the mean age of 39.76 ± 12.18 years. The duration of non-healing ulcers ranged from 6 months to 24 months with a mean duration of 15.05 ± 2.37 months. According to Wagner's ulcer classification scale, 15 ulcers (65.21%) belong to grade 1 and 8 ulcers (34.78%) belong to grade 2. Grade 1 ulcer cases required 3 doses of L-PRF gel application for the ulcer to get contracted while grade 6 ulcer cases required a maximum of 6 doses of L-PRF gel application.

In grade 1 ulcer cases, the mean area and volume improvement observed was 100% at the end of 3 months whereas, in grade 2 ulcer cases, the mean area and volume improvement observed was 95.67% and 97.31% at the end of 3 months (as shown in Figures 2 and 3). Both the grade cases have not to be supplemented with any adjuvant treatment. At the end of the study, we observed no adverse events in any of our cases. The volume of 15 ml of venous blood was sufficient to cover an ulcer with a maximum area of 23.12 cm^2 with 1 mm thickness of L-PRF.

4. Discussion

Non-healing ulcers pose a major challenge in successful management. Various treatment modalities have played a major role in the treatment of non-healing ulcers from conservative management, adjuvant management to surgical management.^{9–11} Due to the robust increase in the evidence of regenerative modality, various researchers utilized platelet concentrates for non-healing ulcer management.^{12,13} The differences among various platelet concentrates have been appended in Table 1.

Newer insights and appraisals in the dimension of regenerative medicine have offered the world exhilarating treating modalities and one such is the advances in utilizing platelet concentrates for managing complex wounds. We used L-PRF in our study to treat non-healing ulcers of small to medium-sized present at lower extremities. These usually present with exposed underlying tissues and further complicates the process of granulation tissue whilst simple

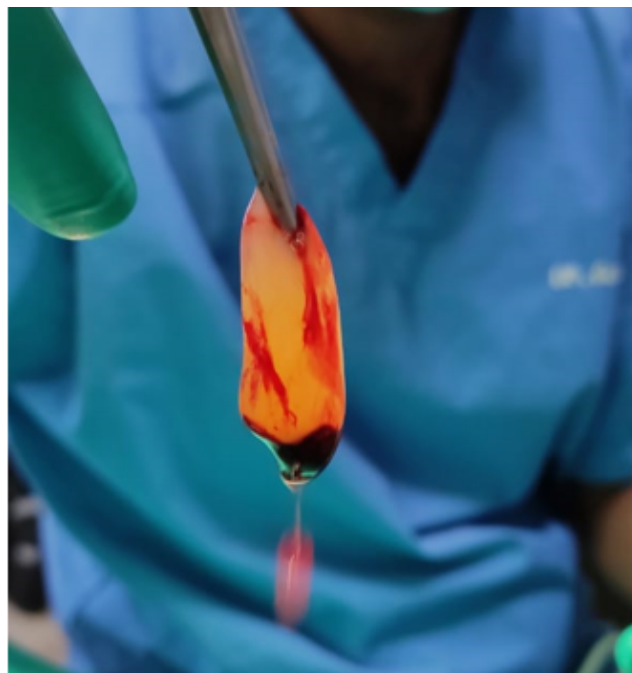


Fig. 1: Leucocyte Platelet-Rich Fibrin gel

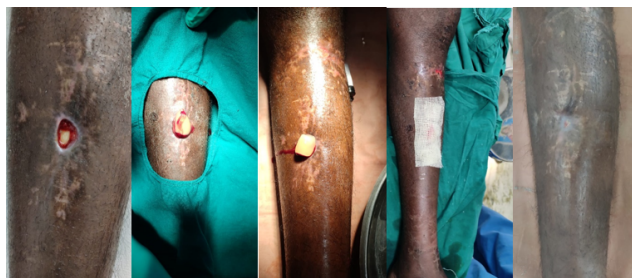


Fig. 2: Case 1 showing a non-healing ulcer on the middle 1/3rd of right tibia (4 months duration). The images showing L-PRF gel application and progressive healing of the ulcer in 6 weeks.



Fig. 3: Case 17 showing a non-healing ulcer on the dorsal aspect of the right foot (7 months 3 weeks duration). The images showing L-PRF gel application and progressive healing of the ulcer in 6 weeks.

Table 1: Schematic tabulation of biological characterization of the first and second generation of platelet concentrates⁸

Generation of Platelet Concentrate	First Generation PRP	PRGF	Second Generation L-PRF	A-PRF
Use of anticoagulants	CPDA	Sodium citrate	Not use	Not use
Activating coagulation	Calcium glutamate/bovine thrombin	Calcium chloride	Not use	Not use
Fibrin membrane	-	Yes (induced)	Yes (physiological)	Yes (physiological)
Leukocytes	Non-determined	0%	50-65%	50-65% (Increase neutrophils)
Growth factors	Present in a small amount	Present in moderate amount	Present in a comparatively very good amount	Present in an excellent amount
Regeneration of bone	Minimally	Moderately	Good	Good
Form of presentation	Gel	Liquid; Clot; Supernatant; Fibrin membrane	Plugs used for alveolar filing; Exudate; Fibrin membrane	Plugs used for alveolar filing; Exudate; Fibrin membrane

dressings. Additionally, at the same time, it presents with an equivocal challenge to maintain the viability of surrounding tissue. Individuals failing to respond within 4 weeks of treatment have been regarded as an ideal candidate for advanced therapy as per the literature. Moreover, pre-existing co-morbid conditions present with other sets of complications in case of treating complex wounds with surgery. Thereby, we believe that such dilemmatic circumstances can be best approached with alternative therapy using platelet concentrates.

In our study, we have treated 23 cases of non-healing ulcers of various etiology with L-PRF gel application. At the end of 3 months, in grade 1 ulcer cases, the mean area and volume improvement observed was 100% whereas, in grade 2 ulcer cases, the mean area and volume improvement observed was 95.67% and 97.31%. The biology of platelet and their concentrate have a beneficial effect in the management of non-healing ulcers due to the high concentration of platelets, leucocytes, bioactive micromolecules such as fibrin, and growth factors (TGF- β , VEGF, PDGF, EGF, IGF).^{7,14,15} In vitro studies have proven the maximal release of growth factors and cytokines in the media in the presence of fibrin meshwork for consequent 7 days.¹⁵

In-vitro on cell cultures, L-PRF induces proliferation and stimulates differentiation of all cell lineages (osteogenesis, chondrogenesis, and adipogenesis).¹⁶ L-PRF membranes behave in vitro like living tissue and hence used as a covering tissue graft in non-healing ulcers.^{17,18} L-PRF gel is an “optimized blood clot,” and is a very simple treatment without any risk for the patient with no financial cost.^{19,20}

Platelet-rich fibrin (PRF) was initially developed by dental surgeons as a surgical adjuvant for bone and soft tissue regeneration in oral surgery and implant dentistry.^{21,22} The advanced forms of PRFs (A-PRF, A-PRF+, T-PRF, and i-PRF) have been documented with promising results.²³ With the available evidence in

dentistry, the same protocol has been extrapolated to treat non-healing ulcers in our study. We observed an excellent outcome in all the ulcers without any adverse side effects.

L-PRF gel possesses antimicrobial and anti-inflammatory properties. The beneficial effects of L-PRF gel have been proven in osteonecrosis of the jaw due to bisphosphonate usage. The treatment of non-healing ulcers with L-PRF gel is a breakthrough in terms of challenges faced during the treatment and morbidity in the quality of life of the patients. In this study, all cases were successfully treated with a 100% ulcer contraction rate. Regenerative medicine has provided a better therapeutic option, autologous, safe, and easy to use adjuvant without any risk to the individuals undergoing such procedures.

The major limitation of our study is no inclusion of a control group for comparing L-PRF applications and therefore considering it as a ‘holy grail’ for treating non-healing complex wounds solely seems indefensible. The other notable limitations include smaller sampling size, no patient stratification, and single-centered study respectively.

5. Conclusion

Our study adds to the string of positive evidence for treating non-healing ulcers using L-PRF wherein this autologous preparation is available at greater ease to facilitate the process of granulation tissue formation and epithelization. This serves as a good alternative to manage wounds of small to medium size and minimizes the need to plan for soft tissue surgeries.

6. Conflict of Interests

All authors have declared no conflict of interests.

7. Conflict of Interest

None.

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