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ORIGINAL ARTICLE

Leucocyte- and platelet-rich fibrin (L-PRF) as a regenerative medicine strategy for the treatment of refractory leg ulcers: a prospective cohort study

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Abstract

Chronic wounds (VLU: venous leg ulcer, DFU: diabetic foot ulcer, PU: pressure ulcer, or complex wounds) affect a significant proportion of the population. Despite appropriate standard wound care, such ulcers unfortunately may remain open for months or even years. The use of leukocyte- and platelet-rich fibrin (L-PRF) to cure skin ulcers is a simple and inexpensive method, widely used in some countries but unknown or neglected in most others. This auto-controlled prospective cohort study explored and quantified accurately for the first time the adjunctive benefits of topical applications of L-PRF in the management of such refractory ulcers in a diverse group of patients. Forty-four consecutive patients with VLUs (n = 28, 32 wounds: 17 ≤ 10 cm^2 and 15 > 10 cm^2), DPUs (n = 9, 10 wounds), PUs (n = 5), or complex wounds (n = 2), all refractory to standard treatment for ≥3 months, received a weekly application of L-PRF membranes. L-PRF was prepared following the original L-PRF method developed more than 15 years ago (400g, 12 minutes) using the Intra-Spin L-PRF centrifuge/system and the XPression box kit (Intra-Lock, Boca Raton, FL, USA; the only CE/FDA cleared system for the preparation of L-PRF). Changes in wound area were recorded longitudinally via digital planimetry. Adverse events and pain levels were also registered. All wounds showed significant improvements after the L-PRF therapy. All VLUs ≤ 10 cm^2, all DFUs, as well as the two complex wounds showed full closure within a 3-month period. All wounds of patients with VLUs > 10 cm^2 who continued therapy (10 wounds) could be closed, whereas in the five patients who discontinued therapy improvement of wound size was observed. Two out of the five PUs were closed, with improvement in the remaining three patients who again interrupted therapy (surface evolution from 7.35 ± 4.31 cm^2 to 5.78 ± 3.81 cm^2). No adverse events were observed. A topical application of L-PRF on chronic ulcers, recalcitrant to standard wound care, promotes healing and wound closure in all patients following the treatment. This new therapy is simple, safe and inexpensive, and should be considered a relevant therapeutic option for all refractory skin ulcers.

Keywords

Diabetic foot, leukocyte- and platelet-rich fibrin, pressure ulcer, venous ulcer, wound healing

History

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Introduction

The term "skin ulcers" refers to a heterogeneous group of wounds including venous leg ulcer (VLU) [1], diabetic foot ulcer (DFU) [2,3], pressure ulcer (PU) [4–10], and arterial and neurotrophic ulcers. Such wounds, especially if recalcitrant despite appropriate wound care, have a dramatic impact on patients' quality of life, productivity, and life expectancy. They are associated with high treatment costs and are a significant cause of morbidity. Nonhealing ulcers are affecting, for example, more than 6000 000 persons in the USA (a number that might even further increase as the population ages), and represent a substantial financial burden on the health care systems, the families affected by these wounds, and the human societies in general [11,12].

Despite the relative diversity of their etiology, these skin ulcers share similar biological patterns: deep impaired healing mechanisms, pathological and disruptive inflammatory equilibrium, dysfunctional local vascularization, tissue necrosis, and infection. The standard treatment for the above-mentioned chronic ulcers may include debridement of necrotic tissues, revascularization surgery, infection control, mechanical offloading, management of blood glucose, foot care education, mechanical compression, or limb elevation [12]. Full wound closure, after standard VLU care, can take months or even years in some patients, and in up to half of the patients wound closure even fails

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[13,14]. For DFUs, similar wound closure rates have been reported (from 24.2% to 30.9% at 12 and 20 weeks, respectively)[15]. For PU, there is no good evidence to support the use of any particular wound-cleansing solution or technique, and wound closure remains extremely difficult [8]. If such treatment fails, "advanced wound care" is recommended. In the last decade, a large array of advanced therapies has been proposed, but their efficacy, comparative effectiveness, and eventual harms are not well established. Unfortunately, most of these advanced therapies are expensive, and not necessarily clearly superior as compared to standard optimal wound care [3,8,12,16].

The use of blood derivatives (fibrin glues, or platelet concentrates for surgical use regrouped under the acronym PRP, platelet-rich plasma)[17] was often suggested in the last 50 years for the treatment of skin wounds [18,19], and the use of platelet-fibrin concentrates is one of the oldest approaches of regenerative medicine in modern medicine [20]. Fibrin matrix and platelet components (particularly growth factors) offer interesting healing properties as surgical adjuvants [17]. For this reason, many PRP methods are still marketed and find applications in the treatment of skin wounds [21]. Unfortunately, despite interesting results [21], these techniques offer limited benefits for refractory ulcers, and do not allow closing the extended and complex wounds [22–25]. Moreover, their complexity and cost of use per application make them not accessible to most patients, and in most countries worldwide.

Leukocyte- and platelet-rich fibrin (L-PRF), a second-generation platelet concentrate for topical use, is an autologous blood-derived product, which can be obtained, quickly and at low cost [17,20,26]. It is classified as one of the four families of platelet concentrates for surgical use, and is therefore a different class of products than traditional PRPs [17]. L-PRF is produced from a small peripheral blood sample (9–10 mL per tube), which is immediately centrifuged without any anticoagulant. Coagulation starts during the centrifugation, and three parts quickly appear in the tube: a red blood cell base at the bottom, acellular plasma as a supernatant (platelet-poor plasma), and the L-PRF clot in-between. The latter, rich in fibrin, platelets (±95% of initial blood) and leukocytes (±50% of initial blood), is further transformed into a membrane, circa 1 mm in thickness, by careful compression [27]. L-PRF membranes release significantly large amounts and for a long period (during at least 7 days) of many different growth factors and matrix proteins [28]. Moreover, L-PRF membranes remain intact for more than 7 days *in vitro* (even more than 28 days in culture)[29], due to a specific polymerization and architecture of the fibrin matrix [30], and they possess some antibacterial effects [31]. *In vitro*, L-PRF showed a very strong stimulation of proliferation of all tested cell lines [32], particularly fibroblasts and prekeratinocytes during more than 28 days [29]. L-PRF appeared therefore as a very interesting healing biomaterial to use for the coverage of skin wounds, and preliminary results showed its positive effect in chronic ulcer wounds [21].

This study aimed to follow, to our knowledge for the first time, the benefits of L-PRF application on recalcitrant leg ulcers (DFU, VLU, PU) by measuring the changes in wound area through planimetry, and by recording adverse events and changes in pain level over time.

Materials and methods

Study design and patient selection

This prospective, auto-controlled, cohort study enrolled a consecutive group of patients, treated at the University of the Andes Health Center, with lower-extremity ulcers (VLU, DFU, PU or complex wounds), which failed to improve after optimal standard wound care. In this study, all patients received weekly a topical application of L-PRF membranes until wound closure. For VLU, DFU, and complex wounds, wound dressing and compression therapy was also applied. Afterwards, patients were recalled every 3 months up to 1 year to verify the healing. The study was approved by the Ethics Committee of Universidad de los Andes, Santiago, Chile. Patients were enrolled after informed consent was obtained, and the protocol was conforming to the ethical guidelines of the 1975 Declaration of Helsinki. During this prospective study, the treatment was offered for free during the first 3 months.

A wound was classified complex if more than one causal factor was involved without knowing which of them was the main contributing one. An ulcer was considered refractory if an optimal standard treatment (conform to the international recommendations) had failed during a period of at least 3 months. Such treatment involved at least weekly clinical evaluation and could include compressive leg therapy, exploration of local wound infection using quantitative cultures and installation of systemic antibiotic therapy on the basis of antibiogram, use of chemical and mechanical debridement methods minimizing tissue damage, avoidance of damage on surrounding skin, medication (pentoxifylline and rutoside), chronic venous insufficiency therapy individualized in each case, and maintenance of a good nutritional status and glycemic control.

In order to mimic clinical reality as close as possible, only minor selection criteria were used. The following inclusion criteria were respected: age above 18, able to read, understand, and accept the background information and the study protocol, signature of informed consent, and a mental status adequate to comply with the treatment. In the case of VLUs, chronic venous insufficiency treatment, when indicated with surgery or vein sclerosing agents, was completed prior to the L-PRF treatment.

Exclusion criteria were as follows: suboptimal standard wound care, peripheral artery disease (distal pulses absent or ankle-brachial index <0.8 and/or >1.2), active cancer, pyoderma gangrenosum, connective tissue disorders, cutaneous granulomatous diseases, mycobacterial or fungal infection, monoclonal gammopathy, leukemia, chronic steroidal, and/or immunosuppressive drugs.

Wound preparation and L-PRF application

Prior to wound cleaning, a peripheral blood sample was obtained from a forearm vein, with a volume depending on the wound area. Blood was collected into specific 9 mL glass-coated plastic tubes without anticoagulant and immediately centrifuged with an adequate stable table centrifuge (using the only CE-marked and FDA-cleared system for L-PRF on the market, Intra-Spin system, Intra-Lock, Boca Raton, FL, USA) at 2700 rpm for 12 minutes (≈400g) at room temperature [33]. Each L-PRF clot was removed from the tube, separated from the red cell part, extended over a metallic perforated surface (XPression preparation box, Intra-Lock, FL, USA), and gently compressed by gravity to obtain 1.0 mm thick L-PRF membranes, ready to apply in the wound (Figure 1A).

At the first visit and every follow-up visit (at a weekly interval), the same protocol was repeated. First, the wound was gently cleaned through irrigation with a saline solution in order to remove all exudate (Figure 1B). Remaining devitalized tissue and fibrin membranes were removed mechanically. Further ulcer debridement was not performed. Subsequently, L-PRF membranes were placed on the entire wound area (Figure 1C). Finally, the wound was covered with a knitted cellulose acetate non-adherent dressing, impregnated with a petrolatum emulsion (Adaptic, Systagenix Wound Management Limited, North Yorkshire, UK) in order to prevent maceration. This dressing was sealed with a Tegaderm Transparent Film (3M, Medical Division, St Paul, USA; Figure 1D) and covered by a dry dressing followed by elastic bandages in two layers, from the toes up to the

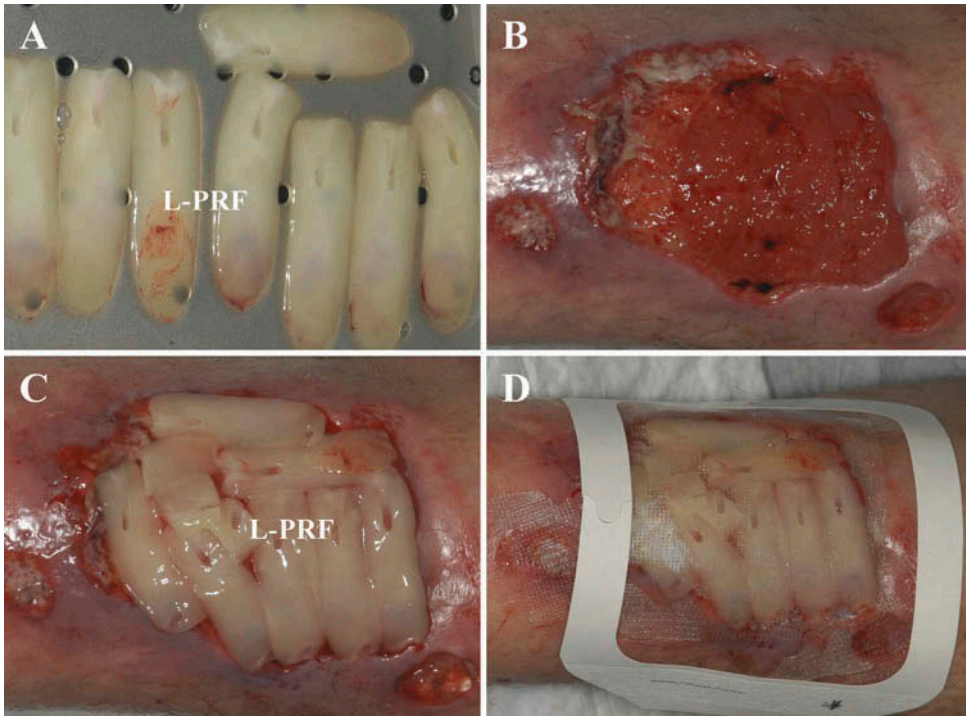

Figure 1. Description of the general protocol to regenerate a chronic skin ulcer using L-PRF. (A) Eight L-PRF clots were prepared from venous blood, and compressed into eight 1.0 mm thick L-PRF membranes on a metallic perforated surface. (B) The wound was gently cleaned through irrigation with a sterile saline solution, and necrotic tissues and fibrin residues were removed mechanically. (C) L-PRF membranes were placed in the entire wound area. (D) Finally, the wound was covered with a knitted cellulose acetate non-adherent dressing, impregnated with a petrolatum emulsion and protected by a Tegaderm Transparent Film. Afterwards a dry dressing was applied, followed by elastic bandages.

knee, to achieve a pressure gradient from distal to proximal. This procedure was repeated until complete wound closure. Afterwards, patients were followed for one year. During these follow-up consultations, patients were given instructions to prevent recurrences, including daily use of graduated compression stockings, and dermal ointment application to the skin.

Wound healing recording and statistical analysis

At baseline, and every 2nd–3rd follow-up visit, digital photographs (Nikon D3000 Reflex, Nikon, Tokyo, Japan) of the target ulcer were taken, at a fixed focal length and with automatic, flash-adjusted white balancing. All ulcer photographs were framed with a 3–6 cm scale calibration sticker, fixed just outside the wound margin. The resolution of the digital photographs was at least 300 × 300 dpi (>900 kb). The ulcer area was calculated by means of the Pictzar Pro 7.0 software (Advanced Planimetric Services, NJ, USA).

This article primarily presents descriptive data. For the comparison between standard therapy and the effect of the L-PRF application, the initial wound area was compared with the final area utilizing a paired *t*-test (with the patient as statistical unit).

Results

Forty-four consecutive patients (18 females, 26 males, mean age at first L-PRF application 63.7 ± 14.3 years) were enrolled in this cohort study. Their demographics are summarized in Table I. They were suffering from a chronic ulcer which was at least 3 months refractory to optimal standard wound care. Twenty-eight patients suffered from VLUs (32 wounds), nine from DFUs (10 wounds), five from PUs, and two from a complex wound. The average age at the start of the therapy was quite similar for the VLU and DFU groups (65 and 68 years, respectively), while the patients in the PU and complex wound groups were younger (55 years). The majority of patients suffered from a systemic disease, especially diabetes or hypertension (for details see Table I).

Table I. Patient demographics and ulcer characteristics for VLUs, DFUs, PUs, and complex chronic ulcers, respectively.

	VLUs $n = 28$	DFUs $n = 9$	PUs $n = 5$	Complex $n = 2$
Gender (female/male)	15/13	2/7	1/4	0/2
Age (mean /SD)	65.0 ± 12.5	67.8 ± 8.0	55.8 ± 29.0	54.5 ± 4.9
General health				
No systemic disease	10	0	3	0
Diabetes	8	9	2	0
Kidney insufficiency	0	0	0	0
Hypertension	17	5	1	0
Cancer	0	0	0	1
Arthritis	0	1	0	1
Smoker	3	0	0	0
Medication				
No medication	0	0	3	2
Anticoagulants	8	3	0	0
Antihypertensive drugs	11	5	1	0
Hyperglycemic drugs	7	9	2	0
NSAID	28	2	0	0
Ulcer characteristics				
Chronic ulcers	32	10	5	2
Initial ulcer size (cm^2)	15.7 ± 17.0	6.7 ± 8.2	5.4 ± 4.8	3.5 ± 1.6
0–10 cm^2	17	8	4	2
>10–20 cm^2	5	1	1	0
>20 cm^2	10	1	0	0

Table II. Wound parameters after standard wound care (Initial) and after L-PRF application (After L-PRF) including ulcer area in cm^2, the proportion of full wound closure, and the number of L-PRF applications, per ulcer type (VLU, DFU, PU, or complex) and more in details per initial ulcer size ($\leq$10 cm^2 or >10 cm^2).

		Ulcer size	VLUs $n = 32$	DFUs $n = 10$	PUs $n = 5$	Complex $n = 2$
Ulcer area (cm^2)	**Initial**	All	15.7 ± 17.0	6.7 ± 8.2	5.4 ± 4.8	3.5 ± 1.6
Mean ± S.D.	**After L-PRF**		2.9 ± 10.1	0.0	3.5 ± 4.6	0.0
	Initial	≤10 cm^2	4.9 ± 2.9	2.6 ± 1.7	3.6 ± 3,1	3.5 ± 1.6
	After L-PRF		0.0	0.0	1.6 ± 2.2	0.0
	Initial	>10 cm^2	27.9 ± 18.2	20.9 ± 0.8	12.4	–
	After L-PRF		6.2 ± 14.2	0.0	10.9	–
Proportion full wound closure		All	27/32	10/10	2/5	2/2
		≤10 cm^2	17/17	8/8	2/4	2/2
		>10 cm^2	10/15	2/2	0/1	–
Number of L-PRF applications		All	9.3	6.8	3.8	12.5
		≤10 cm^2	6.3	5.8	4.3	12.5
		Range	2–15	2–16	1–7	10–15
		>10 cm^2	12.6	11	2	–
		Range	6–25	10–12	–	–

Thirty-two VLUs (subdivided in 17 small (≤10 cm^2) and 15 large wounds (>10 cm^2)) were followed longitudinally (Table II, Figures 2A,B). All small wounds (mean baseline area of 4.9 cm^2, S.D. 2.9 cm^2) reached full closure, mostly within 9 weeks (mean number of L-PRF applications = 6.3). For two of them, the healing took 15 weeks.

All 15 larger VLUs (mean area 27.9 cm^2, S.D. 18.2 cm^2) showed significant improvement over time (Figure 2B). For 10/15 full wound closure could be obtained (Figures 3 and 4), mostly after 15 or less applications (mean 12.6, range: 6–25). Five patients discontinued the L-PRF therapy before final wound closure, two of them for financial reasons, one moved to another city, and for two of them no explanation could be found. The healing tendency for these five patients (slope in their curves) was, however, similar to the successful cases.

All 10 DFUs (mean baseline size 6.7 cm^2, S.D. 8.2 cm^2; with 8 wounds ≤ 10 cm^2, and 2 > 10 cm^2) reached full closure (Figure 2C), mostly within less than 9 weeks (mean number of L-PRF applications = 6.8). For four of them with the largest wounds (>4 cm^2), the healing took 10 weeks or longer (maximum was 16 weeks).

From the five PUs (mean baseline size 5.4 cm^2, S.D. 4.8 cm^2), two could be closed in a short period of time (Table II, Figure 2D). The remaining three patients unfortunately discontinued the L-PRF therapy, because they moved to another hospital/country. Their wounds, however, showed a similar healing tendency as the other chronic wounds (Figure 2).

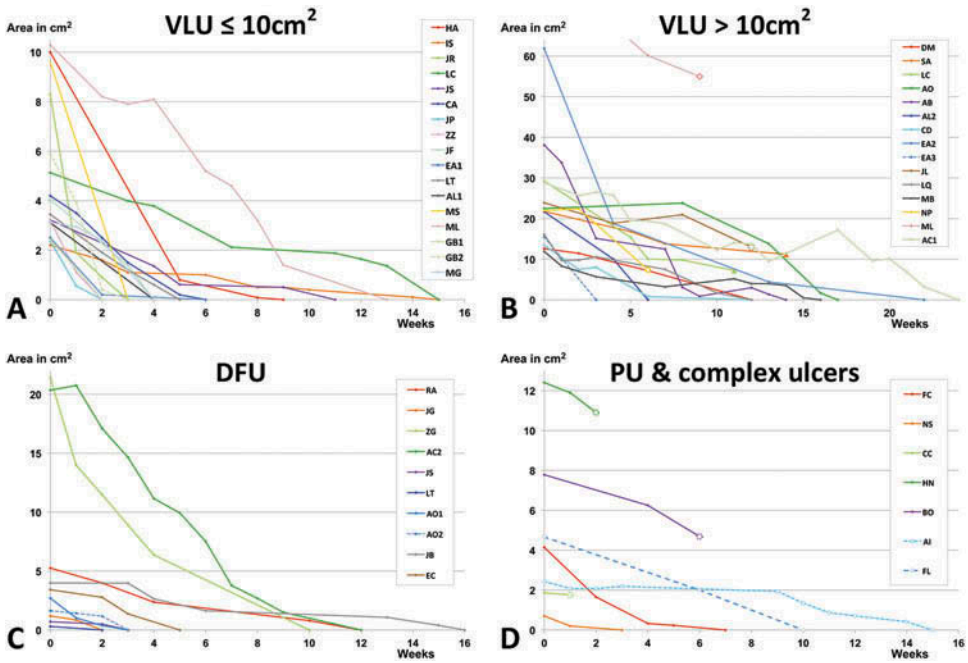

Figure 2. Reduction in wound area (expressed in cm^2) over time for: (A) VLUs ≤ 10 cm^2 (16 patients, 17 wounds), (B) VLUs > 10 cm^2 (14 patients, 15 wounds—note that patient ML started with an initial wound area of 74,5 cm^2, but in order to have a better view on the other wounds, the *y*-axis was cut off at 64 cm^2), (C) DFUs (9 patients, 10 wounds), (D) PU (5 patients, 5 wounds), and 2 complex wounds (2 patients). L-PRF was applied weekly, and pictures to analyze changes in wound area were taken at some weeks' interval (each mark represents a wound size analysis). For patients who did not reach full wound closure, the reason was mentioned (Δ: interruption because of financial reasons, O: moved to different area/hospital, or ◊: no info).

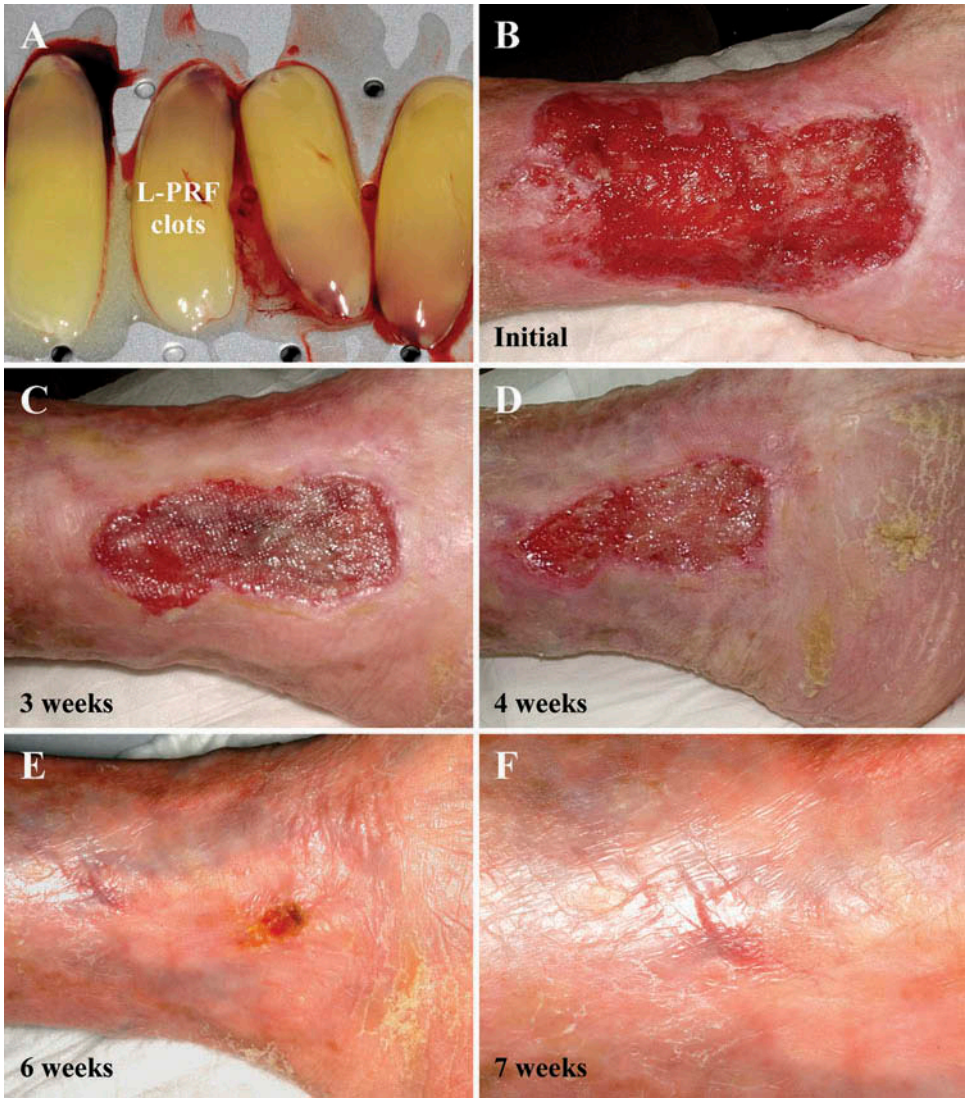

Figure 3. Follow-up pictures of a typical VLU case treated with L-PRF membranes. (A) L-PRF clots were prepared carefully, and then transformed into membranes. (B) Initial wound. (C) Wound after 3 weeks of treatment (two applications of L-PRF). (D) Wound after 4 weeks of treatment (three applications). (E) Wound after 6 weeks of treatment (three applications of L-PRF). (F) Final wound closure after 7 weeks.

The two complex chronic ulcers (2.4 and 4.7cm^2 in size, Figure 2D) could be closed after 10 and 15 weeks, respectively.

At the beginning of the study, all patients with VLUs were suffering from severe pain requiring analgesic management (a combination of acetaminophen plus non-steroidal anti-inflammatory drugs, and in some cases opioids drugs). After a few applications of L-PRF, these patients reported a significant decline in pain and in the need of analgesic drugs, and after 3 months none of them needed analgesic treatment any more. Also, the bad smell arising from the ulcers, a very common complaint for these patients, disappeared in the first few weeks of treatment. There was no recurrence of the wound during the first year after therapy. Adverse events, related to the therapy, were not observed.

Discussion

When VLUs, DFU, or PU do not respond to standard treatment within 4 weeks, with a significant wound reduction (30–50%), it is recommended to apply an advanced therapy [12,16,34,35]. A recent meta-analysis on advanced therapies of VLU or DFU indicated only minor benefits for such advanced treatment strategies [12]. Most of these advanced therapies resulted in not more than 50% wound closure [12]. In view of the aforementioned results for advanced therapies, the results with the L-PRF application for VLUs seem promising. The L-PRF application in our pilot group gave a 100% wound closure for ulcers $\leq$10 cm^2, and at least 10/15 large wounds could be closed. The remaining five patients showed similar healing tendencies (same slope in the defect size reduction, Figure 2B), but have quit the treatment early. For DFUs again the L-PRF seems extremely efficient, with a 100% wound closure in sites where standard wound care had failed. Smith and co-workers identified and reviewed 89 studies on the effectiveness of local wound applications for PUs and found no strong evidence for significant benefits [8]. The application of L-PRF in our patient population with PUs resulted in two wound closures, whereas the others, even though the treatment was only very short, already showed significant improvements. Even in complex wounds, a wound closure could be obtained.

The beneficial effect of L-PRF membranes in the healing of chronic leg ulcers can be explained by its high concentration of platelets and leukocytes, together with the long-term release of growth factors specific to L-PRF fibrin matrix [30]. The L-PRF clot indeed contains nearly all platelets and more than 50% of the

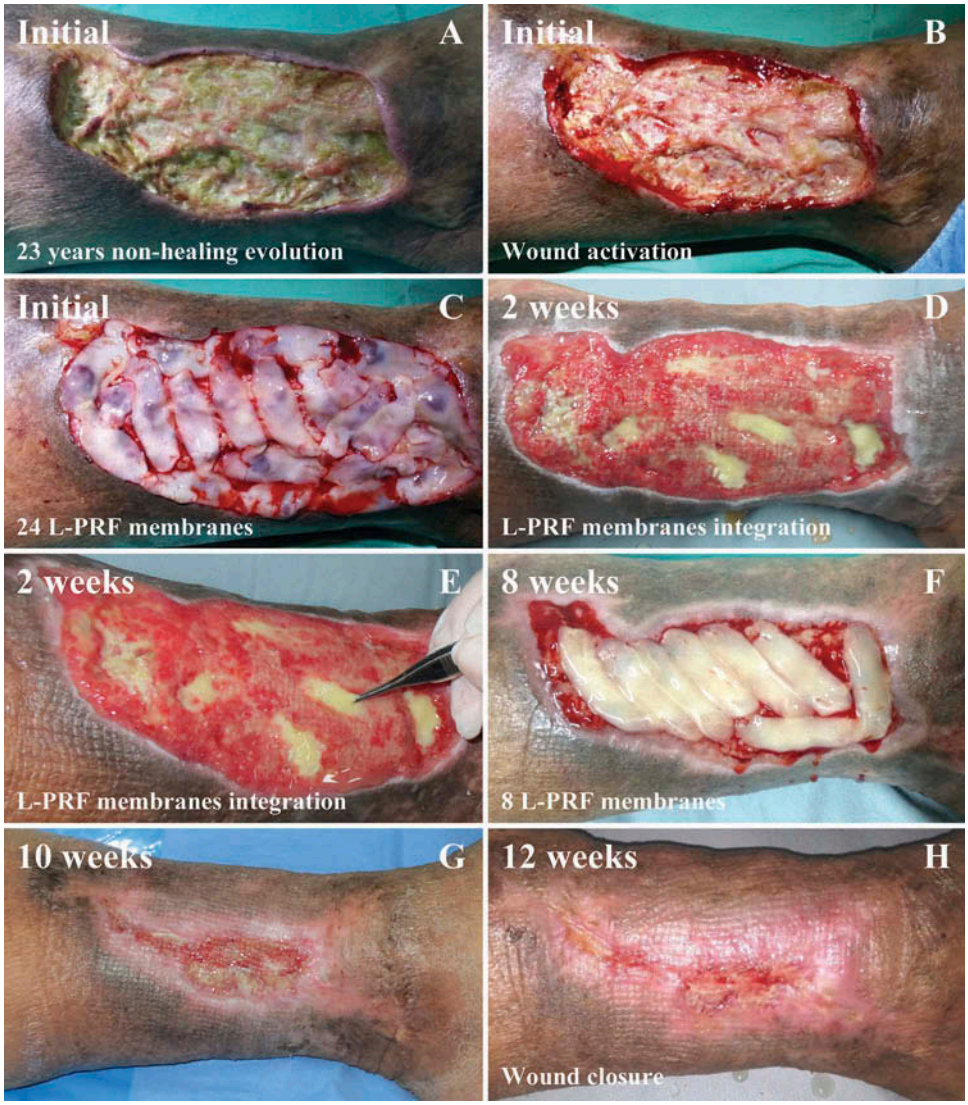

Figure 4. Follow-up pictures of a deep extended long-term chronic VLU case treated with weekly L-PRF membranes. (A) The initial wound was the consequence of a 23-year nonhealing evolution of a chronic ulcer in a diabetic female patient—65 years old. (B) Before starting the first L-PRF application, the wound was cleaned and lightly activated. A small bleeding was provoked on the surface. (C) Twenty-four L-PRF membranes were placed on the wound surface, and the bandage was then done to stabilize the membranes and to protect the area. (D) Evolution of the wound after 2 weeks of treatment (two applications of L-PRF). The wound floor was covered with a first regenerated granulation tissue. (E) Some L-PRF membranes were still partially visible on the wound, showing the integration and remodeling of the membranes into a neo-tissue. (F) Evolution of the wound after 8 weeks. At this time, only eight membranes were needed to cover the wound. (G) Evolution of the wound after 10 weeks. (H) The wound could be considered closed after 12 weeks, even if some more applications were needed to stabilize completely this result.

leukocytes (majority of lymphocytes) from the initial blood [27,36,37]. As it was proven *in vitro*, the membranes, with a special fibrin network, progressively release a significant amount of growth factors (e.g., transforming growth factor β1 (TGFß-1), platelet-derived growth factor AB (PDGF-AB), vascular endothelial growth factor (VEGF), and insulin-like growth factors (IGF)), matrix glycoproteins (thrombospondin-1 (TSP-1)), fibronectin and vitronectin), and sequences of cytokines (e.g., IL-1ß, IL-6, TNF-α, and IL-4) for at least 7 days [30,36,38]. The effects of L-PRF *in vitro* on cell cultures are very strong during at least 28 days, with a strong stimulation of proliferation of all tested cell lines (fibroblasts, pre-keratinocytes, preadipocytes, osteoblasts, and mesenchymal stem cells) and also a stimulation of differentiation of bone cells [29,32]. L-PRF membranes behave *in vitro* like a living tissue interacting in co-cultures with cells (with the release of the leukocytes from the membrane)[29], and this specific behavior reinforced the idea of using L-PRF membranes like a covering tissue graft in skin wounds [21]. Actually, L-PRF can be considered as an autologous blood tissue graft, L-PRF being often described as an "optimized blood clot," ideally prepared to be handled during surgery. In this sense, L-PRF is a very simple treatment without any risk for the patient that could be tried in all cases: it is only an optimized blood clot used to cover a wound, at almost no financial cost.

The use of L-PRF for the treatment of skin ulcers is both very new and already quite ancient. Several members of our team developed and started to use this treatment more than 10 years ago, and this therapeutic option is nowadays frequently used in several South American countries, particularly in Chile. In Costa Rica, the Ministry of Health now supports this efficient and inexpensive method, and almost all chronic ulcer patients are directed to specialists trained in the use of L-PRF. At the time of the preparation of this study, this technique was already widely advertised in Chile with excellent results (almost 100% when managed correctly), and therefore this study was classified and registered as a cohort follow-up and not as a real clinical trial,

sensu stricto, by the Ethics Committee of the University. However, this protocol is still largely unknown by wound specialists in North America and Europe.

L-PRF was initially developed in the dental world as an inexpensive and user-friendly surgical adjuvant to improve healing and promote tissue regeneration, particularly in oral surgery and implant dentistry [39,40]. The published data from the clinical trials within the oral cavity (wound healing, bone regeneration, and ridge preservation) are promising and growing extensively [39,40], and L-PRF is nowadays widely used worldwide in this field. However, despite several interesting applications in tumor surgery and sports medicine [41,42], it did not develop yet so actively in the medical field, probably due to the lack of founding international publications.

To the knowledge of the authors, this article is the first complete study reporting the results of the use of L-PRF membranes to treat chronic skin wounds, with the exception of a concept description with a single case report [21]. On other platelet concentrates, with higher costs in preparation and with the use of additives (PRP, platelet-rich fibrin patches, or matrix), less favorable clinical observations were reported [22–25]. This can be explained by differences in physical, antimicrobial, biochemical, and cellular properties between different platelet concentrates [17,30,31,38,43]. These beneficial factors may also explain the significant development of the use of L-PRF in oral applications, particularly in clinical situations with a compromised healing (such as bisphosphonate-related osteonecrosis lesions of the jaw), while most other platelet concentrates have been abandoned in the dental field [39].

Finally, the use of L-PRF for the treatment of skin wounds is a major breakthrough in terms of health policies. The human and financial cost of skin wounds is very high for those affected by such pathologies, but also for their environment as a whole. This L-PRF regenerative medicine treatment is both efficient and inexpensive (about one US dollar per membrane in expendable materials—mostly the price of a glass-coated plastic tube). In this cohort study, all patients following the treatment were successfully treated; the sole limit to a 100% closure rate appeared to be the discontinuation of the therapy by some patients (in general patients from the countryside, who resigned to visit the physician repetitively once the wound started to close and to look better). The use of L-PRF developed extremely quickly in developing countries, both in dentistry and in medical applications – even quicker than in developed countries. This method offers a very interesting therapeutic option, easy to use, inexpensive, and extremely efficient for millions of individuals in developing countries (but also in developed countries) who would never afford more sophisticated treatments for skin wounds.

As a conclusion, L-PRF represents a safe, convenient, easy-to-use adjuvant therapy with significant potential for closing chronic wounds without adverse events. L-PRF treatment seems a promising alternative to the above-mentioned advanced treatment strategies, if standard therapy fails. Moreover, ulcer recurrence, a frequent problem in these patients, was not observed in the first year of follow-up. This probably implies that L-PRF is not only a good promoter of wound closure, but helps to achieve a better quality of the regenerated tissue. Considering the efficiency, low cost, and safety of the use of such an autologous "optimized blood clot" to cover these wounds, this therapeutic option should be known and considered for the treatment of refractory skin ulcers.

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Contributors

NRP was principal investigator of the study, codeveloped the L-PRF treatment strategy, undertook treatment and monitoring, had full access to data, reviewed and analyzed data, wrote the Article, and had final responsibility for the decision to submit for publication. MU, YZ, and VDR undertook treatment and monitoring, had full access to data, reviewed and analyzed data, and reviewed the article. MQ had oversight of treatment and monitoring, had full access to data, reviewed and analyzed data, wrote the article, and had final responsibility for the decision to submit for publication. DDE codeveloped the L-PRF treatment strategy, had oversight of treatment and monitoring, had full access to data, reviewed and analyzed data, wrote the article, and had final responsibility for the decision to submit for publication.

Declaration of interest

All authors declare no competing interests. DDE and NRP are initial co-inventors of the L-PRF open-access technique more than 15 years ago and co-developers of the current optimized protocol and material to prepare L-PRF clots and membranes (centrifuge, tubes, L-PRF box); however, they do not hold any patent, trademark nor any financial interests in any company selling devices or kits.

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