Platelet-Rich Fibrin (PRF) as a Regenerative Treatment for Chronic Diabetic Osteomyelitis and Its Therapeutic Potential

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ABSTRACT

The hypothesis is that the procedure of fibrin rich in leukocytes and platelets advanced (A-PRF) in ulcerative osteomyelitis of the diabetic foot (DFU) allows therapy from this critical illness. In this exploration, the focus was to standardize the use of platelet-rich fibrin (PRF) in patients not amputated with osteomyelitis, to use this second-generation platelet concentrate as a regeneration enabler. The researchers submitted and utilized A-PRF membranes (1300 g × 8 min) in 10 patients (all diabetics) with osteomyelitis and cutaneous injury for 6 months at least. The membranes, in amalgamation with the supernatant fluid produced by stress, have been integrated into the skin lesion down to the bone after surgical debridement. The progression of the lesions after some period of time has been analyzed. All 8 subjects had a probe-to-bone positive assay; magnetic resonance imaging indicated cortico-periosteal coagulation and/or foci of cortico-spongeous osteolysis contiguous to the lesion. Gram-positive bacteria were identified in our procedures in 52% of cases. Gram+ Cocci, for example, *Staphylococcus aureus* (15.6%), β-hemolytic *Streptococci* (12.1%), *Streptococcus viridans* (7.1%), and Gram-negative bacteria, for example, *Pseudomonas* (10.6%), *Proteus* (7.8%), *Enterobacter* (5.7%) were present. *Candida albicans* was active in 2.8% of cases. The blood count showed no relevant differences among different patients. To date, cutaneous lesions have been cured in 9 of the 10 subjects treated (1 patient for more than 8 years) without any evidence of infection or repetition. The results obtained on our subjects indicate that PRF membranes may be a therapeutic option in this problematic disease. In fact, this clinical approach may have the potential to support the healing of diabetic skin lesions with osteomyelitis.

Keywords: Chronic Osteomyelitis, Buffy Coat, Growth Factor Content, Platelet-Rich Fibrin

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INTRODUCTION

Platelet-rich fibrin (PRF) [1] is a second-generation platelet concentrate (PC), substantially an overcoming of platelet-rich plasma (PRP), is, by these lines, another step forward in the therapeutic idea of platelet gel with a simplification formulation and small fake biochemical adjustments. Differently from other PCs, this procedure does not necessitate anticoagulants, thrombin, or some other gelificant, which makes the blood close to a natural centrifugation containing no additives. PRF can be created by simply activating the naturally occurring coagulation pathway without the assistance of anticoagulants or clotting promoters [2]. Although leukocyte platelets and cytokines play a relevant role in the biology of this biological material, the support matrix of fibrin is undoubtedly the unmistakable factor of the strong therapeutic capacity of leukocyte-and platelet-rich fibrin (L-PRF). Within a short time, the exclusion of an anticoagulant allows the activation of the vast majority of platelets included in the model to start the coagulation sequence. PRF contains mitogenic and chemotactic compounds that promote and modulate cell proliferation and attraction [3]. It can also enhance, in vitro, the expression of various markers of osteogenic differentiation, such as bone alkaline phosphatase, osteocalcin, osteopontin, and osteonectin [3].

PRF cannot be stored in tissue banks because it contains circulating immune cells and antigenic plasma molecules; being strictly donor-specific, it cannot be used as material for allogeneic grafts.

Furthermore, PRF cannot be stored in tissue banks. Due to its antigenic potential, PRF membranes are recipient-specific and cannot be used as universal graft material [4].

The osteomyelitis (OM) expressly alludes to bone marrow disease as contrasted with an osteitis in which the periosteum or cortical surface becomes contaminated through a penetrative lesion or wound. Regardless of these differentiations, the two are medically analyzed or treated in a corresponding way. Much has been reported on the diagnosis of OM in the long term and, even more critically, on how it attaches to diabetic foot ulcers (DFUs). OM involving the diabetic foot frequently results from a collateral lesion or ulcer of the foot [5]. The global impact is from 1:1000 to 1:20 000 people in Italy 19 000 cases/year, in Europe 100 000 cases/year. The proportion of men to women is 2:1.

Bone and articulation infections are today problematic for patients and humiliating for them and the medical service operators who treat them. The high success rates of antibiotic management in many infectious pathologies have not yet been encountered in this condition. The variety of OM types necessitates distinctive clinical and surgical therapeutic methods. The different types of this syndrome incorporate, in a decreasing order of incidence: OM auxiliary to an adjacent concentration of contamination (after injury, surgery, or addition of a joint prosthesis) that is subordinate to vascular deficiency (in diabetic foot infections); finally, the OM of the hematogenic origin. Chronic OM is associated with avascular bone necrosis (dead bone), at present, and the surgical procedures are important for therapy despite antibiotic management. Contrary to what might be expected, acute OM can only respond to antibiotics. All in all, a multidisciplinary treatment approach is desirable for a successful outcome, including the abilities of musculoskeletal surgery, infectious diseases, and plastic surgery, as well as vascular surgery, especially for complex presentations with delicate tissue disasters [6,7].

The use of second-generation PCs in DFU with OM was not known to the authors until recently and was ascertained by them unexpectedly (2018) [8]. This research describes therefore, a new study hypothesis involving the use of solid PRF membranes as an autologous product for the regeneration of disorganized soft and bone tissues in DFUs (diabetic foot ulcers) with chronic osteomyelitis. The results presented were obtained on 10 subjects with chronic DFU OM of the lower extremities (DFO).

MATERIAL AND METHODS

Patients Collection

Blood was collected with the informed approval of each of the 10 volunteers screened. All the contributions of the participants in this project were performed according to the ethical principles of the Institutional and Public Research Advisory Group and the 1964 Helsinki Declaration and its subsequent revisions. The Ethics Committee has waived an ethical order for this survey because blood was not used as a recognizable resource [7] (Research Registry: No. 5927). It has been confirmed that all donors are carriers of chronic OM caused by diabetic lower limb ulcers. Predictive assessment of OM in the subjects considered was performed by the probe-to-bone (PTB) technique and then by magnetic resonance

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imaging (MRI) and bone cell culture for bacterial testing. All selected patients were classified into Level 3, Grade A or B according to the Texas University Classification [9]. This study population excluded patients with a complicated hospital stay who stayed in the intensive care unit. The blood count of donors was also examined before starting the studies to determine the standard blood cell enumeration interval. In order to quantitatively describe and follow the clinical sequence of reparative, a severity rating of the lesion was set by examining the lesion and marking the various clinical and anatomical patient factors (Wound Severity Score, Tables 1-4). All patients presenting with peripheral arteriopathy (PAD) with no vascular opacification at AngioTAC underwent Percutaneous Angioplasty (PTA).

Primary Endpoint: Healing from chronic OM ulcers in the diabetic foot as a result of PRF treatment.

Based on this, it will be determined whether or not the scientific hypothesis of PRF use has been verified. Secondary Endpoint: any side effects due to the use of PRF.

Preparing the PRF

Factors affecting fibrin coagulation growth and structure incorporate hereditary components, stimulating factors (eg, irregular convergence of thrombin and factor XIII in plasma, blood flow, platelet activation, oxidative stress, hyperglycemia, hyperhomocysteinemia, drugs, and tobacco smoke), and various parameters (eg, microgravity, pH, and temperature) [10,11].

The blood was then collected in A-PRF glass tubes (advanced PRF) without anticoagulant or gel separator (9.0 ml A-PRF Serum Vacutainer, PRF-Process, Nice, France) to create clots and Advanced-PRF membranes. The blood was immediately collected with a needle in sterile tubes (average value 22'', less than 25'' per tube) and instantly (after 2 minutes of rest of the full tube) centrifuged at a temperature above 21 °C (somewhere in the range of 21 °C to 30 °C). Using the L-PRF wound box, the membrane stress procedure in coagulation is performed through light, homogeneous stress, and the resulting membrane remains consistently moist and evenly wet in serum. The PRF fabrication method is extremely simple and requires only a blood test and centrifugation speed with a swinging centrifuge (CTL420, CenLee, Beijing, China) a DUO Quattro per PRF tabletop centrifuge specifically designed for this method (DUO Quattro for PRF, PRF-Process, Nice, France) [8-12].

The subsequent protocol is as described below: blood samples are collected in 9 mL glass tubes, without anticoagulant or splitting gel, and are rapidly spinned according to the recommended time interval: 30" acceleration, 8 min at 1300 rpm (189g), 36" deceleration and stop. After spin drying, 3 sections are located in the tube: the red cells at the bottom, the fibrin coagulation that corresponds to the PRF in the center, and the acellular plasma at the top. The fibrin clotting is separated from the tube with sterile clips and the PRF is obtained by removing the red cloth from its lower end. The realization of this method is completely based on the ease of blood collection and the speed of movement in the centrifuge [13,14].

Platelet storage is limited to 3 and 5 days in Japan and the rest of the world, respectively. This limit is based on the fact that platelets are sensitive to temperature and pH fluctuations: when samples are stored at temperatures between 2 and 6°C, the platelets become unsuitable for the production of platelet concentrates, and a pH below 6.2 is associated with reduced **in vivo** platelet efficacy. However, given that platelet concentrates must deliver significant amounts of growth factors and fibrin (or fibrinogen) to implantation sites, stored platelets are not necessarily expected to function exactly like fresh ones (for instance, in terms of aggregation). Instead, the expectation is that stored platelets do not lose growth factors during the storage period, while coagulation factors—particularly those involved in the endogenous coagulation cascade—should retain the activity required to convert and polymerize fibrinogen and form well-cross linked fibrin fibers.

The entire procedure should be carried out consistently in a sterile manner, based on the fact that the growth factors contained stimulate tissue regeneration and, therefore, presumably also cell regeneration with risk of infection. This delicate technique preserves the extraction and acute significant amount of growth factors. The available PRF boxes will be accessible in a set of shapes and will exercise, through the stress plate, various exercises depending on the weight, offering to climb to a membrane of varying compactness, variable width, and length. The wound L-PRF box for compression is planned by AA [16]. It consists of a 17.5 × 7.6 ×2 cm metal container containing a perforated steel plate measuring 150 × 68 × 1.5 mm. There is a second steel plate that acts as a compressor, measuring 150 × 68 × 1.5 mm, weighing 148 g. This second type of U-shaped plate uses a mass of 142.437 Pa/cm2. In this investigation, the stress to create the membranes was applied to the clot for 2 min. Each

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membrane is separated into 3 equivalent size areas: proximal (head), central (body), and distal (tail) through a sterile scalpel cut. Only the proximal portion of the membrane has been used [17,18] and the central portion only if important.

With the PRF method preparation, blood coagulation occurs immediately after sampling, directly after contact with the glass surface of the tube, due to the elimination of anticoagulation. On the off chance that the time required for blood collection and initiation of centrifugation (several revolutions per minute, g/min) is greatly delayed, the polymerization of the fibrin is of such an extent that only a small fraction will coagulate and a inconsistent result (similar to PRF). Next, blood acquisition must be quick and easy, driven by rapid centrifugation, and is essential in the PRF yield specification. It is defined to provide a thickness of ~3 mm (3.08±0.5), [18] an evenly hydrated membrane with an exudate rich in platelets, leukocytes, vitronectin, and fibronectin, expressed in the structure of the fibrin network with CD34+ hematopoietic stem cells (Figure 1) [19].

The fibrinogen is originally focused in the upper part of the tube until it causes the creation of the autologous circulating thrombin that transforms it into the fibrin mesh, through a polymerization process. The result is the coagulation of fibrin containing platelets located in the middle, directly between the lower membrane of the red cell deposition and the upper acellular part of the plasma. The PRF coagulation thus acquired is then placed on the bottom of the L-PRF box and compacted with the compressor cover. This approach forms an autologous fibrin membrane.

The L-PRF box is intended for the creation of constant thickness membranes that remain hydrated for a few hours and allow the recovery of serum exudate expressed by fibrin coagulation, which is rich in proteins, such as vitronectin and fibronectin [8]. The L-PRF clot has all the potential to be responsible for a slow release of growth agents and glycoproteins from the matrix (≥7 dd, up to 28 dd) [16].

Adherent proteins: Fibrinogen (Fg), fibronectin (Fn), vitronectin (Vn), and thrombospondin-1 (TSP-1) are copious in the fibrin structure. Growth factors that accumulate in platelets and are essential for wound regeneration include PDGF, with - AB - C; they are also present as VEGF-A, TGF-b1, EGF (vascular endothelial growth factor, transforming growth factor -1, epidermal growth factor, respectively) (Figure 2), FGF-2, HGF, and insulin-like growth factor-1 (IGF-1). Analyzing 3 pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), 1 inflammatory cytokine (IL-4), and 1 angiogenesis activator (VEGF), it was suggested that PRF might also be a focus of immune modeling with the potential to control inflammation and multiplication of non-differentiated adult cells, comprising CD34+ precursor cells, MSCs (mesenchymal stem cells), SMC progenitors (smooth muscle cells), and endothelial precursors [16-19]. The multiple power of these types of stem cells and their increased capacity to increase vascular tissue repair and through paracrine mechanisms also make them as therapeutic vehicles in restorative medicine. In addition, tissue damage produces severe chemo-attractive signals for stem cells, creating the prerequisite for their restorative activity. Platelets control the recruitment of adult stem cells to damaged cells and can, in this way, be a considerable tool in the execution of curative cell reactions. Activated platelets discharge HGF and have been depicted to advance MSC absorption in human endothelial cells. Human stem cell proliferation (hMSCs) is related to platelet count in A-PRF [3].

Table 1: Patient specifications

Patient No.	Aging	Gender	Length of stay (days)	Concomitant diseases	DDY	Wound Size LxWxH (cm^3)	Position of the wound	Therapy Period	Follow up days	Result	Total Severity Score
1	68	Male	103	PAD, DFO	25	2X2X5	Lower third left	40	2920	Automatic closing	25
2	71	Female	64	PAD, DFO,HTN	40	1X1X2	Right V0 finger foot	25	1095	Automatic closing	24
3	63	Female	56	PAD, DFO,ESRD	24	2X2X2	Right Plantar	32	180	Automatic closing, deceased for CAD	40
4	60	Male	46	PAD, DFO,HTN	20	1X3X5	Left toe finger foot	27	360	Automatic closing	15
5	66	Male	45	PAD, DFO,ESRD	15	1X2X5	Right toe finger foot	33	633	Automatic closing	20
6	58	Male	94	PAD, DFO,HTN	23	1X1X5	Left toe plantar	33	1164	Automatic closing	35
7	70	Male	86	PAD, DFO,HTN	35	2X2X5	Right toe plantar	30	2715	Automatic closing	23
8	55	Male	90	PAD, DFO	12	1X5X1	Left fifth toe	30	40	Automatic closing	12
9	71	Female	45	PAD, DFO	20	2X5X2	First toe on the left foot	35	640	Automatic closing	20

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10	60	Male	60	PAD, DFO	30	1X1X2	First toe on the left foot	30	300	Automatic closing	35
Average ± D.S	64.2 ± 5.8	7.3	68.9 ± 22.3		24.4 ± 8.6	1.4x2.4x3.4		31.2 ± 4.5	1004.7 ± 1023.13		24.9 ± 9.1
Median	64.5		62.0		23.5	1x23.5		31	636.5		23.5
Normal-ity	Yes		Yes		Yes						Yes

*The improvement is defined as the regeneration of the limb wound within a year and a half, but without the wound closure.
DDY: Year of Diagnosis of Diabetes, **PAD:** Peripheral Arteriopathy, **ESRD:** End Stage Renal Disease, **HTN:** Arterial Hyertension, **CAD:** Coronary Artery Disease, **DFO:** Diabetic Foot Osteomyelitis

Table 2: Complete the general criteria of the injury

	None	Mild	Marked	Severe
Periwound erythema	0	2	5	7
Periwound edema	1	2	4	7
Injury purulence	0	3	4	7
Injury fibrin	0	2	5	7
Limb fitting edema	1	2	4	7
Limb Brawn v edema	0	3	4	7
Injury x Q: granulation	0	0	0	0

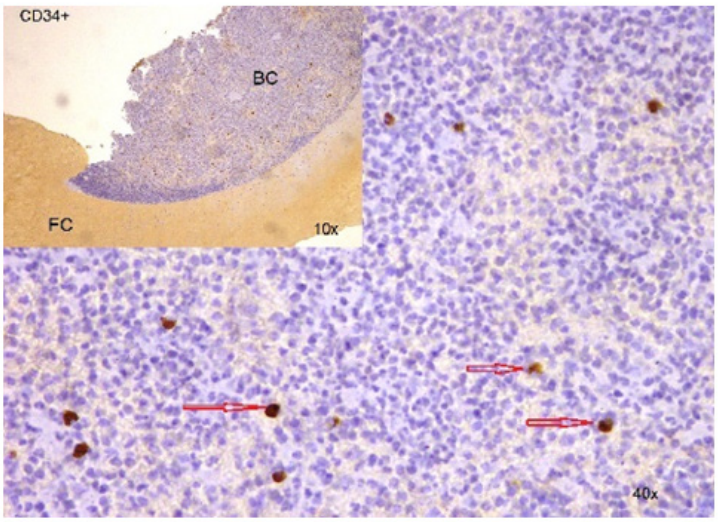


Figure 1. CD34+ stem cells (arrows) found in the intermediate portion (body) of a horse's auto-compressed A-PRF membrane (ingr.10, 40 x).
BC: Buffy Coat; **FC:** Fibrin Coat

Table 3: Anatomical wound score considerations

				Dorsalis		Posterior	
Visible bone	**Score**	**Visible tendon**	**Score**	**Pedis pulse**	**Score**	**Tibial pulse**	**Score**
Yes	10	Yes	7	0-1^{+}	5	0-1+	5
No	0	No	0	2^{+}	2	2^{+}	2
				3-4^{+}	0	3-4^{+}	0

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Table 4: Wound score and wound measurements

Measure (cm^2)	Score	Width (mm)	Score	Indefinite (mm)	Score	Lifetime	Score
<1	0	<5	0	<2	3	<8 weeks	0
	1	5-10	3	2-5	5	8 weeks-6 months	1
	3	10-20	7	>5	8	6-months-1 year	2
	6	>20	10			2-3 years	5
	8					5-10 years	7
	10					10 years	9

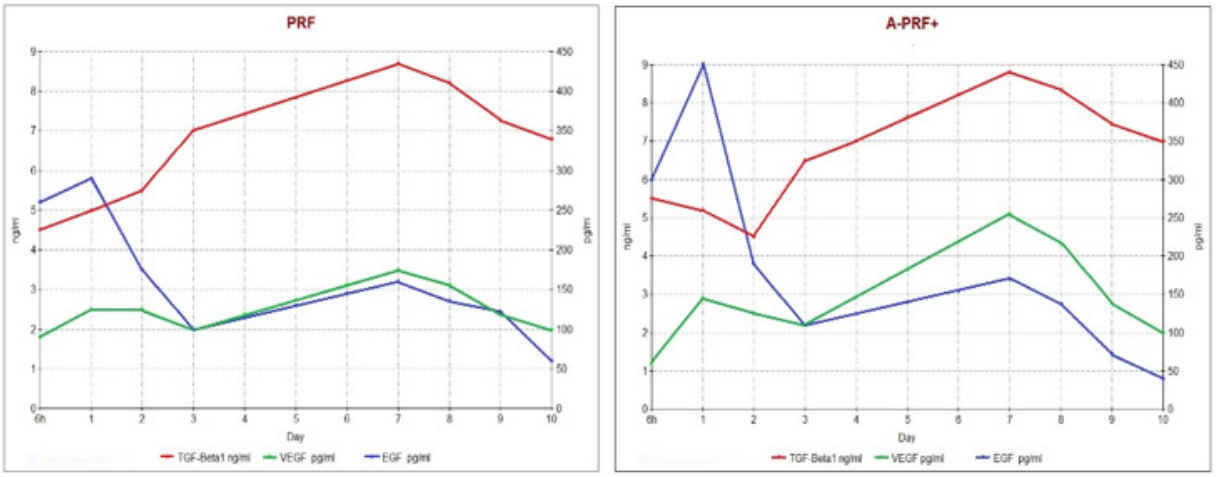


Figure 2. Several concentrations of growth agents TGF-β1, VEGF, EGF over time generated by PRF and A-PRF+

PRF: Platelet-Rich Fibrin, **A-PRF+:** Advanced Platelet-Rich Fibrin Plus, **VEGF:** Vascular, Endothelial Growth Factor, **TGF-β1:** Transforming Growth Factor β1, **EGF:** Epidermal Growth Factor

The number of PRF applications at the recipient site depended on the size of each wound. One PRF clot was applied for every 2.5 cm^2 of wound surface area.

PRF treatment was administered twice and three times during the first week for cases 1 and 2, respectively, and subsequently reduced to once a week. PRF application was discontinued once granulation tissue covered the wound bed.

All patients were re-evaluated 4 and 6 months after wound closure.

Recently, some authors have been trying to use allogeneic PRF as a blood derivative material obtained from selected canine donors under experimental conditions, but without blood typing tests. Their ability to induce the formation of granulation tissue, as observed within the lesions after each subsequent PRF application, has been confirmed. Therapy with allogeneic PRF had the same results as the autologous methodology. PRF therapy also reduced the presence of both fibrin and exudate without the need for mechanical debridement [20].

Allogeneic administration of platelet-derived products offers an advantage over autologous approaches in cases where medical conditions preclude blood collection from the patient or when such collection is not advisable (e.g., low-volume or small-sized donors, or uncooperative or sick animals). However, allogeneic therapies entail a risk of transmissible diseases, necessitating rigorous donor screening that takes into account the geographical prevalence of blood-borne diseases. Cross-contamination or bacterial contamination is minimal during PRF production due to the limited handling involved, although clinical application must always be carried out using strictly aseptic techniques [21,22].

Inclusion Criteria

No intake of anticoagulants or functional equivalents for one month before enrollment and platelet count in the range of 150,000 to 450,000/μL and coagulation index determined as normal [prothrombin time (PT value) between 11 and 16 seconds]. Hemoglobin concentration (Hb) >9 mg/dl, total serum protein concentration >6 grams/dl, and serum albumin

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>3 grams/dl.

Absolute contraindications for PRF production include Sindrome from platelet dysfunction, critical thrombocytopenia, hemodynamic instability, and sepsis. Relative contraindications include heavy smokers, drug and alcohol users, patients with chronic liver disease, severe metabolic or systemic disorders, patients with cancer of hematopoietic origin, with low hemoglobin (<10g/mL) or platelet count (<1.2 x 10^5/ L). Also, patients taking NSAIDs, prednisolone >20 mg/day, and anticoagulant therapy were excluded (Table 1).

Blood Chemical Tests

Blood samples of each patient were also taken to perform complete blood count cells (CBC) using K3E tubes with 5.4 mg EDTA (VacuMed). As reported by the previous investigation [8,14]. 3 blood samples were taken from the left brachial vein of each person through an 18-gauge needle, 2 samples for PRF generation, and 1 sample for cellular blood count. The samples were processed with a HECO 5 hematology analyzer (Seac Radim Company). The predictive evaluation of OM in the considered subjects was delivered by PTB technique and then by MRI and bone cell culture for bacterial testing.

A-PRF Grafting Protocol

Each of the 10 patients, after adequate preparation (suspension of anticoagulant drugs for at least 7 days and low molecular weight subcutaneous heparin) underwent surgical debridement, under subarachnoid anesthesia, in the operating room, with the evacuation of imperative and conceivable bone portions in the deep part of the wound, including the execution of the planned bacterial cultivation tests.

Extremity vasodilator drugs (Iloprost, Alprostadil) have not been used. After cleaning the surgical wound with a half combination of hydrogen peroxide (50%) and iodopovidone (50%) and proper control of hemostasis by electrocautery, A-PRF was transformed into membranes after compression stress for 2 min. The supernatant obtained from the compression was taken from the wound L-PRF box with a sterile 10 cc syringe and was completely incorporated into the lesion in depth together with the A-PRF membrane [12-14] which acts as a biological glue through which cells can migrate. Before the insertion of PRF, the wound was washed with hydrogen peroxide, as the active drainage inhibits the activity of growth factors. The dressing was performed with oily gauze, sterile gauze, germanic cotton, and flexible cement cloth. The postsurgical drug therapy was with levofloxacin 500 mg cp, 1 cp per day for 5 days, and low molecular weight heparin (enoxaparin sodium) for 7 days, though the drugs that each subject constantly takes for several pathologies have been hired. Based on lifestyle and antibiogram consequences, specific antibiotics for generic use for 15 days have been included. The main drugs were administered for 7 days. Subjects were inspected every week in the ambulatory until they healed. In the remote possibility that there was no signal of wound recovery, the PRF was reapplied 5-8 weeks after the first time. All trace of PRF were removed with water and sterile cloth at the first dressing. Patients proceeded with the refreshment dressing routine between PRF treatments, as it had already been used. Alone three subjects had to perform the surgical procedure a second time after 45-60 days. No direct patient identifiers were maintained in the study database.

Wound Gravity Index Score

The wound severity score is shown in Table 1 for each patient.

The severity score of the lesion was calculated according to clinical and anatomical conditions and by considering the lesion and the patient's factors. The points were awarded in a self-assertive and considered manner using the usual clinical experience on wound repair. These overall parameters of the lesion are recorded in Table 2.

Anatomical contemplations have been recorded and obtained, for example, the presence of uncovered bone or a wound area of the ligament, and the nature of the pulsation of the pedidial artery and posterior tibial (and the area compared with the wound area) (Table 3). The lesions have been estimated to decide the absolute territory of the lesion, the depth and the degree of openness.

The estimation of the lesion surface was dictated by photographing the lesion and comparing it with a strip graded to the millimeter and then analyzed with an estimation software (IC Measure 2.0.0.133), discovered free of charge on the web.

Three estimates were made and the average surface area was the normal of the 3 valuations. The evolution of the lesion was controlled by the set of measurements on the patient.

The scores attributed to these different assessments of the lesion can be found in Table 4. Initial and subsequent wound

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scores were recorded and organized at each visit to each facility by 2 clinical agents and medical personnel in charge of wound recovery. These conclusions were periodically cherished by the examiner who proposed them.

Description of Successful Therapy

All patients were asked to observe rest without limb loading for 20 days. The use of unloading devices was not suggested.

A lesion was called recovered when it was repaired with a new epithelium. This was externally resolved during the evaluation of the lesion performed during the next routine procedure. At each visit, estimates and photos of the lesion were taken to report progression. The result of the treatment is determined by the rate of variance of the area and volume of the surface, determined as the least estimation each day of the preliminary assessment separated from the baseline estimation (Daily Re-epithelialization Index - IDR) [16].

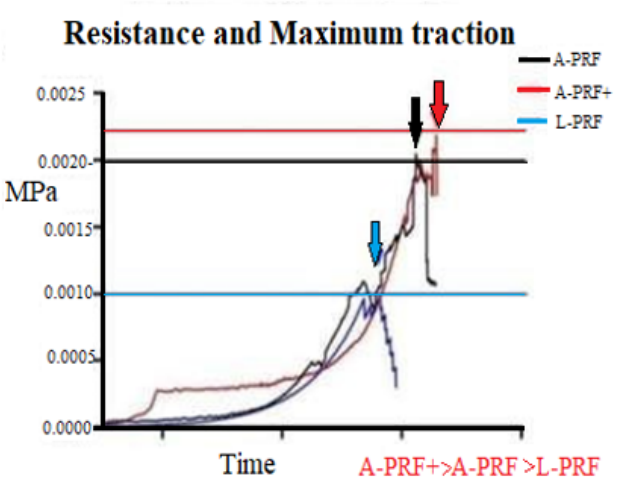


Figure 3. Resistance and maximum traction of the PRF



Figure 4. *Staphylococcus aureus* highlighted in 15.6% of patients

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RESULTS

The authors have produced and used A-PRF membranes based on venous blood, in patients not-amputated suffering from OM, with skin lesions that have been present, in any case, for a semester at least. The membranes, together with the fluid obtained from the stress of the wound L-PRF box, were inserted into the injured skin, deep down, after surgical debridement. The progress of the lesions has been studied after some time.

The results obtained with this method are given in Table 1, together with the general features of the processed patients.

The average age of patients processed is 64.2 years ± 5.84, and a median of 64.5 years, with a male/female relationship of 6 of 2, all subjects were affected by Chronic OM and Chronic Obstructive Arteriopathy in noninsulin-subordinate Diabetic Disease (T2DM) presented for 24.4 years ± 8.6 of medium. The average duration of OM was 73.0 ± 22.9 days. The standard score of absolute severity of treated subjects was 24.9 ± 9.1 (Table 1).

All patients indicated positivity in the PTB test, and the nuclear MRI showed cortico-periostal necrosis or potentially with cortico-spongeous osteolysis, adjacent to the lesion. Osteonecrosis was also present with bone fracture and dissolution (Figure 5-9).

Gram-positive microorganisms were found in our subjects in 52% of cases. Other discovered germs include Gram-positive cocci, for example, *Staphylococcus aureus* (15.6%)(Fig.4), *β-hemolytic Streptococci* (12.1%), Streptococcus viridans (7.1%), and Gram-negative bacillus, for example, *Pseudomonas* (10, 6%), *Proteus* (7.8%), *Enterobacter* (5.7%). Candida was present in 2.8% of cases.

The normal therapy time after PRF grafting was 31.2 ± 4.5 days. The average follow-up to date was 1004.7 ± 1023.13 days (median 636.5 dd).

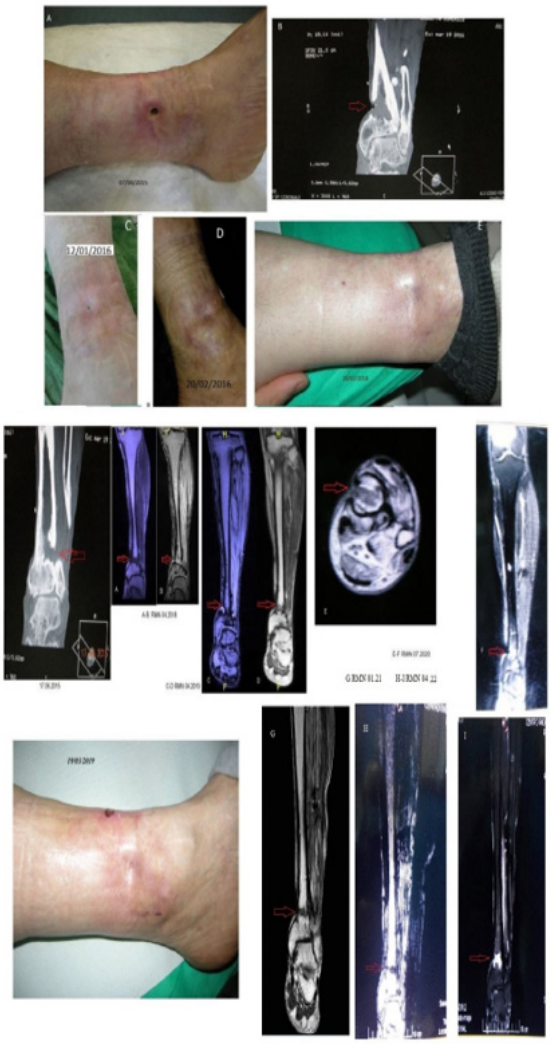


Figure 5. Patient No. 1.(C.F.) In A-C-D-E-F-G-H-I, MRI at various stages of wound development until healing, stable after more than 8 years old. Some skeletal regrowth is also appreciated at the recent MRI [16,17].

MRI: Magnetic Resonance Imaging

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Figure 6. Patient No. 4. (A.F.) (A) In a fifth digital X-ray imaging situation. (B) Intraoperative status. (C) PRF graft deep in the wound. (D) Wound progression to injury healing after 4 months and only 1 PRF graft. (E) RNM from July 16, 2020 once healing is obtained [16,17].



Figure 7. Patient No. 5. (D.S.M.) (A and B) In MRI status of first digit. (C) Intraoperative status. (D) L: PRF graft deep in the wounds. (E, F, and G) Injury progression until closure after 6 months and only one PRF graft [16,17].

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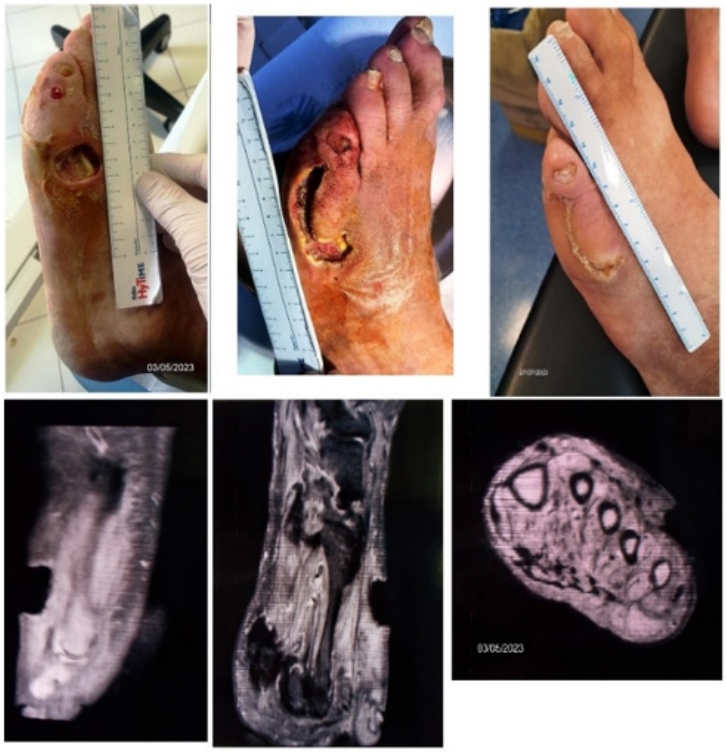


Figure 8. Patient No. 8.(B.M.) (A) Preoperative status. B) Postoperative status after 45 days. In MRI status of first digit (D, E, F). Injury progression until closure after 45 dd. (C) and only one PRF graft.

MRI: Magnetic Resonance Imaging



Figure 9. Patient No. 10. (D.R.M.) (A) Preoperative status. Rx- status of first digit. (B) and only one PRF graft. (C) Injury progression until closure after 45 dd

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DISCUSSION

A key limitation of this study was the small sample size of the DFO population studied (N=10), limited by the number of DFO patients that can undergo regenerative surgery available within the hospital. Up to this point, skin OM injuries have been recuperated in all treated subjects (only 1 subject died of cardiovascular causes 2 years after surgery, however, the injury was completely healed at that point), without any evidence of infectivity or backslide. In 1 of the patients (No. 1) we are witnessing a specific bone regrowth more than 6 years after the closure of the skin lesion (Figure 5). The use of PRF in the treatment of skin lesions of the foot by the authors has led to the announced results, with a moderate effort in terms of surgical strategy and monetary expenses for the medical organization where patients were treated. In addition, the surgical hazard to the patients was low.

The treatment of DFU is tied to a huge budgetary burden, and the cost increases with the delay remain together with the need for surgery. The presence of OM is a basic factor for significant expenses, the longer duration of the pathology remains with the long-range use of anti-infection agents and the need for removal. Moreover, of the apparent multitude of factors that influence the qualitative scar condition, what seems to have the best effect is the time needed to repair a lesion [18]. The extensive writing supports the case that the injury that recovers within 21 days limits abnormal healing. Secondly, one of the basic areas of research on consumption and recovery of wounds is to explain the pathophysiology of the wound correction measure, shortening the time of healing of the wound, the danger factors defined by the scarring procedure, and the transformation of this information into therapeutic provisions.

The use of A-PRF solid in compressed format as a speeding up wound repair agent seems to warrant its use. The integration of leukocytes into PRF should be carefully examined especially when the biomaterial is used for injury repair and when scar formation is a significant preoccupation.

One aspect to consider is the inclusion of leukocytes. Not long ago, this was the subject of discussion: some specialists, including the AA, argue that leukocytes should be incorporated to encourage wound debridement, wound recovery, and subsequent tissue regeneration, while some are concerned about the surprising intensification of the worsening [23,24]. In this way, additional researches and investigations should culminate in solutions.

In this research, each of the 10 patients performed the "Probe-To-Bone Test" with positive results, MRI revealed cortico-periosteal colliquation, and the central areas of cortico-spongeous osteolysis with reduced sign strength in the skin lesion region. Edemas due to septic irritation and delicate tissue abscesses have also been discovered (Table 4).

The contraction rate of all wounds treated with PRF was higher than that of untreated wounds, with the highest values observed in cases of repeated PRF application.

In our subjects, as often happens in chronic injury, some germs have been found at the same time: microorganisms are the most known cells, but fungal contamination has also been identified. Treatment of constant OM currently includes surgical treatment, anti-infective treatment, hyperbaric oxygen treatment (OTI), active antibacterial stimulation (ITSB). Surgical therapy, however, is the basic of treatment [22]. The focus is the removal of contamination and the practical reconstruction of the treated bone fragment. With the surgeries received so far for the treatment of OM, the possibility of annihilating the disease is to eliminate the infected bone and all contiguous tissue down to the essential healthy tissue. Occasionally, however, a small evacuation may serve the purpose, which guarantees neither the stability nor the capacity of the treated limb, yet most of the time, after the removal of the infected bone, a rational surgical restoration is necessary.

Together with the contaminated fabrics, it is equally appropriate to eliminate all internal fixation systems (plates, screws, nails, staples, etc) present in the infected area, and fall back on another external adjustment. All modern surgical practices can offer amazing results, despite the length and order of the incredibly long medications and the not inconsiderable danger of complications and disappointments. The use of solid A-PRF in OM lesions treated by us has given the announced results with a moderate involvement in the surgical method and the economic commitment of the health-care facility where the patient is operated. In addition, the surgical danger to the patient is even lower (our patients have been fully treated under subarachnoid anesthesia) [25,26].

CONCLUSIONS

Finally, the effect of PRF on bone cells should not be due to the effect of a solitary growth factor, but to the combined impact of several platelet growth factors.

Both the primary and secondary endpoints are considered to have been met in this trial.

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Additional medical, histological, verifiable investigations with control group studies are required to understand the benefit of this new procedure. Nevertheless, it cannot be ignored that, that is obtained from an autologous blood withdrawal, the delivered PRF is scarce and only a limited volume can be used. This situation limits the efficient use of PRF in huge OM lesions. Although the possible uses of PRF are extensive, larger, statistically controlled studies are needed to validate these observations, as well as precise information on the functioning of the biomaterial, its science, expertise, and cutting points is needed to improve its use in daily clinical application.

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