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Advance Research on Foot and Ankle

Review Article

Crisci A, et al. Adv Res Foot Ankle 2: 111.

DOI: 10.29011/ARFA-111.1000011.

Second-Generation Platelet Concentrates (L-PRF, A-PRF, *i*-PRF, *i*-PRF M, *i*-PRF+) in Cutaneous Wound Surgery of the Foot

Alessandro Crisci[1-3*], Francesco Crisci[2], Michela Crisci[4]

[1]Department of Medicine, Surgery and Dentistry "Salernitan Medical School", University of Salerno, Fisciano (SA), Italy

[2]Unitsd of Derma surgery, Skin Transplants and Difficult Wounds, "Villa Fiorita" Nursing Home, Aversa (CE), Italy

[3]Institute for Studies and Treatment of Diabetes, Abetaia, Casagiove (CE), Italy

[4]Faculty of Medicine and Surgery, Vasile Goldis Western University of Arad, Arad, Romania

[*]**Corresponding author:** Alessandro Crisci, Department of Medicine, Surgery and Dentistry "Salernitan Medical School", University of Salerno, Fisciano (SA), Italy. Tel: +39 3388722799; E-mail: alcrisci@unisa.it

Citation: Crisci A, Crisci F, Crisci M (2019) Second-Generation Platelet Concentrates (L-PRF, A-PRF, *i*-PRF, *i*-PRF M, *i*-PRF+) in Cutaneous Wound Surgery of the Foot. Adv Res Foot Ankle 2: 111. DOI: 10.29011/ARFA-111.1000011.

Received Date: 8 March, 2019; **Accepted Date:** 15 March, 2019; **Published Date:** 26 March, 2019

Abstract

The aim of the growing multidisciplinary tissue engineering field is the regeneration, amelioration or substitution, in an expectable fashion, of damaged or missing tissues, due to a variety of conditions, caused by traumas, diseases and aging. To guarantee the widespread availability of tissue engineering methods, in clinical fields, it is necessary to modify them in order to have them readily available and relatively easy to apply in everyday clinical routine. Choukroun's Platelet Rich Fibrin (PRF) and its derivatives have been employed in a widespread selection of medical fields, as a supra-physiological autologous growth factors concentrate, able to stimulate tissue regeneration. Platelets have been found inside the clot, *in toto*, in all its groups, even if inside A-PRF group the platelet count was higher in the distal portion, far off the Buffy Coat (BC). T and B lymphocytes, stem cells and monocytes have been found near the BC. Lowering the number of spins while increasing the centrifugation duration in A-PRF group gave a higher neutrophilic count in the distal clot portion. In conclusion, this systematic review results highlight the positive PRF and its derivatives (A-PRF, *i*-PRF) effects on wound healing, after regenerative therapy for cutaneous wounds of the foot management.

Keywords: Advanced Platelet Rich Fibrin; *Blood Derivatives,* Growth Factors; Injectable Platelet Rich Fibrin; *L-PRF Wound Box;* Platelet and Leukocyte Rich Fibrin; Stem Cells

Introduction

If a wound does not heal in an orderly and timely manner, or if the healing process does not have structural integrity, the wound can be considered chronic. Chronic wounds constitute a significative problem, not only in specialized structures, but also in everyday practice inside general physician line of work. Chronic wound healing takes place through the same processes of an acute wound, but in this case, abundant granulation tissue usually forms, with excessive fibrosis that leads to scar contraction and function loss. 1 % of Danish population suffers from chronic wounds and in USA, more than 2 millions of people suffer from pressure ulcers, and 600,000 to 2.5 millions of people have more than one chronic ulcer on their legs and feet, suffering of diabetes and venous insufficiency as a common etiology. 2-10 % of all people diagnosed with diabetes mellitus also suffer from foot ulcers. The incidence rate is 2.2-5.9 % per year. Often amputation is the last resort and the risk of a second amputation taking place is high. Wound healing involves a complex cascade of events, ordered and complex, involving many types of cells, which are driven by the release of soluble mediators and signals able to influence these circulating cells and cause them to home back to damaged tissues. Platelets proved themselves to be important cells regulating hemostasis phases through the vascular occlusion and facilitating fibrin clot formation.

It is known that they are also responsible for activation and release of important biomolecules, including specific platelet proteins, growth factors, including Platelet-Derived Growth Factors (PDGF), coagulation factors, adhesion molecules, cytokines/chemokines and antigenic factors, able to stimulate proliferation

and activation of cells involved in wound healing processes, among which fibroblasts, neutrophils, macrophages and stem cells are included. Crisci A (2015), Criscid A, et al. (2015), Crisci A, et al. (2017) [1-3] Notwithstanding the diffuse usage of Human Platelet Concentrates (HPC), like Platelet-rich plasma (PRP) (Figure 1), one of the reported drawbacks is the usage of anticoagulation factors that are able to delay normal wound healing phases. Crisci A, et al. (2017), Marotta G, et al. (2018) [4,5] due to these limitations, further research is intent on the development of a second generation platelet concentrate excluding the usage of anticoagulation factors. As such, a platelet concentrate without coagulation factors, subsequently defined as Platelet-rich fibrin (PRF), was developed by Choukroun, with particular reference to its abilities to speed up wound healing and tissues regeneration.

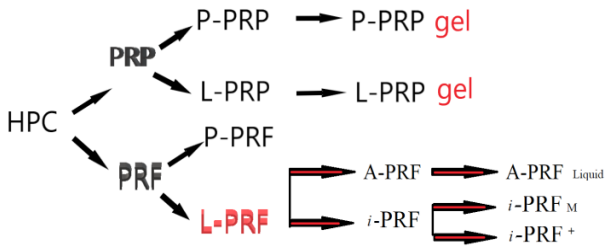

Figure 1. Platelets concentrates (HPC). PRP: Platelet rich-plasma.

This fibrin scaffold, devoid of any cytotoxic property, is obtained from 9 ml of patient's blood, after centrifugation performed with a PRF-DUO centrifuge (Figure 2) and the use of gel-free vials; it contains a variety of blood cells - including platelet, B and T lymphocytes, monocytes, stem cells and neutrophilic granulocytes - other than growth factors. L-PRF (leukocyte-PRF) and its derivatives (A-PRF, *i*-PRF and so on) (Figure 3), furthermore, contain white blood cells, necessary during wound healing processes. Crisci A, et al. (2018) [6] also, since white blood cells, including neutrophils and macrophages, are among the first types of cells present in wound sites, their role includes also cell debris, microbes and necrotic tissue phagocytosis, hence preventing infection. Macrophages are also cells derived from myeloid lineage and are considered one of the cell types involved in growth factor secretion during wound healing, including Transforming growth factor β (TGF-β), PDGF and Vascular-Endothelial Growth Factor (VEGF). These cells, together with neutrophils and platelets, are main players in wound healing and, in association with their secreted growth factors/cytokines, they are able to facilitate tissue regeneration, formation of new blood vessels (angiogenesis) and infection prevention (antimicrobial action). In 2008, Lundquist [7] was one of the first to evaluate PRF effects on fibroblast coming from human dermis. It was seen that PRF proliferative effect on dermic fibroblasts was significantly higher than fibrin glue and recombinant PDGF-BB. Furthermore, PRF induced rapid collagen I release, and the prolonged release, and protection against proteolytic degradation, of endogenous fibro genic factors, which is important for would healing.

Figure 2. Clinical Centrifuge PRF-DUO. PRF, fibrin rich in platelets.

A-PRF+	A-PRF+	1300 rpm, 8min
i-PRF	I-PRF	700 rpm, 3min
i-PRF M	I-PRF *M*	700 rpm, 4min
i-PRF+	I-PRF+	700 rpm, 5min
A-PRF Liquid	A-PRF Liquid	1300 rpm, 5min
Custom	Custom	1300 rpm, 3min
Manual	Manual	FREE SETTINGS

Figure 3. Types of platelet concentrates obtainable by means of Clinical Centrifuge PRF-DUO.

In a second *in vitro* study performed by Lunquist et al. in 2013 [8], PRF induced the mitogenic and migratory effect on human dermic fibroblasts in culture and also demonstrated that fibrocytes (a type of cell important in acute wound healing) might be cultured on PRF disks, favoring even more wound healing and soft tissue regeneration. Subsequently, Clipet F, et al. (2012) [9] found that PRF induces survival and proliferation of fibroblasts and keratinocytes. It was discovered that PRF induces mitogenic effects on endothelial cells through the extracellular activation pathway of signal regulated kinase. It was observed a slow and steady release of growth factors from the PRF matrix, which released VEGF, a known growth factor responsible for endothelial mitogenic response.

L-PRF and Its Derivatives in Chronic Foot Wound Healing

L-PRF

In the longitudinal section of L-PRF clot, produced following the standard centrifugation protocol (30" of acceleration, 2' at 2700 rpm, 4' at 2400 rpm, 3' at 3000 rpm and 36" of deceleration and arrest) a dense clot of fibrin with a minimal inter-fibrous space is found. Cells are observed along the whole clot, even if they diminish in the distal PRF clot portions. Crisci A, et al. (2017) [4].

Advanced-PRF

PRF clots formed with the Advanced-PRF (A-PRF) centrifugation protocol, in its A-PRF+ (1300 rpm, 8 minutes) and A-PRF Liquid (1300 rpm, 5 minutes) variants [10] following Choukroun indications, demonstrated a freer structure, with an increased inter-fibrous space and a higher count of cells inside the fibrin rich clot. Furthermore, cells are more uniformly distributed inside the clot, compared to L-PRF, and some cells might also be found in the distal part of the clot. A representative image for cell distribution inside A-PRF is provided in (Figure 4).

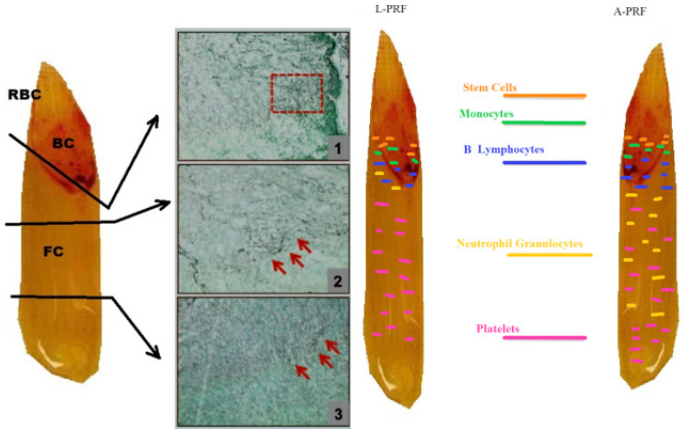

Figure 4: Advanced-PRF (A-PRF) total scan of a fibrin clot along its longitudinal axis (Masson-Goldner staining). RBC represents the fraction of red blood cells. The buffy coat (BC) is the transformation zone between the fraction of RBC and the fibrin clot and FC represents the fibrin clot. The three bars within the scan and the arrows show the first floors of the respective areas. The red arrows mark cells that are trapped inside the fibrin network [11].

Injectable PRF formulation (*i*-PRF)

Development of a PRF injectable solution (named *i*-PRF) [12,13] (centrifuged at 700 rpm [60 g] for 3 minutes) and its derivatives *i*-PRF M (700 rpm for 4 minutes) and *i*-PRF+ (700 rpm for 5 minutes) (Figure 3) was pursued with the aim to deliver a platelet concentrate to physician which proved easy to use in a liquid formulation, to be used alone or easily combined with various biomaterials. Taking advantage of a slower and shorter centrifugation velocity, it is possible to observe an increased count of regenerative cells with an increased concentration of growth factors, compared to other PRF formulations obtained at higher centrifugation velocity. Ghanaati, et al. (2014) [10] referred that velocity and time do not influence monocyte and stem cell concentrations, but they are able to influence platelet and neutrophils concentrations. Consequently, A-PRF contains an increased count of platelets, mostly found in the distal membrane portion, and includes more neutrophils compared to L-PRF. This type of concentrate has the potential to improve angiogenesis by expressing the enzymatic matrix metalloproteinase-9. Therefore, the neutrophilic inclusion in PRF membrane, with the use of A-PRF, might be taken in account if angiogenesis is one of the aims.

The analyses of Ghanaati, et al. (2014) [10] study also revealed that platelets were the only cells present in each clot area up to 87 ± 13 % in L-PRF group, and up to 84 ± 16 % in A-PRF group (Figure 4). Furthermore, results showed that T lymphocytes (L-PRF: 12 ± 5 %, A-PRF: 17 ± 9 %), B lymphocytes (L-PRF: 14 ± 7 %, A-PRF: 12 ± 9 %), CD34+ stem cells (L-PRF: 17 ± 6 %, A-PRF: 21 ± 11 %) and monocytes (L-PRF: 19 ± 9 %, A-PRF: 22 ± 8 %) were not found past a certain point at maximum 30 % of the total clot length, since they're distributed near the BC created by the centrifugation process (Figure 4).

PRF Various Types' Effects on Growth Factors Release

It was long observed that PRF release a series of growth factors for the microenvironment. The TGF-β has a wide efficacy of over 30 factors, known as fibro genetic agents, with TGF-β1 being the most described in literature. It is a known stimulator of various types of cells' proliferation, including osteoblasts, and it constitutes the most powerful fibro genetic agent among all cytokines. It plays a prominent role in matrix molecule synthesis, like collagen-1, and fibronectin, both from osteoblasts and fibroblasts. Even though its regulatory mechanisms are particularly complex, TGF-β1 plays an active role in cutaneous wound healing, in all different districts. VEGF is the most powerful growth factor in tissue angiogenesis. It has powerful effects on tissue remodeling and VEGF incorporation by its own in various osseous biomaterials demonstrated an increase in the novel formation of bone, thus pointing out the rapid and powerful effects of VEGF. Insulin-Like Growth Factors (IGF) is a positive regulator of proliferation and differentiation for the majority of mesenchymal cell types, acting as cellular protection agents. Even though these cytokines are cell proliferation mediators, they also constitute the main axis of planned cell death (apoptosis), [14] inducing survival signals that are able to protect cells from many apoptotic stimuli. Bayer, et al. (2016) [15] explored for the first time the PRF properties, which might contribute to its anti-anti-inflammatory/antimicrobial activities. It was found that in human keratinocytes, PRF was able to induce hBD-2 (β-defensin 2).

Effects of PRF on Cutaneous Wound Healing of Foot and *in vivo* Angiogenesis

The tissue growth factors effect, and in particular PRF and its derivatives, have been particularly studied concerning soft tissue wound healing and angiogenesis in various animal models. In many medical procedures, PRF usages were principally combined to achieve successful management of leg ulcers, previously proved difficult to heal, including the diabetic foot ulcers, venous ulcers and arteriopathic ulcers of lower limbs. Furthermore, PRF was studied by authors in the management of diabetic hand ulcers and in foot tissue scarring defects (Figure 5-8). Crisci A, et al, (2018), Crisci A, et al. (2018) [16,17].

Our work group proposed the use of platelet and leukocyte rich fibrin (L-PRF) also in ulcer-related osteomyelitis in diabetic foot, hypothesizing the recovery from this severe pathology. In this study, the aim was to standardize the use of L-PRF in patients with osteomyelitis, to use this second-generation platelet concentrate, facilitating healing processes. The authors produced and used L-PRF membranes created from peripheral blood, in patients with osteomyelitis, with cutaneous lesions standing for at least 6 months. Membranes, together with the liquid derived by Wound L-PRF Box compression, were inserted in the cutaneous lesion, up to the bone, after surgical debridement. The evolution of the lesions was subsequently analyzed through time. All patients showed positivity to Probe-to-Bone test, and Nuclear Magnetic Resonance showed cortico-periosteal thickening and/or osteolysis foci of the cortico-spongious, adjacent to the ulcer. Gram-positive bacteria were found in our patients in 52 % of cases. Agents found included Gram-positive cocci like S. Aureus (15.6 %), β-hemolytic Streptococci (12.1 %), S. Viridans (7.1 %) and Gram-negative bacilli like Pseudomonas (10.6 %), Proteus (7.8 %), Enterobacter (5.7 %). Candida was present in 2.8 % of cases. To this day, cutaneous osteomyelitic lesions are healed in all treated patients, with no signs of infection nor relapses. The use of L-PRF in cutaneous foot lesions treatment by the authors have the reported results, with a moderate effort in terms of surgical technique, and economical costs for the health structure where the patients was treated. Moreover, also the surgical risk to which the patient is subjected is low (our patients were all treated under local anesthesia).

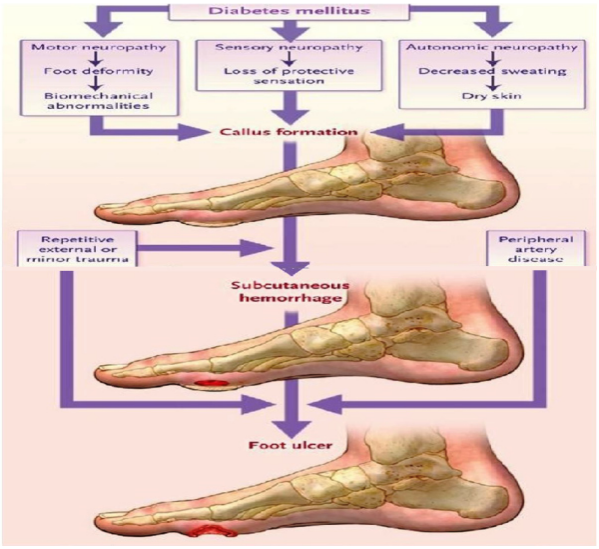

Figure 5: Diabetic foot cutaneous ulcers pathogenesis.

Figure 6: Post-surgical dehiscence following Achilles' tendon reconstruction (A) treated with L-PRF (B) after seven days from therapy (C).

Discussion

Regenerative properties of L-PRF and its derivatives (A-PRF, *i*-PRF) (Figure 1) as a surgical adjuvant received a notable attention ever since the material was introduced in the first years of the millennium. On the other hand, there is no clear evidence to

explain the antimicrobial potential of this biomaterial, which differs both structurally and biologically from other HPC forms. Ghanaati, et al. [10] have histologically described A-PRF™ as a cellular matrix seeded on fibrin, which contains various blood cells, including: platelets, lymphocytes (B and T), monocytes, stem cells and neutrophilic granulocytes, which are able to release a series of growth factors. Crisci A, et al. (2018), Kobayashi E, et al. (2016) [17,18] Theoretically, biological components and physiological mechanisms to exert antimicrobial activity are similar among all the various HPC types, and also similar to clotted blood. However, all these autologous biomaterials differ among themselves for: I) the variable mix of cell types; II) vitality of the contained cells; III) their activation method, natural or chemical; IV) density of the fibrin meshwork; V) interactions between cellular and extracellular components; VI) the release of a variety of proteins.

All these differences might have a significative outcome of the respective anti-inflammatory and antimicrobial activities. Fujioka-Kobayashi M, et al. (2016), Del Fabbro M, et al. (2016), Burnouf T, et al. (2013), Cieslik-Bielecka A, et al. (2012), Dohan Ehrenfest DM, et al. (2009) [19-23] Furthermore, the mechanisms and the dynamics of the single antimicrobial components found in these biomaterials are scarcely understandable.

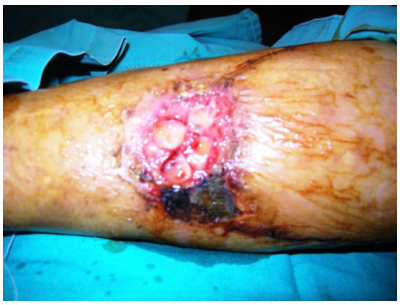

Figure 7. L-PRF clots, used on a cutaneous leg wound.

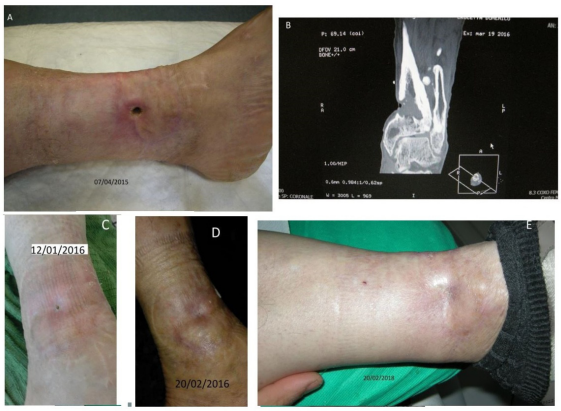

Figure 8: Use of Leukocyte Platelet (L-PRF) Rich Fibrin in diabetic foot ulcer with osteomyelitis. (**A**,**C**,**D**,**E**) Different moments of the wound healing, stable after two years; (**B**) NMR of the patient with the bone lesion [16].

A-PRF™ shows antimicrobial activity against all the single organisms tested in this study on a timespan of 24 hours. These results are consistent with those obtained from past studies, evaluating the antimicrobial properties of other HPC preparations. Fujioka-Kobayashi M, et al. (2016), Del Fabbro M, et al. (2016), Burnouf T, et al. (2013), Cieslik-Bielecka A, et al. (2012) [19-22] Since A-PRF™ shows antimicrobial properties, there is the need to establish if this activity is significantly higher than a whole blood clot. Future research is needed to explore the antimicrobial spectrum of A-PRF™ and all L-PRF derivatives, and to export the possibility that it might act as substrate to facilitate the growth of specific organisms. In particular, for the surgeon, it is important to remember that Staphylococcus Aureus is one of the main causes of acquired nosocomial infections, infections correlated to internal medical devices and infections of surgical wounds. Significative research is nowadays focused on alternative S. Aureus infection treatments, to lower the risk of selecting antibiotic resistant strains. Cieslik-Bielecka A, et al. (2012), Dohan Ehrenfest DM, et al. (2009), Bielecki TM, et al. (2007), Sause WE, et al. (2016) [22,25] This is the reason S. Aureus is still the most frequently tested organism in literature, examining PC antimicrobial activity. Fujioka-Kobayashi M, et al. (2016) [19] Many different HPC preparations demonstrated antimicrobial activity, for both methicillin-resistant and methicillin-sensitive strains of S. Aureus. Fujioka-Kobayashi M, et al. (2016), Del Fabbro M, et al. (2016), Burnouf T, et al. (2013), Cieslik-Bielecka A, et al. (2012) [19-22] Candida albicans is the most frequently fungal species isolated in microbiome. Immune response impairment might allow these opportunistic fungi to give infection. Jabra-Rizk MA, et al. (2016), Marsh PD, et al. (2017) [26,27] A-PRF™ has got a higher capability to constantly inhibit *C. Albicans* growth, compared to a whole blood clot. Moreover, C. albicans is less susceptible to antimicrobial components of platelets and confirms the discoveries made in 2002 by Tan et al. (2002) [28] who noticed how antimicrobial peptides of human platelets were more powerful against bacteria than fungi. A-PRF™ shows a greater potential inhibiting Streptococcus mutans compared to natural blood clot. However, since no other HPC was tested against this organism, the inhibition mechanism and of its clinical potential requires additional studies.

Even though results of various studies suggest that A-PRF™ shows an antimicrobial activity, several limitations are present. In first instance, the *in vitro* examination does not mimic a clinical situation where A-PRF™ would be employed in an environment, surrounded by tissues that react to a surgical event. In this scenario, A-PRF™ is able to interact with several cells and cytokines, involved in wound healing processes and it can modify the initial immune response and the healing phases. Activated platelets' growth factors release inside the fibrin meshwork might modify the expression of antimicrobial peptides from surrounding tissues. It is possible that numerous patient related factors might influence

A-PRF™ quality. Yajamanya, et al. (2016) [29] demonstrated that the fibrin matrix formed in their PRF version in elderly patients was more generically organized compared to fibrin matrix formed in younger patients. The magnitude of this discovery still needs to be determined. Cell types, number of cells and plasma component concentration differ inside each clot and between single clots, each sample disk cannot be identical to another.

One of the problems to be assessed is that there is still no way to determine if the tested material is bactericidal or bacteriostatic. On this argument, our study group is working at the very moment. Drawbacks set aside, the disk diffusion method proved enough to demonstrate that A-PRF™, like all others L-PRF derivatives, shows antimicrobial activity.

Conclusion

Still too little is known about PRF and its derivatives (A-PRF, *i*-PRF) antimicrobial properties and a scant amount of studies have shed light, to the date, on this phenomenon. Under a tissue engineering point of view, it is interesting to point out that no research project focused on PRF strength, rigidity and resilience, notwithstanding its clinical usage this past 15 years. Hence, an interesting future prospect is to better characterize its biomaterial properties, and future research should focus on those factors that might improve further its characteristics, for its various biomedical applications. It's of fundamental importance that future researches, concentrating on PRF usage as co-adjuvant in soft tissue regenerative therapies, would design appropriate studies, with the required controls, to further estimate the regenerative potential of PRF in soft tissue wound healing, in particular concerning foot wound healing. The use of A-PRF™ in clinical practice showed a great potential to improve healing and to improve surgical outcomes, since it works as an autologous scaffold, able to host cells and bioactive compounds. However, the antimicrobial potential of this material was demonstrated, and it can be an important property, which contributes to clinically ascertained accelerated and non-complicated healing events. Results of this revision point out that A-PRF™ shows, nonetheless, an antimicrobial activity against *S. Aureus*, *S. Mutans*, *Enterococcus faecalis* and *C. albicans*. Furthermore, spectrum and potency as antimicrobial agent are far less than those of an established surgical antimicrobial (specific antibiotic). It is necessary, thus, future investigations that would involve A-PRF™ and its derivatives to ascertain the entire spectrum of its antimicrobial activity *in vitro*, its participation *in vivo*, and the influence of patient's characteristics on its biological activity. Moreover, the clinical potential as administration vehicle for local drugs in infected sites should be explored. Future studies should increase patient variability and the sample dimensions for all HPC based studies.

Further clinical, histological and statistical studies are required to comprehend the advantages of this new technique. However, it's wouldn't be feasible to ignore that, once obtained from autologous blood samples, L-PRF and its derivatives have a reduced volume, and only a limited quantity can be used. This limits the systematic use of PRF in greater cutaneous lesions. Even if there are ample potential applications of PRF, a deep knowledge of this biomaterial functioning is needed, as well as knowledge of its biology, efficacy and limits, to better optimize its use in everyday practice.

References

1. Crisci A (2015) Le membrane L-PRF utili in chirurgia. J Plast Dermatol 2: 75-90.
2. Crisci A, Placido F, Crisci M, Bosco A (2015) A new instrument aid of plastic surgeon: membranes L-PRF (Platelet-Rich- Fibrin). Update Plast Surg 3: 162-172.
3. Crisci A, Serra E, Cardillo F, Crisci M (2017) Selezione di un modello animale pertinenteper la prova degli effetti in vitro della fibrina ricca di leucociti e piastrine di Choukroun (L-PRF equino). Nota su un protocollo standardizzato proposto per l'uso clinico e l'uso di L-PRF Wound Box®. V.P.E 1: 41-50.
4. Crisci A, Lombardi D, Serra E, Lombardi G, Cardillo F, et al. (2017) Standardized protocol proposed for clinical use of L-PRF and the use of L-PRF Wound Box®. J Unexplored Med Data 2: 77-87.
5. Marotta G, Licito A, Serra E (2018) Evaluation of genotyping methods and costs for IL1a polymorphisms in Platelet Rich Plasma (PRP); view point for therapy on the diabetic foot ulcers. Eur Rev Med Pharmac Sci 22: 575-577.
6. Crisci A, Benincasa G, Crisci M, Crisci F (2018) Leukocyte Platelet-Rich Fibrin (L-PRF), a new bio membrane useful in tissue repair: basic science and literature review. Bio interface Res Appl Chem 8: 3635-3643.
7. Lundquist R, Dziegiel MH, Agren MS (2008) Bioactivity and stability of endogenous fibrogenic factors in plateletrich fibrin. Wound Repair Regen 16: 356-363.
8. Lundquist R, Holmstrøm K, Clausen C, Jørgensen B, Karlsmark T (2013) Characteristics of an autologous leukocyte and platelet rich fibrin patch intended for the treatment of recalcitrant wounds. Wound Repair Regen 21: 66-76.
9. Clipet F, Tricot S, Alno N, Massot M, Solhi H, et al. (2012) *In vitro* effects of Choukroun's platelet-rich fibrin conditioned medium on 3 different cell lines implicated in dental implant ology. Implant Dent 21: 51-56.
10. Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, et al. (2014) Advanced Platelet-Rich Fibrin: a new concept for cell based tissue engineering by means of inflammatory cells. J Oral Implantol 40: 679-689.
11. Choukroun J (2014) Advanced-PRF and *i*-PRF: platelet concentrates or blood concentrates? J Periodont Med Clin Pract 1: 1-3.
12. Miron RJ, Fujioka-Kobayashi M, Hernandez M, Kandalam U, Zhang Y, et al. (2017) Injectable platelet rich fibrin (*i*-PRF): opportunities in regenerative dentistry? Clin Oral Invest 21: 2619-2627.
13. Crisci A, De Crescenzo U, Crisci M (2018) Platelet-Rich Concentrates (L-PRF, PRP) in tissue regeneration: control of apotosis and interactions with regenerative cells. J Clin Mol Med 1: 5-12.

14. Bayer A, Lammel J, Rademacher F, Groß J, Siggelkow M, et al. (2016) Platelet-released growth factors induce the antimicrobial peptide human beta-defensin-2 in primary keratinocytes. Exper Dermatol 25: 460-465.

15. Crisci A, Marotta G, Licito A, Serra E, Benincasa G, et al. (2018) Use of leukocyte platelet (L-PRF) rich fibrin in diabetic foot ulcer with osteomyelitis (three clinical cases report). Diseases 6: 30.

16. Crisci A, Marotta G, Benincasa G, Crisci M (2018) L-PRF (fibrina ricca in leucociti e piastrine): uso in tre casi di ulcera diabetica con osteomielite cronica. JAMD 21: 197-203.

17. Kobayashi E, Flückiger L, Fujioka-Kobayashi M, Schaller B, Miron RJ, et al. (2016) Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clin Oral Invest 20: 2353-2360.

18. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, e t al. (2016) Optimized platelet-rich fibrin with the low-speed concept: growth factor release, biocompatibility, and cellular response. J Periodontol 88: 112-121.

19. Del Fabbro M, Bortolin M, Taschieri S, Ceci C, Weinstein RL (2016) Antimicrobial properties of platelet-rich preparations. A systematic review of the current pre-clinical evidence. Platelets 27: 276-285.

20. Burnouf T, Chou M-L, Wu U-W, Su CY, Lee LW (2013) Antimicrobial activity of platelet (PLT)-poor plasma, PLT-rich plasma, PLT gel, and solvent/detergent-treated PLT lysate biomaterials against wound bacteria. Transfusion 53: 138-146.

21. Cieslik-Bielecka A, Dohan EDM, Lubkowska A, Bielecki T (2012) Microbicidal properties of leukocyte- and platelet-rich plasma/fibrin (L-PRP/L-PRF): new perspectives. J Biol Regul Homeost Agents 26: 43-52.

22. Crisci A, Rescigno C, Crisci M (2019) The L-PRF membrane and its derivatives useful in Wound Care. Italian Journal of Wound Care 3: 19-26.

23. Dohan EDM, Rasmusson L, Albrektsson T (2009) Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 27: 1578-1567.

24. Bielecki TM, Gazdzik TS, Arendt J, Szczepanski T, Król W, et al. (2007) Antibacterial effect of autologous platelet gel enriched with growth factors and other active substances: An *in vitro* study. J Bone Joint Surg B 89: 417-420.

25. Sause WE, Buckley PT, Strohl WR, Lynch AS, Torres VJ, et al. (2016) Antibody-Based biologics and their promise to combat staphylococcus aureus infections. Trends Pharmacol Sci 37: 231-241.

26. Jabra-Rizk MA, Kong EF, Tsui C, Nguyen MH, Clancy CJ, et al. (2016) Candida albicans pathogenesis: fitting within the host-microbe damage response framework. Infect Immun 84: 2724-2739.

27. Marsh PD, Zaura E (2017) Dental biofilm: ecological interactions in health and disease. J Clin Periodontol 44: S12-S22.

28. Tan Y-Q, Yeaman MR, Selsted ME (2002) Antimicrobial Peptides from Human Platelets. Infect Immun 70: 6524-6533.

29. Yajamanya SR, Chatterjee A, Babu CN, Karunanithi D (2016) Fibrin network pattern changes of platelet-rich fibrin in young versus old age group of individuals: A cell block cytology study. J Indian Soc Periodontol 20: 151-156.