Journal of
Stem Cell Reports

JScholar
Fostering Scholarly Communication

Research Open Access

The L-PRF Membrane (Fibrin Rich in Platelets and Leukocytes) And Its Derivatives Useful as A Source of Stem Cells in Wound Surgery

Alessandro Crisci*[1,2,3], Silvana Manfredi[2], Michela Crisci[4]

[1]Unit of Dermosurgery Cutaneous Transplantations and Hard-to-HealWound,"Villa Fiorita"Private Hospital, 81031Aversa CE, Italy
[2]School of Medicine, University of Salerno Italy, 84084 Fisciano SA, Italy
[3]Institute for the Studies and Care of Diabetics, Abetaia, 81020 Casagiove CE, Italy
[4]School of Medicine and Surgery, Vasile Goldis Western University of Arad, 310025 Arad, Romania

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

Received Date: February 02, 2019; **Accepted Date:** March 26, 2019; **Published Date:** March 28, 2019

Citation: Alessandro Crisci (2019) The L-PRF Membrane (Fibrin Rich in Platelets and Leukocytes) And Its Derivatives Useful as A Source of Stem Cells in Wound Surgery. J Stem Cell Rep. 1: 1-11.

Abstract

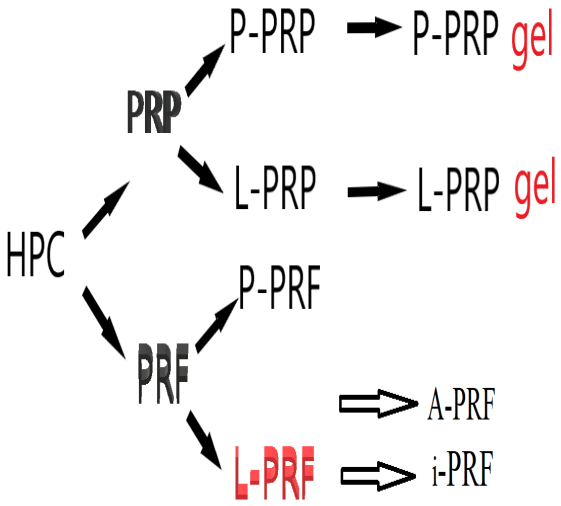
Growing multidisciplinary field of tissue engineering aims to regenerate, improve or replace predictably damaged or missing tissues for a variety of conditions caused by trauma, disease and old age. To ensure that tissue engineering methods are widely applicable in the clinical setting, it is necessary to modify them in such a way that they are readily available and relatively easy to use in daily clinical routine. Therefore, the steps between preparation and application must be minimized and optimized to make them realistic implementation. General objective of developing platelet concentrates of natural origin can be produced "close" to the patient and accelerate the implantation process, being financially realistic for the patient and the health system. PRF and its derivatives have been used in a wide variety of medical fields for soft tissue regeneration. In conclusion, the results of this systematic review highlight the positive effects of PRF on wound healing after regenerative therapy for the management of various soft tissue defects found in wound care. Factors freed by platelets contained in L-PRF induce and control the proliferation and migration of other cell types, involved in tissue repair, like smooth cell muscles (SMCs) and mesenchymal stem cells (MSCs).

This review article focuses on the development of various platelet concentrates, their fabrication procedure, advantages and disadvantages for use in regenerative surgery and healing process.

Keywords: Growth factors; Fibrin-rich in leukocytes and platelets; Fibrin-rich injectable platelets, Stem cells

Introduction

The multidisciplinary field of tissue engineering aims to repair, regenerate or restorably repair damaged and supportive tissues, including cells, tissues and organs, due to an assortment of biological conditions, including congenital anomalies, lesions, diseases and/or aging. [1,2] During their regeneration, a key aspect concerns the growth of a vascular source that is able to support cell function and the future development of tissues by maintaining a vital nutrient exchange through vessels blood. Although most tissue engineering scaffolds are avascular in nature, it remains essential that all regenerative strategies focus on developing a vascular network to achieve positive clinical outcomes and regeneration in both soft and hard tissues. [3] Wound healing involves a cascade of complex, orderly and elaborate events involving many cell types driven by the release of soluble mediators and signals that are able to influence the return of circulating cells to damaged tissues. Platelets have proven to be important cells that regulate the hemostasis phase through vascular obliteration and facilitating the formation of fibrin clots. It is known that they are responsible for the activation and release of important biomolecules, including specific platelet proteins, growth factors including platelet-derived growth factor (PDGF), coagulation factors, adhesion molecules, cytokines/chemokines and angiogenic factors that are able to stimulate proliferation and activation of cells involved in wound healing, including fibroblasts, neutrophils, macrophages and mesenchymal stem cells (MSC). Despite the widespread use of platelet concentrates (HPC) (Figure.1) such as PRP (Platelet-rich plasma), one of the drawbacks reported is the use of anticoagulation factors that delay normal wound events. [4, 5] Because of these limitations, further research has been focused on the development of a second-generation platelet concentrate without using anticoagulation factors. As such, a platelet concentrate free of coagulation factors, subsequently termed platelet-rich fibrin (PRF), was developed because of its properties of anticipating tissue regeneration and wound healing. This fibrin scaffold, which has no cytotoxic potential, is obtained from 9 ml of the patient's blood after 1 phase of centrifugation and contains a variety of blood cells - including platelets, B and T lymphocytes, monocytes, stem cells, and neutrophil granulocytes - in addition to growth factors. Furthermore, L-PRF (also called leukocyte-PRF) contains white blood cells, necessary cells that are important during the wound healing process. [6] Moreover, since white blood cells, including neutrophils and macrophages, are among the first types of cells present in wound sites, their role also includes phagocytic fragments, microbes, and necrotic tissue, thus preventing infection. Macrophages are also key cells derived from the myeloid lineage and are considered one of the key cells involved in growth factor secretion during wound healing, including the transforming growth factor beta (TGF-β), PDGF and growth factor vascular endothelium (VEGF) (Figure.2). These cells, together with neutrophils and platelets, are key players in wound healing and in combination with their growth factors/secreted cytokines are able to facilitate tissue regeneration, the formation of new blood vessels (angiogenesis) and the infection prevention.



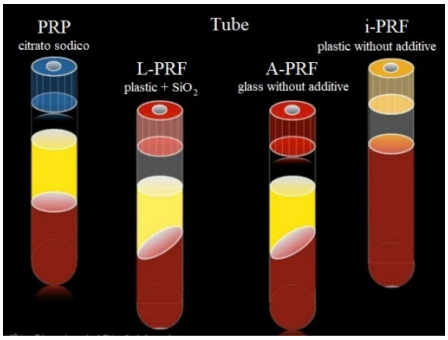


Figure. 1 Platelet concentrates (HPC)

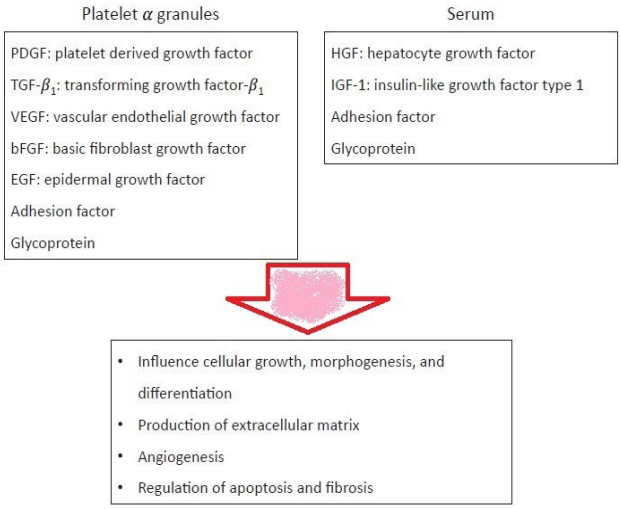


Figure.2 Function of the platelets in wound healing

In 2008, Lundquist [7] was one of the first to evaluate the effects of PRF on human dermal fibroblasts. It was found that the proliferative effect of PRF on dermal fibroblasts was significantly greater than fibrin glue and recombinant PDGF-BB. Furthermore, PRF induced rapid release of collagen 1 and prolonged release and protection against proteolytic degradation of endogenous fibrogenic factors that are important for wound healing. In a second in vitro study conducted by Lundquist et al. in 2013 [8], PRF induced the mitogenic and migratory effect on cultured human dermal fibroblasts and also showed that fibrocytes (a type of cell important for healing acute wounds) could be cultured within disks PRF, further promoting wound healing and soft tissue regeneration. Subsequently, Clipet et al. [9]. found that PRF induces the survival and proliferation of fibroblasts and keratinocytes. The PRF has been found to induce endothelial cell mitogenesis via the extracellular pathway of signal-regulated kinase activation. A slow and steady release of growth factors from the PRF matrix was observed that releases VEGF, a known growth factor responsible for the endothelial mitogenetic response.

L-PRF And Its Derivatives in The Healing of Chronic Wound Ulcers

L-PRF

In the longitudinal section of the L-PRF coagulum, produced according to the standard centrifugation protocol (30" of acceleration, 2' at 2700 rpm, 4' at 2400 rpm, 3' at 3000 rpm, and 36" of deceleration and stopping) [4], a thick fibrin clot is present with minimal inter-fibre space. Cells are observed throughout the blood clot, although decreasing towards the most distal parts of the PRF clot (Figure.3).

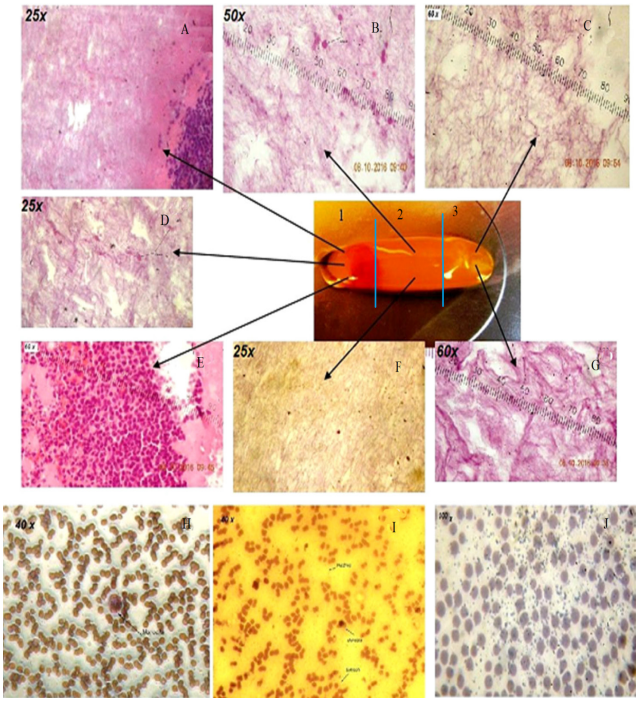


Figure.3 Horse L-PRF membrane at 0 minutes from compression (Eosin-Hematoxylin color). The L-PRF layers were fixed in 10% formalin buffered neutral solution at pH 7.2 for 48 hours and incorporated in paraffin according to the standard procedure. Twenty serial sections (7 µm thickness) of each sample were cut using a microtome. A) III proximal ingr. 25x White Blood Cells- Fibrin Reticulum; B) III average ingr. 60x Erythrocytes- Fibrin pattern; C) III distal ingr. 60x Fibrin Reticulum; D) III proximal ingr. 25x Erythrocytes-Fibrin; E) III proximal ingr. 60x Fibrin on the right, Lymphocytes in the center, Erythrocytes and neutrophil granulocytes on the left; F) III medium ingr. 25x fibrin lattice; G) III distal ingr. 60x Fibrin Reticulum; H) Red clot smear ingr. 40x presence of monocita in a carpet of erythrocytes; I) smear red clot ingr. 40x presence of erythrocytes, monocytes and platelets; J) smear red clot ingr. 100x platelets in a carpet of erythrocytes (May-Grunwald- Giemsa stain). (Crisci et al. 2017) [4].

A-P RF

The PRF clots formed with the A-PRF centrifugation protocol (Advanced-PRF) (1500 rpm, 14 minutes) [10] showed a freer structure with more inter-fibre space and more cells can be counted in the fibrin-rich clot. Furthermore, the cells are more evenly distributed in the clot than L-PRF, and some cells can also be found in the most distal parts of the clot. A representative image for cellular distribution within A-PRF is shown in Figure. 4.

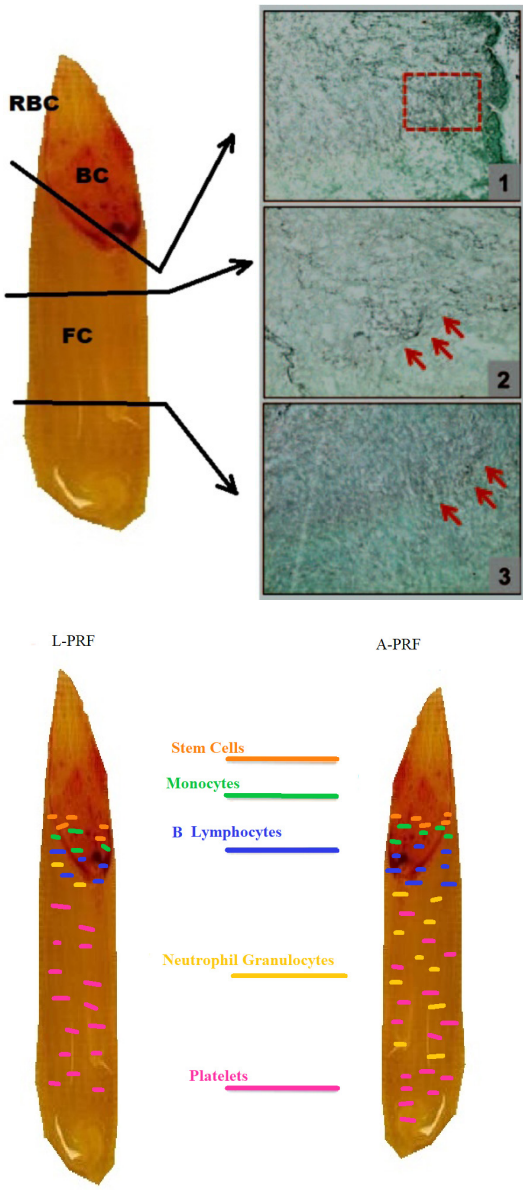


Figure. 4. A-PRF (Advanced-PRF) Total scan of a fibrin clot along its longitudinal axis (Masson-Goldner staining).
RBC represents the fraction of red blood cells. The buffy coat (BC) is the transformation zone between the fraction of RBC and the fibrin clot and FC represents the fibrin clot. The three 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.

i-PRF

The development of an injectable formulation of PRF (referred to as i-PRF) [11, 12] (centrifuged at 700 rpm

[60 g] for 3 minutes) was pursued with the goal of delivering a platelet concentrate easy to use to doctors in liquid formulation that can be used alone or easily combined with various biomaterials. Taking advantage of slower and shorter centrifugation speeds, a greater presence of regenerative cells with higher concentrations of growth factors can be observed compared to other PRF formulations using higher centrifugation rates.

PRF Effects in Tissue Engineering

Platelet localization inside the PRF gel was examined through immunostaining and with the aid of Scanning Electron Microscope from Kobayashi et al. 2016 [13].

Burnouf [14] demonstrated that a copious amount of growth factors was discarded when pressing took place. Hence, pressing processes could influence efficacy and clinical quality of PRF preparations, to be used as graft material.

Platelet derived mediators induce and regulate fibroblasts' late action, and leukocytes' recruitment, neutrophils first, followed by macrophages, consequently eliminating dead cells and cellular debris. Moreover, factors derived from platelets induce and control proliferation and migration of other types of cells, which are critically involved in tissue repair, like smooth muscle cells (SMCs) and mesenchymal stem cells (MSCs).

Activated platelets release a whole range of chemokines and promote adult stem cells' absorption, adhesion and proliferation, including progenitor CD-34 positive cells, MSCs, SMC progenitors and endothelial progenitors. The multipotent nature of these cells and their capability to increase vascular tissue repair, due to paracrine mechanisms, makes them good candidates as therapeutical vehicles to be employed in regenerative medicine fields. Moreover, tissue damages themselves are able to generate strong chemoattractant signals, affecting stem cells, and providing their regenerative action basis. Platelets regulate adult stem cells recruitment toward damaged cells and could therefore constitute an essential mechanism for regenerative cellular processes. Activated platelets release HGF and have been linked to MSCs passage through endothelial cells, lining human arteries. Human mesenchymal stem cells' proliferation (hMSCs) is proportional to platelet concentration inside PRF concentrates.

Among tested growth factors, PRF contained PDGF constitutes the major portion, and stimulates, significantly, cell proliferation and neovascularization. An important PRF characteristic is the resulting fibrin gel, shown to be denser than the gel prepared with thrombin addition (PRP).

Thus, the establishment of a standard protocol for PRF preparation was necessary, satisfying the following criteria:

1) Platelet-contained growth factors should be preserved to stimulate surrounding host cells;
2) Platelets should be stored inside the fibrin mesh with minimal damage or activation;
3) The tridimensional fibrin mesh must be used as scaffold by surrounding host cells.

The PRF was subdivided in 3 regions, of equal length (Figure.3) and platelet presence in each region was observed through S.E.M. and through Optic Microscope in horse-derived preparations (Crisci A.et al.2017) [3, 4].

Region 1 is the region closest to the red clot, and shows a conspicuous number of platelets aggregates, displaying some lymphocytes and other white blood cells. Platelet count is reduced as the distance from the red clot is increased. Inside region 2 (central region), we observe fibrin fibers (primary and secondary fibers) and some platelets. Inside region 3, the fibrin mesh is extremely evident, while the platelet count is low (Figure.3).

We identified some anti-CD41 antibody positive cells, through immunocytochemistry, and, as a matter of fact, in L-PRF, on one side of the membrane, many CD41-positive

platelets were gathered, and some platelets could be found inside the membrane.

On the membrane's opposite side, only few platelets were observed. The discovery explained in Kobayashi et al. [13] studies is constituted by the fact that platelets are not equally distributed inside and on the surface of the PRF clot, even if the clot is considered as a gel with uniform platelet concentration. Therefore, in a clinical setting where platelet-derived growth factors are expected and desired, the red clot-adjacent region must be used, being richer in platelets.

Basing our actions on the assumption that PRF-retained serum could contain elevated GFs levels, released by platelets, which are more or less active during centrifugation phases, we didn't try to squeeze all the plasma with a complete compression of PRF clots.

This obtained result could be due to fibrin, since the fibrin mesh could directly absorb GFs or could entrap serum albumin or heparin, hence indirectly retaining GFs. It is almost impossible counting and regulating the platelet count in PRF preparations before clinical usage. Therefore, the clinically most effective protocol to check result quality is using the PRF region closest to RBC clot.

Cell migration was performed through the employment of MSCs (mesenchymal stem cells), derived from human bone marrow, and human umbilical vein endothelial cells (HUVECs). MSCs migrated principally at day 3 for L-PRF preparations. A higher migration rate was observed for L-PRF compared to L-PRP at day 3, day 7 and day 14. HUVECs migration also reached its peak at day 3, day 7 and day 14 for PRF preparations Figure.5.

In a first set of experiments, the release of growth factors from PRP and i-PRF was investigated by ELISA including PDGFAA, PDGF-AB, PDGF-BB, TGF-β1, VEGF, EGF, and IGF-1. Interestingly, all growth factors investigated demonstrated a significantly higher early (15 min) release of growth factor from PRP when compared to i-PRF. Thereafter, the total release of growth factors was quantified up to a 10-day period. It was found that PDGF-AA, PDGF-AB, EGF, and IGF-1 all demonstrated higher total growth factors released from i-PRF when compared to PRP, but lower than the L-PRF. Interestingly, however, total growth factor release of PDGF-BB, VEGF, and TGF-β1 were significantly higher in PRP when compared to i-PRF. These results point to the fact that various spin protocols/cell types found in PRP/i-PRF are likely responsible for the variations.

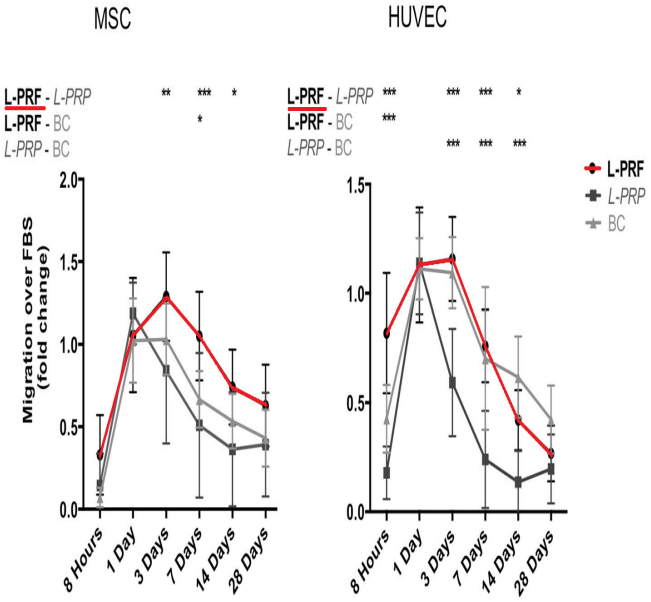


Figure.5. MSC and HUVEC migration is shown in response to factors released by L-PRF, L-PRP and blood clot (BC).

Migration of MSC and HUVEC was assessed in Boyden chambers with media collected after 8 hours and 1, 3, 7, 14 and 28 days of L-PRP, L-PRF and blood clot compared with soils containing 10% FBS and expressed change as a turn. Data are presented as a mean ± SD from a triple of 11 samples. Statistical evaluation was performed using the repeated two-way ANOVA and the Bonferroni post hoc test. Significant differences for the migration of MSC and HUVEC between platelet concentrates at different time points are indicated: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Ghanaati et al. (2014) [10] reported that velocity and time do not affect monocyte and stem cell concentrations, but influence platelet and neutrophil concentrations. As a result, A-PRF contains more platelets, most were found in the distal part of the PRF and L-PRF membrane include more neutrophils. This type of concentrate has the potential to improve angiogenesis by expressing the enzymatic matrix metalloproteinase-9. Therefore, the inclusion of neutrophils in the PRF could be considered if angiogenesis is of interest.

Analysis of the study by Ghanaati et al. 2014 also revealed that the platelets were the only ones present in each coagulum area up to 87 ± 13% in the L-PRF group and up to 84 ± 16% in the A-PRF group (Figure. 4). Furthermore, the results showed that T lymphocytes (L-PRF: 12 ± 5%, A-PRF: 17 ± 9%), B lymphocytes (L-PRF: 14 ± 7%, A-PRF: 12 ± 9 %), CD34 positive stem cells (L-PRF: 17 ± 6%, A-PRF: 21 ± 11%), and Monocytes (L-PRF: 19 ± 9%, A-PRF: 22 ± 8%) not more than 30% of the total length of the clot have been found beyond a certain point, since they are distributed near the BC generated by the centrifugation process (Figure.4).

Effect of PRF on the Release of Growth Factors

It has long been observed that the PRF releases a number of growth factors for the microenvironment.

The TGF-β (Transforming Growth Factor β) has a broad efficacy of over 30 factors known as fibrosis agents, with TGF-β1 which is the most described in the literature. It is a known stimulator of the proliferation of various types of mesenchymal cells, including osteoblasts, and is the most powerful fibrotic agent among all cytokines. It plays a pre-eminent role in the synthesis of the matrix molecule such as collagen1 and fibronectin, both from osteoblasts and fibroblasts. Although its regulatory mechanisms are particularly complex, TGF-β 1 plays an active role in wound healing.

VEGF (vascular endothelial growth factor) is the most powerful growth factor responsible for tissue angiogenesis. It has powerful effects on tissue remodeling and the incorporation of VEGF alone into various bone biomaterials has shown increases in new bone formation, thus indicating the rapid and powerful effects of VEGF.

IGF (Insulin-like growth factor) is a positive regulator of proliferation and differentiation for most types of mesenchymal cells, which also act as cell protection agents. Although these cytokines are cell proliferative mediators, they also constitute the main axis of programmed regulation of cell death (apoptosis) [15], inducing survival signals that protect cells from many apoptotic stimuli. Bayer et al [16]. explored for the first time the properties contained in the PRF that can contribute to its anti-inflammatory/antimicrobial activities. It was discovered that in human keratinocytes, PRF induced the expression of hBD-2 (β-defensin 2).

Effects of PRF on Wound Healing And In Vivo Angiogenesis

The effects of PRF have in particular been studied on the healing of soft tissue wounds and on angiogenesis in various animal models. In other medical procedures, the use of PRF has mainly been combined for success in the management of leg ulcers that are difficult to heal, including diabetic foot ulcers, venous ulcers, and leg ulcers. Furthermore, the PRF has been studied for the management of hand ulcers and soft tissue defects [17-18].

Further Randomized Clinical Trials

One of the advantages reported by the PRF is the ability of the fibrin network to contain leukocytes, to resist and fight infections. Chronic unhealed wounds represent a significant medical challenge and the pathogenesis of unhealed wounds, therefore, requires new therapeutic options to improve clinical outcomes. Macrophages have proven to be key actors during

tissue regeneration, wound healing and infection prevention. Furthermore, they contain antimicrobial effects that are able to reduce bacterial contamination after surgery.

Discussion

The regenerative capacities of the PRF and its derivatives (A-PRF, i-PRF) (Figure.1) as a surgical adjuvant, have received considerable attention since its introduction in the early years of the new millennium. In contrast, no clear evidence remains to clarify the antimicrobial potential of this particular biomaterial that differs both structurally and biologically from other forms of HPC. Ghanaati et al. (2014) [10] described histologically A-PRF ™ as a matrix of cells on fibrin-containing a variety of blood cells including: platelets, lymphocytes (B and T), monocytes, stem cells and neutrophil granulocytes able to release a set of growth factors (Kobayashi et al., 2016 [13]; Fujioka-Kobayashi et al., 2017 [19]). In theory, the biological components and physiological mechanisms for antimicrobial activity are similar within various types of HPC and even coagulated blood. However, these autologous biomaterials differ in terms of 1) the variable mix of cell types; 2) the vitality of the contained cells; 3) their mode of activation, natural or chemical; 4) the density of the fibrin network; 5) interactions between cellular and extracellular components; 6) and the release of a variety of proteins. These differences may have a significant impact on their respective anti-inflammatory and antimicrobial properties (Del Fabbro et al., 2016 [20]; Burnouf et al., 2013 [21]; Cieslik-Bielecka et al., 2012 [22]; al., 2011 [23]; Dohan Ehrenfest et al., 2009 [24]. Furthermore, the mechanisms and dynamics of the individual antimicrobial components contained in these biomaterials are poorly understood.

A-PRF ™ shows antimicrobial activity against all single organisms tested within this study over a 24-hour period. These results are consistent with those of previous studies evaluating the antimicrobial properties of other HPC preparations [20-23] (Bielecki et al., 2007 [25]). Because A-PRF ™ shows antimicrobial properties, the need to determine whether this activity is significantly greater than that of a natural blood clot has emerged. Future investigations are needed to explore the antimicrobial spectrum of A-PRF™ and explore the possibility that it may act as a substrate to facilitate the growth of specific organisms.

Of particular relevance to the surgeon is that Staphylococcus Aureus (SA) is a major cause of hospital-acquired infections, infections related to internal medical devices and infection of surgical wounds (Zalavras et al., 2004 [26]). Significant research is focused on alternative treatment strategies in SA-guided infections to reduce the risk of developing antibiotic-resistant strains (Sause et al., 2015 [27]; Anitua et al., 2011 [23]). For this reason, SA remains the most frequently tested organism in the literature examining the antimicrobial activity of PC (Del Fabbro et al., 2016 [20]). Many different HPC preparations have shown antimicrobial activity for both methicillin-resistant and methicillin-susceptible SA strains (Del Fabbro et al., 2016 [20]; Anitua et al., 2011 [23]; Bielecki et al., 2007 [25]).

Candida Albicans (CA) is the most frequently isolated of the fungal species in the microbiome. The impairment of an individual's immune response may allow these opportunistic fungi to cause infections (Jabra-Rizk et al., 2016 [28]; Marsh et al., 2017 [29]). A-PRF ™ has a greater ability to consistently inhibit AC growth than a normal blood clot. Furthermore, CA is less susceptible to the antimicrobial components of platelets and confirms the findings of Tang et al. (2002) [30] who noted that human platelet antimicrobial peptides are more potent against fungi bacteria. A-PRF™ shows greater potential to inhibit Streptococcus Mutans (SM) than a natural blood clot. However, since no other HPC has been tested against this organism, the mechanism of its inhibition and clinical

potential requires further exploration.

Limitations

Although the results of many studies indicate that A-PRF ™ shows an antimicrobial activity, several limitations have emerged. Firstly, the in vitro investigation does not mimic a clinical situation in which A-PRF ™ will be placed in an environment surrounded by tissues that respond to a surgical event. In this scenario, A-PRF ™ can interact with a series of cells and cytokines involved in the wound healing process and modify initial immune responses and healing events (Miron et al., 2016 [13]; Burnouf et al., 2013 [21]; El-Sharkawy et al., 2007 [31]). The release of activated platelet growth factors within the fibrin matrix may also modify the expression of antimicrobial peptides from surrounding tissues (Bayer et al., 2016 [15]). It is possible that many patient factors can influence the quality of A-PRF™. Yajamanya et al. (2016) [32] demonstrated that the fibrin matrix formed by their version of PRF in elderly patients was more generally organized than the fibrin matrix of younger subjects. The impact of this discovery has yet to be determined. The cell type, the number of cells and the concentration of the plasma components differ within each coagulum and between each coagulum (El Bagdadi et al., 2017 [33]; Ghanaati et al., 2014 [10]), each sample disk cannot be identical to the other. One problem to be defined is that it is not yet possible to determine whether the tested material is bactericidal or bacteriostatic. Regardless of these drawbacks, the disc diffusion method was sufficient to demonstrate that A-PRF™ shows antimicrobial activity.

Conclusions

Very little is known about the antibacterial properties of the PRF and its derivatives (A-PRF, i-PRF) and very few studies have investigated this phenomenon. From a tissue engineering point of view, it is interesting to note that so far, no research has focused on the strength, rigidity or resistance of the PRF despite its clinical use for over 15 years. Therefore, interest remains to better characterize its biomaterial properties and future research should focus on which factors could further improve its characteristics for various biomedical applications. It is essential that the next wave of research using PRF as an adjunct to soft tissue regenerative therapies develop appropriate studies with the necessary controls to further evaluate the regenerative potential of PRF for the healing of soft tissue wounds.

The use of A-PRF ™ in clinical practice has shown potential to improve healing and improve surgical outcomes as it serves as an autologous scaffold that hosts cells and bioactive compounds (Castro et al., 2017 [34]; Miron et al., 2016 [12]; Moraschini et al., 2016 [35]; Del Corso et al., 2012 [36], Crisci et al., 2019 [37]). However, the antimicrobial potential of the material has been demonstrated and may be an important property contributing to clinically detected accelerated and uncomplicated healing events. The results of this review indicate that A-PRF™ shows, however, an antimicrobial activity against Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis and Candida Albicans. Furthermore, the spectrum and potency as an antimicrobial agent are far lower than those of an established surgical antimicrobial (specific antibiotic). Future investigations involving A-PRF ™ are therefore necessary to determine the full spectrum of it's in vitro antimicrobial activity, it's in vivo participation and the influence of the patient's characteristics on its biological activity. Furthermore, its clinical potential should be explored as a vehicle for the local administration of drugs within infected sites (Del Fabbro et al., 2016 [20]). Future studies should increase both patient variation and sample sizes for all future HPC-based studies.

References

1. Crisci A. Le membrane L-PRF utili in chirurgia. (2015) Journal of Plastic Dermatology 2:75-90

2. Crisci A, Placido F, Crisci M, Bosco A. (2015) A new instrument aid of plastic surgeon: membranes L-PRF (Pletelet-Rich-Fibrin). Update in Plastic Surgery 3:162-72

3. Crisci A, Serra E, Cardillo F, Crisci M. (2017) Selezione di un modello animale pertinente per 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, Crisci M. (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, Benincasa G, Crisci A. (2018) Evaluation of genotyping methods and costs for IL1 α polymorphisms in Platelet Rich-Plasma (PRP); viewpoint for therapy on the diabetic foot ulcers. Europ. Rev. Med. Pharmac. Sci. 22: 575-77

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 Research in Applied Chemistry 5: 3635-43

7. Lundquist R, Dziegiel MH, Agren MS. (2008) Bioactivity and stability of endogenous fibrogenic factors in platelet rich fibrin. Wound Repair Regen 16: 356

8. Lundquist R, et al. (2013) Characteristics of an autologous leukocyte and platelet-rich fibrin patch intended for the treatment of recalcitrant wounds. Wound Repair Regen 21: 66

9. Clipet F, et al. (2012) In vitro effects of Choukroun's platelet-rich fibrin conditioned medium on 3 different cell lines implicated in dental implantology. Implant Dent 21: 51

10. Ghanaati S, Booms P, Orlowska A, Kubesch A, Lorenz J, Rutkowski J, Landes C, et.al. (2014) Advanced Platelet-Rich Fibrin: A New Concept for Cell- Based Tissue Engineering by Means of Inflammatory Cells. Journal of Oral Implantology 40: 679-689

11. Choukroun J. (2014) Advanced-PRF and i-PRF: platelet concentrates or blood concentrates? Journal of periodontal medicine and clinical practice JPMCP 1: 1- 3

12. Miron RJ, Fujioka-Kobayashi M, Hernandez M, Kandalam U, Zhang Y, Ghanaati S, Choukroun J. (2017) Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry? Clin Oral Invest

13. Kobayashi E, Flückiger L, Fujioka-Kobayashi M, Sawada K, Sculean A, Schaller B, Miron RJ. (2016) Comparative release of growth factors from PRP, PRF, and advanced-PRF. Clin Oral Invest

14. Burnouf T, Chou, M-L, Wu U-W, Su C-Y, Lee L-W. (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: 138-46

15. Crisci A, De Crescenzo U, Crisci M, (2018) Platelet-Rich Concentrates (L-PRF, PRP) in Tissue Regeneration: Control of Apoptosis and Interactions with Regenerative Cells. J Clin Mol Med 3: 5-2

16. Bayer A, Lammel J, Rademacher F, Grob J, Siggelkow M, Lippross S, et.al. (2016) Platelet-released growth factors induce the antimicrobial peptide human beta-defensin-2 in primary keratinocytes. Experimental Dermatology 25: 460–465

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

18. 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. Journal A.M.D. 213: 197-203

19. Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J. (2016) Optimized Platelet-Rich Fibrin with the Low-Speed Concept: Growth Factor Release, Biocompatibility, and Cellular Response. J Periodontol. 88: 112-21

20. 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 27:276–85

21. Burnouf T, Chou, M-L, Wu U-W, Su C-Y, Lee L-W. (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: 138-46

22. Cieslik-Bielecka A, Dohan Ehrenfest DM, Lubkowska A, Bielecki T. (2012) Microbicidal properties of leukocyte- and platelet-rich plasma/fibrin (L-PRP/L-PRF): new perspectives. Journal of Biological Regulators & Homeostatic Agents 26: 43-52

23. Anitua E, Muruzabal F, Orive G. (2011) Antimicrobial properties of plasma rich in growth factors (PRGF-ENDOREST). Science against microbial pathogens; A. Méndez-Vilas (Ed) FORMATEX 414-21

24. Dohan Ehrenfest DM, 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 in Biotechnology 27

25. Bielecki TM, Gazdzik TS, Arendt J, Szczepanski T, Król W, Wielkoszynski T. (2007) Antibacterial effect of autologous platelet gel enriched with growth factors and other active substances: An in vitro study. J Bone Joint Surg 89-B: 417-20

26. Zalavras CG, Patzakis MJ, Holton P. (2004) Local antibiotic therapy in the treatment of open fractures and osteomyelitis. Clin Orthop 427: 86-93

27. Sause WE, Buckley PT, Strohl WR, Lynch AS, Torres VJ. (2016) Antibody-Based Biologics and Their Promise to Combat Staphylococcus aureus Infections. Trends in Pharmacological Sciences 37: 231-41

28. Jabra-Rizk MA, Kong EF, Tsui C, Nguyen MH, Clancy CJ, Fidel PL Jr, et.al. (2016) Candida Albicans pathogenesis: fitting within the host-microbe damage response framework. Infect Immun 84: 2724 –739

29. Marsh PD, Zaura E. Dental biofilm: ecological interactions in health and disease. (2017) J Clin Periodontol 18: S12–S22.

30. Tan Y-Q, Yeaman MR, Selsted ME. (2002) Antimicrobial Peptides from Human Platelets Infection and Immunity 70 12: 6524–33

31. El-Sharkawy H, Kantarci A, Deady J, Hasturk H, Liu H, Alshahat M, et.al. (2007). Platelet-rich plasma: Growth factors and pro- and anti-inflammatory properties. J Periodontol 78: 661–9

32. 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: 151–56

33. El Bagdadi K, Kubesch A, Yu X, Al Maawi S, Orlowska A, Dias A, Booms P, Dohle E, Sader R, Kirkpatrick CJ, Choukroun J, Ghanaati S. (2017) Reduction of relative centrifugal forces increases growth factor release within solid platelet-rich-fibrin (PRF)- based matrices: a proof of concept of LSCC (low speed centrifugation concept). Eur J Trauma Emerg Surg

34. Castro AB, Meschi N, Temmerman A, Pinto N, Lambrechts P, Teughels W, Quirynen M. (2017) Regenerative potential of leucocyte- and platelet-rich fibrin. Part B: sinus floor elevation, alveolar ridge preservation, and implant therapy. A systematic review. J Clin Periodontol 44: 225–34

35. Moraschini V, dos Santos Porto Barboza E. (2016) Use of Platelet-Rich Fibrin Membrane in the Treatment of Gingival Recession: A Systematic Review and Meta-Analysis. J Periodontol 87: 281-90

36. Del Corso M, Vervelle A, Simonpieri A, Jimbo R, Inchingolo F, Sammartino G, et.al. (2012) Current Knowledge and Perspectives for the Use of Platelet- Rich Plasma (PRP) and Platelet-Rich Fibrin (PRF) in Oral and Maxillofacial Surgery Part 1: Periodontal and Dentoalveolar Surgery. Current Pharmaceutical Biotechnology 13: 1207- 30

37. Crisci A., Rescigno C., Crisci M. (2019) La membrana L-PRF e suoi derivati utili nella chirurgia del wound care, Italian Journal of Wound Care 1:19-26