History of Medicine, 2022, 8(2): 638-651

DOI: 10.48047/HM.V8.I2.2022.61

EFFICACY OF LEUKOCYTE PLATELET RICH FIBRIN (L-PRF) IN THE HEALING OF MANDIBULAR THIRD MOLAR SOCKETS

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ABSTRACT

This study assessed the effects of leukocyte-platelet-rich fibrin (L–PRF) on mandibular 3rd molar sockets healing.

Methods

Fifty patients belonging to age group ranging from 20-40 years, with bilateral impacted mandibular third molars, were included in the study.

Results

This study showed that PRF significantly reduced the postoperative pain, especially in the first 1–3 days after tooth extraction. Soft tissue healing was significantly improved in the group of PRF compared to the spontaneous wound healing after 1 week. Dimensional bone loss was significantly lower in the PRF group compared to the spontaneous wound healing after 8–15 weeks but not after 6 months. Socket fill was in 85% of the studies significantly higher in the PRF group compared to the spontaneous wound healing.

Conclusions

Based on the analysed studies, PRF is most effective in the early healing period of 2–3 months after tooth extraction. A longer healing period may not provide any benefits. The currently available data do not allow any statement regarding the long-term implant success in sockets treated with PRF or its combination with biomaterials. Due to the heterogeneity of the evaluated data no meta-analysis was performed.

Keywords: PRF, Platelet-rich fibrin, Socket preservation, Ridge preservation, Socket healing, Pain management, soft tissue healing

INTRODUCTION

The use of blood and blood-derived products to seal wounds and improve healing in different clinical situations started with fibrin glues, which are mainly fibrinogen concentrates. Addition of platelets to fibrin glue not only improved their strength but also promoted neo angiogenesis and regeneration. These benefits are attributed to the release of a variety of peptide growth factors from the alpha-granules of platelets upon activation.[8]

Platelet concentrates (PC) were seen as a practical way to deliver growth factors and was strongly driven by commercial interests rather than research characterization.[16] In fact, PCs are difficult to characterize because unlike homogenous and defined pharmacological preparations, they are a potpourri of signalling molecules and blood cells (platelet and leukocytes) entrapped within a fibrin matrix.[11]

Many studies have revealed that platelet concentrates for surgical use can be used as efficient adjuvant for tissue repair. The growth factors (particularly platelet-derived growth factors (PDGF), transforming growth factors (TGF-β), and vascular endothelial growth factors (VEGF)) and the other molecules (fibrinogen, fibronectin, and vitronectin) contained in platelets (α-granules) give to these products the ability to modulate many phases of the healing process like the haemostasis and the neoangiogenesis.[7] The clinical results of these products are interesting but remain quite mixed and controversial in the literature, depending on the kind of preparation.[9]

Platelet concentrates are classified into 4 main families depending on their leukocyte and fibrin content: pure platelet-rich plasma (P-PRP), leukocyte- and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (PPRF), and leukocyte- and platelet-rich fibrin (L-PRF). Each family of products has different aspect, biological content, and potential application.[12] The PRPs were already tested in many oral surgery applications, with mixed results depending on the kind of preparations [13-16]. Numerous protocols have attempted to optimize the preparation of the autologous factors, using various performances standards and centrifugation parameters.[1-8]

The processes of blood coagulation (fibrinogen polymerization), platelet enrichment and activation occur simultaneously in the preparation of L-PRF. The coagulation cascade is triggered when whole blood contacts the walls of a dry glass tube and continues throughout the centrifugation process. This results in the formation of a mechanically-strong blood clot (L-PRF) that can be surgically handled and used. L-PRF has been shown to have dense fibrin structure and delayed growth factor release profile.[3-13]

MATERIALS AND METHODS

This study was conducted in the Department of oral and maxillofacial surgery, Rama Dental College-hospital and research centre, Kanpur

SOURCE OF DATA

Fifty patients belonging to age group ranging from 20-40 years, with bilateral impacted mandibular third molars, were included in the study. Informed consent was taken from the subjects prior to treatment.

STUDY DESIGN

This is a Split-mouth study where impacted lower third molars on either side will be divided in to two groups.

GROUP A – Those in which Leukocyte -Platelet Rich Fibrin (LPRF) will be placed into the extraction socket.

GROUP B – Those in which normal bone healing is allowed in the extraction socket. In each patient, Right side will belong to Case and the other will be Control for normal bone healing.

INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria:

- ❖ Patients with bilateral unerupted mandibular third molar requiring similar surgical methods for their removal.
- ❖ Patients free of significant systemic diseases.
- ❖ Patients between 20 – 40 years of age.

Exclusion Criteria:

1. Patient's not giving consent for treatment.
2. Patient's having local soft tissue infections and poor oral hygiene.
3. Patient's undergoing bisphosphonates therapy.
4. Patient's undergoing radiation therapy.
5. Patient's having psychiatric illness or pregnant

METHOD OF PREPARATION OF PLATELET RICH FIBRIN:

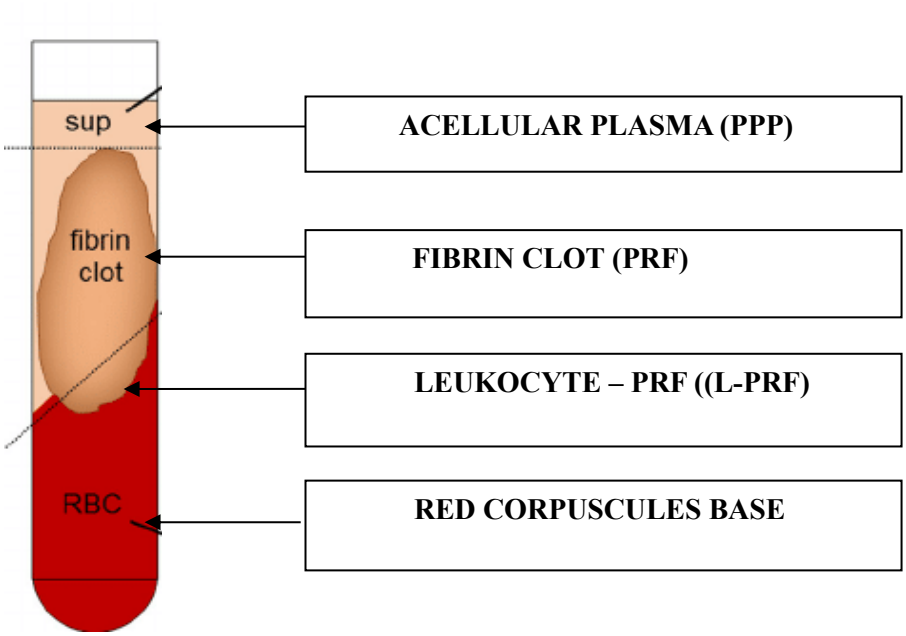
ARMAMENTARIUM:

- ❖ 10 ml syringe 24-gauge needle
- ❖ Test tube
- ❖ Centrifuge machine
- ❖ Surgical gloves and disinfectant swab.

L-PRF Preparation Protocol

The L-PRF will be prepared through a single centrifugation of blood according to the protocol of Dohan Ehrenfest et al. for a period of 12 minutes at 2700 rpm. Blood was taken in 9 mL tubes, 30 minutes before the surgery, immediately centrifuged, and used for the filing of the experimental sites. The L-PRF clot is then condensed and modeled on a sterile surgical plate before the application in the sockets.

L-PRF membranes with a consistent thickness of 1 mm. A piston and cylinder assembly (right site) can be used for the creation of L-PRF plugs, suitable for filling extraction sockets. L-PRF membranes after gentle compression, the red area of the membrane represents the face side, where most leucocytes, platelets and stem cells are concentrated.



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SURGICAL TECHNIQUE

- Written informed consent was obtained from all the patient.
- For preparation of PRF, the blood obtained transferred into the vial which did not contain Citrate phosphate dextrose (anticoagulant).
- Patient was asked to gargle with betadine mouthwash 10 minutes before starting the procedure.
- Surgical procedure was carried out after drapping and painting the surgical area with betadine solution.
- Local anesthetic agent administered was 2% lignocaine Hydrochloride with adrenaline in 1:100000 concentrations (Astra – Zinc a)
- Both right & left impacted molars were be treated by the same surgeon within a time interval of minimum 30 days.
- Incision was given with B.P. blade no.15 and envelope flap will be used.
- After reflection of the flap, bone removal was done with straight fissure bur (No. 702 S.S White). If required tooth sectioning was performed.
- Once tooth is removed smoothening of bone margins, irrigation of socket with normal saline was done. Hemostasis was achieved.
- L-PRF (in CASE) was placed into the socket with the help of tweezer.
- Sutures were given with 3-0 black silk (Lifeline) after surgery.
- Immediate post-operative x- ray (RVG) of the extraction socket was taken.
- Post extraction instructions were given and Patient were recalled at regular intervals of 1st, 2nd and 4 months post op to take RVG'S.
- All the patients were instructed to take the following medication:
  - Cap Amoxicillin 500mg thrice daily for 5 days
  - Tab Metronidazole 400 mg thrice daily for 5 days
  - Tab diclofenac sodium 50 mg thrice daily for 5 days.

- Tab Ranitidine 150mg twice daily was prescribed.

CLINICAL PARAMETERS:

Various preoperative, intraoperative and postoperative parameters was used to evaluate the study subjects. They are –

Preoperative assessment:

- By case history

Intraoperative assessment:

- Immediately after the procedure, details of the procedure were documented

Post operative assessment

- Soft tissue healing-
- Bone density

The bone density of the third molar socket was assessed using RVG.

The patient was called for a follow up on the first post op day, 3rd week, second month and fourth months post op. Regeneration of bone was measured using serial radiographs (RVG) at immediate post op, 3rd week, two and fourth months.

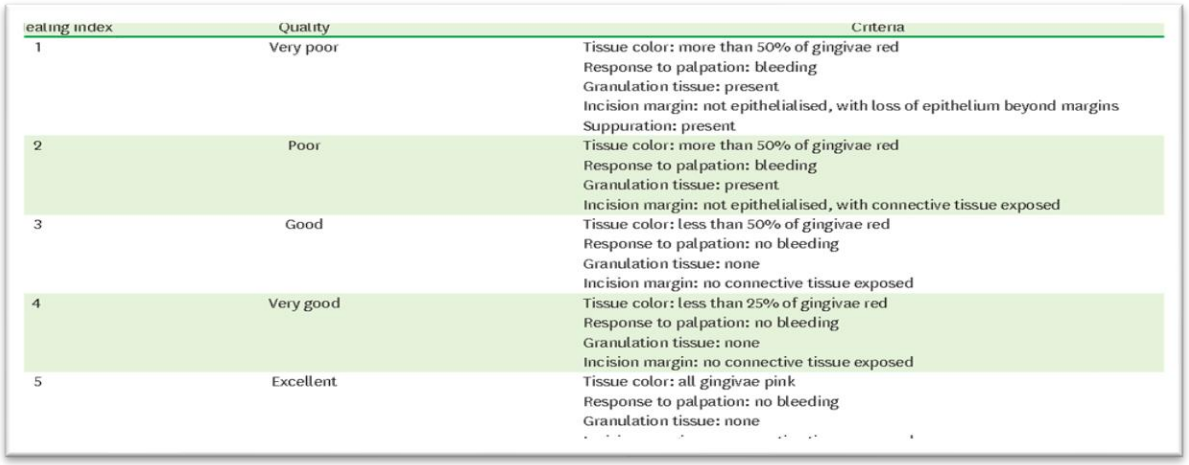
RADIO VISIO-GRAPHIC (RVG) ANALYSIS

After Placing the L-PRf Gel in the Socket region an Immediate RVG was taken. Patient was recalled at regular intervals of 3rd week, 2nd and 4th months post op to take RVG'S. The Radiolucency of the Socket region was measured using the Corel Draw Software. The radiographs were converted to a digital format by a scanner connected to a computer and through the transfer of RVG images to the Corel Draw software. They were then converted to grayscale tonalities of 256 using Corel Photo paint Software.

A person blinded of the L- PRF primary closure marked the size of the residual cavity using the software. The area marked was converted into a histogram which gave the number of pixels in the residual cavity. The number of pixels in the residual cavity was calculated on all RVGs.

This indicated the size of the defect. The number of pixels in the residual defect in the immediate post-operative radiograph was fixed at 100 %. The decreasing number of pixels in the surgical defect overtime gave us the relative bone filling in the area of the lesion. The percentage of bone filling was then calculated. The residual cavity defect and regenerated bone density in both the study group and control group were also calculated using Radio Visio- Graphs to rule out bias. Comparing the bone regeneration results of the participants on test side and Control side with 6 months follow up.

- Soft tissue healing will be assessed based on the criteria given by Landry et al.[2]



ealing index	Quality	Criteria
1	Very poor	Tissue color: more than 50% of gingivae red Response to palpation: bleeding Granulation tissue: present Incision margin: not epithelialised, with loss of epithelium beyond margins Suppuration: present
2	Poor	Tissue color: more than 50% of gingivae red Response to palpation: bleeding Granulation tissue: present Incision margin: not epithelialised, with connective tissue exposed
3	Good	Tissue color: less than 50% of gingivae red Response to palpation: no bleeding Granulation tissue: none Incision margin: no connective tissue exposed
4	Very good	Tissue color: less than 25% of gingivae red Response to palpation: no bleeding Granulation tissue: none Incision margin: no connective tissue exposed
5	Excellent	Tissue color: all gingivae pink Response to palpation: no bleeding Granulation tissue: none

Table 2.1 Comparison of mean soft tissue healing at different time intervals between two study groups using student paired‘t’test

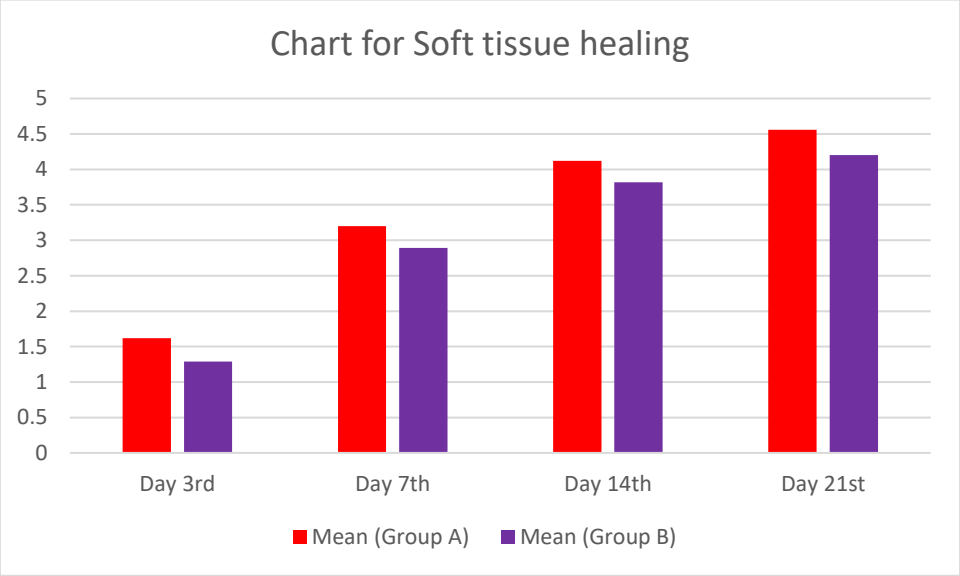
TIME	GROUP	N	Mean	SD	S.E.M	Mean diff.	Pearson Correlation	Significance level(Alpha)	T	DF	P
Day 3rd	A	50	1.62	1.00793	0.14254	0.33	-0.278	0.05	1.99	49	0.0410
	B	50	1.29	1.07305	0.15175						
Day 7th	A	50	3.2	0.67006	0.09476	0.31	0.362	0.05	2.21	49	0.0321
	B	50	2.89	0.43753	0.06188						
Day 14th	A	50	4.12	0.59385	0.08398	0.30	-0.056	0.05	2.53	49	0.0147
	B	50	3.82	0.56025	0.07923						
Day 21st	A	50	4.56	0.50143	0.07091	0.36	-0.114	0.05	3.17	49	0.0026
	B	50	4.2	0.57143	0.08081						

Table 3.1: Comparison of mean pixel value at different time intervals between two study groups using student paired’t’test.

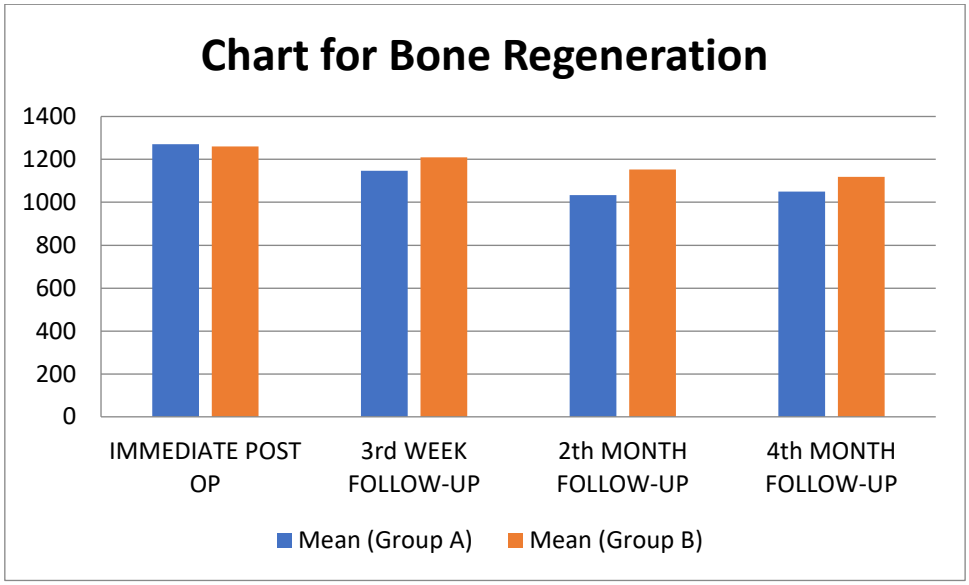
TIME	GROUP	N	Mean	SD	S.E.M	Mean diff.	Pearson Correlation	Significance level (Alpha)	T	DF	P
IMMEDIATE POST OP	A	50	1270.72	5.60072	0.79206	10.04	0.988	0.05	78.23	49	0.00
	B	50	1260.69	5.81281	0.82206						

3rd WEEK FOLLOW-UP	**A**	**50**	**1146.46**	**6.11837**	**0.86527**	**62.65**	**0.306**	**0.05**	**-24.67**	**49**	**0.00**
	B	**50**	**1209.12**	**18.8622**	**2.66752**						
2th MONTH FOLLOW-UP	**A**	**50**	**1034.21**	**6.13743**	**0.86796**	**118.53**	**0.346**	**0.05**	**-63.38**	**49**	**0.01**
	B	**50**	**1152.74**	**14.0278**	**1.98383**						
4th MONTH FOLLOW-UP	**A**	**50**	**1050.16**	**6.0986**	**0.86247**	**68.75**	**0.340**	**0.05**	**-73.49**	**49**	**0.00**
	B	**50**	**1118.91**	**13.9965**	**1.97941**						

Graph 1 : Comparison of mean value of soft tissue healing at different time intervals between two study groups.



Graph 2: Comparison of mean pixel value at different time intervals between two study groups.



Results of comparison of various parameters pain, soft tissue healing and bone density of LPRF site (GROUP A) and Normal healing sites (GROUP B) are described below.

Soft Tissue Healing

The soft tissue healing measurements were obtained on 3rd, 7th day and 14th, 21st day post-operative for both group A and group B in 50 patients (Table 2).

The mean value of soft tissue healing on 3rd, 7th day and 14th, 21st day post-operative for Group A and GROUP B were 1.62, 3.2, 4.12,4.56 and 1.29,2.89,3.82,4.2 respectively (Table 2.1). The healing was less on 3rdpost-operative day and gradually increased from 7th post- operative day to 21st post – operative day for both the groups. In both the groups, there was statistically significant reduction in swelling from Day 3 to Day 7.

The respective 't' value for 3rd, 7th day and 14th, 21st day were 1.99, 2.21, 3.17 and 2.53 respectively. The tables depict that there wasn't any major difference in mean value between both groups.

'P' value is 0.041 and below is significant.

Bone Density

The coral draw pixel measurements for bone formation were obtained on 3rd week, 2nd month and 4th month post – operative weeks for both Group A and Group B in 50 patients (Table 2).

Bone healing which was evaluated after on 3rd week, 2nd month and 4th month weeks postoperatively using the rvg and coral draw software. The scores were tabulated and later evaluated statistically. Study shows statistically significant in inter mediate time and also between both groups.

Group A had less pixel count state higher bone density than group B with mean diff. 63.57, 115.96, 140.02 respectively. The respective 't' value for 3rd week, 2nd month and 4th month were 78.23, -24.67, -63.38, -73.49 respectively (Table 2.1).

'P' value is 0.00 and below is significant.

DISCUSSION

Platelet concentrates are blood derivatives prepared from the patient's own blood in which the activated platelets become trapped within a fibrin matrix scaffold and release growth factors and cytokines involved in the key processes of tissue regeneration, including cell proliferation and differentiation, extracellular matrix synthesis, chemotaxis and angiogenesis.

These processes found to promote healing in both soft and hard tissues which led to successful therapeutic applications in medical and dental fields over the last two decades. Based on differences in centrifugation speeds and times, adding chemicals, and the selection of supernatants and precipitates; various preparations have been produced with different fibrin network structures, leukocyte content and growth factors and subsequent different physical and physiological properties and applications.

Autologous platelet concentrates are promising in the field of regenerative medicine because of the abundance of growth factors. However, these preparations often lacked a defined structure that makes surgical manipulation very difficult. Many times, the suspensions and gels are not retained effectively at the site of delivery, resulting in unpredictable outcomes.

The original concept behind the use of platelet concentrates was based on the fact that autologous concentrated platelets and growth factors could be collected in plasma solutions to mimic the terminal stage of the natural coagulation cascade, which is, the formation of fibrin clot and release of growth factors that could then be utilized in a surgical site as natural scaffold and to promote local healing and lost tissue regeneration .[6] This autologous extract of the circulating blood tissue is considered tissues themselves, and not pharmaceutical preparations thus eliminated concerns about immunogenic reactions and disease transmission.[5]

Platelet concentrates which was self-clotted and was developed by Choukroun J et al.[7] in France in 2006 and termed "Platelet-Rich Fibrin (PRF)" due to the strong fibrin gel polymerization of the preparation and was considered the (Second Generation).[15] In the last years authors started pointing out that these platelet concentrates were also associated with various forms of circulating cells particularly leukocytes that may be a key element in healing process.[35]

For this reason, the "Leukocyte-and Platelet-Rich Fibrin (L-PRF)" was often described as an (Optimized Blood Clot) and allowed to define new therapeutic principles.[9]

LEUKOCYTE - PLATELET RICH FIBRIN (L-PRF)

The L-PRF technique was first described by Choukroun in 2001.[8] It is an inexpensive technique using the patient's blood to create a leukocyte fibrin rich substrate that can be placed in areas of the surgical site in conjunction with supplemental tissue to stimulate bone and soft tissue formation.The use of L-PRF can provide the patient with enhanced healing, possibly fewer appointments, and a faster end result. When compared to other platelet concentrates, L-PRF has been reported to release higher levels of growth factors over a 10-day period.[7] In vitro and animal studies have shown that L-PRF improves soft tissue wound healing by promoting angiogenesis and cell proliferation . Additionally, clinical studies from different medical fields have reported a positive effect of L-PRF in soft tissue regeneration and angiogenesis. Aside from its beneficial action on soft tissues, the

application of L-PRF has also been associated with positive results in bone tissue repair and regeneration. When compared to a widely used porcine collagen membrane, L-PRF appeared to render superior results in terms of the proliferation of human osteoblasts and periosteal cells in vitro.[16]

The inclusion of leukocytes in this autologous blood derivatives have been addressed not only because of their immune and antibacterial properties, but also because of their contribution in the wound healing process through regulation of local factors, cell proliferation and differentiation. Moreover, leukocyte populations had a strong influence on the release of some growth factors, particularly TGFβ1. The fibrin content and architecture of the product were studied also which found to affect the physical properties and thus clinical applications and considerably influence the strength and the growth factor trapping/release potential of the product.

L-PRF represents a huge advance in the evolution of platelet concentrates in that it is essentially a firm fibrin membrane with entrapped platelets. These solid membranes possess excellent handling characteristics, and can be securely sutured at an anatomically desired location during open surgeries. L-PRF is a 3-D autogenous combination of Platelet Rich Fibrin derived from the patient's blood. A simplified chairside procedure results in the production of a thin, compressed layer of platelet rich fibrin that is strong, pliable and suitable for suturing. This natural fibrin network is rich in platelets, growth factors and cytokines that are derived from the blood platelets and leukocytes.

One of the important considerations in generating a good L-PRF membrane is the time delay between blood draw and centrifugation. The success of L-PRF technique entirely depends on the speed of blood collection and immediate transfer to the centrifuge, usually within a minute. It is impossible to generate a well-structured L-PRF clot (with its specific cell content, matrix architecture and growth factor release profile), if blood harvesting is prolonged and not homogenous; a small incoherent, friable mass of fibrin with unknown content is formed instead.[38]

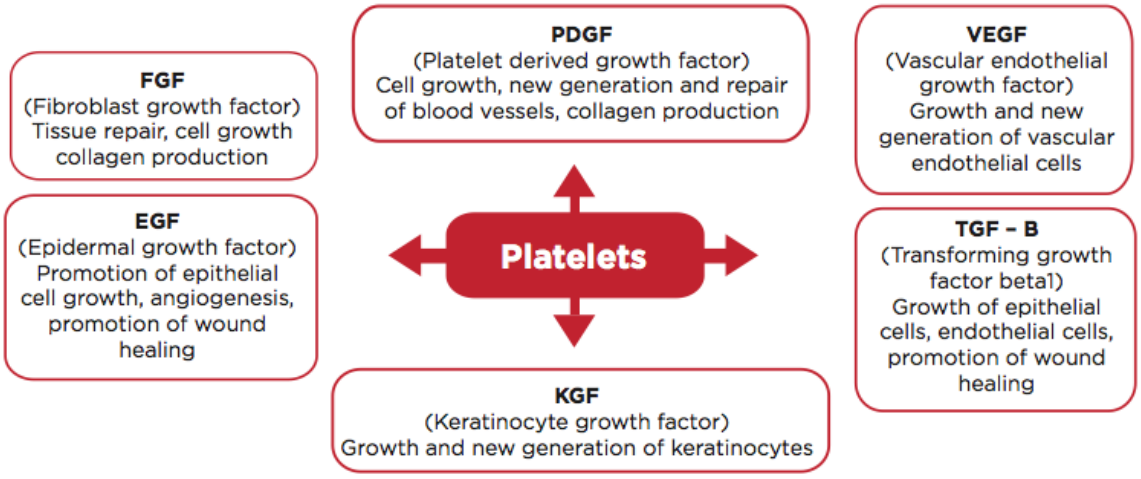
The fibrin architecture of L-PRF, constituted by connected trimolecular junctions (or equatorial), due to a slow polymerization of the platelet concentrate and due to the absence of heterologous thrombin, induces a flexible fibrin network, able to promote the gradual release of growth factors and leukocytes migration.[22] The fibrin membrane promotes the mechanical protection of the surgical site and, biologically, it interacts with the physiological mechanisms of healing favouring the angiogenesis. The fibrin induces the expression of αv-β3 integrin by endothelial cells, allowing the links with structural proteins, such as fibronectin and vitronectin, supporting the process of formation of capillaries.

In relation to the previous properties, the fibrin also allows the association of some growth factors, such as FGFb (fibroblast growth factor basic) and PDGF (platelet-derived growth factor) involved in the angiogenic process and useful as chemotactic factors, favouring diapedesis of white blood cells. The immunological properties of the L-PRF, resulting from its content in leukocytes, could be useful to prevent the surgical site infections, such as post extraction alveolitis, with a consequent reduction of the inflammation symptoms. The presence of leukocytes is a very important parameter to stimulate healing and wound control.[22]

The presence of these proteins has been reported to produce rapid healing, especially during the critical first seven days after placement. This network promotes more efficient cell migration and proliferation without

chemical or bovine thrombin additives. Leukocyte-Platelet Rich Fibrin (L-PRF) is one of the four main families of platelet concentrates for surgical use. L-PRF is used to improve healing and promote tissue regeneration. Blood sample is taken without anti-coagulant and immediately centrifuged to obtain L-PRF clot that can be used directly or compressed into a membrane without damaging the cells and growth factors content.

Because L-PRF is an autologous biologic modifier obtained from the patient's blood, it provides a condensed network of fibrin that is saturated with cytokines, growth factors and platelets. In short, it speeds up the initial healing process of tissue repair and is capable of generating soft tissue and bone; L-PRF can be used alone or in conjunction with a bone substitute. There are many circumstances in which tissue growth needs to be supported for the success of a procedure.



The L-PRF clot or membrane contains most of the platelets and leukocytes present in the initial blood harvest plus the platelet growth factors and stem cells that are also trapped within the fibrin network. With this architecture, L-PRF is the source of a strong and slow release of growth factors such as TGF-b1, VEGF, IGF-1, FGF, EGF, PDGF-AB, IL-1ß for more than three weeks in vitro. Through the release of these growth factors trapped within the fibrin gel or through the production of new molecules by the leukocytes, the L-PRF membranes have strong effects on the stimulation of the proliferation of most cell types (fibroblasts, keratinocytes, pre-adipocytes, osteoblasts, bone mesenchymal stem cells) and on the differentiation of the bone cells.

When compared with a coronally advanced flap alone, a coronally advanced flap in combination with L-PRF was associated with superior root coverage outcomes in the treatment of Miller class I and II gingival recession. L-PRF has also been utilized widely in oral implantology procedures in an effort to enhance and accelerate tissue healing – as a clot, mixed with a bone graft, or as a membrane.[7] In fact, one of the earliest applications of L-PRF in dentistry was in this field. However, variable results have been reported so far in the dental implant literature regarding its benefits. While some studies have shown that the addition of L-PRF in maxillary sinus augmentation and ridge preservation procedures accelerates new bone formation and reduces alveolar bone resorption, others have failed to report any gains in similar applications.

The clinical, immune histochemistry and histological findings (SEM, Confocal Laser and Optical Microscopy) on animals and human's studies over the last 14 years confirm the potential of L-PRF as a biological

scaffold for hard and soft tissue regeneration in acute or chronic wounds. What we thought impossible yesterday could be routine tomorrow, "Natural Guided Regeneration with L-PRF".[33]

Advantages of LPRF

- **Safety:** L-PRF is a by-product of the patient's own blood, therefore, disease transmission is not an issue.
- **Convenience:** L-PRF can be generated in the doctor's office while the patient is undergoing an outpatient surgical procedure, such as placement of dental implants.
- **Faster healing:** The supersaturation of the wound with PRP, and thus growth factors, produces an increase of tissue synthesis and thus faster tissue regeneration.
- **Ease of use:** L-PRF is easy to handle and actually improves the ease of application of bone substitute materials and bone grafting products by making them more gel-like

This study was designed to test the efficacy of L-PRF in fostering socket healing after tooth extractions. The 50 split-mouth case control extractions that constituted our study were statistically enough to prove the ability of L-PRF to improve the early healing phases (haemostasis and epithelial closure), reducing the inflammatory process and check the bone healing. The study compares the clinical effectiveness of LPRF in Group A patients and normal bone healing allow in Group B. Different parameters like pain, soft tissue healing and bone density were considered.

A study was done by Olufemi K et al where pain was evaluated using a 10- point VAS with a score of "0" equal to no pain and "10" equal to "very severe pain". They found significant decrease in pain when 2nd generation platelet aggregation was placed in one socket and other was kept empty. Same scale was used to assess pain in the present study. In both the groups, there was statistically significant reduction in pain from Day 3rd to Day 21st as per. Student t test.

The study of soft tissue healing was measured using the method described by laundry et al reported reduced soft tissue healing after the use of platelet rich concentrates in 3rd molar extraction socket.[39] The second parameter of our study was soft tissue healing which when measured at specific time intervals, the mean scores varied from Day 3rd to Day 21st intervals in Group A post-operatively. Similarly, the mean scores in Group B varied between 1.62, 3.2, 4.12,4.56 and 1.29,2.89,3.82,4.2 from Day 1 to Day 7 intervals post-operatively.

The last parameter of the present study was the bone healing which was evaluated after 7, 12 weeks and 24 weeks postoperatively using the rvg and coral draw software. The scores were tabulated and later evaluated statistically. Study shows statistically significant in inter mediate time and also between both groups. Group A had less pixel count state higher bone density than group B with mean diff. 10.04, 62.65, 118.53 and 68.75 respectively. This implies that Group A showed better outcomes than Group B in bone healing property at different evaluation periods.

Our study proved that there was statistically significant improvement in the efficacy of LPRF when compared to normal bone healing alone. Further, group A was better than group B in term of soft tissue healing, pain and periodontal health but result wasn't statistically significant.

Based on these findings, it is clear that L-PRF is a novel biomaterial with unique attributes: predictable preparation from autologous blood, simplicity of protocol, defined architecture, impressive mechanical properties and abundance of growth factors from activated platelets.[6] The blood is allowed to clot under physiological conditions with no exposure to anti-coagulants, exogenous thrombin and calcium chloride. All of these characteristics make L-PRF promising biomaterial for applications in regenerative medicine.

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