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Role of Platelet Rich Fibrin in Periodontics

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Abstract*: platelet concentrates are known to be rich in growth factors with added antimicrobial efficacy due to incorporation of leukocytes. Growth factors secreted from platelets promote wound healing and tissue formation. They have found varied applications in the field of dentistry for the past decades. In Periodontal regeneration, 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 that 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 healing and tissue regeneration. Among the platelet concentrates PRP (platelet Rich Plasma) is the first generation and PRF (Platelet Rich Fibrin) is the second generation. Nowadays PRF can be regarded as the least expensive and streamlined way to produce platelet concentrate. The aim of present review of literature is to discuss the role of PRF in periodontics in detail.*

Keywords : PRF, Platelet Concentrates, Periodontal regeneration

I. INTRODUCTION

Platelets play a crucial role in hemostasis and wound healing, platelet growth factors are well known source of healing cytokines.Numerous techniques of autologous platelet concentrates have been developed and since 15 years, their use has dramatically increased in many surgical fields, including Periodontics.The concept was developed with the aim of utilizing human blood proteins as a source of growth factors capable of supporting angiogenesis and tissue ingrowth based on the notion that blood supply is a prerequisite for tissue regeneration.

PLATELETS

Platelets also called as thrombocytes are the components of blood whose primary function is coagulation .The normal platelet count in healthy people ranges between 200000-500000/µL. They are non nucleated cells which are derived from megakaryocytes by the process of fragmentation of cytoplasm. It has a half-life of 7- 10 days. Platelets have three reservoir organelles namely Lysosomes, α granules, and dense granules. Alpha granules are the most abundant type and also the largest compartment for protein storage. They also contain clotting factors, growth factors like Platelet derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) and other proteins. Dense granules contain ATP, ADP, Serotonin, and Calcium[1].

Various techniques of autologous platelet concentrates have been developed and applied in periodontics. The first generation incorporates the platelet-rich plasma while the second generation involves the platelet-rich fibrin

CONCEPT BEHIND THE USE OF PLATELET CONCENTRATES

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 that 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 healing and tissue regeneration[2]

HISTORY OF EVOLUTION OF PLATELET CONCENTRATES

1954-Kingsley first used the term Platelet rich plasma [PRP] to earmark thrombocyte concentrate during experiments related to blood coagulation.

1970s-Research works of ***Matras***about the "Fibrin glues"to improve skin wound healing in a rat model based on the concept that fibrin matrix is the final result of the reaction chains of the coagulation and the first matrix of healing[1].

1975-1978-Numerous research works suggested an enhanced concept for using blood extracts and designated them as "platelet-fibrinogen-thrombin mixtures"[3].

1979-Fischer H. introduced "gelatin platelet - gel foam"; role of the platelets was advocated to serve only to reinforce the fibrin matrix architecture[4].

1986-Knighton et al first demonstrated that Platelets successfully promote healing and they termed it as "platelet-derived wound healing factors (PDWHF)", which was successfully tested for the management of skin ulcers.

1988, 1990-Kingsley et al and ***Knighton*** et al used a slightly different term "platelet-derived wound healing formula (PDWHF)".

1997-Whitman et al named their product platelet rich plasma (PRP) during preparation but when the end product had a consistency of a fibrin gel therefore they labeled it as "platelet gel"[5].

1998-The craze for "growth factors" and the use of the term "Platelet-Rich Plasma" (PRP) started with the article of ***Marx et al.*** in a study about the effect of a platelet-rich preparation during maxillofacial bone reconstruction. The platelet suspension was then activated into a gel using bovine thrombin[6].

2001-Choukroun et al developed another form of platelet concentrate in France which was labeled as Platelet rich fibrin (PRF), based on the strong fibrin gel polymerization found in this preparation.It was stamped as a "second-generation" platelet concentrate because it was different from other PRPs. This proved an important milestone in the evolution of terminology[6].

2006-Sacco[7] introduced a new concept of CGF (concentrated growth factors). The fibrin rich blocks that were obtained were much larger, richer and denser.

2008-Everts et al[8] focused on the leukocyte component of the platelet concentrate and introduced two forms - non-activated and activated forms.The inactivated/non-activated product was called "platelet-leukocyte rich plasma (P-LRP) and activated gel was labeled platelet-leukocyte-gel" (PLG).

2009-The first classification about platelet concentrate was proposed by ***DohanEhrenfest***et al[9]based on the cellular content (primarily leukocytes) and the fibrin architecture:

1. Pure platelet-rich plasma (P-PRP)
2. Leukocyte- and platelet-rich plasma (L-PRP)
3. Pure platelet-rich fibrin (P-PRF)
4. Leukocyte- and platelet-rich fibrin (L-PRF)

2010- Sohn[10] introduced → Concept of sticky bone (autologous fibrin glue mixed with bone graft)

2012-Mishra et al[11]proposed another classification which was limited to PRP and applicable to sports medicine only.

Type 1: L-PRP solution
Type 2: L-PRP gel
Type 3: P-PRP solution
Type 4: P-PRP gel.

DeLong et al[12] introduced another classification system called PAW (Platelets quantity, Activation mode, White cells presence).

2014 - Choukroun[13] introduced an advanced PRF called A- PRF (claimed to contain more monocytes).
Tunali et al[14] introduced a new product called T-PRF (Titanium-prepared PRF).

2015-Mourao et al[15] gave detailed technical note on preparation of i-PRF.

2016-Kobayashi et al studied to compare growth factor release over time from PRP, PRF and A-PRF using ELISA.The highest reported growth factor released from platelet concentrates was PDGF-AA followed by PDGF-BB, TGFβ1, VEGF, and PDGF-AB.

Platelet-Rich Plasma (PRP) – First generation plasma concentrates in 1998 Marx et al introduced platelet rich plasma for the first time. It is obtained from autologous blood[16]. It is clinically used to deliver growth factors in high concentrations to the site bone defect or a region Along with bone marrow grafts PRP was used in mandibular reconstruction. According to the data's Max suggested that the rate and degree of bone formation is found to be accelerated by the addition of PRP. PRP was developed to combine the fibrins sealant properties with growth factor effects of platelets, thus providing an ideal growth factor delivery system at the site of injury. These Growth factors exhibit chemotactic and mitogenic properties that promote and modulate cellular

functions involved in tissue healing, regeneration, and cell proliferation[16]. Bone morphogenic proteins, a subtype of TGF, has been shown to induce formation of a new bone at the implant site with bone substitute particle .PRP acts through stimulation of normal healing. A PRP blood clot consist of 4% RBCs, 95%Platelets, and 1% WBCs. It is used in sinus lift procedures in which PRP accelerates the healing and reduces the healing time with stable bone gain. It is also used in ridge augmentation, Socket preservation to maintain the alveolar bone height. Intrabony defects or osseous defects have shown bone fill with the use of PRP[17,18].
The main limitation of PRP is that lack of uniformity in preparation protocol as different platelet concentrations have different storage time. Release of growth factors for a shorter period of time. Antibodies to bovine factor Va may cross react with human factor Va and may produce coagulopathies and rare bleeding episodes[16].

Platelet Rich Fibrin – Second Generation Platelet Concentrates
It is a second generation platelet derivative. In 2001 Choukroun et al developed PRF.it is an autologous leukocyte and platelet rich – fibrin biomaterial with a specific composition and three dimensional architecture[19].PRF is classified as second generation platelet concentrate as it is prepared as natural concentrates without the addition of any anticoagulants to eliminates the risk associated with the use of bovine thrombin .PRF affects cellular activities at genetic and cellular levels. PRF Membrane consist of a fibrin three dimensional polymerized matrix in a specific structure , with the incorporation of almost all the platelets and more than half of leukocytes along with growth factors and circulating stem cells[20]

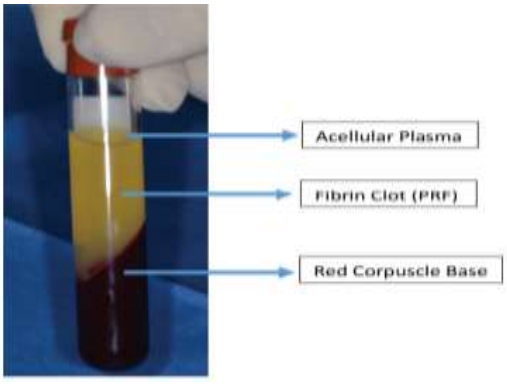
- PRF is classified according to its leukocyte content as either L-PRF or P-PRF.
- Pure platelet-rich fibrin (P-PRF) without leukocytes and with a high-density fibrin network
- Leukocyte- and platelet-rich fibrin (L-PRF) contains up to 90% of the platelets and at least 75% of the leukocytes present in the patient's blood.

Method of preparation
Preparation of PRF follows the protocol developed by Choukroun et al[20]. The protocol tries to accumulate platelets and the released cytokines in a fibrin clot. PRF protocol requires only centrifuged blood without any addition of anti-coagulant and bovine thrombin[21]. It is manufactured just prior to itsapplication. The preparation requires i. table centrifuge ii.10 mL dry glass test tube without anticoagulant iii.blood collection armamentarium
The steps described as follows

1. Blood specimen is drawn from the patient (10 mL) in dry test tubes
2. The blood specimen is placed in the centrifuge immediately at 3000 rpm for 10 minutes
3. Following this the blood sample settles into various layers

The absence of any anticoagulant grants the activation of platelets to set off a coagulation cascade. Due to the absence of the anticoagulant, the blood coagulates immediately upon contact with the glass tube. Initially, fibrinogen occupies the upper part of the tube, only till the circulating thrombin transforms it into a fibrin network[22].
The three layers formed are

- RBCs – lower fraction
- Fibrin clot – middle fraction
- Straw colored acellular plasma

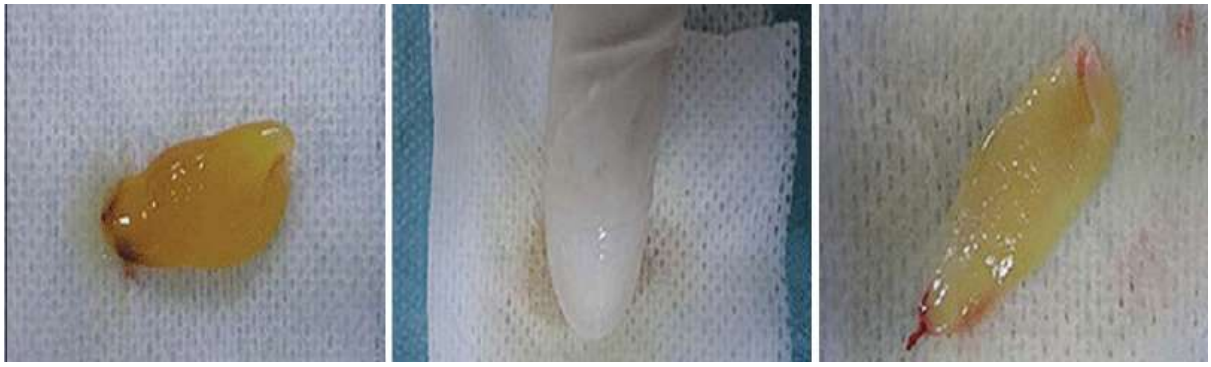


The upper portion of the test tube containing the acellular plasma is removed. The middle portion containing the fibrin clot is then removed and is scrapped off from the lower part containing the red blood cells.

The natural and progressive polymerization results in a fibrin clot formation with substantial embedding of platelets and leukocyte growth factors into the fibrin matrix

PRF Membrane

The clot can be squeezed between two gauge pieces to obtain an inexpensive autologous fibrin membrane. The serum exudate expressed from the clot is rich in proteins such as vitronectin and fibronectin[23]. This exudate may be used to hydrate graft materials, rinse the surgical site, and store autologous graft. The PRF Box (Process Ltd., Nice, France) is commercially available to prepare the PRF membrane. The PRF clot is placed on the grid in the PRF box and covered with compressor lid which squeezes out the fluid from the clot. The membranes formed using this method had constant thickness which remain hydrated for several hours and have recovered the serum exudate expressed from the fibrin clots.



L-PRF provides a scaffold consisting of fibrin that promotes cellular migration, a fundamental aspect in the process of regeneration. L-PRF membranes remain solid and intact in vitro and continuously release large quantities of growth factors for 7 to 14 days.

Contents of L-PRF Membranes

- Platelets
- Leukocytes
- Growth Factors
- Fibrin
- Stem Cells

Platelets in L-PRF

After centrifugation, at least 90% of the platelets derived from the blood sample are present in the fibrin clot. The platelets are mainly present in the lower portion of the clot, at the border between the RBCs and the clot itself. As a result, the lower portion of the clot, also called the *face*, is considered to be the most biologically active.

Leukocytes in L-PRF

Leukocytes are the basic cells responsible for the wound healing process and the first cells to start neoangiogenesis. Have potential antibacterial characteristics but can also regulate cell proliferation and cell differentiation. Monocytes and macrophages modulate the acute inflammatory response, produce growth factors such as bone morphogenetic protein 2 (BMP-2) and PDGF-BB, and induce osteogenesis in mesenchymal stem cells.Macrophages secrete collagenase, which promotes the cleaning of the wound.They are a source of growth factors such as TGF, which stimulates the keratinocytes, and PDGF, which plays an important role in angiogenesis. Granulocytes and macrophages promote the production of inflammatory mediators such as leukotriene B4 and platelet-activating factor, which stimulate the expansion and increased permeability of blood vessels as well as the production of inflammatory cytokines and protolithic enzymes.

Growth Factors in L-PRF

The alpha-granules in platelets contain PDGF, insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), VEGF, and TGF-β, which initiate wound healing by attracting and activating macrophages, fibroblasts, and endothelial cells.PRF preparations seems to have a sustained release of growth factors in a period between 1 and 4 weeks Kobayashi et al 2016

Fibrin in L-PRF

Fibrin is an insoluble clotting protein that plays a major role in platelet aggregation during hemostasis and wound healing.The fibrin wires tend to polymerize and form a biochemical structure with trimolecular or equilateral junctions, providing a fine and flexible fibrin network that favors the entrapment of cytokines and cell migration. This matrix is also able to capture glycosaminoglycans (originating from the blood platelets) which have a high affinity for circulating peptides (e.g., cytokines) and a large capacity to support cell migration and healing processes.

Role of fibrin matrix of PRF

It act as Natural guide for angiogenesis
It constitutes a support for immunity
Fibrin matrix guides the coverage of injured tissues, affecting the metabolism of epithelial cells and fibroblasts

Stem Cells in L-PRF

Dohan et al in 2010 showed a significant stimulation of human bone mesenchymal stem cells when in contact with L-PRF. This effect was dose-dependent during the first weeks in normal conditions and during the whole experiment in differentiation conditions.The combination of oral BMSC and PRF might offer many potential clinical and biotechnological applications, and deserves new studies.

A-PRF (Advanced PRF)

Low-speed concept with modifications to centrifugation speed and time favor an increase in growth factor release from PRF clots developed by ***Kobayashi et al 2017***

Protocol

- A-PRF → 1300rpm for 14 min
- A-PRF+ → 1300rpm for 8 min

Both A-PRF and A-PRF+ demonstrated significantly higher levels of human fibroblast migration and proliferation compared with L-PRF.

I-PRF (Injectable PRF)

Protocol

- 700 rpm for 3 min *(Miron et al 2017)*
- The 1 mL upper plasma layer was then collected using 21 gauge needle and designated as i-PRF.

The objective of this technique is to present a platelet-rich fibrin for use in liquid (injectable) form.Standard PRP and i-PRF were compared for growth factor release, fibroblast biocompatibility, migration and proliferation

by ***Miron et al (2017)***. **i-PRF** showed significant higher levels of total long term release of growth factors. Finding from the study demonstrate that a potent formulation of liquid platelet concentrates could be obtained without the use of anticoagulants.

T-PRF (Titanium-prepared PRF)

Tunali M et al 2014 developed this novel platelet-rich product.T-PRF is based on the hypothesis that titanium may be more effective at activating platelets than the silica activators used with glass tubes in Choukroun's platelet-rich fibrin (PRF).it is almost similar to that of L-PRF except that titanium tubes, are used instead of the glass evacuated collection tubes. Titanium tubes are produced from Grade **IV** titanium.The blood collected, is immediately centrifuged at **2,800 rpm for 12 minutes**

CLINICAL APPLICATION OF PRF

(1) PRF and PRF membrane have been used in combination with bone grafts to hasten the healing in lateral sinus floor elevation procedures[24].
(2) Protection and stabilization of graft materials during ridge augmentation procedures[25]
(3) Socket preservation after tooth extraction or avulsion.
(4) PRF membrane has been used for root coverage with single and multiple teeth recession[26].
(5) Regenerative procedures in treatment of 3-walled osseous defect.
(6) In the treatment of combined periodontic endodontic lesion.
(7) Treatment of furcation defect[27].
(8) PRF enhances palatal wound healing after free gingival graft[28].
(9) Filling of cystic cavity
(10)Better osseo integration capability

Advantages	**Disadvantages**
Anti coagulants are not used	Only a small quantity of PRF is obtained because of autologous blood
Ease of preparation	
Slow natural polymerization , 3D fibrin network forming a matrix aiding in cytokine retention for extended periods	Short handling time – PRF membrane should be usedimmediately after the preparation as it will shrink resulting in dehydration altering the structural integrity of PRF and leukocyte viability will be adversely affect the biological properties
Simple and cost effective	The fibrin matrix contains the circulating immune cells and highly antigenic plasmatic molecules that is why PRF is totally specific to donor
Formulation of a PRF membrane that possesses elasticity and flexibility	PRF when stored in refrigerator can result in risk of bacterial contamination
Able to support cytokines entrapment and cellular migration	

II. CONCLUSION

The application of autologous platelet concentrates are novel approach for enhanced tissue healing and regeneration. Being completely naturalthe chance of immunogenic reactions and disease transmission are less. However the knowledge on this topic is still in preliminary stage, the role of these in regenerative procedures should be evaluated on the basis of more studies.

REFERENCES

[1]. Agrawal AA. Evolution, current status and advances in application of platelet concentrate in periodontics and implantology. World J Clin Cases. 2017;5(5):159.
[2]. Goel A, Jack Windsor L, Blanchard S. Platelet-Rich Fibrin: Utilization in the Treatment of Periodontitis. In: Manakil J, editor. Periodontology and Dental Implantology [Internet]. IntechOpen; 2019 [cited 2022 Dec 9]. Available from: https://www.intechopen.com/books/periodontology-and-dental-implantology/platelet-rich-fibrin-utilization-in-the-treatment-of-periodontitis
[3]. Rosenthal AR, Egbert PR, Harbury C, Hopkins JL, Rubenstein E. Use of platelet-fibrinogen-thrombin mixture to seal experimental penetrating corneal wounds. Albrecht Von Graefes Arch F□r Klin Exp Ophthalmol. 1978;207(2):111–5.
[4]. Sixou JL, Magaud C, Jolivet-Gougeon A, Cormier M, Bonnaure-Mallet M. Evaluationof the Mandibular Third Molar Pericoronitis Flora and ItsSusceptibility to Different Antibiotics PrescribedinFrance. J Clin Microbiol. 2003 Dec;41(12):5794–7.
[5]. Whitman H, Berry L, Green M. P/a telet Gel: An Autologous Alternative to Fibrin Glue With Applications in Oral and Maxillofacial Surgery. :6.
[6]. Halawani SM, Hamdi BN, Abdullah M, Almotawa ZY, Al-Harbi SA. The role of platelet-rich fibrin (PRF) in periodontology.
[7]. Sacco L. Lecture, International academy of implant prosthesis and osteoconnection. Lecture 2006; 12: 4.
[8]. Everts PA, van Zundert A, Schönberger JP, Devilee RJ, Knape JT. What do we use: platelet-rich plasma or platelet-leukocyte gel? J Biomed Mater Res A 2008; 85:

[9]. Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier JB. Three_dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. J Periodontol 2010; 81: 546-555.
[10]. Sohn DS. Lecture titled with sinus and ridge augmentation with CGF and AFG, Symposium on CGF and AFG. Tokyo, June 6, 2010.
[11]. Mishra A, Harmon K, Woodall J, Vieira A. Sports medicine applications of platelet rich plasma. Curr Pharm Biotechnol 2012; 13: 1185-1195.
[12]. DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: the PAW classification system. Arthroscopy 2012; 28: 998-1009.
[13]. Choukroun J. Advanced PRF and i-PRF: Platelet concentrate or blood concentrate? J Periodontal Med Clin Pract 2014; 1: 3.
[14]. Tunalı M, Özdemir H, Küçükodacı Z, Akman S, Fıratlı E. In vivo evaluation of titanium-prepared platelet-rich fibrin (T-PRF): a new platelet concentrate. Br J Oral Maxillofac Surg 2013; 51: 438-443.
[15]. Mourão CF, Valiense H, Melo ER, Mourão NB, Maia MD. Obtention of injectable platelets rich-fibrin (i-PRF) and its polymerization with bone graft: technical note. Rev Col Bras Cir 2015; 42: 421-423.
[16]. Peterson LJ. ORAL AND MAXILLOFACIAL SURGERY. ORAL Surg ORAL Med ORAL Pathol. 1998;85(6).
[17]. Froum SJ, Wallace SS, Tarnow DP, Cho SC. Effect of Platelet-Rich Plasma on Bone Growth and Osseointegration in Human Maxillary Sinus Grafts: Three Bilateral Case Reports. Restorative Dent. 2002;22(1).
[18]. Arora NS, Ramanayake T, Ren YF, Romanos GE. Platelet-Rich Plasma: A Literature Review. Implant Dent. 2009 Aug;18(4):303–10.
[19]. Toler M, Toscano N, Holtzclaw D, Corso MD, Ehrenfest DD. Introducing Choukroun's Platelet Rich Fibrin (PRF) to the Reconstructive Surgery Milieu.
[20]. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontology. 2006 Mar;101(3):e37–44.
[21]. Appel TR, Pötzsch B, Müller J, Von Lindern JJ, Bergé SJ, Reich RH. Comparison of three different preparations of platelet concentrates for growth factor enrichment: Platelet concentrates for growth factor enrichment. Clin Oral Implants Res. 2002 Oct;13(5):522–8.
[22]. Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier JB. Three-Dimensional Architecture and Cell Composition of a Choukroun's Platelet-Rich Fibrin Clot and Membrane. J Periodontol. 2010 Apr;81(4):546–55.
[23]. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part II: Platelet-related biologic features. 2006;101(3).
[24]. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part V: Histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontology. 2006 Mar;101(3):299–303.
[25]. Kassolis JD, Rosen PS, Reynolds MA. Alveolar Ridge and Sinus Augmentation Utilizing Platelet-Rich Plasma in Combination With Freeze-Dried Bone Allograft: Case Series. J Periodontol. 2000 Oct;71(10):1654–61.
[26]. Kumar A, Surya C, Fernandes B. Platelet rich fibrin: A promising approach for root coverage. J Interdiscip Dent. 2011;1(2):115.
[27]. Sharma A, Pradeep AR. Autologous Platelet-Rich Fibrin in the Treatment of Mandibular Degree II Furcation Defects: A Randomized Clinical Trial. J Periodontol. 2011 Oct;82(10):1396–403.
[28]. Mehta D, Jain V, Triveni M, Tarun Kumar A. Role of platelet-rich-fibrin in enhancing palatal wound healing after free graft. Contemp Clin Dent. 2012;3(6):240.