AIMS Bioengineering

http://www.aimspress.com/journal/Bioengineering

AIMS Bioengineering, 10(1): 53–61.
DOI: 10.3934/bioeng.2023005
Received: 22 December 2022
Revised: 20 January 2023
Accepted: 06 February 2023
Published: 14 February 2023

Review

An overview on platelet concentrates in tissue regeneration in periodontology

Kapil Bhangdiya[1,*], Anand Wankhede[1], Priyanka Paul Madhu[2] and Amit Reche[2]

[1] Department Of Periodontology, Sharad Pawar Dental College and Hospital, Datta Meghe Institute Of Higher Education and Research (Deemed To Be University) Sawangi (Meghe) Wardha, Maharashtra 442001, India
[2] Department Of Public Health Dentistry, Sharad Pawar Dental College and Hospital, Datta Meghe Institute Of Higher Education and Research (Deemed To Be University) Sawangi (Meghe) Wardha, Maharashtra 442001, India

* **Correspondence:** Email: kapilbhangdiyas@gmail.com.

Abstract: Recent research on the use of platelet concentration in medicinal dentistry has enhanced the potential for tissue regeneration. The ability of platelets to enhance tissue regeneration by using different forms of platelet concentration (blood component) such as platelet-rich plasma (PRP), platelet-rich fibrin (PRF), standard platelet-rich fibrin, advanced platelet-rich fibrin, etc. PRP became widely used once its potential for tissue regeneration was discovered; however, the use of anticoagulants restricted the easy use of PRP as compared to PRF; however, after the discovery of PRF because of the easy formulation, it became widely used in medicinal dentistry. The purpose of this review is to elaborate on the importance of platelet concentration in periodontal regeneration.

Keywords: platelets; platelet-rich fibrin; regeneration; concentrate; bone

1. Introduction

The periodontium regenerates continuously through physiological processes. Wear and tear repair is the process of continuously forming new cells and tissues to replace those that mature and die under normal circumstances. Periodontal disorders are chronic inflammatory conditions caused by periodontal microorganisms and the toxins released by them, which cause the destruction of the periodontium.

The gingival epithelium is replaced by epithelium tissue, while the periodontal ligament and supporting connective tissue are comprised of connective tissue, which is the precursor to both bone and cementum, and it replaces them. Undifferentiated mesenchymal connective tissue cells transform into the cementoblasts and osteoblasts that form cementum and bone, respectively.

Periodontal treatment helps with repair and regeneration by eliminating bacterial plaque. Healing only involves re-establishing an adequate gingival sulcus at the root on the precise location of the bottom of the prior periodontal pocket; this is referred to as scar healing. This stops bone degradation but does not increase the attachment of the gingiva or the height of the bone. However, complete regeneration occurs in the periodontium through the use of various materials like guided tissue regeneration, platelet concentrates and bone grafts [1].

Regeneration is the process of regenerating damaged tissue from its precursor or a similar type of tissue that has already been damaged. The process of a structure growing and differentiating new cells and inter-cellular compounds to develop new tissue is known as regeneration [1].

The purpose of this review is to elaborate the importance of platelet concentrates in periodontal regeneration.

2. Role of platelet concentrates in tissue regeneration in periodontology

Platelets' ability to regenerate was initially discovered in 1974 [2]. Platelets are small blood cells with a number of physiological tasks. They are critical to preserving adequate blood volume in individuals with vascular injury through their clotting activities and stimulation of the coagulation factors.

The α-granules contain large adhesive proteins (vWF, TSP1, vitronectin, fibronectin), mitogenic factors (PDGF, VEGF, TGFβ), coagulation factors (factors V, VII, XI, XIII), and protease inhibitors (protein C, PAI-1, TFPI), which are released immediately after platelet activation. [3]. Platelets are produced by megakaryocytes in the bone marrow.

Schwertz et al. discovered that platelets outside of the bone marrow enhance the tissue regeneration process [4]. Platelets are well known for their thrombotic function. Platelets, in addition to reducing bleeding, contribute to various mechanisms such as inflammation, angiogenesis, cell proliferation and differentiation.

Platelets can be utilized in tissue engineering in vivo and in vitro [5,6]. The significance of platelets in long-term tissue healing has high clinical value. Aside from their ability to cover vascular abnormalities in the event of tissue injury, platelets also contain a source of cytokines, chemokines and growth factors, allowing platelets to potentially modulate tissue regeneration in a paracrine fashion [7]. The utilization of platelet concentrates in in vivo tissue engineering is one of its most recent advancements in dentistry [8].

3. Different types of platelet generation

Different types of platelets concentrates are described in Table 1 and their preparation according to revolutions per minute (RPM) and time are described in Table 2.

Table 1. Various generations of platelet concentrates.

First generation	Second generation
Plasma rich in growth factors	Platelet-rich fibrin (PRF)
Platelet-rich plasma (PRP)	Leucocyte- and platelet-rich fibrin (L-PRF)
	Advanced platelet-rich fibrin (A-PRF)
	Injectable platelet-rich fibrin (I-PRF)
	Titanium-prepared platelet-rich fibrin (T-PRF)

Table 2. Preparation of different types of PRF according to revolutions per minute (RPM) and time.

SR. NO	Types	RPM	Time	References
1	PRP	2000	10 min	[10]
2	PRF	2700	12 min	[8]
3	S-PRF	2700	12 min	[8]
4	A-PRF	1500	14 min	[8]
5	I-PRF	700	3 min	[29]
6	L-PRF	2700	12 min	[26]
7	T-PRF	3500	15 min	[16]

3.1. Platelet-rich plasma

The first application of platelet-rich plasma (PRP) was used in oral and maxillofacial surgery by Whitman et al. [9].

The potential of PRP is rapid tissue revitalization and wound healing, which includes new vasculature and tissue regeneration [10]. PRP is a biological component prepared from the whole blood of the subject that has a higher amount of platelets with anti-inflammatory and pro-regenerative qualities that help the body to heal tissue wounds more quickly and effectively [10].

PRP stimulates angiogenesis, cell proliferation, chemotaxis and paracrine actions of neighboring stem cells [10]. The two major PRP activators are calcium hydrochloride and thrombin, which trigger growth factor release within an hour [11]. This second activator allows the growth factors to be released gradually for up to 7 days following PRP injection. However, evidence suggests that PRP uses an anticoagulant component, which causes normal wound healing to be delayed [12].

Thrombin, calcium or other biologically acceptable anticoagulants are frequently needed for PRP preparation [13–15]. These components may interfere with the coagulation process and trigger an immunological reaction. The presence of fibrinogen in platelet-rich fibrin (PRF), which is transformed to fibrin under the effect of physiologically accessible thrombin, does not necessarily necessitate the use of any additive. This considerably reduces the possibility of complications following surgery.

3.2. Platelet-rich fibrin

In 2001, Choukroun et al. utilized PRF for the very first time. PRF has attracted a good amount of attention recently, as it is widely used in dentistry and medicine [16–19]. This PRF has been extensively used in hard and soft tissue healing [12]. PRF has several benefits over PRP, including its ease of preparation, low cost and lack of an additional anticoagulant or bovine thrombin, which reduces biochemical alteration and the hazards associated with the application of bovine thrombin [20]. Furthermore, unlike PRP, which creates a liquid end product with a short-term effect, the PRF network forms a homogeneous three-dimensional organization that has a persistent influence on tissue repair by gradually releasing cytokines.

3.2.1. PRF preparation

PRF is an autologous healing biomaterial containing the majority of leukocytes, platelets and growth factors isolated out of a simple blood sample in an autologous fibrin matrix [21–24]. At the moment, the PRF protocol is the simplest and least expensive method of producing platelet concentrates. The blood sample is taken from the patient during the surgical procedure and treated with a single centrifugation by using a specific centrifugation and collection kit, with no blood manipulation. There is no anticoagulant used during blood collection, and no bovine thrombin or calcium chloride was used for fibrin polymerization.

Three distinct fractions are produced at the end of the centrifugation process.

1) The red cells are concentrates at the test tube's bottom (easily discarded).

2) The surface layer is a platelet-depleted plasma liquid serum.

3) The intermediate fraction is a dense PRF clot that can be used clinically as a membrane [25].

Preparation of advanced PRF:

10 ml of venous blood is taken into the tube without anticoagulant and centrifuged at 1500 rpm for 14 minutes before being divided into the three layers described below:

Acellular plasma in the surface layer, PRF in the intermediate layer and red blood cells in the tube's base. The PRF is collected 2 mm below the lower dividing line after the above straw-colored layer was removed.

3.2.2. Standard platelet rich fibrin

A solid fibrin clot with little interfibrous space was visible in the longitudinal slice of the S-PRF clot, which was formed using the normal centrifugation process (2700 rpm, 12 minutes). Cells were seen throughout the clot using the normal histochemical staining techniques, however they were less visible in the more distant areas of the PRF clot.

3.3. Titanium-prepared platelet-rich fibrin

T-PRF denotes titanium-prepared platelet-rich fibrin. The basic idea behind the procedure is that titanium tubes have a considerably greater ability to stimulate clotting factors than glass tubes, contrary to Choukroun's approach [6,16,20,21]. The above material would be utilized to remove parched glass or crystal plastic tubes, as well as the long- or short-term detrimental consequences of silicon-related problems. Researchers found that clots produced in titanium pipes were identical to those generated in glass vials, and that co-aggregation caused by titanium had been analogous to that. Because titanium particles, rather than silica particles, are used to activate platelets, T-PRF offers unique features, such as enhanced biocompatibility.

3.4. Leukocyte- and platelet-rich fibrin

One recently developed approach is to implant leukocyte- and platelet-rich fibrin (L-PRF) immediately after tooth extraction. Previous studies have revealed that this autologous biomaterial can help to reduce postoperative complications in third molar extractions [26]. L-PRF is an autologous fibrin-based biomaterial that contains platelets, leukocytes and cytokines. L-PRF has been demonstrated to enhance biological functions such as chemotaxis, angiogenesis and cell proliferation and differentiation, all of which may aid in wound healing. Unlike PRP, L-PRF is an additive-free biomaterial that does not require chemical platelet activation. In addition, unlike PRP, L-PRF is a solid biomaterial that does not disperse soon after application. Solid-state L-PRF has been demonstrated to dramatically embed platelet and leukocyte growth factors into the fibrin matrix, resulting in an enhanced cytokine life span.

3.5. Injectable PRF

By altering spin centrifugation pressures, injectable PRF (I-PRF) was produced in 2014. Blood centrifuged in non-glass centrifuge tubes at lower centrifugation speeds produced a flowable PRF known as I-PRF [27]. I-PRF is a newly created platelet concentrate supplemented with leukocytes that have the ability to promote both soft and hard tissue regeneration [27,28]. Because I-PRF is a liquid for around 15 minutes, it will afford dental practitioners with another useful form of PRF [29]. After application, the human liquid fibrinogen in I-PRF gradually transforms into a PRF clot rich in growth factors that release continuously for 10–14 days [30].

3.6. Concentrated PRF

Recent research has shown that using conventional I-PRF methods only slightly improves platelet and leukocyte concentrates. New harvesting methods were recently created to obtain larger concentrates of platelets and leukocytes from the buffy coat layer (i.e., concentrated PRF (C-PRF)) after using quicker centrifugation procedures. When compared to regular I-PRF, it was discovered that C-PRF collected precisely from the buffy coat layer by using more stringent centrifugation methods showed up to a threefold increase in growth factor release.

4. Horizontal centrifugation

Leukocyte and platelet counts and concentrates both significantly increased after horizontal centrifugation (up to 3.5 times higher for either solid or liquid PRF). Compared to an angled or fixed centrifuge (IntraSpin, i.e., a process for PRF). A relevant study demonstrated a novel/accurate approach for counting cells using PRF protocols. In addition, PRF made by using horizontal centrifugation gathered more platelets and leukocytes than PRF made by using fixed-angle centrifugation. Due to a bigger difference in RCF values between the RCF-min and RCF-max, it provides a superior capacity to distinguish between different cell types depending on density. When compared to all other protocols, the horizontal centrifuge produced the most platelets and leukocytes, with a roughly twofold increase in leukocyte numbers than the fixed-angle centrifuge.

There are two noted benefits for using horizontal centrifugation. First, the greatest disparity can be achieved by using a completely horizontal tube made from a swing-out bucket to produce the smallest and largest radius that can be found inside a centrifugation tube. As a result, it is possible to more easily distinguish between cell layers based on differences in the RCF-min and RCF-max produced inside a tube. A fixed-angle centrifuge also causes more damage to cells. When employing fixed-angle centrifuges, cell layer separation is always observed in angulated form since centrifugation typically pulls cells outward and downward.

5. Biological actions of PRF

There are various biological effects of PRF, which include angiogenesis, immunomodulatory effects, mitogenesis, osteogenic effect, entrapment of stem cells and wound recolonization [31].

5.1. Angiogenesis

Angiogenesis is mediated by basic fibroblast growth factor, VEGF, PDGF and angiopoietin. The actions are migration, cell division and phenotypic changes in the proximity of the wound. It increases the endothelial cells' production of the α5β3 integrins, which then encourages the adherence of endothelial cells to fibrin, fibronectin and vitronectin.

5.2. Mitogenesis

The action of PRF in mitogenesis is mediated by TGF-β (fine & flexible trimolecular/ equilateral junctions).

The action of prf in mitogenesis effect are as follows:

1. Faster cellular migration is encouraged through increased cytokine trapping.
2. Inhibitory effect on osteoclasts.

5.3. Immunomodulatory effects

The action of PRF in terms of immunomodulatory effects is mediated by fibrin and its degradation products, IL-4, leukocytes and fibronectin, and the action is to stimulate, by phagocytosis, migration and enzymatic degradation via neutrophils. The effects include the following:

• Enhanced neutrophil CD11C/CD18 receptor expression, which facilitates endothelial as well as fibrinogen adhesion.

• Certain chemotactic factors are released, which govern macrophage wound colonization.

• Increased degranulation results in the production of numerous molecules, such as IL-1, IL-4, IL-6 and TNFα.

• Coherent healing without inflammatory excess.

5.4. Wound recolonization

The action of PRF in terms of wound recolonization is mediated by fibrinogen, fibronectin, vitronectin, tenascin and fibrin, and the actions degrade and facilitate epithelial cell migration at wound edges. Would recolonization involves the following action

• PRF binds to several molecules, including fibronectin, PDGF and TGF-β through the αVβ3 integrin

• Encourages fibroblast migration.

5.5. Osteogenic effect

PRF yields osteogenic effects, which include the following:

• Enhance the expression of phosphorylated extracellular signal-regulated protein kinase and osteoprotegerin, as well as alkaline phosphatase activity.

• Alkaline phosphatase and osteoprotegerin expression may be increased.

5.6. Entrapment of stem cells

The fibrin clot has been hypothesized to function as a trap for circulating stem cells, allowing them to congregate to a secretory phenotype and aid in vascular and tissue regeneration regardless of the fact that stem cells have a lesser intrinsic composition.

6. Conclusion

PRF and its modifications have enhanced regenerative potential in the field of dentistry. The PRF membrane develops, which supports the healing process. PRP and PRF are used or tested as surgical adjuvants or regenerative medicine preparations in most medical fields. Platelet concentrates naturally contain the growth factors that help physiological tissue healing.

Conflict of interest

The authors declare no conflict of interest.

References

1. Carranza FA, Newman MG, Takei H, et al. (2006) Carranza's Clinical periodontology 10th edition. Philadelpia: Elsevier Inc, 2006.
2. Ross R, Glomset J, Kariya B, et al. (1974) A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. *Proc Natl Acad Sci USA* 71: 1207–1210. https://doi.org/10.1073/pnas.71.4.1207
3. Jurk K and Kehrel BE (2005) Platelets: physiology and biochemistry. *Semin Thromb Hemost* 31: 381–392. https://doi.org/10.1055/s-2005-916671
4. Schwertz H, Koster S, Kahr WH, et al. (2010) Anucleate platelets generate progeny. *Blood* 115: 3801–3809. https://doi.org/10.2174/157016112801784648
5. Amaral RJ and Balduino A (2015) Platelets in tissue regeneration, In: Steve Kerrigan, Niamh Moran, *The Non-Thrombotic Role of Platelets in Health and Disease,* London: IntechOpen, 221–237.
6. Kiran NK, Mukunda KS, Tilak Raj TN (2011) Platelet concentratess: A promising innovation in dentistry. *J Dent Sci Res* 2: 50–61.

7. Stellos K, Kopf S, Paul A, et al. (2010) Platelets in regeneration. *Semin Thromb Hemost* 36: 175–184. https://doi.org/10.1055/s-0030-1251502
8. Ghanaati S, Booms P, Orlowska A, et al. (2014) Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. *J Oral Implantol* 40: 679–689. https://doi.org/10.1563/aaid-joi-D-14-00138
9. Whitman DH, Berry RL, Green DM (1997) Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. *J Oral Maxil Surg* 55: 1294–1299. https://doi.org/10.1016/S0278-2391(97)90187-7
10. Roubelakis MG, Trohatou O, Roubelakis A, et al. (2014) Platelet-rich plasma (PRP) promotes feta mesenchymal stem/stromal cell migration and wound healing process. *Stem Cell Rev Rep* 10: 417–428. https://doi.org/10.1007/s12015-013-9494-8
11. Marx RE, Carlson ER, Eichstaedt RM, et al. (1998) Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodontics* 85: 638–646. https://doi.org/10.1016/S1079-2104(98)90029-4
12. Rozman P and Bolta Z (2007) Use of platelet growth factors in treating wounds and soft-tissue injuries. *Acta Dermatoven APA* 16: 156–165.
13. Kilian O, Flesch I, Wenisch S, et al. (2004) Effects of platelet growth factors on human mesenchymal stem cells and human endothelial cells in vitro. *Eur J Med Re*s 9: 337–344.
14. Fréchette JP, Martineau I, Gagnon G (2005) Platelet-rich plasmas: growth factor content and roles in wound healing. *J Dent Res* 84: 434–439. https://doi.org/10.1177/154405910508400507
15. Kour P, Pudakalkatti PS, Vas AM, et al. (2018) Comparative evaluation of antimicrobial efficacy of platelet-rich plasma, platelet-rich fibrin, and injectable platelet-rich fibrin on the standard strains of Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans. *Contemp Clin Dent* 9: S325–S330. https://doi.org/10.4103/ccd.ccd_367_18
16. Rouwkema J and Khademhosseini A (2016) Vascularization and angiogenesis in tissue engineering: beyond creating static networks. *Trends Biotechnol* 34: 733–745. https://doi.org/10.1016/j.tibtech.2016.03.002
17. Dambhare A, Bhongade ML, Dhadse PV, et al. (2019) A randomized controlled clinical study of autologous platelet rich fibrin (PRF) in combination with HA and beta-TCP or HA and beta-TCP alone for treatment of furcation defects. *J Hard Tissue Biol* 28: 185–190. https://doi.org/10.2485/jhtb.28.185
18. Marx RE (2004) Platelet-rich plasma: evidence to support its use. *J Oral Maxillofac Surg* 62: 489–496. https://doi.org/10.1016/j.joms.2003.12.003
19. Meheux C, McCulloch P, Lintner D, et al. (2016) Efficacy of intra-articular platelet-rich plasma injections in knee osteoarthritis: a systematic review. *Arthroscopy* 32: 495–505. https://doi.org/10.1016/j.arthro.2015.08.005
20. Choukroun J, Adda F, Schoeffler C, et al. (2001) Uneopportunite enparo-implantologie: Le PRF. *Implantodontie* 42: e62.
21. Ghanaati S, Booms P, Orlowska A, et al. (2014) Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells. *J Oral Implantol* 40: 679–689. https://doi.org/10.1563/aaid-joi-D-14-00138
22. Dohan DM, Choukroun J, Diss A, et al. (2006) Platelet-rich fibrin (PRF): a second-generation platelet concentration. Part I: technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodontics* 101: e37–e44. https://doi.org/10.1016/j.tripleo.2005.07.008

23. Dohan DM, Choukroun J, Diss A, et al. (2006) Platelet-rich fibrin (PRF): a second-generation platelet concentration. Part II: platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodontics* 101: e45–e50. https://doi.org/10.1016/j.tripleo.2005.07.009
24. Dohan DM, Choukroun J, Diss A, et al. (2006) Platelet-rich fibrin (PRF): a second-generation platelet concentration. Part III: leucocyte activation: a new feature for platelet concentrations? *Oral Surg Oral Med Oral Pathol Oral Radiol Endodontics* 101: e51–e55. https://doi.org/10.1016/j.tripleo.2005.07.010
25. Farid Shehab M, Hamid NM, Askar NA, et al. (2018) Immediate mandibular reconstruction via patient-specific titanium mesh tray using electron beammelting/CAD/rapid prototyping techniques: One-year follow-up. *Int J Med Robot Comp* 14: e1895. https://doi.org/10.1002/rcs.1895
26. Pimentel T, Ritto F, Canellas JV, et al. (2020) Re: Randomized double-blind clinical trial evaluation of bone healing after third molar surgery with the use of leukocyte- and platelet-rich fibrin. *Int J Oral Maxillofac Surg* 49: 692. https://doi.org/10.1016/j.ijom.2019.10.018
27. Choukroun J and Ghanaati S (2018) Reduction of relative centrifugation force within injectable platelet-rich-fibrin (PRF) concentrates advances patients' own inflammatory cells, platelets and growth factors: the first introduction to the low speed centrifugation concept. *Eur J Trauma Emerg Surg* 44: 87–95. https://doi.org/10.1007/s00068-017-0767-9
28. Wang X, Zhang Y, Choukroun J, et al. (2017) Behavior of gingival fibroblasts on titanium implant surfaces in combination with either injectable-PRF or PRP. *Int J Mol Sci* 18: 331. https://doi.org/10.3390/ijms18020331
29. Lei L, Yu Y, Ke T, et al. (2019) The application of three-dimensional printing model and platelet-rich fibrin technology in guided tissue regeneration surgery for severe bone defects. *J Oral Implantol* 45: 35–43. https://doi.org/10.1563/aaid-joi-D-17-00231
30. Kyyak S, Blatt S, Pabst A, et al. (2020) Combination of an allogenic and a xenogenic bone substitute material with injectable platelet-rich fibrin–A comparative in vitro study. *J Biomater Appl* 35: 83–96. https://doi.org/10.1177/0885328220914407
31. Shah R, Thomas R, Mehta DS (2017) An update on the protocols and biologic actions of platelet rich fibrin in dentistry. *Eur J Prosthodont Re* 25: 64–72. https://doi.org/10.1922/ejprd_01690shah09