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Research Article

Efficacy of i-PRF as a Local Drug Delivery with Scaling and Root Planing in the treatment of Chronic Periodontitis

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Abstract

Aim: The present study was planned to evaluate clinically the efficacy of i-Platelet Rich Fibrin injected locally into the periodontal pocket.

Materials and Methods: A total of 30 sites with pocket depths 5 - 6 mm were chosen as a split mouth design. Selected sites were randomly divided into two groups to receive scaling and root planing with injectable Platelet Rich Fibrin (SRP+i-PRF) (Test) and scaling and root planing (SRP) alone (Control). Clinical parameters like modified sulcular bleeding index (mSBI) and probing pocket depth (PPD) was recorded at baseline and 3 months interval.

Results: A significant reduction in mSBI, probing pocket depth (PPD), was observed from base line to 3 months in both the groups. However, results were found to be statistically significant in the SRP+i-PRF group (test).

Conclusion: SRP+i-PRF group showed positive results, however further studies are needed to evaluate i-PRF as a local drug delivery.

Clinical Significance: There is currently widespread interest for the use of various biomaterials to locally deliver biomolecules for regeneration. This article focuses on the use of liquid version of platelet rich fibrin as a local drug delivery for the fast delivery of growth factors.

Keywords: *Chronic Periodontitis; i-PRF; Local Drug Delivery; Scaling and Root Planing*

Introduction

Periodontal disease is a general term which encompasses several pathological conditions affecting the tooth supporting structures. These conditions are characterized by a destruction of the periodontal ligament, resorption of the alveolar bone and the migration of the junctional epithelium along the tooth surface [1]. It is a localized inflammatory response caused by bacterial infection of a periodontal pocket associated with subgingival plaque [2]. Antimicrobial therapy has also been directed at specific bacteria associated with clinically diseased sites to help augment the mechanical treatment aimed at the removal of sub-gingival calculus and toxins. However, the inability

to achieve and maintain therapeutic concentrations of the antibiotic in the crevicular fluid with systemic administration can limit its effectiveness [3]. Also, the administration of antimicrobial agents requires frequent dosing which is associated with the risk of developing resistant organisms and super infection [4].

Platelet concentrations have been utilized in dentistry for over three decades as a regenerative tool capable of releasing supra physiological doses of growth factors responsible for inducing tissue regeneration derived from autologous sources [5,6]. Platelet-rich fibrin (PRF) was developed as a second generation autologous platelet concentrate without the use of anticoagulants or other additives, which was first termed by Choukroun., *et al*. in 2001 [7]. Recently the effects of PRF have been documented in several systematic reviews demonstrating its long-term effects on tissue-wound healing [8-12]. Two of the main documented advantages of PRF include the fact that it contains host immune defense cells (leukocytes) which act to fight infection [13].

Interestingly, the pioneering development of the low speed centrifugation concept introduced the development of a new formulation of PRF whereby a liquid formulation of PRF could be obtained for injectable purposes (i-PRF) without using anti-coagulants [14-16]. This new platelet concentrate does not contain any anti-coagulants, maintains a liquid viscosity for roughly 15 minutes following centrifugation.

This injectable formulation of PRF (termed i-PRF) has been pursued with the aim of delivering to clinicians an easy to use platelet concentrate in liquid formulation which can be either utilized alone or combined easily with various biomaterials. Taking advantage of slower and shorter centrifugation speeds, a higher presence of regenerative cells with higher concentrations of growth factor can be observed when compared to other formulations of PRF utilizing higher centrifugation speeds [17,18]. Various mechanisms have been hypothesized regarding the mechanism of antibacterial effect of platelet-derived preparations such as generation of oxygen metabolites, including superoxide, hydrogen peroxide, and hydroxyl free radicals; binding, aggregation, and internalization of microorganisms, thereby enhancing the clearance of pathogens from the bloodstream and release of potent antimicrobial peptides [19]. Utilizing the liquid formulation of platelet concentrate and antimicrobial effect of platelets the study was designed to evaluate the effect of i-PRF on reduction of periodontal pocket depth with scaling and root planing in the treatment of periodontal pockets.

Materials and Methods

A total 24 patients were screened out of which 15 patients in the age group of 25 - 55 years (males and females) were enrolled in the trial. Patients with untreated periodontitis satisfying the inclusion and exclusion criteria were enrolled in the study selected from the outpatient section, Department of Periodontics and Implantology. A detailed case history was recorded, the nature and purpose of the study was explained to the patients in their native language, and written informed consent was obtained. Helsinki guidelines were been followed, after which the institutional ethical committee approved the study.

Inclusion criteria:

- Systemically healthy patients.
- Age group between 25 - 55 years.
- Presence of horizontal bone loss and at least two sites with probing pocket depth ≥ 5 mm.

Exclusion criteria:

- Pregnant and lactating females.
- Patients with a history of any kind of periodontal therapy within past 6 months.
- Medically compromised patients.
- Teeth showing endo-periodontal lesions.
- Restorations and other plaque retentive factors.
- Smokers.
- Tobacco chewers and importantly patients not compliant with oral hygiene procedures.

Sample size

Sample size was calculated using G power 3.1 software. 30 sites would be sufficient to achieve the required significance under 95% power with alpha error value set at 0.05.

Preparation of i-PRF

Blood samples were collected from antecubital vein. i-PRF was prepared via a protocol as previously described [20]. For i-PRF preparation, 10 ml of whole blood without anticoagulant were centrifuged using plastic tubes at 700rpm for 3 minutes at room temperature by REMI centrifuge. The upper liquid layer was collected as i-PRF (Figure 1a-1c).

Figure 1a-1c: *1a: Sample collection. 1b: Remi centrifuge. 1c: Upper layer i-PRF.*

Study design

For each patient, two contralateral target sites were selected using simple randomization procedure. Consort guidelines were followed. Randomization was done using predetermined computer generated randomization scheme to receive balanced random allocation of patients (Figure 4). Patients were assigned to one of the two treatment modalities as follows:

- **Test group:** Sites treated with SRP + i-PRF (Figure 2a-2c).
- **Control group:** Sites treated with SRP alone (Figure 3a and 3b).

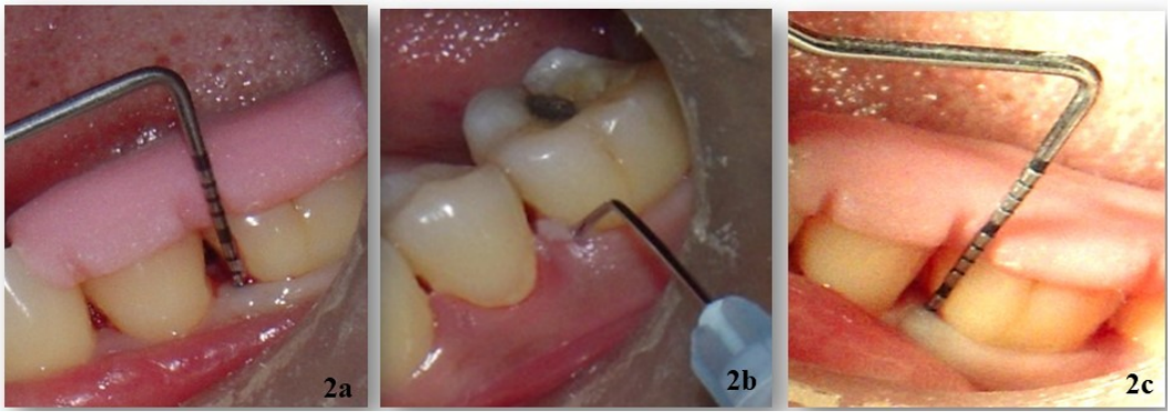


Figure 2a-2c: *2a: Baseline (Test group). 2b: i-PRF injected. 2c: 3 months (Test group).*

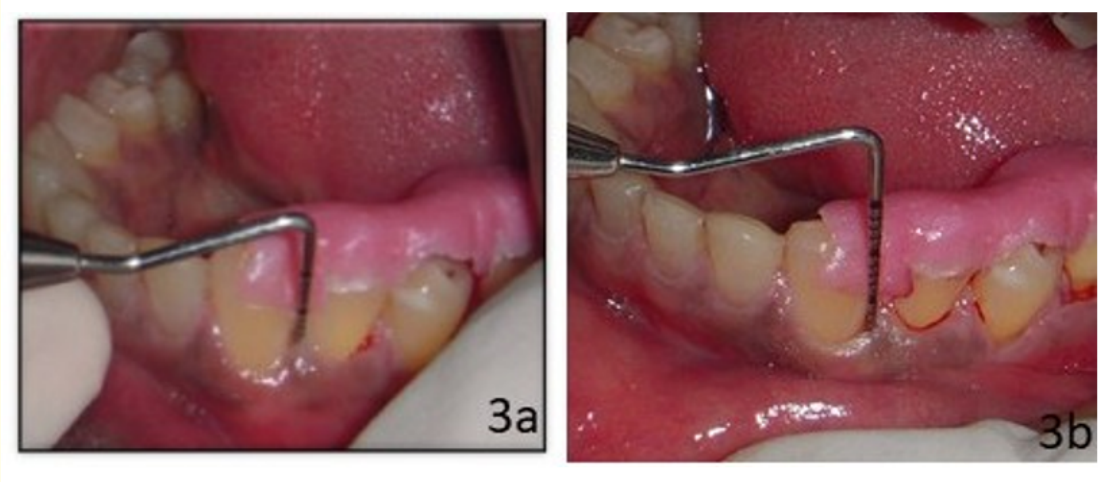


Figure 3a-3b: *3a: Baseline (Control group). 3b: 3 months (Control group).*

The study was carried out by the single operator. Patients were instructed to avoid flossing, toothpicks or hard, crunchy or sticky food in the experimental area for 7-10 days and to record any adverse events.

Figure 4 shows study design.

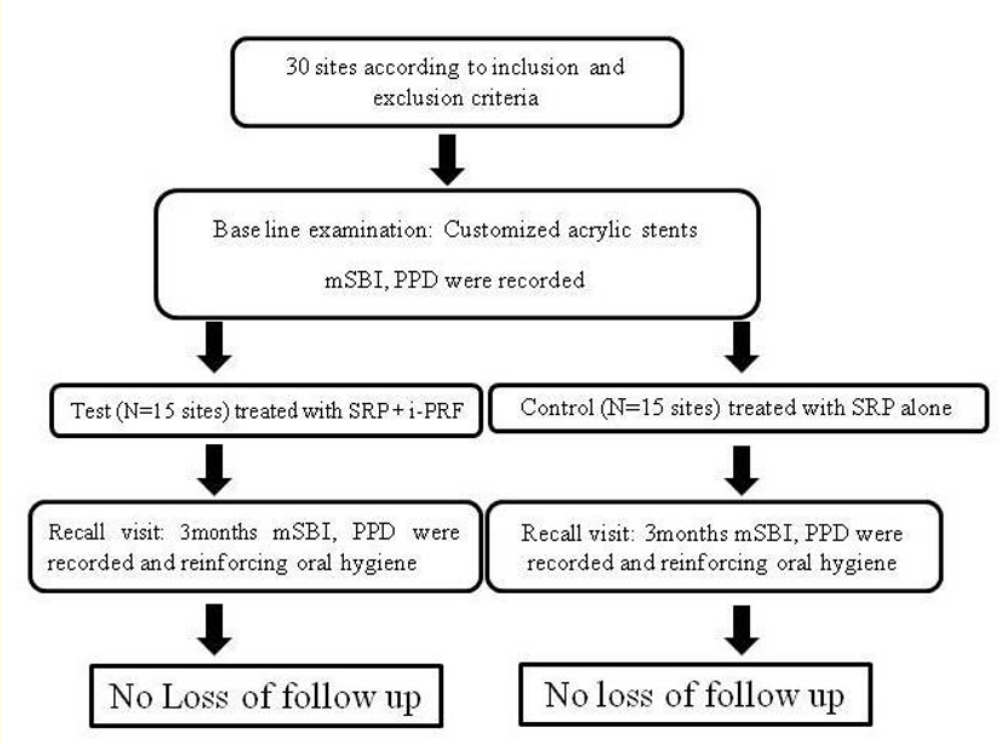


Figure 4: *Study design.*

Statistical analysis

Statistical analysis was performed by using SPSS Software (version 19, IBM Corporation, New York, NY, USA). Paired t-test was used for intergroup comparison of mSBI and PPD and Wilkinson signed rank test was used for intragroup comparison. Statistical significance was considered at $p < 0.05$. All data were expressed as the Mean ± SD.

Results

The mean age of all the individuals enrolled in study was found to be 30 years. Of these 30 subjects, 9 (60%) were females and 6 (40%) were males. Intra group comparison of mean mSBI score and probing pocket depth score at base line were 1.53 ± 0.488 and 5.53 ± 0.516 respectively, whereas mean mSBI score and probing pocket depth score at 3 months were 0.33 ± 0.33 and 3.40 ± 0.507 respectively (Table 1 and graph 1). Intra group comparison of mean mSBI score and probing pocket depth score at base line were 1.40 ± 0.507 and 5.73 ± 0.4586 whereas mean mSBI score and probing pocket depth score at 3months were 0.03 ± 0.258 and 2.40 ± 0.507 respectively (Table 2 and graph 1). Inter group comparison of mean values for test and control for mSBI score at base line showed no statistically significant difference between the control group and test group ($p > 0.001$) (Table 3 and graph 2). Inter group comparison of mean values for test and control for mSBI score at 3months showed no statistically significant difference between the control group and test group ($p > 0.001$) (Table 3 and graph 2). Inter group comparison of mean values for test and control for pocket probing depth at base line showed no statistically significant difference between the control group and test group ($p > 0.001$) (Table 3 and graph 2). Inter group comparison of mean

values for test and control for pocket probing depth at 3 months showed statistically significant difference between the control group and test group ($p < 0.001$) (Table 3 and graph 2).

Aspect	mSBI		Probing Pocket Depth	
	Baseline	3 Months	Baseline	3 Months
Mean ± SD	1.53 ± 0.488	0.33 ± 0.33	5.53 ± 0.516	3.40 ± 0.507

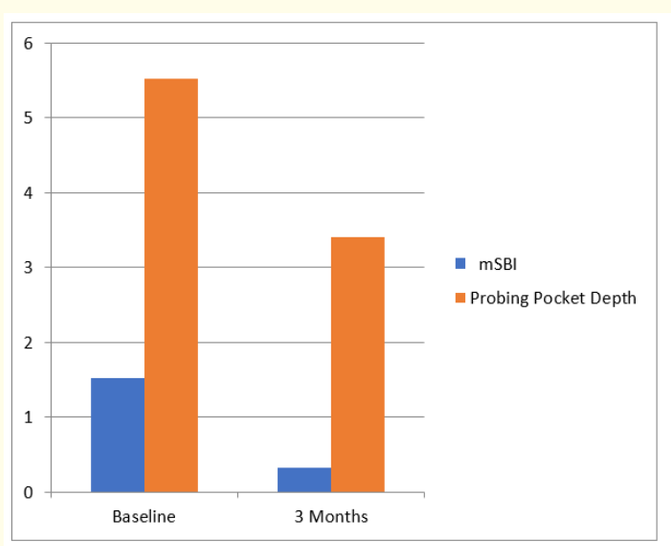
Table 1: *Comparison of intragroup mean mSBI score and probing pocket depth changes at baseline and 3 months in control group.*

Aspect	mSBI		Probing Pocket Depth	
	Base line	3 month	Base line	3 months
Mean ± SD	1.40 ± 0.507	0.07 ± 0.258	5.73 ± 0.458	2.40 ± 0.507

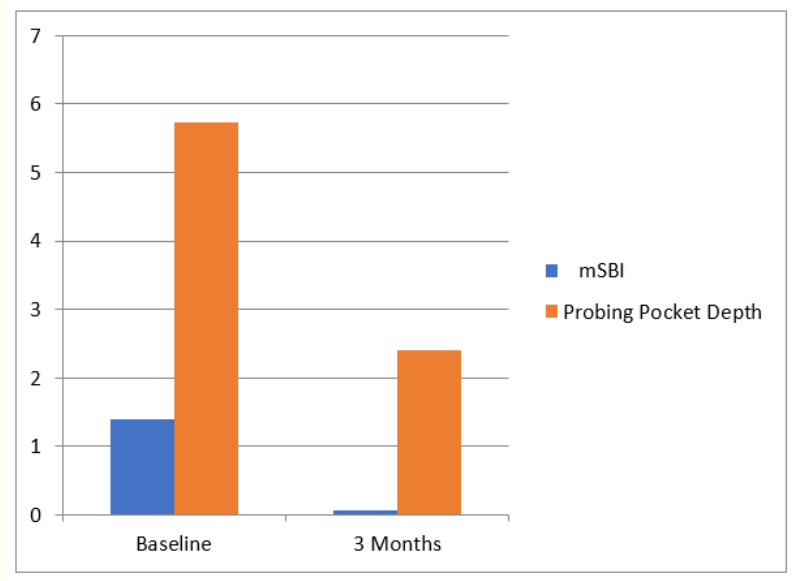
Table 2: *Comparison of intragroup mean mSBI score and probing pocket depth changes at baseline and 3 months in test group.*

		df	Sig. (2-tailed)
Pair 1	Baseline mSBI Control Baseline mSBI Test	14	0.582
Pair 2	3 Months mSBI Control 3 month mSBI Test	14	0.041
Pair 3	Baseline PPD Control Baseline PPD Test	14	0.082
Pair 4	3 Months PPD Control 3 Months PPD Test	14	0.000

Table 3: *Comparison of intergroup mean mSBI score and probing depth differences at baseline and 3 months.*



Graph 1: *Comparison of intragroup mean mSBI score and probing pocket depth changes at baseline and 3 months.*



Graph 2: *Comparison of intergroup mean mSBI score and probing pocket depth changes at baseline and 3 months.*

Discussion

The present split mouth study design reduces interpatient systemic differences (e.g. age, systemic health, and gender) that are known to confound the effects of periodontal treatment on clinical parameters and bacterial counts. Also, maintaining the same oral environment for the test and control sites provides similar bacterial load, salivary pH, and oral hygiene maintenance etc. The presence of bacterial plaque represents the principal etiological factor involved in the initiation and progression of periodontitis. These bacterial plaque or biofilms are difficult therapeutic targets as they are not easily disrupted [21]. In a non-sterile environment such as the mouth, it is virtually impossible to completely prevent their formation [22].

Adequate blood supply is required for optimal healing of bone and cartilage. When utilized alone liquid-PRF promote regeneration in various tissues [23]. Over the last few decades several antibiotics like penicillin's, metronidazole, tetracycline's, and cephalosporin's are utilized [24]. However, due to microbial resistance alternatives or combination approaches are necessary [25]. While most studies to date have focused on local delivery of antibiotics [26-29] new strategies are needed to reduce the amount of tissue lost due to infectious disease. Because liquid PRF can be delivered locally to defective tissues in a minimally invasive manner, it presents regenerative potential to act as a local delivery system for various biomolecules.

Several antimicrobials in local drug delivery form include tetracycline, ofloxacin, clindamycin, chlorhexidine, etc. in the treatment of periodontal infections [30-32].

Wound healing is a central concept in regenerative surgical sciences. It occurs as a result of complex interplay between various cells and signaling molecules. Clinicians continuously strive to provide rapid and enhanced healing to the patients. The quest to enhance the natural healing response, leave us in a constant search for alternatives. One such alternative is platelet concentrates have been applied since more than half a century. With the increase in understanding of the biological properties of these concentrates, the initial protocols

have undergone immense change. Among the various platelet concentrates, currently, the most widely used concentrate in dentistry is platelet rich fibrin (PRF) [33]. There are several blood derived growth factors in PRF and it can be produced within short time by centrifugation and without any additives [34]. The PRF matrix has the ability to trap circulating cytokines [35]. In PRF formulations which were prepared initially there was a lack of liquid concentrate of proteins. Hence recently a liquid form of PRF (i-PRF) was developed which does not contain any anticoagulants. A study by Ghanaati., *et al*. [36] showed the slow-speed concept for blood centrifugation whereby lower centrifugation speeds were shown to contain higher numbers of cells including leukocytes prior to the formation of a fibrin clot. Leukocytes are immune cells having vast importance in tissue regeneration by directing and recruiting various cell types during the wound healing process [37-39]. The scientific rationale behind the use of platelet preparations lies in the fact that the platelet alpha granules are a reservoir of many growth factors that are known to play a crucial role in hard and soft tissue repair mechanism. These include platelet-derived growth factors (PDGFS), transforming growth factor beta (TGFB), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor-β1 (IGF-B1), etc. Platelet growth factors exhibit chemotactic and mitogenic properties that promote and modulate cellular functions involved in tissue healing, regeneration and cell proliferation [40,41]. The kinetic release of growth factors from i-PRF is longer, up to 14 days, because of its preserved valuable components, including platelets and leukocytes [42]. i-PRF potentially allows more growth factor attachment to the heparin-binding domain, which is the high-affinity growth factor-binding site of the fibrinogen that causes prolonged retention of the growth factor within fibrin [43]. In this way, platelets play an important role in proliferation of cells, collagen synthesis and formation of osteoid resulting in regeneration of lost periodontal tissues.

However, antimicrobial properties of platelets are scarcely studied. The present study was carried to evaluate the antimicrobial property of i-PRF similar to local drug deliver and aimed to compare the efficacy of i-PRF with scaling and root planing versus scaling and root planing alone. In test group there was a significant reduction in mSBI bleeding index from baseline (1.40 ± 0.507) to (0.07 ± 0.258) at 3 months and probing pocket depth from baseline (5.73 ± 0.458) to (2.40 ± 507) at 3 months when compared to control group where mSBI bleeding index reduced from baseline (1.53 ± 0.488) to (0.33 ± 0.33) at 3 months and pocket probing depth from baseline (5.53 ± 0.516) to (3.40 ± 0.507) at 3 months. Inter group comparison of mean values for test and control for pocket probing depth at 3 months showed statistically significant difference between the control group and test group ($p \leq 0.001$). In a study by Karde., *et al.* [44] where the antibacterial activity of PRP, PRF, and iPRF were tested on the supragingival plaque and it was observed that iPRF showed a maximum zone of inhibition followed by PRP and then PRF. It was observed that i-PRF showed maximum zone of inhibition around oral microflora, i.e. 1.42 ± 0.25 (in cm). In another study by Prabhdeep., *et al.* where PRP, PRF and i- PRF were compared for their antibacterial effect against *Pg* and *Aa*. i- PRF had maximum antibacterial activity [45].

Apart from platelets even leukocytes that are present in much greater concentrations in this i-PRF concentrates as compared to the whole blood. Neutrophils exhibit antibacterial action by secreting the myeloperoxidase present in the granules. Furthermore, monocytes produce the cytokines and chemotactic factors that participate in inflammation [46]. The platelet microbicidal proteins (PMPs) include various materials such as platelet factor 4, connective tissue activating peptide 3, thymosin beta-4, platelet basic protein, and fibrinopeptide B which have an antibacterial activity. These PMPs could exhibit the antimicrobial activity by coming in contact with the bacterial membrane, altering the permeability of the membrane, entering the cell, and blocking the synthesis of important molecules [47]. SRP was shown to remove all granulation tissue and on gingiva and root surface whereas i-PRF was capable of inducing higher cell migration and mRNA expression of TGF-β, PDGF, and COL1a2. Another interesting finding was the fact that i-PRF formed a small clot likely as a result of fibrin components that acted as a dynamic gel with cells likely contained within its hydrogel. These could be the probable reasons for improvement in clinical parameters in test group. Since there is limited literature related to this novel i-Platelet Rich Fibrin, results could not be compared. Bacterial infection can impair wound healing and periodontal regeneration and so antibiotics are usually prescribed after the surgery. However, due to increased incidences of antibiotic resistance, alternative methods are being sought after and platelet concentrates seem to be a very useful adjunct [48,49].

Conclusion

To our knowledge this is the first study to evaluate the treatment outcome of i-PRF in the periodontal pocket depth reduction. As it is natural biomaterial, easy, efficient, safety, low risk, increased clinical performance, increased healing potential, reduced morbidity, cost effective and requires no additives for its preparation has grabbed attention of clinicians worldwide. The study showed positive results, suggesting i-PRF may be an alternative for local drug delivery of antibiotics, but their antibacterial activity is limited as compared to antibiotics, studies need to be done to evaluate i-PRF as a local drug delivery.

Conflict of Interest

No conflict of interest.

Bibliography

1. Carranza FA., *et al*. "Carranza's Clinical Periodontology". 10th Edition 494.
2. Ximenez-Fyvie LA., *et al*. "Comparison of the microbiota of supra- and subgingival plaque in health and periodontitis". *Journal of Clinical Periodontology* 27.9 (2000): 648-657.
3. Newman MG., *et al*. "A 6-Month Multi-Center Evaluation of Adjunctive Tetracycline Fiber Therapy Used in Conjunction With Scaling and Root Planing in Maintenance Patients: Clinical Results". *Journal of Periodontology* 65.7 (1994): 684-688.
4. Sadaf N., *et al*. "Evaluation of efficacy of tetracycline fibers in conjunction with scaling and root planing in patients with chronic periodontitis". *Journal of Indian Society of Periodontology* 16.3 (2012): 392-397.
5. Chow TW., *et al*. "Importance of plasma fibronectin in determining PFP and PRP clot mechanical properties". *Thrombosis Research* 29.2 (1983): 243-248.
6. Delaini F., *et al*. "Enhanced affinity for arachidonic acid in platelet-rich plasma from rats with adriamycin induced nephrotic syndrome". *Thrombosis and Haemostasis* 48.3 (1982): 260-262.
7. Choukroun J., *et al*. "Une opportunite en paro-implantologie: le PRF". *Implantodontie* 42.55 (2001): 62.
8. Miron R., *et al*. "Platelet Rich Fibrin and Soft Tissue Wound Healing: A Systematic Review". *Tissue Engineering Part B: Reviews* 23.1 (2017): 83-99.
9. Ali S., *et al*. "Platelet-Rich Fibrin in Maxillary Sinus Augmentation: a Systematic Review". *The Journal of Oral Implantology* 41.6 (2015): 746-753.
10. Lopez-Jornet P., *et al*. "Medication-related osteonecrosis of the jaw: Is autologous platelet concentrate application effective for prevention and treatment? A systematic review". *Journal of Cranio-Maxillofacial Surgery* 44.8 (2016): 1067-1072.
11. Malavolta EA., *et al*. "Comments on: Evaluation of platelet-rich plasma and fibrin matrix to assist in healing and repair of rotator cuff injuries: A systematic review and meta-analysis". *Clinical Rehabilitation* 30.7 (2016): 726-727.
12. Moraschini V and Barboza Edos S. "Use of Platelet-Rich Fibrin Membrane in the Treatment of Gingival Recession: A Systematic Review and Meta-Analysis". *Journal of Periodontology* 87.3 (2016): 281-290.

13. Owen CA and Campbell EJ. "The cell biology of leukocyte-mediated proteolysis". *Journal of Leukocyte Biology* 65.2 (1999): 137-150.

14. Choukroun J and Ghanaati S. "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". *European Journal of Trauma and Emergency Surgery* 44.1 (2018): 87-95.

15. El Bagdadi K., *et al*. "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)". *The European Journal of Trauma and Emergency Surgery* 45.3 (2019): 467-479.

16. Miron RJ., *et al*. "Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry?" *Clinical Oral Investigations* 21.8 (2017): 2619-2627.

17. Ghanaati S., *et al*. "Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells". *The Journal of Oral Implantology* 40.6 (2014): 679-689.

18. Fujioka-Kobayashi M., *et al*. "Optimized platelet rich fibrin with the low speed concept: growth factor release, biocompatibility and cellular response". *Journal of Periodontology* 88.1 (2017): 112-121.

19. Joshi CP., *et al*. "Autologous platelet rich fibrin as a potential antiperiopathogenic agent: An in-vitro study". *IP International Journal of Periodontology and Implantology* 1.2 (2016): 50-54.

20. Miron RJ., *et al*. "Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry?" *Clinical Oral Investigations* 21.8 (2017): 2619-2627.

21. Listagarten MA., *et al*. "Effects of tetracycline and/or scaling on human periodontal disease, Clinical, microbiological and histopathological observation". *Journal of Clinical Periodontology* 5.4 (1978): 246-271.

22. Lindhe J., *et al*. "Local tetracycline delivery using hollow fiber devices in periodontal therapy". *Journal of Clinical Periodontology* 6.3 (1979): 141-149.

23. Rao N., *et al*. "Treating osteomyelitis: antibiotics and surgery". *Plastic and Reconstructive Surgery* 127.1 (2011): 177s-87s.

24. Heitz-Mayfield LJ. "Systemic antibiotics in periodontal therapy". *Australian Dental Journal* 54.1 (2009): S96-101.

25. Bidault P., *et al*. "Risk of bacterial resistance associated with systemic antibiotic therapy in periodontology". *Journal of the Canadian Dental Association* 73 (2007): 721-725.

26. Tonetti MS., *et al*. "A controlled multicenter study of adjunctive use of tetracycline periodontal fibers in mandibular class II furcations with persistent bleeding". *Journal of Clinical Periodontology* 25 (1998): 728-376.

27. Mombelli A., *et al*. "Treatment of peri-implantitis by local delivery of tetracycline. Clinical, microbiological and radiological results". *Clinical Oral Implants Research* 12 (2001): 287-294.

28. Rathbone CR., *et al*. "Effect of various concentrations of antibiotics on osteogenic cell viability and activity". *Journal of Orthopaedic Research: Official Publication of the Orthopaedic Research Society* 29 (2011): 1070-1074.

29. Botelho MA., *et al*. "Nanotechnology in ligature induced periodontitis: protective effect of a doxycycline gel with nanoparticules". *Journal of Applied Oral Science: Revista FOB* 18 (2010): 335-342.

30. Cattabriga M., *et al*. "Tetracycline fiber used alone or with scaling and root planing in periodontal maintenance patients: Clinical results". *Quintessence International* 27.6 (1996): 395-400.

31. Hanes PJ and Purvis JP. "Local anti-infective therapy: Pharmacological agents. A Systematic Review". *Annals of Periodontology* 8.1 (2003): 79-98.

32. Baker PJ., *et al*. "Tetracycline and its derivatives strongly bind to and are released from the tooth surface in active form". *Journal of Periodontology* 54.10 (1983): 580-585.

33. Miron RJ., *et al*. "Use of platelet-rich fibrin in regenerative dentistry: a systematic review". *Clinical Oral Investigations* 21.6 (2017): 1913-1927.

34. Choukroun J., *et al*. "Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 101.3 (2006): 56-60.

35. Dohan DM., *et al*. "Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 101.3 (2006): 37-44.

36. Ghanaati S., *et al*. "Advanced platelet-rich fibrin: a new concept for cell-based tissue engineering by means of inflammatory cells". *The Journal of Oral Implantology* 40.6 (2014): 679-689.

37. Bielecki T., *et al*. "The role of leukocytes from L-PRP/L-PRF in wound healing and immune defense: new perspectives". *Current Pharmaceutical Biotechnology* 13.7 (2012): 1153-1162.

38. Martin P. "Wound healing–Aiming for perfect skin regeneration". *Science* 276.5309 (1997): 75-81.

39. Barrick B., *et al*. "Leukocyte proteinases in wound healing: roles in physiologic and pathologic processes". *Wound Repair and Regeneration* 7.6 (1999): 410-422.

40. Fujioka-Kobayashi M., *et al*. "Optimized platelet rich fibrin with the low speed concept: growth factor release, biocompatibility and cellular response". *The Journal of Periodontology* 88.1 (2017): 112-127.

41. Kobayashi E., *et al*. "Comparative release of growth factors from PRP, PRF, and advanced-PRF". *Clinical Oral Investigations* 20.9 (2016): 2353-2360.

42. Schar MO., *et al*. "Platelet-rich concentrates differentially release growth factors and induce cell migration in vitro". *Clinical Orthopaedics and Related Research* 473.5 (2015): 1635-1643.

43. Martino MM., *et al*. "Heparin-binding domain of fibrinogen binds growth factors and promotes tissue repair when incorporated within a synthetic matrix". *Proceedings of the National Academy of Sciences of the United States of America* 110.12 (2013): 4563-4568.

44. Badade PS., *et al*. "Antimicrobial effect of platelet-rich plasma and platelet-rich fibrin". *Indian Journal of Dental Research* 27.3 (2016): 300-304.

45. Kour P., *et al*. "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*". *Contemporary Clinical Dentistry* 9.2 (2018): S325-330.

46. Badade PS., *et al*. "Antimicrobial effect of platelet-rich plasma and platelet-rich fibrin". *Indian Journal of Dental Research* 27.3 (2016): 300-304.

47. Tang YQ., *et al*. "Antimicrobial peptides from human platelets". *Infection and Immunity* 70.12 (2002): 6524-6533.

48. Anitua E. "Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants". *The International Journal of Oral and Maxillofacial Implants* 14.4 (1999): 529-535.

49. Anitua E., *et al*. "Implementation of a more physiological plasma rich in growth factor (PRGF) protocol: anticoagulant removal and reduction in activator concentration". *Platelets* 27.5 (2016): 459-466.

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