



ANTIMICROBIAL PROPERTIES OF PLATELET-RICH FIBRIN-A SYSTEMATIC REVIEW

Periodontology

Dr Nawamee Phadnis*	3 rd year Post graduate student, Department of Periodontology, YMT Dental College and Hospital, Kharghar, Navi Mumbai. *Corresponding Author
Dr Amit Benjamin	MDS Professor and HOD, Department of Periodontology, YMT Dental College and Hospital, Kharghar, Navi Mumbai
Dr Pankti Gangar	2 nd year Post graduate student, Department of Periodontology, YMT Dental College and Hospital, Kharghar, Navi Mumbai.
Dr Vishaj Maru	1 st year Post graduate student, Department of Periodontology, YMT Dental College and Hospital, Kharghar, Navi Mumbai.
Dr Niharika Puppalwar	3 rd year Post graduate student, Department of Periodontology, YMT Dental College and Hospital, Kharghar, Navi Mumbai.

ABSTRACT

Background: Platelet rich fibrin (PRF) has gained great popularity in the field of regenerative medicine as it is capable of releasing growth factors, promoting tissue repair, angiogenesis, inflammation and immunomodulatory agents with antimicrobial activity. The aim of this systematic review was to evaluate the antimicrobial efficacy of PRF through in-vitro studies.

Methods: An electronic search was carried out on PubMed/MEDLINE and EBSCO till December 2019 in English language of in-vitro studies assessing the antimicrobial potential of the PRF. The review was registered in PROSPERO (CRD42020202167)

Results: Five in-vitro studies were included, which investigated the effects of PRF on various periodontal microorganisms. In all the included studies, it was found that PRF led to a decrease in the growth and not complete breakdown of the microorganisms.

Conclusion: Although the exact mechanism of interaction of PRF with oral microorganisms is unclear and needs further investigation, PRF has proved to have antimicrobial properties. Thus, PRF could represent a useful natural substance for combating postoperative infections with an additive effect of regeneration.

KEYWORDS

Antimicrobial, Platelet rich fibrin, antimicrobial agents

INTRODUCTION

Periodontitis is a biofilm induced chronic inflammatory and an infectious disease affecting the supporting structures around the teeth resulting in the progressive attachment loss and bone loss.^{1,2} Various studies have demonstrated the close association between the initiation of periodontitis and some bacterial species such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Fusobacterium nucleatum*.^{3,4}

In all fields of medicine, post-surgical infection is a serious complication as it hampers the wound healing as well as tissue regeneration.⁵ The main therapeutic goal of the periodontal therapy is to eliminate the dental biofilm and includes approaches such as mechanical debridement by scaling and root planing, periodontal surgery and local and systemic antimicrobials. Even with stringent disinfection protocols for controlling the infection there are chances of bacterial contamination with surgical therapy as the bacteria can infiltrate and survive into the deeper tissues resulting in failure of the surgery.⁶

In the past two decades, the use of autologous platelet concentrates (PC) has gained great popularity in multidisciplinary field of regenerative medicine.⁷ PC refers to a group of biomaterials derived from processing of the whole blood by various laboratory or chairside protocols involving centrifugation of blood with or without the use of biochemical additives.⁸ The rationale for its use is that the platelets when activated release growth factors (GFs) and other molecules that promote tissue repair, angiogenesis, inflammation, and immune response.⁹

Platelet rich fibrin (PRF), is a second generation of PC obtained by removing the biochemical additives and modifying the centrifugation protocols. PRF is a complex network of micro fibrins with entrapped platelets and leucocytes. PRF has a dense fibrin network with leukocytes, cytokines, structural glycoproteins, and growth factors.⁹ Leukocytes that are concentrated in PRF scaffold play an important role in growth factor release, immune regulation, anti-infectious activities, and matrix remodeling during wound healing. The slow polymerization mode of PRF and cicatricial capacity create a physiologic architecture favorable for wound healing.^{10,11,12,13}

The regenerative potential of platelet concentrates has been extensively studied since their introduction during the late 1990s.¹⁴ In spite of the increase in the number of publications in field of regenerative medicine occurred in recent years, only a few studies have investigated the antimicrobial effect of Platelet rich fibrin (PRF).

The objective of this systematic review was to evaluate the available evidence regarding the antimicrobial efficacy of Platelet rich fibrin procured from autologous blood samples of systemically healthy individuals through in-vitro studies.

METHODS

Protocol and registration:

This systematic review was registered under the number CRD 42020202167 in the PROSPERO database and carried out according to the PRISMA guidelines.

ELIGIBILITY CRITERIA:

INCLUSION CRITERIA-

In vitro studies assessing the antimicrobial activity of platelet rich fibrin, full text articles published in English till December 2019

EXCLUSION CRITERIA-

Case reports, Unpublished research, Animal model studies, Clinical studies

Information Sources and Search

A systematic search was performed using the following search terms, alone and in combination, by means of Boolean operators: "platelet rich fibrin", "PRF", "microorganisms," "antibacterial," "antimicrobial" and "infection". The electronic search was carried out by two independent reviewers through two electronic databases, PubMed/MEDLINE and EBSCO till December 2019 for in-vitro studies in English language.

Study selection

The articles were retrieved based on titles and abstracts meeting the inclusion criteria. The obtained titles were checked for duplicates and independently screened by 2 reviewers. Wherever insufficient

information was provided, full-text article was obtained. Additional studies were retrieved through searching the references from relevant articles. Disagreements if present, were resolved by discussion. Meta-analysis was not conducted because of the heterogeneity of the included studies.

Data collection and data items

The extracted data was recorded in a pre-designed data extraction table by two reviewers. The recorded variables were citation, year of publication, type of PRF, microorganisms tested and their source, study results and the authors conclusion.

ASSESSMENT OF THE RISK OF BIAS

The risk of bias of the included articles was evaluated through the CRIS Guidelines, developed specially for improving the quality of reporting of in vitro studies during experimental dental research.¹⁵ The following criteria were considered: sample size calculation; meaningful difference between groups; sample preparation and handling; allocation sequence, randomization and blinding; conflict of interest statement; statistical analysis. All these criteria were judged as adequate (yes), unclear or non-adequate (no). To summarize the validity of studies, they were grouped into the following categories: A) Low risk of bias if none of the quality criteria was judged as inadequate and no more than two of them were judged as unclear; B) Moderate risk of bias if one to three criteria were judged as inadequate; C) High risk of bias if four or more criteria were judged as inadequate. In case of discrepancy between the two reviewers, an agreement was obtained by discussion.

Table 1: Risk of bias in individual studies

Criteria	Badade et al. 2016 ¹⁶	Karde et al., 2017 ¹⁷	Kour P et al, 2019 ¹⁸	Jasmine et al, 2019 ¹⁹	Mamajiwa la et al, 2019 ²⁰
Sample size calculation	No	No	No	No	Yes
Meaningful difference between groups	Yes	Yes	Yes	Yes	Yes
Sample preparation and handling	No	No	No	No	No
Randomization	No	No	No	No	No
Concealment of sequence allocation	No	No	No	No	No
Blinding	No	No	No	No	No
Conflict of interest statement	Yes	Yes	Yes	No	Yes
Statistical analysis	Yes	Yes	Yes	Yes	Yes
RISK OF BIAS	HIGH	HIGH	HIGH	HIGH	HIGH

RESULTS

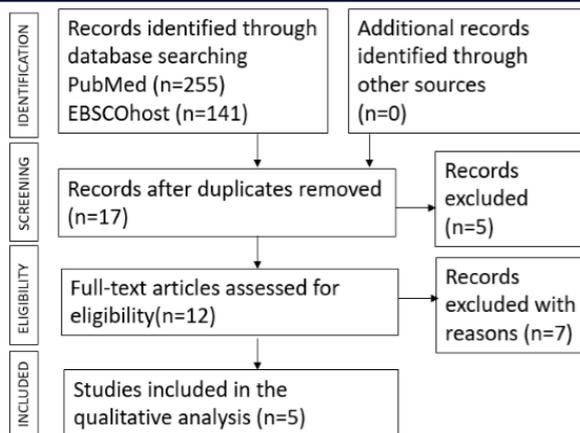


Figure 1: Prisma flow diagram

The initial electronic search provided 396 articles. Figure 1 is a flowchart summarizing the article selection process. After screening the titles and abstracts, 12 studies evaluating the antimicrobial effect of PC were identified. After review of the full text, 7 publications were excluded for the following reasons. In one study, Leukocyte-Platelet Rich Fibrin was modified using silver nanoparticles instead of using pure PRF²¹; two articles were systematic reviews.^{22,23} One publication was a letter to the editor²⁴, one was a scientific report²⁵ and the other two were reviews.^{26,27} Five articles fulfilled the inclusion criteria and were included in the present analysis. The included articles were published in a period ranging from 2016 to 2019. The articles adopted a broad range of variable outcomes and evaluated several microorganisms for assessing the antimicrobial potential of PRF.

Invitro studies

In vitro studies evaluating the antimicrobial activity of PRF are listed in Table 1. There was a considerable difference in experimental design among the included studies. Several bacterial types such as *P. gingivalis*, *A. actinomycetemcomitans*, *Staph. aureus*, *Staph. epidermis*, *P. intermedia* and *F. nucleatum* were tested. In two studies, microorganisms present in the supragingival plaque samples were evaluated. In all the studies blood samples were obtained from adult patients. PRF was procured using centrifugation following the protocol of Choukroun et al. and its antimicrobial activity was evaluated by microbial culturing. According to the classification proposed by Joseph Choukroun et al., three studies compared PRF with other platelet concentrates and whole blood, one study compared amongst the different centrifugation protocols of PRF and one used only injectable PRF.²⁸ Regarding quality assessment, all in vitro studies were classified at high risk of bias as they had more than four criteria being judged as inadequate. In general, the sample preparation and handling were always well described, the statistical analysis was mostly appropriate, but no details were provided regarding the sample size calculation, randomization procedures, allocation concealment and blinding, which might lead to some concern on the validity of the results.

Table 2: Table of in vitro characteristics

Author, year	Platelet concentrate	Micro-organisms tested	Results	Conclusion
Badade et al. 2016 ¹⁶	PRF/PRP	<i>P. g.</i> , <i>A.a</i>	<i>P.g</i> and <i>A.a</i> were inhibited by PRP but not by PRF.	PRP had greater inhibitory effect against periodontal pathogens compared to PRF
Karde et al., 2017 ¹⁷	iPRF/ PRF/ PRP/ WHOLE BLOOD	Supragingival plaque microorganism incubated aerobically on agar plates for 48h.	Mean zone of inhibition was highest in around i-PRF followed by PRF, and PRP.	i-PRF showed significant inhibitory effect on growth of oral bacteria in comparison to other PC
Kour et al, 2019 ¹⁸	PRP, PRF, and i-PRF	<i>P. g.</i> , <i>A. a</i>	<i>P. g.</i> : I-PRF had the widest zone of inhibition compared to PRF. Also PRP had significantly wider zone of inhibition against PRF. <i>A. a</i> : PRP had wider zone of inhibition which was significantly wider as compared to that of PRF and i-PRF	PRP and I-PRF have greater antimicrobial activity as compared to PRF
Jasmine et al, 2019 ¹⁹	i-PRF	<i>Staph. aureus</i> , <i>Staph. epidermis</i>	i-PRF exhibited bactericidal activity against both non biofilm and biofilm producing bacteria.	i-PRF could be used as an antimicrobial peptide to combat postoperative infections caused by staphylococcus.

Mamajiwala et al, 2019 ²⁰	PRF of different age groups & different centrifugation protocols	Supragingival plaque microorganisms incubated aerobically on agar plates for 48h.	Highest platelet count and antimicrobial activity in PRF membranes, was obtained at 1400 rpm for 8 min	Lower centrifugation speed & time can increase the platelet concentration & antimicrobial activity of the PRF membrane
--------------------------------------	--	---	--	--

DISCUSSION

Autologous Platelet Concentrates (APC) have been investigated widely over their regenerative potential but only few studies have investigated their antimicrobial potential. Furthermore, the available literature has considered the antimicrobial effects of APC's as whole and not PRF alone.

PRF contains an increased concentration of platelets that serve as a vehicle for delivery of growth factors to the surgical site such as platelet-derived growth factor, transforming growth factor, platelet factor 4, platelet-derived angiogenesis factor, vascular endothelial growth factor, epidermal growth factor, platelet-derived endothelial growth factor, epithelial cell growth factor, and insulin-like growth factor. Platelets have been demonstrated to have the ability to recognize, sequester and neutralize the invading pathogens, and indirectly contribute to recruit leukocytes to site of infection and inflammation thus enhancing their ability to phagocytose and kill microorganisms by triggering different types of signaling pathways.²⁹ Platelets are also involved in generating reactive oxygen species including superoxide, hydrogen peroxide, and hydroxyl-free radicals; and release of an array of antimicrobial peptides such platelet factor 4, regulated upon activation of normal T-cell expressed and secreted protein, connective tissue-activating peptide 3, platelet basic protein, thymosin beta-4, fibrinopeptide A, and fibrinopeptide contributing to the antimicrobial effect.^{30,31} There is presence of receptors on the platelets that are known to interact with bacteria thus participating in the antibody-dependent cell cytotoxicity and engulfment by entrapped white blood cells within PRF which could have a lethal action on bacteria.³² The various possible antimicrobial mechanisms of PRF are mentioned above.

The reason for only considering PRF was to eliminate the effects of the biochemical additives which could influence its antimicrobial potential. The aim of this systematic review was to gather the available preclinical evidence, through in vitro studies evaluating the antibacterial efficacy of PRF. The results of the invitro studies suggested that platelet concentrates may be effective in inhibiting the growth of a wide variety of oral microflora. However, when specific bacterial species were considered, the results were contradictory. Badade et al.¹⁶ reported inhibition of *P. gingivalis* and *A. actinomycetemcomitans* by PRP but not PRF. Kour P et al.,¹⁸ found maximum inhibitory effect against *P. gingivalis* by i-PRF followed by PRP and PRF and for *A. actinomycetemcomitans* PRP followed by PRF and i-PRF. Lower centrifugation speeds have shown to increase the platelet concentration and antimicrobial activity of PRF.²⁰ The discrepancies in the results may be due to types of platelet concentrates used, their form in which they have been used (liquid/gel), concentration of platelets, leukocytes; density of fibrin meshwork or due to intrinsic nature of the bacterial strains used that may exhibit a different susceptibility to different platelet concentrates. In studies comparing PRP and PRF, the discrepancies may be due to the usage of thrombin or calcium chloride in PRP.

Similar systematic reviews of in-vitro and animal studies have been conducted to investigate the antimicrobial properties of platelet concentrates. This study includes only PRF and its modifications amongst all the PC to exclude the effects of the biochemical processing which could alter their antimicrobial effect.

The results of this systematic review suggest that PRF has significant antimicrobial effects against oral pathogens. The CRIS guidelines, developed specially for experimental lab research were used for qualitative assessment of the studies. The results indicated that the studies were not conducted in conformity with the CRIS guidelines and hence the overall quality was considered poor with high risk of bias. Thus, the results of this study should be interpreted with caution and confirmed by further studies.

The limitations of this review are the high risk of bias present in all the included studies and the heterogeneity among the data with respect to the study aims, methodology, protocols and microbial species selected affecting the antimicrobial action of the PRF. Although the considered studies in the present review showed that PRF has considerable

antimicrobial properties, the diversities among the various study design and lack of standardization makes it difficult to draw definitive conclusions.

CONCLUSION

Thus, it can be established that PRF has antimicrobial properties and hence can be used to combat periodontal infections. Antibiotics in the form of systemic or local delivery systems have been used to treat periodontal infections but have a disadvantage of developing resistance. Thus, PRF can overcome this disadvantage if used as an adjunct by the virtue of its antimicrobial effect and with an additive benefit of regeneration.

Future research should be focused on determining the extents of its antimicrobial spectrum, role of other components of the PRF on its antimicrobial effects. Various other treatment modalities such as antibiotics in combination with PRF should be investigated to assess if there are any synergistic effects on its antimicrobial properties.

FUNDING SOURCES

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

- Hajishengallis, G. (2014). Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. *Trends in immunology*, 35(1), 3-11.
- Carranza FA, Newman MG, Takei HH, Klokkevoeld PR. Carranza's Clinical Periodontology, 10th Edition, Saunders Co., Lindo. (2006).
- Torrungruang, K., Jitpakdeebordin, S., Charatkulungku, O., & Gleeubua, Y. (2015). Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Treponema denticola/Prevotella intermedia co-infection are associated with severe periodontitis in a Thai population. *PLoS one*, 10(8), e0136646.
- Loesche, W. J., & Grossman, N. S. (2001). Periodontal disease as a specific, albeit chronic, infection: diagnosis and treatment. *Clinical microbiology reviews*, 14(4), 727-752.
- Burnouf, T., Chou, M. L., Wu, Y. W., Su, C. Y., & Lee, L. W. (2013). Antimicrobial activity of platelet (PLT)-poor plasma, PLT-rich plasma, PLT gel, and solvent/detergent-treated PLT lysate biomaterials against wound bacteria. *Transfusion*, 53(1), 138-146.
- Drago, L., Bortolin, M., Vassena, C., Taschieri, S., & Del Fabbro, M. (2013). Antimicrobial activity of pure platelet-rich plasma against microorganisms isolated from oral cavity. *BMC microbiology*, 13(1), 1-5.
- Miron, R. J., Fujioka-Kobayashi, M., Bishara, M., Zhang, Y., Hernandez, M., & Choukroun, J. (2017). Platelet-rich fibrin and soft tissue wound healing: a systematic review. *Tissue Engineering Part B: Reviews*, 23(1), 83-99.
- Ehrenfest, D. M. D., Rasmuson, L., & Albrektsson, T. (2009). Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leukocyte-and platelet-rich fibrin (L-PRF). *Trends in biotechnology*, 27(3), 158-167.
- Dohan Ehrenfest, D. M., de Peppo, G. M., Doglioli, P., & Sammartino, G. (2009). Slow release of growth factors and thrombospondin-1 in Choukroun's platelet-rich fibrin (PRF): a gold standard to achieve for all surgical platelet concentrates technologies. *Growth factors*, 27(1), 63-69.
- Ehrenfest, D. M. D., Diss, A., Odin, G., Doglioli, P., Hippolyte, M. P., & Charrier, J. B. (2009). In vitro effects of Choukroun's PRF (platelet-rich fibrin) on human gingival fibroblasts, dermal prekeratinocytes, preadipocytes, and maxillofacial osteoblasts in primary cultures. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 108(3), 341-352.
- Moojen, D. J. F., Everts, P. A., Schure, R. M., Overdeest, E. P., van Zundert, A., Knape, J. T., ... & Dhert, W. J. (2008). Antimicrobial activity of platelet-leukocyte gel against *Staphylococcus aureus*. *Journal of Orthopaedic Research*, 26(3), 404-410.
- Anitua, E., Andia, I., Ardanza, B., Nurdan, P., & Nurdan, A. T. (2004). Autologous platelets as a source of proteins for healing and tissue regeneration. *Thrombosis and haemostasis*, 91(01), 4-15.
- Anitua, E. (1999). Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. *International journal of Oral and maxillofacial Implants*, 14(4), 529-535.
- Marx, R. E., Carlson, E. R., Eichstaedt, R. M., Schimmele, S. R., Strauss, J. E., & Georgeff, K. R. (1998). Platelet-rich plasma: Growth factor enhancement for bone grafts. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 85(6), 638-646.
- Krithikadatta, J., Gopikrishna, V., & Datta, M. (2014). CRIS Guidelines (Checklist for Reporting In-vitro Studies): A concept note on the need for standardized guidelines for improving quality and transparency in reporting in-vitro studies in experimental dental research. *Journal of conservative dentistry: JCD*, 17(4), 301. Karde, P. A., Sethi, K. S.,
- Badade, P. S., Mahale, S. A., Panjwani, A. A., Vaidya, P. D., & Warang, A. D. (2016). Antimicrobial effect of platelet-rich plasma and platelet-rich fibrin. *Indian Journal of Dental Research*, 27(3), 300.
- Mahale, S. A., Khedkar, S. U., Patil, A. G., & Joshi, C. P. (2017). Comparative evaluation of platelet count and antimicrobial efficacy of injectable platelet-rich fibrin with other platelet concentrates: An in vitro study. *Journal of Indian Society of Periodontology*, 21(2), 97.
- Kour, P., Pudukalkatti, P. S., Vas, A. M., Das, S., & Padmanabhan, S. (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*. *Contemporary clinical dentistry*, 9(Suppl 2), S325.
- Jasmine, S., Thangavelu, A., Janarthanan, K., Krishnamoorthy, R., & Alshatwi, A. A. (2020). Antimicrobial and antibiofilm potential of injectable platelet rich fibrin—a second-generation platelet concentrate—against biofilm producing oral staphylococcus isolates. *Saudi journal of biological sciences*, 27(1), 41-46.

20. Mamajiwala, A. S., Sethi, K. S., Raut, C. P., Karde, P. A., & Mangle, N. M. (2020). Impact of different platelet-rich fibrin (PRF) procurement methods on the platelet count, antimicrobial efficacy, and fibrin network pattern in different age groups: an in vitro study. *Clinical oral investigations*, 24(5), 1663-1675.
21. Khorshidi, H., Haddadi, P., Raofi, S., Badiie, P., & Dehghani Nazhvani, A. (2018). Does adding silver nanoparticles to leukocyte-and platelet-rich fibrin improve its properties?. *BioMed research international*, 2018.
22. Varshney, S., Dwivedi, A., & Pandey, V. (2019). Antimicrobial effects of various platelet rich concentrates-vibes from in-vitro studies-a systematic review. *Journal of oral biology and craniofacial research*, 9(4), 299-305.
23. Fabbro, M. D., Bortolin, M., Taschieri, S., Ceci, C., & Weinstein, R. L. (2016). Antimicrobial properties of platelet-rich preparations. A systematic review of the current pre-clinical evidence. *Platelets*, 27(4), 276-285.
24. Cieslik-Bielecka, A., Gazdzik, T. S., Bielecki, T. M., & Cieslik, T. (2007). Why the platelet-rich gel has antimicrobial activity?. *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics*, 103(3), 303-5.
25. Castro, A. B., Herrero, E. R., Slomka, V., Pinto, N., Teughels, W., & Quirynen, M. (2019). Antimicrobial capacity of Leucocyte-and Platelet Rich Fibrin against periodontal pathogens. *Scientific reports*, 9(1), 1-8.
26. Cieslik-Bielecka, A., Dohan Ehrenfest, D. M., Lubkowska, A., & Bielecki, T. (2012). Microbicidal properties of leukocyte-and platelet-rich plasma/fibrin (L-PRP/L-PRF): new perspectives. *J Biol Regul Homeost Agents*, 26(2 Suppl 1), 43S-52S.
27. Bielecki, T., M Dohan Ehrenfest, D., A Everts, P., & Wiczowski, A. (2012). The role of leukocytes from L-PRP/L-PRF in wound healing and immune defense: new perspectives. *Current pharmaceutical biotechnology*, 13(7), 1153-1162.
28. Dohan, D. M., Choukroun, J., Diss, A., Dohan, S. L., Dohan, A. J., Mouhyi, J., & Gogly, B. (2006). 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), e37-e44.
29. Jenne, C. N., & Kubers, P. (2015). Platelets in inflammation and infection. *Platelets*, 26(4), 286-292.
30. Tang, Y. Q., Yeaman, M. R., & Selsted, M. E. (2002). Antimicrobial peptides from human platelets. *Infection and immunity*, 70(12), 6524-6533.
31. Joshi, C. P., Patil, A. G., Karde, P. A., Khedkar, S. U., & Arunkum, S. (2021). Autologous platelet rich fibrin as a potential antiperiopathogenic agent: An in-vitro study. *IP International Journal of Periodontology and Implantology*, 1(2), 50-54.
32. Yeaman, M. R. (2014). Platelets: at the nexus of antimicrobial defence. *Nature Reviews Microbiology*, 12(6), 426-437.