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In vitro Evaluation of the antibacterial activity of Platelet-rich Plasma against Selected Oral and Periodontal Pathogens

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ABSTRACT

Background: Despite the availability of a wide range of antibiotics, bacterial infections are among important challenges for the healthcare system. Therefore, the introduction of new antimicrobial preparations such as platelet-rich plasma (PRP) could be suggested theoretically as a preventive measure for bacterial infections. We aimed to evaluate the in vitro antibacterial activity of PRP against three common oral bacteria.
Methods: In vitro antibacterial activity of PRP against Streptococcus Mitis, Streptococcus Mutans, and Neisseria Lactamica as three common oral/dental bacteria was determined by disc diffusion method. Baseline antibacterial activity was assessed by measuring the diameter zone of inhibition after 24 hours of incubation at 37 °C.
Results: PRP had strong in vitro antibacterial activity against *Streptococcus Mitis*, *Streptococcus Mutans* and *Neisseria Lactamica* with the mean zone of inhibition diameters of 6.73±0.52, 5.8±0.43 and 6.67±0.43 mm, respectively.
Conclusion: PRP is an effective antibacterial agent along with conventional antibiotic treatments against oral and periodontal infections.

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Introduction

The practice of platelet-derived preparations has spread to various medical fields such as dentistry, maxillofacial surgery, orthopedics, sport medicine, ophthalmology, dermatology, and cosmetics. Platelets, aside from their role in coagulation and hemostasis, have other useful properties including antimicrobial effects and healing properties.[1-6] The healing properties of platelets are related to the release of several growth factors.[7-10] While their antimicrobial activities can be attributed to multiple factors such as release of platelet microbicidal proteins (PMPs), generation of cytotoxic oxygen metabolites and free radicals, direct interaction with microorganisms, modulation of complement activation and augmentation of leukocyte activities.[11-14]

Since the prevalence of bacterial infections is one of the major issues in the field of dentistry and on the other hand, routine antibiotic administration should be limited in oral surgery,[15] the introduction of new antimicrobial products is worthwhile.[3, 16-21]

Assuming that platelet-rich plasma (PRP) could be considered as an important antibacterial preparation due to its high platelet content, autogenous origin, biocompatibility, safety profile and ease of preparation,[3, 22] this study was conducted to evaluate the *in vitro* antimicrobial effect of PRP against three common oral bacteria.

Materials and Methods

Bacteria and Preparation of Inoculums

Streptococcus Mitis (ATCC: 6249) and *Streptococcus Mutans* (ATCC: 35668) as gram positive bacteria and *Neisseria Lactamica* (ATCC: 23970) as a gram negative

bacterium were selected for this observational study. The bacterial strains were obtained from Pasteur institute (Tehran, Iran) and maintained on Brain heart infusion (BHI) at 4 °C, at Islamic Azad University laboratory. To prepare inoculums of bacteria culture, the stock culture from BHI was sub-cultured on blood agar (Merck, Germany) and incubated over night at 37 °C. Then a suspension of freshly grown bacteria in sterile distilled water was prepared for each strain with an optical density equal to 0.5 McFarland (1×10^8 CFU/mL).

Platelet-Rich Plasma Preparation

Each of 10 random PRPs was obtained from Tehran Blood Transfusion Center on the day of experiment. PRPs, prepared from whole blood of healthy blood donors using platelet-rich plasma method,[23] were stored and shipped at 20 to 24ºC along with continuous agitation during storage.

Determination of Antibacterial Activity

In vitro laboratory susceptibility to PRP was determined by disc diffusion method [24] on blood agar. For this purpose, agar plates were coated with one of the bacterial strains including *Streptococcus Mitis* or *Streptococcus Mutans* as gram positive bacteria and *Neisseria Lactamica* as gram negative bacterium. Then standard 6 mm discs soaked with PRP, positive and negative controls were placed on the coated agar media. The inoculated agar plates were then incubated at 37 °C for 24 hours. Then antimicrobial activity was assessed by measuring the diameter zone of inhibition after 24 hours of incubation at 37 °C and results were expressed as mean±SD. It should be noted that the assay was performed in triplicate for each strain and Co-amoxiclav and Ceftriaxone were used in all assays as positive control for Gram-positive and Gram-negative bacteria, respectively. Blood broth was used as a negative control.

Results

As shown in Table 1 and Figure 1, PRP had antibacterial activity against *Streptococcus Mitis*, *Streptococcus Mutans* and *Neisseria Lactamica* with the mean diameter zone of inhibition of 6.73±0.52, 5.8±0.43 and 6.67±0.43 mm, respectively.

Discussion

Despite the availability of a wide range of antibiotics, bacterial infections remain as an important challenge for the healthcare system. This is mainly due to increasing rate of antibiotic resistance and adverse effects of antibiotics.[15-21, 25] Therefore, it has recently been attempted to overcome these issues by using alternative methods such as PRP technology which utilizes the antimicrobial properties of PRP and results in less microbial resistance.

Based on the positive results of several conducted studies,[1, 3, 26-28] the antimicrobial properties of the PRP can be due to the release of platelet microbicidal proteins (PMPs), direct interaction of platelets with microorganisms, generation of antimicrobial oxygen metabolites, modulation of complement activation and augmentation of the antimicrobial functions of leukocytes.[1, 2, 11, 12, 27] Moreover, the anti-inflammatory and healing-promoting properties of PRP may have a synergistic effect on infection prevention.[3]

Oral infections and periodontal diseases have been implicated in the pathogenesis of several chronic systemic diseases.[29] On the other hand, the antimicrobial properties of PRP against some periodontal pathogens such as *Enterococcus faecalis*, *Candida albicans*, *Streptococcus agalactiae*, *Streptococcus oralis*, *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* have already been studied and proven.[1, 30, 31] Therefore, this study was designed to determine the *in vitro* antibacterial activity of PRP against *Streptococcus Mitis*, *Streptococcus Mutans* and *Neisseria Lactamica* as

Table 1: Zones of inhibition, exerted by platelet-Rich Plasma, positive control and negative control against three bacteria after 24 hours of incubation

Bacteria / Sample	*Streptococcus Mitis*	*Streptococcus Mutans*	*Neisseria Lactamica*
Platelet-Rich Plasma	6.73±0.52 mm	5.8±0.43 mm	6.67±0.43 mm
Positive Control	8.6±0.99 mm	10±2.1 mm	16±0.81 mm
Negative Control	-	-	-

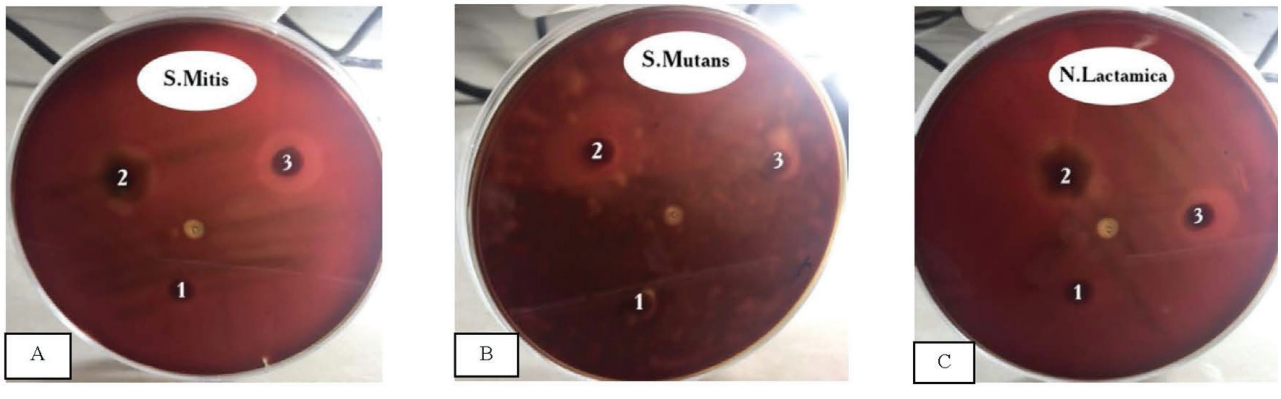

Figure 1: The zones of inhibition exerted by negative control (1), positive control (2) and Platelet-Rich Plasma (3) against *Streptococcus Mitis* (A), *Streptococcus Mutans* (B) and *Neisseria Lactamica* (C), after 24 hours of incubation at 37 °C.

three common oral and dental bacteria.[32-35] Based on the results of this study, PRP has strong *in vitro* antimicrobial properties against aforementioned bacteria comparable to co-amoxiclav and ceftriaxone. However, since this study was limited to *in vitro* evaluations of ATCC bacterial strains, performing *in vivo* evaluations of the clinical isolates is recommended.

Conclusion

PRP, a biocompatible product can be considered as an effective antibacterial preparation with activity against oral and periodontal infections. However, further *in vitro* and *in vivo* studies using clinical isolates are needed to assess whether the antimicrobial effects of PRP could have positive effects against other periodontal pathogens.

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Conflict of Interest: None declared.

References

1. Drago L, Bortolin M, Vassena C, Taschieri S, Del Fabbro M. Antimicrobial activity of pure platelet-rich plasma against microorganism isolated from oral cavity. BMC Microbiol. 2013; 13: 47-51. doi: 10.1186/1471-2180-13-47. PubMed PMID: 23442413. PubMed Central PMCID: PMC3599521.
2. Burnouf T, Chou ML, Wu YW, Su CY, Lee LW. Antimicrobial activity of platelet (PLT)-poor plasma, PLT-rich plasma, PLT gel, and solvent/detergent-treated PLT lysate biomaterials against wound bacteria. Transfusion.2013; 53(1):138-46.doi: 10.1111/j.1537299 5.2012.03668.x. PubMed PMID: 22563709.
3. Li H, Li B. PRP as a new approach to prevent infection: Preparation and in vitro antimicrobial properties of PRP. J Vis Exp. 2013; 74: e50351. doi: 10.3791/50351. PubMed PMID: 23609458.
4. YuanT, Zhang CQ, Tang MJ, Guo SC, Zeng BF. Autologous platelet-rich plasma enhances healing of chronic wounds. Wounds. 2009; 21(10):280–5. PubMed PMID: 25902712.
5. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost. 2004; 91(1):4-15. doi: 10.1160/TH03-07-0440. PubMed PMID: 14691563.
6. Hom DB, Linzie BM, Huang TC. The healing effects of autologous platelet gel on acute human skin wounds. Arch Facial Plast Surg. 2007; 9(3):174-83. doi: 0.1001/archfaci.9.3.174. PubMed PMID: 17519207.
7. El-Sharkawy H, Kantarci A, Deady J, Hasturk H, Liu H, Alshahat M, et al. Platelet-rich plasma: growth factors and pro- and anti-inflammatory properties. J Periodontol. 2007; 78(4):661–9. doi: 10.1902/jop.2007.060302. PubMed PMID: 17397313.
8. Assoian RK, Grotendorst GR, Miller DM, Sporn MB. Cellular transformation by coordinated action of three peptide growth factors from human platelets. Nature. 1984; 309 (5971):804-6. PubMed PMID: 6330562.
9. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev. 2003; 83(3):835-70. doi: 10.1152/physrev.2003.83.3.835. PubMed PMID: 12843410.
10. Toscano N, Holtzclaw D. Surgical considerations in the use of platelet-rich plasma. Compend Contin Educ Dent. 2008; 29(3):182–5. PubMed PMID: 18468304.
11. Tang YQ, Yeaman MR, Selsted ME. Antimicrobial peptides from human platelets. Infect Immun. 2002; 70(12): 6524–33. doi: 10.1128/IAI.70.12.6524-6533.2002. PubMed PMID: 12438321. PubMed Central PMCID: PMC132966.
12. Yeaman MR. The role of platelets in antimicrobial host defense. Clin Infect Dis. 1997; 25(5):951-68; quiz 969-70. PubMed PMID: 9402338.
13. The generation of free radicals by platelets. In: Immunopharmacology of platelets. Joseph M, ed. London: Academic Press; 1995. pp. 209-225.
14. Dziarski R, Gupta D. Mammalian PGRPs: novel antibacterial proteins. Cell Microbiol. 2006; 8(7):1059-69. doi: 10.1111/j.1462-5822.2006.00726.x. PubMed PMID: 16819960.
15. Asenjo-Lobos C, Jofre J, Cortés-Jofré M, Manterola C. Use of antibiotics in dental implant surgery: a decision based on evidence from systematic review. Int J Odontostomat. 2015; 9(1):137-47. doi: 10.4067/S0718-381X2015000100021.
16. Laxminarayan R, Matsoso P, Pant S, Brower C, Rottingen JA, Klugman K, et al. Access to effective antimicrobials: a worldwide challenge. Lancet 2016; 387(10014): 168-75. doi: 10.1016/S0140-6736(15)00474-2. PubMed PMID: 26603918.
17. Balsalobre LC, Dropa M, Matté MH. An overview of antimicrobial resistance and its public health significance. Braz J Microbiol. 2014; 45(1):1-5. doi: 10.1590/S1517-83822014005000033. PubMed PMID: 24948906. PubMed Central PMCID: PMC4059282
18. Maragakis LL, Perencevich EN, Cosgrove SE. Clinical and economic burden of antimicrobial resistance. Expert Rev Anti Infect Ther. 2008; 6(5):751–63. doi: 10.1586/14787210.6.5.751. PubMed PMID: 18847410.
19. World Health Organization. WHO global strategy for containment of antimicrobial resistance [Internet]. Geneva: WHO; 2001 [cited 2010 Jun 17]. Available from: http://www.who.int/csr/resources/publications/drugresist/en/EGlobal_Strat.pdf.
20. Carlet J, Jarlier V, Harbarth S, Voss A, Goossens H, Pittet D. Ready for a world without antibiotics? The Pensieres antibiotic resistance call to action. Antimicrob Resist Infect Control. 2012; 1:11. doi: 10.1186/2047-2994-1-11. PubMed PMID: 22958833. PubMed Central PMCID: PMC3436635.
21. Andersson DI, Hughes D. Persistence of antibiotic resistance in bacterial populations. FEMS Microbiol Rev. 2011; 35(5): 901–11. doi: 10.1111/j.1574-6976.2011.00289.x. PubMed PMID: 21707669.

22. Lopez-Vidriero E, Goulding KA, Simon DA, Sanchez M, Johnson DH. The use of platelet-rich plasma in arthroscopy and sports medicine: optimizing the healing environment. Arthroscopy. 2010; 26(2):269-78. doi: 10.1016/j.arthro.2009.11.015. PubMed PMID: 20141991.
23. Fung MK, Grossman BJ, Hillyer CD, Westhoff CM. Technical Manual, 18TH ed. Bethesda, Maryland: American Association of Blood Banks (AABB); 2014.
24. Black JG. Microbiology Principles and Exploration textbook. 5th ed. USA: John Wiley and Sons Inc.; 2002. p. 137-52.
25. Ahmad N, Saad N. Effects of Antibiotics on Dental Implants: A Review. J Clin Med Res. 2012; 4(1):1–6. doi: 10.4021/jocmr658w. PubMed PMID: 22383920. PubMed Central PMCID: PMC3279494.
26. Jafarzadeh E, Pourmokhtar M, Tavili S. In Vitro evaluation of the anti-bacterial effect of human platelet concentrate. IJBC. 2016; 8 (1):5-8.
27. Anitua E, Alonso R, Girbau C, Aguirre JJ, Murozabal F, Orive G. Antibacterial effect of plasma rich in growth factors (PRGF-Endoret) against Staphylococcus aureus and Staphylococcus epidermidis strains. Clin Exp Dermatol. 2012; 37(6):652-7.doi: 10.1111/j.1365-2230.2011.04303.x. PubMed PMID: 22329713.
28. Intravia J, Allen DA, Durant TJ, McCarthy MB, Russell R, Beitzel K, et al. In vitro evaluation of the anti-bacterial effect of two preparations of platelet rich plasma compared with cefazolin and whole blood. Muscles Ligaments Tendons J. 2014; 4(1):79-84. PubMed PMID: 24932452; PubMed Central PMCID: PMC4049655.
29. Lee A, Wang HL. Biofilm related to dental implants. Implant Dent. 2010; 19:387–93. doi: 10.1097/ID.0b013e3181effa53.
30. Aggour RL, Gamil L. Antimicrobial Effects of Platelet-rich Plasma against Selected Oral and Periodontal Pathogens. Pol J Microbiol. 2017; 66(1):31-37. doi: 10.5604/17331331.1235227. PubMed PMID: 29359696.
31. Yang LC, Hu SW, Yan M, Yang JJ, Tsou SH, Lin YY. Antimicrobial activity of platelet-rich plasma and other plasma preparations against periodontal pathogens. J Periodontol. 2015; 86(2):310-8. doi: 10.1902/jop.2014.140373. PubMed PMID: 25345340.
32. Zheng W, Tan TK, Paterson IC, Mutha NVR, Siow CC, Tan SY, et al. StreptoBase: An oral streptococcus mitis group genomic resource and analysis platform. PLoS One. 2016; 11(5):e0151908. doi: 10.1371/journal.pone.0151908. PubMed PMID: 27138013. PubMed Central PMCID: PMC485445.
33. Metwalli KH, Khan SA, Krom BP, Jabra-Rizk MA. Streptococcus mutans, candida albicans, and the human mouth: A sticky situation. PLoS Pathog. 2013; 9(10):e1003616. doi: 10.1371/journal.ppat.1003616.
34. van Houte J, Lopman J, Kent R. The predominant cultivable flora of sound and carious human root surfaces. J Dent Res. 1994; 73(11):1727-34. doi: 10.1177/00220345940730110801. PubMed PMID: 7983259.
35. Everts RJ, Speers D, George ST, Ansell BJ, Karunajeewa H, Ramos RD. Neisseria lactamica Arthritis and Septicemia Complicating Myeloma. J Clin Microbiol. 2010; 48(6):2318. doi: 10.1128/JCM.02132-09. PubMed PMID: 20410341. PubMed Central PMCID: PMC2884484.