ANTIMICROBIAL EFFECT OF ADVANCED PLATELET-RICH FIBRIN PLUS AGAINST METHICILLIN-SUSCEPTIBLE AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*: AN *IN VITRO* STUDY

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ABSTRACT

Objectives: The virulence of methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) differs significantly; however, the antimicrobial effects of advanced platelet-rich fibrin plus (A-PRF+) on these subspecies remain unclear. This study aimed to evaluate the efficacy of A-PRF+ against MSSA and MRSA.

Methods: Fifteen male participants volunteered for this study. Solid and liquid forms of A-PRF+ were produced using the DUO Quattro centrifuge machine following the recommended protocol. The inoculum of MSSA and MRSA was prepared from reference samples obtained from the American Type Culture Collection. Both inocula were adjusted to a McFarland standard of 0.5. The antimicrobial effects of A-PRF+ against MSSA and MRSA were evaluated using disk diffusion assays, minimum inhibitory concentration (MIC) tests, and biofilm formation experiments.

Results: The disk diffusion assay demonstrated weak antimicrobial activity against both MSSA and MRSA, with inhibition zones measuring 0.68 ± 0.44 mm and 0.69 ± 0.35 mm, respectively. However, MIC testing revealed that A-PRF+ did not exhibit antimicrobial effects against either subspecies following dilution. Finally, A-PRF+ significantly reduced the biofilm-forming capacity of both MSSA and MRSA to approximately 70%.

Conclusion: A-PRF+ exhibited weak antimicrobial activity against both MSSA and MRSA in agar diffusion assays. Additionally, A-PRF+ reduced the biofilm-forming capacity of both MSSA and MRSA. However, no significant differences were detected in the antimicrobial effects of A-PRF+ between MSSA and MRSA.

Keywords: *platelet-rich fibrin; antimicrobial agent; in vitro techniques; methicillin-susceptible staphylococcus aureus; methicillin-resistant staphylococcus aureus.*

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I. INTRODUCTION

Oral surgeries, such as surgical tooth extractions, alveoloplasty, and implant surgeries, are becoming increasingly common in dental practice. These procedures are classified as minor to moderately invasive interventions affecting both soft and hard tissues and carry a potential risk of infected wounds (1). Such infections not only delay healing but can also progress to more severe complications, including fascial space infections and septicemia. Antibiotics are commonly prescribed by oral surgeons to prevent oral infections. However, while antibiotic prophylaxis is both relevant and cost-effective, it is associated with adverse effects, including disruption of the resident microbiome, nausea, stomach discomfort,

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and limited efficacy in completely preventing post-surgical oral infections.

One of the most prominent bacteria associated with postsurgical infections in the oral cavity is *Staphylococcus aureus*. This facultative anaerobic, Gram-positive coccus forms biofilms, which enhance its survival under unfavorable conditions. Methicillin-resistant *Staphylococcus aureus* (MRSA) is notably more virulent than methicillin-susceptible *Staphylococcus aureus* (MSSA) due to its resistance to β-lactam antibiotics. Recent studies have reported an increasing prevalence of MRSA in oral infections (2).

Advanced platelet-rich fibrin plus (A-PRF+) is widely recognized for its superior efficacy in promoting oral wound healing. It is extensively utilized in various dental procedures, including wisdom tooth extractions, treatment of osteonecrosis of the jaw, and bone regeneration (3). Several case reports have indicated that grafting A-PRF+ may also reduce post-surgical oral infections. Consequently, the antimicrobial activity of A-PRF+ against specific microorganisms has gained increasing attention. However, most existing studies have concentrated on periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Pseudomonas aeruginosa* (4). To our knowledge, limited research has examined the effects of A-PRF+ on *Staphylococcus aureus*, notably methicillin-resistant *Staphylococcus aureus* (MRSA).

In this *in vitro* study, we aimed to investigate the antimicrobial activity of A-PRF+ against MSSA and MRSA isolated strains that were obtained from the American Type Culture Collection (ATCC). The antimicrobial potential was assessed using agar diffusion assays, minimum inhibitory concentration (MIC) testing, and biofilm formation experiments.

II. MATERIALS AND METHODS

From November 2022 to July 2023, this *in vitro* study was carried out. Fifteen healthy male adults volunteered to participate in this study. The inclusion criteria were as follows: (1) age between 20 to 65 years; (2) no medical history of hematologic diseases; (3) non-smokers; (4) no use of antibiotics and antithrombotic medication within the past three months; and (5) absence of infection. Participants with abnormal platelet or leukocyte counts in blood tests were excluded. Before enrollment, the purpose of the study was verbally explained to the participants, and signed informed consent was obtained from all participants.

MRSA (ATCC 6518) and MSSA (ATCC 33591) strains were used in this study. The bacteria were grown on Luria-Bertani agar (1.5%) under aerobic conditions at 37°C. After 24 h of incubation, bacterial colonies were collected and diluted in 1X phosphate-buffered saline (PBS; Thermo Fisher Scientific, Waltham, MA, USA) to a McFarland standard of 0.5, which corresponds to approximately 1.5×10^8 colony-forming units (CFU)/mL. These inocula were then used for agar diffusion, minimum inhibitory concentration (MIC), and biofilm formation assays.

A-PRF+ was prepared according to the protocol described by Fujioka-Kurobayashi et al (5). For each participant, 20 mL of venous blood was drawn into one A-PRF+ tube (red cap) and one S-PRF tube (green cap) to produce solid and liquid A-PRF+, respectively. The tubes were centrifuged at 1,300 rpm for 8 min using a DUO Quattro centrifuge (Process for PRF, Nice, France).

Subsequently, the A-PRF+ was collected and used in the following experiments.

The solid A-PRF+ was removed from the A-PRF+ tubes using sterile tweezers and scissors. The A-PRF+ mass was gently pressed with standard equipment to obtain the A-PRF+ membrane. The membrane was then cut into two equal parts and placed on 90 mm Luria-Bertani agar plates (Merck KGaA, Darmstadt, Germany) pre-coated with 100 µL of MSSA or MRSA inocula (1.5×10^8 CFU/mL). The plates were incubated overnight under aerobic conditions at 37°C. Paper disks soaked in PBS served as control group. After 24h of incubation, the plates were photographed with a standard ruler for scale. ImageJ software (version 1.53; University of Wisconsin, Madison, WI, USA) was used to to measure the widths of the inhibition zones in the recorded images. This assay protocol followed the standard guidelines provided by the American Society for Microbiology. Figure 1 shows the procedure of the agar diffusion assay.

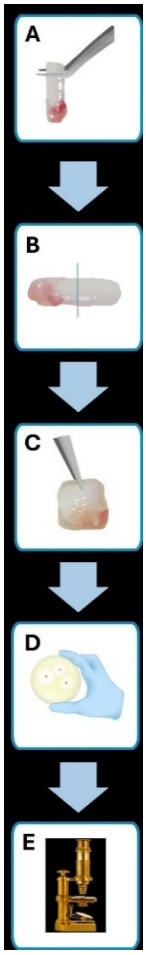

Figure 1. ***The protocol of agar diffusion assay.*** *A. Remove A-PRF+ from the tube with sterile tweezer. B. Cut A-PRF+ into two equal parts. C. Place two halves of A-PRF+ on the agar plate. D. Collect the images of agar diffusion assay after incubation. E. Analyze the images with Image J software.*

The minimum inhibitory concentration (MIC) test was conducted using the resazurin assay method. MSSA and MRSA were incubated in Tryptone Soya Broth (TSB; Thermo Fisher Scientific, Waltham, MA, USA) medium overnight at 37°C. The bacterial inocula were then diluted to a concentration of 2×10^5 CFU/mL. A 96-well plate containing A-PRF+ liquid was prepared with two-fold serial dilutions ranging from 1:2 to 1:64 of the original concentration. The final volume of A-PRF+ in each well was adjusted with PBS 1X to 100 µL before adding the bacterial inocula. Then, 20 µL of MSSA or MRSA inocula was added to each A-PRF+ well. The total liquid volume in each well was 120 µL. Chlorhexidine 2% (CanalPro, Coltene, Altstätten, Switzerland) was used as the positive control, while MSSA and MRSA without A-PRF+ served as negative controls. The plates were incubated overnight at 37°C. Then, 20 µL of resazurin (Thermo Fisher Scientific, Waltham, MA, USA) was added to each well and incubated at 37°C for 24h. A color change from blue to pink indicated bacterial growth, while no color change signified inhibition by A-PRF+. The procedure of the MIC test is illustrated in Figure 2.

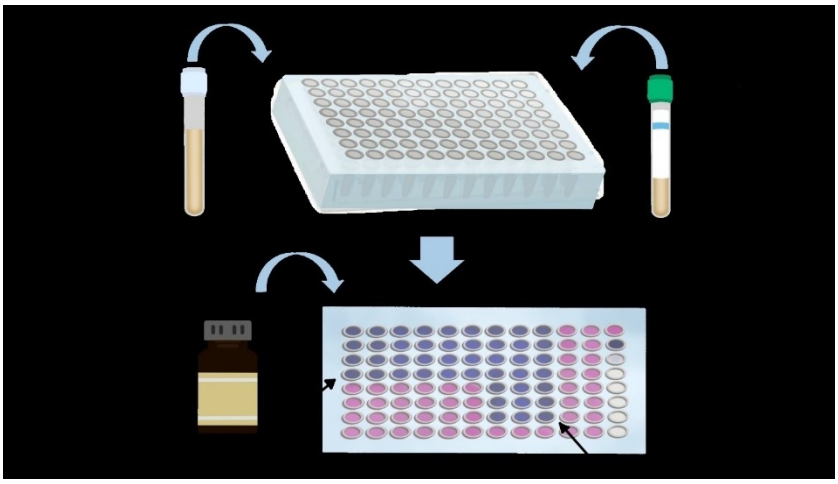

Figure 2. ***The protocol of minimum inhibitory concentration test.*** *A. The appropriate volumes of A-PRF+ and PBS were used to achieve the two-fold serial dilutions ranging from 1:2 to 1:64 of the original concentration. B. After overnight incubation, resazurin was added to the 96-well plate. The phenomenon of changing resazurin color from blue to pink indicates bacteria growth.*

The experiment was conducted in a 96-well plate to evaluate the efficiency of A-PRF+ in inhibiting the biofilm formation of *Staphylococcus aureus* (Figure 3). Each well contained 100 µL of liquid comprising 10 µL of MSSA or MRSA bacterial broth and 90 µL of TSB with 1% glucose. Subsequently, 100 µL of A-PRF+, 2% chlorhexidine, or TSB with 1% glucose was added to the experimental, positive control, and negative control wells, respectively. The final volume in each well was 200 µL. The plates were incubated at 37°C for 24h. After incubation, the medium was removed from each well, and the plate was washed three times with distilled water to remove non-adherent bacteria. Finally, the plate was dried in a cabinet at 37°C.

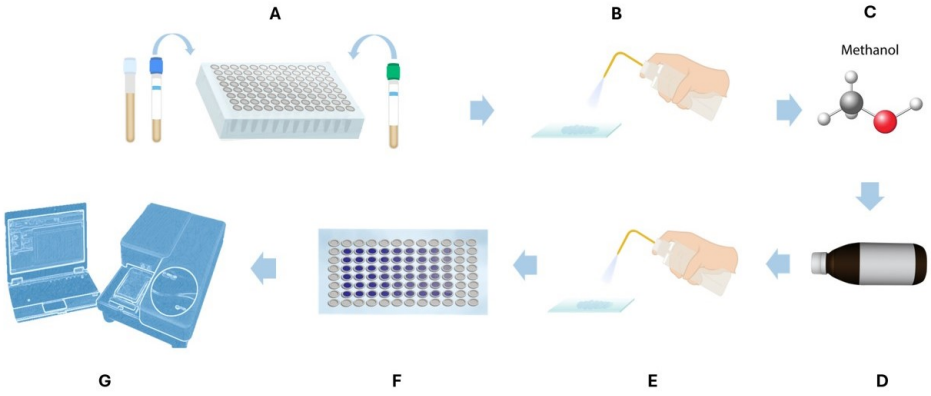

Figure 3. ***The protocol of biofilm formation experiment.*** *A. A-PRF+ and bacterial inocula were incubated in the appropriate condition. B. The 96-well plate was washed to remove the remaining bacteria. C. The bacterial biofilm was fixed with methanol 99%. D. 0.1% crystal violet was added to stain biofilm. E. Excessive staining medium was removed. F. The final stained 96-well plate. G. EZ Read 400 ELISA Reader (Biochrom, Holliston, MA, USA) was used to measure optical density.*

Next, each well was filled with 200 µL of 99% methanol (Merck KGaA, Darmstadt, Germany) and incubated at room temperature for 20 min. The methanol was then removed, and the wells were air-dried for 15 min. Subsequently, 200 µL of 0.1% crystal violet (Sigma–Aldrich, St. Louis, MO, USA) was added to each well to stain the biofilms. After waiting for 15 min, the wells were cleansed with distilled water and immersed in 200 µL of 95% ethanol (Merck KGaA, Darmstadt, Germany) for 10 min. Finally, 150 µL of 33% glacial acetic acid (Merck KGaA, Darmstadt, Germany) was added to each well, and the optical density at 570 nm was measured using an EZ Read 400 ELISA Reader (Biochrom, Holliston, MA, USA).

Statistical analyses were performed using JASP (version 0.19.0; University of Amsterdam, Amsterdam, Netherlands). The Wilcoxon signed-rank test was used to compare the widths of inhibition zones and the percentage of the incubated well surface between the A-PRF+ group and control groups, as well as between the MSSA and MRSA groups. A p-value < 0.05 was considered statistically significant.

III. RESULTS

Fifteen male adults with an average age of 27.88 ± 0.78 (years old) volunteered to participate in the study. The complete blood count of the participants varied within normal ranges. The average number of platelets was 285.00 ± 36.83 (x 10^3 cells/mm^3). The average number of leukocytes was 5.68 ± 1.23 (x 10^3 cells/mm^3).

The results of the agar diffusion assay (Figure 4) showed that A-PRF+ can produce antimicrobial effects against *Staphylococcus aureus*. The widths of the inhibition zones of A-PRF+ against MSSA and MRSA were 0.68 ± 0.44 and 0.69 ± 0.35 mm, respectively. The Wilcoxon signed-rank test showed that the widths of inhibition zones of A-PRF+ against MSSA and MRSA were not significantly different with $p = 0.575$. No inhibition zones was noted in the control group.

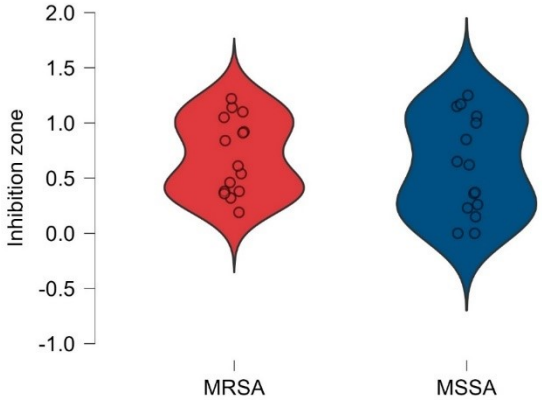

Figure 4. ***The widths of inhibition zones of A-PRF+ against MSSA and MRSA.***

The color of the resazurin changed from blue to pink after incubation. Thus, A-PRF+ did not show antimicrobial activity against either MSSA or MRSA after dilution.

Figure 5 shows the percentage of the incubation well surface covered by the *Staphylococcus aureus* biofilm. The experimental results revealed a significant reduction in biofilm formation by MSSA and MRSA on the well surface when incubated with A-PRF+ with the percentage of 66.78 ± 16.45 and 70.72 ± 20.77, respectively. Moreover, the antimicrobial effects of A-PRF+ against MSSA and MRSA are similar (Wilcoxon signed-rank test, p = 0.034). The MSSA and MRSA biofilm formation covered on all of the well surface (100%) in the control group.

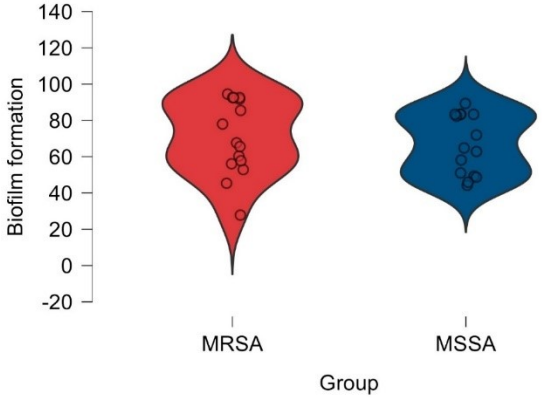

Figure 5. ***The percentage of well surface that was covered by MSSA and MRSA biofilm after incubating with A-PRF+.***

IV. DISCUSSION

Recently, the antimicrobial potential of the PRF family has garnered significant interest due to its autologous origin, healing properties, and instantaneous availability. Previous studies have evaluated the antimicrobial effects of PRF against several common bacterial species, including *Staphylococcus aureus*, *Escherichia coli*, and *Fusobacterium nucleatum* (4). However, none of these studies have specifically examined the antimicrobial capacity of A-PRF+ against MRSA or compared it with MSSA under the same conditions. The findings of this study revealed that A-PRF+ exhibits weak and comparable antimicrobial activity against MSSA and MRSA, as indicated by the limited width of the inhibition zones and the reduction of bacterial adhesion.

A-PRF+ is an advanced product of the platelet-rich fibrin family, containing approximately 2.3 times more platelets and 1.3 times more leukocytes than whole blood. The high concentration of these cells is hypothesized to enhance the antimicrobial effect of A-PRF+, particularly against *Staphylococcus aureus*. Previous studies have shown that *Staphylococcus aureus* can bind to and activate platelets most effectively through staphylococcal proteins and peptidoglycan. Following this activity, platelets generate reactive oxygen species and release platelet-derived microbicidal proteins (PMPs) and β-defensin. Notably, PMPs exhibit antimicrobial activity against *Staphylococcus aureus* that differs from other peptides.[6] These PMPs disrupt the microbial cytoplasmic membrane and significantly influence the binding affinity between platelets and *Staphylococcus aureus* strains. Platelets can also enhance the antimicrobial potential of leukocytes, which are among the most critical cells responding to bacterial invasion. The primary and secondary granules of neutrophils release various antimicrobial peptides, enzymes, and proteins that interact with bacteria. Previous studies have demonstrated that phospholipase A2, calprotectin, and α-defensin support host immune responses against *Staphylococcus aureus* by inhibiting bacterial growth or upregulating selective cytokines.[7-9] Therefore, the authors hypothesize that the antimicrobial activity of A-PRF+ against *Staphylococcus aureus* results from the synergistic effects of concentrated platelets and leukocytes.

The results of this study showed that healthy human A-PRF+ exhibited limited antimicrobial effects against both MSSA and MRSA, as indicated by the small inhibition zones. Additionally, A-PRF+ reduced the capacity for biofilm formation by both MSSA and MRSA. These findings align with previous studies reporting weak antimicrobial activity of A-PRF+ against *Staphylococcus aureus*.[10-15] Notably, Straub et al. observed no inhibition zone when examining A-PRF+ without supplemented antibiotics in an agar diffusion test.[16] Although A-PRF+ and other members of the PRF family often exhibit significant antimicrobial activity against tested oral bacteria, such as *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans*, their efficacy against *Staphylococcus aureus* appears to be limited.[4] *Staphylococcus aureus* is one of the most virulent bacteria, with a more rapid and saturable binding capacity to platelets than other species.[6] Moreover, the thick membrane structure composed of layers of peptidoglycan protects this Gram-positive bacterium from antimicrobial agents like A-PRF+. Biofilm formation is another critical factor that enhances the toxicity and invasiveness of *Staphylococcus aureus*. The results of this study showed that A-PRF+ could reduce biofilm formation by both MSSA and MRSA. Jasmine et al. (2020) reported similar findings that *Staphylococcus aureus* obtained from clinical patients with oral abscesses could be inhibited the antibiofilm activity by i-PRF.[10]

Interestingly, the antimicrobial activity of A-PRF+ against MSSA and MRSA was not significantly different across the three experiments. MRSA exhibits resistance to methicillin antibiotics due to the presence of the *mecA* gene.[17] This gene encodes transpeptidase penicillin-binding protein 2a, which reduces the affinity of *Staphylococcus aureus* for β-lactam antibiotics. Consequently, the antimicrobial activity of A-PRF+ does not appear to be influenced by this gene expression. As a result, A-PRF+ demonstrated comparable antimicrobial effects against both MSSA and MRSA *in vitro*.

This study was conducted using standard *Staphylococcus aureus* colonies provided by

the ATCC. The experimental protocols were adapted from official guidelines to ensure that the in vitro results are relevant to *in vivo* or clinical conditions. However, several limitations should be addressed in future studies. Notably, A-PRF+ should be standardized before further evaluation of its antimicrobial potential. Based on this hypothesis, platelet and leukocyte numbers may play vital roles in the antimicrobial effects of A-PRF+. Therefore, future studies should quantify platelet and leukocyte counts or use A-PRF+ samples of consistent size to address this issue. Additionally, the sample size in this study was relatively small. Future research with a larger and more diverse participant pool will provide more reliable results for clinical application.

V. CONCLUSIONS

Within the limitations of this *in vitro* study, A-PRF+ demonstrated weak antimicrobial activity against both MSSA and MRSA. Furthermore, A-PRF+ inhibited biofilm formation by these *Staphylococcus aureus* subspecies. The antimicrobial activity of A-PRF+ was similar to MSSA and MRSA in this study.

REFERENCES

1. **Dallaserra M, Poblete F, Vergara C, Cortés R, Araya I, Yanine N, et al.** Infectious postoperative complications in oral surgery. An observational study. J Clin Exp Dent. 2020;12(1):e65-e70.
2. **Donkor ES, Kotey FC.** Methicillin-Resistant Staphylococcus aureus in the Oral Cavity: Implications for Antibiotic Prophylaxis and Surveillance. Infect Dis (Auckl). 2020;13:1178633720976581.
3. **Xiang X, Shi P, Zhang P, Shen J, Kang J.** Impact of platelet-rich fibrin on mandibular third molar surgery recovery: a systematic review and meta-analysis. BMC Oral Health. 2019;19(1):163.
4. **Moraschini V, Miron RJ, Mourão C, Louro RS, Sculean A, da Fonseca LAM, et al.** Antimicrobial effect of platelet-rich fibrin: A systematic review of in vitro evidence-based studies. Periodontol 2000. 2024;94(1):131-42.
5. **Fujioka-Kobayashi M, Miron RJ, Hernandez M, Kandalam U, Zhang Y, Choukroun J.** Optimized Platelet-Rich Fibrin With the Low-Speed Concept: Growth Factor Release, Biocompatibility, and Cellular Response. J Periodontol. 2017;88(1):112-21.
6. **Yeaman MR.** 29 - The Role of Platelets in Antimicrobial Host Defense. In: Michelson AD, editor. Platelets (Fourth Edition): Academic Press; 2019. p. 523-46.
7. **Yamasaki K, Gallo RL.** Antimicrobial peptides in human skin disease. Eur J Dermatol. 2008;18(1):11-21.
8. **Stríz I, Trebichavský I.** Calprotectin - a pleiotropic molecule in acute and chronic inflammation. Physiol Res. 2004;53(3):245-53.
9. **Levy O.** Antimicrobial proteins and peptides: anti-infective molecules of mammalian leukocytes. J Leukoc Biol. 2004;76(5):909-25.
10. **Jasmine S, A T, Janarthanan K, Krishnamoorthy R, Alshatwi AA.** Antimicrobial and antibiofilm potential of injectable platelet rich fibrin-a second-generation platelet concentrate-against biofilm producing oral staphylococcus isolates. Saudi J Biol Sci. 2020;27(1):41-6.
11. **Ozcan M, Kabaklı SC, Alkaya B, Isler SC, Turer OU, Oksuz H, et al.** The impact of local and systemic penicillin on antimicrobial properties and growth factor release in platelet-rich fibrin: In vitro study. Clin Oral Investig. 2023;28(1):61.
12. **Bennardo F, Gallelli L, Palleria C, Colosimo M, Fortunato L, De Sarro G, et al.** Can platelet-rich fibrin act as a natural carrier for antibiotics delivery? A proof-of-concept study for oral surgical procedures. BMC Oral Health. 2023;23(1):134.
13. **Polak D, Clemer-Shamai N, Shapira L.** Incorporating antibiotics into platelet-rich fibrin: A novel antibiotics slow-release biological device. J Clin Periodontol. 2019;46(2):241-7.
14. **Ercan E, Suner SS, Silan C, Yilmaz S, Siddikoglu D, Sahiner N, et al.** Titanium platelet-rich fibrin (T-PRF) as high-capacity doxycycline delivery system. Clin Oral Investig. 2022;26(8):5429-38.
15. **Feng M, Wang Y, Zhang P, Zhao Q, Yu S, Shen K, et al.** Antibacterial effects of platelet-rich fibrin produced by horizontal centrifugation. Int J Oral Sci. 2020;12(1):32.
16. **Straub A, Vollmer A, Lâm TT, Brands RC, Stapf M, Scherf-Clavel O, et al.** Evaluation of advanced platelet-rich fibrin (PRF) as a bio-carrier for ampicillin/sulbactam. Clin Oral Investig. 2022;26(12):7033-44.
17. **Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, et al.** Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. Nat Rev Microbiol. 2019;17(4):203-18.