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RESEARCH

The impact of local and systemic penicillin on antimicrobial properties and growth factor release in platelet-rich fibrin: In vitro study

Mustafa Ozcan[1] · Seda Ciritci Kabaklı[1] · Bahar Alkaya[1] · Sıla Cagrı Isler[2,3] · Onur Ucak Turer[1] · Hale Oksuz[4] · Mehmet Cenk Haytac[1]

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Abstract

Objective This study evaluates the impact of local and systemic administration of penicillin on the antimicrobial properties and growth factors of platelet-rich fibrin (PRF) under in vitro conditions.

Materials and methods The study involved 12 volunteers. Four tubes of venous blood were collected before systemic antibiotic administration. Two tubes were centrifuged at 2700 RPM for 12 min to obtain PRF, while 0.2 ml of penicillin was locally added into other two tubes. After systemic administration, blood samples were again collected and subjected to centrifugation. The release of growth factors (IGF-1, PDGF, FGF-2, and TGFβ-1) was determined using the Enzyme-Linked Immunosorbent Assay (ELISA), and an antibiotic sensitivity test was performed for *S. aureus* and *E. coli* bacteria.

Results Results showed that local antibiotic addition before PRF centrifugation had a significant antimicrobial effect without affecting growth factor releases. There was no statistically significant difference in antimicrobial properties between PRF prepared with systemic antibiotic administration and PRF prepared without antibiotics.

Materials and methods The study suggests that incorporating localized antibiotics into PRF results in strong antimicrobial effects without compromise of growth factor release. However, the combination of PRF with systemic antibiotics did not significantly enhance its antimicrobial properties compared to PRF prepared without antibiotics.

Clinical relevance Local addition of penicillin into PRF provides strong antimicrobial properties which may help reduce dependence on systemic antibiotic regimens, mitigating antibiotic resistance and minimizing associated side effects.

Keywords Platelet-rich fibrin · Growth factors · Local/systemic antibiotics · ELISA

Introduction

Surgical procedures commonly employed in dentistry, such as tooth extraction, periodontal surgery, and dental implant applications, often necessitate accelerated wound healing in both soft and hard tissues. In this context, the use of platelet-rich fibrin (PRF) has become routine to facilitate this process [1, 2]. PRF, derived from the patient's own blood and prepared through a single centrifugation protocol, emerges as a biomaterial that can be directly applied to the surgical site.

Historically, thrombocyte concentrates have evolved from fibrin adhesives to various forms. Contemporary thrombocyte concentrates such as PRF exhibit greater success in terms of biological activity and preparation methods compared to earlier techniques [3]. These concentrates encompass a variety of leukocytes embedded within a dense fibrin matrix [4]. Host defense cells within the fibrin matrix contribute to preventing bacterial contamination within the surgical area by eliminating bacteria and pathogens present in the wound [5, 6].

Despite meticulous precautions, postoperative wound healing always carries the risk of infection [7, 8]. As a result, many practitioners lean towards prescribing antibiotics to prevent potential complications. However, unnecessary antibiotic usage can elevate the risk of antibiotic resistance

✉ Mustafa Ozcan
mustafaozcan@cu.edu.tr

1 Department of Periodontology, Faculty of Dentistry, Cukurova University, Adana, Turkey

2 Department of Periodontology, School of Dental Medicine, Bern University, Bern, Switzerland

3 Department of Periodontology, Faculty of Dentistry, Gazi University, Ankara, Turkey

4 Department of Medical Biology, Faculty of Medicine, Cukurova University, Adana, Turkey

and introduce adverse side effects [9]. Therefore, local and slow-release administration of antimicrobials via biological carriers directly in the wound may prevent this issue with minimal adverse side effects [10, 11]. However, the ideal agent for the local delivery of antibiotics and antiseptics is yet to be developed [11].

Autologous platelet concentrates demonstrated to enhance bone and soft tissue healing in periodontal regenerative procedures [12]. A recent comprehensive review concluded that PRF also exerts a significant antimicrobial activity and this effect is usually attributed to platelet proteins and reactive oxygen species [13]. Besides this natural antimicrobial activity, studies have explored the use of PRF as a drug delivery system [14]. PRF has been combined with drugs like metformin, statins, and bisphosphonates for evaluation [15–18]. In another study, silver nanoparticles were added to improve antibacterial activity, and their antibacterial, histological, and mechanical features were assessed [19]. In addition, certain antibiotics such as penicillin, clindamycin, metronidazole, gentamicin, linezolid, and vancomycin have been incorporated into various forms PRF with varying outcome levels of antimicrobial activity [7, 20, 21].

This study aims to evaluate the impact of local and systemic administration of antibiotic (penicillin) on the antimicrobial properties and growth factors of PRF under in vitro conditions.

Materials and methods

This study was conducted at the Department of Periodontology, Faculty of Dentistry, Cukurova University, with the participation of 6 male and 6 female volunteers (age between 22 and 26) with no previous history of periodontal disease. Prior to the study, all individuals were provided with information about the purpose, methodology, and procedures of the research, and written informed consent was obtained. The protocol of the study was approved by the Ethics Committee of Cukurova University Faculty of Medicine (Approval No: 86/59, Date: 08.03.2019).

The study included participants without systemic diseases and good cooperation. The individuals with systemic diseases, penicillin allergy, pregnancy or lactation, antibiotic treatment in the last 6 months, or medication causing bleeding disorders were excluded.

Study groups

Blood samples were collected from a total of 6 male and 6 female participants, and 6 tubes were obtained for each participant, and three study groups were formed:

1. Group (P-PRF): Two samples of pure PRF were prepared from 7 ml of blood obtained before systemic antibiotic administration. The PRF was obtained with centrifuging at 2700 rotations per minute (RPM) for 12 min.
2. Group (LAB-PRF): Two samples of PRF prepared from 7 ml of blood were prepared before systemic antibiotic administration with the addition of 0.2 ml antibiotic (penicillin G, 1.000.000 IU solution, Pfezier, Istanbul, Turkey) in the blood tube before centrifuging with the same protocol.
3. Group (SAB-PRF): Then, the patients received penicillin (2 g penicillin, 1.000.000 IU film-coated tablets, Pen-Os, Sandoz, Istanbul, Turkey) orally. PRF was prepared from 7 ml of blood obtained 1 h after systemic antibiotic administration with the same centrifuge protocol.

One PRF sample of each individual was used for microbiological tests, while the other one was analyzed for the growth factor release.

PRF preparation protocol

PRF membranes were produced using a protocol of 2700 RPM for 12 min (Relative Centrifugal Force- RCF-avg 708 g). PRF membranes were produced with 10-ml glass tubes using a Duo centrifugation device with a 40° rotor angulation with a radius of 88 mm at the clot and 110 mm at the max (Process for PRF, Nice, France).

Determination of local antibiotic dose

A pilot study was conducted prior to the main research to determine the appropriate local antibiotic dosage. Blood samples were collected from four volunteers, and before centrifugation, varying amounts of penicillin solution (0.5, 0.4, 0.3, and 0.2 ml) were added sequentially. After centrifugation at 2700 RPM for 12 min, the macroscopic physical characteristics of the resulting PRF were observed. It was observed that in groups where 0.5 ml, 0.4 ml, and 0.3 ml of penicillin were added, the PRF did not gel after centrifugation. In the group with 0.2 ml added, no noticeable physical changes were detected. Consequently, the local antibiotic dosage was determined as 0.2 ml of penicillin for our study.

Determination of systemic antibiotic dose

The commonly used systemic antibiotic in dentistry and periodontology, penicillin, was selected, and a prophylactic dose (1gr X 2 tablets) was administered according to the guidelines of the American Heart Association (AHA) [22]. After 60 min, venous blood was collected and subsequently centrifuged to obtain PRF.

Determination of growth factors

The obtained PRF samples from 12 volunteer participants were carefully separated from the red blood cell layer using sterile scissors and weighed on a precision balance. A volume of cell culture medium (HyClone™, RPMI-1640 MEDIUM) was added to each PRF sample in proportion to its weight. At the end of each time period (24, 48, and 72 h), small portions of the PRF samples containing the cell culture medium were collected from the top of the PRF and transferred to separate Eppendorf tubes for further use in the ELISA test. Throughout the incubation period, the samples were maintained on an orbital shaker under gentle conditions for 72 h after the addition of the cell culture medium. At the conclusion of each incubation period, the samples were subjected to ELISA assays (Fine Test®, Wuhan Fine Biotech Co., Ltd.) for the assessment of growth factors (IGF-1, PDGF, FGF-2, and TGFβ-1). All ELISA tests were performed according to the manufacturer's instructions.

Microbiological analysis

Bacterial suspensions of *S. aureus* and *E. coli* were prepared. Platelet-rich plasma samples were mixed with bacterial suspensions, and dilutions were prepared for each group. The samples were incubated, and colony counts were determined after 18–24 h.

In accordance with reference studies, antimicrobial activity testing was conducted using strains of Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli* [125, 151]. Strains of *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 were separately inoculated onto 5% sheep blood agar (Beckton Dickinson, Darmstadt, Germany) medium, followed by incubation at 37 °C in an incubator (Nüve EN 500, Turkey) overnight. After incubation, the cultured pure isolates of *E. coli* and *S. aureus* were subcultured onto Mueller Hinton Agar (MHA) and incubated overnight at 37 °C.

After incubating the samples in the incubator at 37 °C for 24, 48, and 72 h, 10 µl of each sample from the respective tubes of each group was taken and diluted in sterile broth to achieve a dilution ratio of 10^ − 8 (total dilution ratio of 10^ − 16). From the diluted suspension, 10 µl was further taken and inoculated onto three separate sheep blood agar plates for quantitative assessment. The cultures were incubated for 18–24 h, and colony counting was performed at the end of the incubation period. The arithmetic means of colonies from the three plates were calculated. The upper limit for countable colonies was set at 1000.

Statistical analysis

Numerical measurements were summarized as medians with minimum and maximum values, given the non-normal distribution of the data. The Kruskal–Wallis test was employed to compare more than two groups in terms of non-normally distributed numerical measurements, and the Dunn test was applied for pair-wise comparisons between groups where significant differences were detected. The Friedman test was used for comparison of non-normally distributed numerical measurements taken from the same samples at different time points. IBM SPSS Statistics Version 20.0 software package was utilized for statistical analysis. A significance level of 0.05 was considered.

Results

Evaluation of antimicrobial properties

Evaluation for *S. aureus*

Significant differences ($p < 0.001$) were observed in *S. aureus* growth (($\times 100 \times 106$) cfu/ml) at 24, 48, and 72 h among the study groups. No *S. aureus* growth was observed at any time point in the LAB-PRF group, resulting in a bacterial count of zero ($p < 0.001$). In contrast, *S. aureus* growth was observed in the P-PRF and SAB-PRF groups throughout all 3 days. Although the SAB-PRF group exhibited less bacterial growth compared to the P-PRF group, no statistical significance was observed between these two groups ($p > 0.05$). Intra-group comparisons showed consistent *S. aureus* growth levels across all time periods within each group ($p > 0.05$) (Table 1).

Evaluation for *E. coli*

Statistically significant differences ($p < 0.001$) were found in *E. coli* growth (($\times 100 \times 106$) cfu/ml) at 24, 48, and 72 h among the study groups. Similar to *S. aureus*, no *E. coli* growth was observed at any time point in the LAB-PRF group, resulting in a bacterial count of zero ($p < 0.001$). Conversely, *E. coli* growth was observed in the P-PRF and SAB-PRF groups throughout all 3 days. Similar to the *S. aureus* evaluation, no statistical significance was found between the P-PRF and SAB-PRF groups ($p > 0.05$). Intra-group comparisons indicated consistent *E. coli* growth levels across all time periods within each group ($p > 0.05$) (Table 1).

Evaluation of growth factors

TGF-β1

Inter-group comparisons showed no statistically significant differences in TGF-β1 measurements (pg/ml) at any time period among the study groups ($p > 0.05$) (Table 2). Intra-group comparisons revealed that TGF-β1 levels in the P-PRF

Table 1 Microbiological analysis

		Group			*p**	Inter–group comparison *p* value		
		P–PRF	LAB–PRF	SAB–PRF				
		Median (Min–Max)	Median (Min–Max)	Median (Min–Max)		1 vs. 2	1 vs. 3	2 vs. 3
S. aureus	24 h	250 (50–400)	0 (0–0)	200 (50–400)	< 0.001	< 0.001	0.662	< 0.001
	48 h	300 (100–300)	0 (0–0)	175 (50–500)	< 0.001	< 0.001	0.689	< 0.001
	72 h	200 (30–300)	0 (0–0)	175 (50–400)	< 0.001	< 0.001	0.984	< 0.001
p+		0.231	0.999	0.423				
E. coli	24 h	375 (50–400)	0 (0–0)	175 (50–500)	< 0.001	< 0.001	0.749	< 0.001
	48 h	200 (50–500)	0 (0–0)	200 (50–400)	< 0.001	< 0.001	0.999	< 0.001
	72 h	100 (20–600)	0 (0–0)	125 (10–300)	< 0.001	< 0.001	0.842	< 0.001
p+		0.290	0.999	0.096				

* *p* value for inter-group comparisons

+ *p* value for time-dependent comparisons

[a] significant compared to 24 h ($p < 0.05$)

[b] significant compared to 48 h ($p < 0.05$)

Table 2 Growth factor analysis

		Group			*p**	Inter–group comparison *p* value		
		P–PRF	LAB–PRF	SAB–PRF				
		Median (Min–Max.)	Median (Min–Max.)	Median (Min–Max.)		1 vs. 2	1 vs. 3	2 vs. 3
TGF-B1	24 h	0.82 (0.21–0.2.88)	0.79 (0.38–2.94)	0.77 (0.28–3.17)	0.655	0.776	0.792	0.698
	48 h	0.72 (0.29–3.29)	0.80 (0.27–3.18)	1.29 (0.28–3.36)	0.945	0.821	0.916	0.810
	72 h	0.61 (0.28–3.14)	0.72 (0.16–3.38)[a]	0.48 (0.19–2.89)[b]	0.637	0.753	0.772	0.688
p+		0.338	0.009	0.004				
PDGF	24 h	2.51 (2.06–2.67)	2.46 (1.45–2.67)	2.39 (2.08–2.86)	0.895	0.854	0.929	0.910
	48 h	2.28 (1.95–2.64)	2.25 (1.82–2.54)	2.35 (1.85–2.51)	0.602	0.755	0.741	0.669
	72 h	2.22 (1.88–2.46)[a]	1.86 (1.28–2.43)[a]	2.39 (1.20–2.85)	0.009	0.120	0.342	0.008
p+		0.017	0.013	0.472				
IGF-1	24 h	1.47 (0.18–2.05)	1.62 (1.21–2.20)	1.47 (0.79–2.11)	0.353	0.912	0.852	0.980
	48 h	1.47 (1.29–2.29)	1.71 (1.27–2.38)	1.88 (1.44–2.46)[a]	0.070	0.841	0.754	0.880
	72 h	1.68 (1.41–2.34)[ab]	1.69 (1.40–2.38)[a]	1.63 (0.76–2.20)	0.680	0.935	0.941	0.959
p+		0.005	0.039	0.004				
FGF-2	24 h	2.19 (0.56–2.98)	0.99 (0.58–2.77)	2.22 (0.44–2.96)	0.179	0.082	0.850	0.094
	48 h	2.67 (0.65–2.99)	1.71 (0.55–2.85)[a]	2.63 (0.45–3.11)[a]	0.193	0.095	0.681	0.124
	72 h	2.66 (0.59–3.06)[ab]	2.17 (0.04–3.12)[a]	2.73 (0.35–3.28)[a]	0.318	0.129	0.542	0.158
p+		< 0.001	0.005	0.018				

* *p* value for inter-group comparisons

+ *p* value for time-dependent comparisons

[a] significant compared to 24 h ($p < 0.05$)

[b] significant compared to 48 h ($p < 0.05$)

group were consistent across all time points ($p > 0.05$), while in the LAB-PRF group, TGF-β1 level was statistically higher at 24 h compared to 72 h ($p < 0.05$). Similarly, in the SAB-PRF group, TGF-β1 level at 48 h was significantly higher than at 72 h ($p < 0.05$).

PDGF

Inter-group comparisons indicated no statistically significant differences in PDGF measurements (pg/ml) at 24 and 48 h ($p > 0.05$) (Table 2). However, at 72 h, PDGF level in the

SAB-PRF group was statistically higher than in the LAB-PRF group ($p < 0.05$). Intra-group comparisons showed that PDGF level at 24 h was significantly higher in the P-PRF and LAB-PRF groups compared to 72 h ($p < 0.05$). Conversely, in the SAB-PRF group, no statistically significant difference was observed in PDGF levels at 24, 48, and 72 h ($p > 0.05$).

IGF-1

Inter-group comparisons revealed no statistically significant differences in IGF-1 measurements (pg/ml) at 24, 48, and 72 h among the study groups ($p > 0.05$) (Table 2). Intra-group comparisons indicated higher IGF-1 levels at 72 h in the P-PRF group compared to the other two time periods and in the LAB-PRF group at 72 h compared to 24 h ($p < 0.05$). Conversely, in the SAB-PRF group, higher IGF-1 levels were found at 48 h compared to the first day ($p < 0.05$).

FGF-2

Inter-group comparisons showed no statistically significant differences in FGF-2 measurements (pg/ml) at 24, 48, and 72 h among the study groups ($p > 0.05$) (Table 2). Intra-group comparisons revealed that the highest FGF-2 levels were observed at 72 h in all three groups, and this increase was statistically significant compared to the first 2 days ($p < 0.05$).

Discussion

In recent years, efforts to enhance wound healing in periodontal surgical procedures have led to the exploration of the antimicrobial properties of PRF and its potential synergies with local or systemic antibiotics. This study aimed to evaluate the impact of systemically administered and locally added penicillin before PRF centrifugation on the antimicrobial efficacy and growth factor release of the resulting tissue engineering scaffold. The results showed that the local addition of penicillin prior to PRF centrifugation exhibited robust antimicrobial activity without adversely affecting the release of growth factors, while systemic penicillin did not provide additional benefit.

Limited research exists focusing on the use of PRF in combination with antimicrobials. Polak et al. utilized PRF as a drug delivery system for antimicrobials (metronidazole, clindamycin, and penicillin solutions) in different volumes before centrifugation to evaluate their antibacterial activities [7]. It has been found that adding antibiotics locally to the tube before centrifugation causes changes in the physical properties of PRF. The authors reported that adding 2 ml and 1 ml penicillin solutions caused significant changes in the physical properties of PRF, while adding a 0.5 ml solution did not result in any macroscopic physical changes [7]. Before starting our study, a pilot study was conducted to determine the ideal antibiotic concentration. The results showed that PRF with 0.5 ml, 0.4 ml, and 0.3 ml penicillin solution displayed noticeable physical degradation, while PRF with 0.2 ml penicillin solution maintained its physical properties. Hence, our study utilized a 0.2 ml penicillin solution.

Polak et al. investigated the antimicrobial effectiveness of PRF using different forms (clot and membrane) and locally added solutions of metronidazole, clindamycin, and penicillin against F. *nucleatum* and *S. aureus.* The best antibacterial activity was observed with penicillin at 24, 48, 72, and 96 h [7]. Hence, in our study, we used penicillin, which is commonly used in dental and periodontal surgery for prophylactic, preventive, and therapeutic purposes.

In a similar study, vancomycin, clindamycin, and cefazidime were added to PRP, and their effectiveness against *S. aureus*, *E. coli*, and *P. aeruginosa* was assessed at 1, 4, 8, 24, 48, and 72 h. Ultimately, it was found that these combinations exhibited higher antimicrobial activity compared to the pure PRP group and continued to release above the MIC value even after 3 days, despite releasing the majority of antibiotics within the first 10 min [23].

Siawash and colleagues conducted a study where they systemically and locally added metronidazole solution before centrifugation to examine the antimicrobial effectiveness of PRF against *P. gingivalis*, *P. intermedia*, and *F. nucleatum* bacteria. It was found that locally added metronidazole significantly increased [20] the antibacterial capacity. In this study, the changes in growth factors for the locally added group were also assessed. The release of growth factors at 4 h, 1, 3, 7, and 14 days showed that the maximum release for PDGF-AB, VEGF, and TGF-β1 occurred at 4 h, 1st day, and 3rd day. When comparing the changes in growth factors with the antibiotic amounts, no significant differences were found [20].

There are limited articles exploring the contribution of systemically administered antibiotics to the antimicrobial activity of PRF. In our study, it was found that systemic antibiotic administration prior to the PRF preparation protocol did not result in additional antibacterial effects, aligning with previous research findings. Peck et al. assessed the antimicrobial activity of L-PRF against *S. mutans* following a single dose of systemic antibiotic. Inhibition was observed at 24 h, but not at 48 h and beyond [24]. Similarly, Saiwasch et al. investigated the antimicrobial properties of PRF prepared 2 h after systemic administration of 2 g amoxicillin and 500 mg metronidazole. In terms of pre- and post-antibiotic inhibition zones, amoxicillin exhibited the highest inhibition zone against *P. gingivalis* and the lowest against *P. intermedia*. Amoxicillin

demonstrated significant effects against three pathogens. On the other hand, metronidazole was effective against *P. gingivalis* and *F. nucleatum* but showed no effect against *P. intermedia* [20].

Although extensive research has been performed on the clinical effectiveness of PRF, there are still controversies related to the preparation protocols which complicates the accurate interpretation of the research results. A consensus report of 2019 listed that the RCF values, dimensions of the centrifuge rotor and rotor angulation, RPM, time, and composition of the blood tubes are the main parameters of the production process of PRF [25]. In his detailed and authoritative review, Miron et al. [26] have described that the RCF should be included in all reports as this force significantly affects the outcome of PRF in terms of cell counts and growth factor release. In addition, the type of the blood tube may also cause substantial changes as tubes with silica and silicon coatings have shown to be cytotoxic leading to cell apoptosis [27].

The main disadvantage of the angle centrifugation system which is used in this study is that the blood cells are driven at the back walls of the tubes which causes difficulty during separation due to their different cell densities. In contrast, horizontal centrifugation systems cells are evenly distributed in the upper layers up to four times of concentration with minimal cell damage [26]. Other potential improvements of PRF preparation protocols include the concentrated PRF protocol in which the cells are specifically sent to the buffy coat region at higher RCF values [28], the development of PRF tubes hydrophobic inner surface to intentionally delay clotting, and the use of cooling devices to increase working time [29].

The main finding of the current study is that the local application of penicillin significantly enhanced the natural antibacterial effect of the PRF, preventing the growth of both tested microorganisms throughout all evaluation periods entirely. This observation may be attributed to the integration of penicillin into the PRF matrix structure, allowing for a slow and sustained release as the PRF is gradually resorbed. This characteristic may enable the antimicrobials to reach high concentrations locally, especially in contaminated wound areas such as periodontal defects. In both dentistry and medicine, fibrin-based matrices are commonly used in tissue engineering due to their receptor-binding capacity for various types of cells, proteins, and growth factors [30]. In addition, it was observed that the use of antibiotics in combination with PRF did not compromise the release of growth factors, a vital concern in tissue engineering applications. The preservation of growth factors' integrity is crucial for successful tissue regeneration. Our study aligns with prior investigations that reported growth factor release patterns to be stable despite the incorporation of antibiotics into the scaffold. This concordance underscores the viability of our approach in maintaining tissue healing capabilities while augmenting antimicrobial effects.

The main limitations of the study include the lack of microbiological analysis for potent pathogens such as *P. gingivalis*, *A. actinomycetemcommitans*, and *F. nucleatum* and the use of only type of antibiotic without any analysis of the release of incorporated antibicrobial. In addition, PRF structure was assessed solely on a macroscopic level following local antibiotic application. Evaluating the mechanical properties histologically through techniques such as SEM would provide more comprehensive insights. Therefore, future studies with extended evaluation periods, along with more comprehensive microbial analyses and detailed mechanical property assessments, are needed to show the antimicrobial effects of PRF with local addition of different types and concentrations of antibiotics. Furthermore, certain individualistic characteristics (such as age, sex, systemic conditions) which may potentially influence the properties of PRF should also be investigated.

Conclusion

In conclusion, the results of the current study show the potential of incorporating antibiotics into PRF scaffolds to enhance their antimicrobial properties without compromising growth factor release. This localized approach offers a promising strategy for addressing oral infections and promoting tissue regeneration. Depending on the desired outcome, such as strong antimicrobial efficacy in cases of periodontal bone defects/tissue regeneration and immediate implant placement after extractions, the incorporation of antibiotics into PRF can be tailored accordingly. However, the complex interplay between antimicrobial effects, growth factor release, and potential antibiotic resistance necessitates further clinical investigations to refine and optimize this approach. The clinical extrapolation of the present in vitro findings necessitates a prudent approach, as the effectiveness of the formulation and any alterations in the properties of PRF must be substantiated through clinical investigations and animal trials. As the field of tissue engineering continues to evolve, the integration of antibiotics into PRF matrices holds promise for advancing both periodontal and broader regenerative therapies.

Conflict of interest

The authors declare no competing interests.

Author contribution M.O. and M.C.H. contributed to study conception and design. S.C.K. was responsible for obtaining and preparing

the samples for data collection. H.O. performed ELISA analysis. B.A., O.U.T and S.C.I. performed the data analysis and prepared the manuscript. All authors critically revised the manuscript.

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Data availability The data of this study are available on request from the corresponding author.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval The protocol of the study was approved by the Ethics Committee of Cukurova University Faculty of Medicine, Adana, Turkey (Approval No: 86/59, Date: 08.03.2019).

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