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Antibacterial effects of leukocyte and platelet-rich fibrin against Escherichia coli and Enterococcus faecalis

Lökosit ve Trombositten Zengin Fibrinin Escherichia Coli'ye ve Enterococcus Faecalis'e Karşı Antibakteriyel Etkileri

Mehtap Bilgin Çetin[1], Yasemin Sezgin[1]*, Ebru Evren[2], Şule Bulut[1]

1.Department of Periodontology, Faculty of Dentistry, Baskent University, Ankara, Turkey
2.Department of Medical Microbiology, Ankara University Faculty of Medicine, Ankara, Turkey

ABSTRACT

Aim: The main goal of this research was to explore the in-vitro antibacterial characteristics of leukocyte and platelet-rich fibrin (L-PRF) against Escherichia coli (E. Coli) and Enterococcus faecalis (E. Faecalis).
Materials and methods: The study was conducted on 21 patients (10 females, 11 males, age range 21-32 years). L-PRF was prepared from the participants' own blood. Antibacterial activity of L-PRF against E. coli and E. faecalis ATCC standard strains was analyzed using the Kirby Bauer disk diffusion method.
Results: The inhibition zones with PRF had not been detected even though the results obtained using control discs were in accordance with the expectations.
Conclusion: L-PRF demonstrated no inhibition zone against E. coli and E. faecalis.

Key words: Antibacterial, Escherichia Coli, Enterococcus Faecalis, Leukocyte and Platelet-Rich Fibrin, postoperative infection.

ÖZ

Amaç: Bu çalışmanın amacı, lökosit ve trombositten zengin fibrinin (TZF) Escherichia coli'ye (E. Coli) ve Enterococcus faecalis'e (E. Faecalis) karşı in vitro antibakteriyel etkilerini araştırmaktır.
Gereç ve yöntemler: Çalışma 21 hasta (10 kadın, 11 erkek, yaş aralığı 21-32 yaş) üzerinde yürütülmüştür. Katılımcılardan elde edilen kan örnekleri kullanılarak TZF hazırlanmıştır. TZF'nin E. coli ve E. faecalis ATCC standart suşlarına antibakteriyel aktivitesi Kirby Bauer disk difüzyon yöntemi kullanılarak test edilmiştir.
Bulgular: Beklenen sonuçlar kontrol diski ile elde edilmesine rağmen, TZF'nin değerlendirilen bakterilere karşı inhibisyon zonu göstermediği tespit edilmiştir
Sonuç: TZF, E.coli'ye ve E. faecalis'e karşı inhibisyon bölgesi göstermemiştir.

Anahtar Kelimeler: Antibakteriyel, Escherichia Coli, Enterococcus Faecalis, lökosit ve trombositten zengin fibrin, postoperatif enfeksiyon

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*Corresponding Author: Yasemin Sezgin. Department of Periodontology, Faculty of Dentistry, Baskent University, Ankara, Turkey, +903122151336, yasemin_tocak@hotmail.com

ORCİD: 0000-0002-4732-805X

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INTRODUCTION

Platelet concentrates are utilized extensively in a wide variety of medical areas in order to promote tissue regeneration. They are classified into four groups based on their fibrin structure and leucocyte content, and all have their respective areas of use and various characteristics. Platelet-rich fibrin (PRF) is a second-generation platelet concentrate that has been proposed by Choukroun et al. In contrast to platelet-rich plasma (PRP), this procedure does not necessitate anticoagulant or bovine thrombin, making it easy and rapid to prepare [1,2]. In addition, due to its dense and mature three-dimensional architecture, L-PRF releases higher quantities of growth factors for longer periods than PRP [3,4]. In the literature, PRF has demonstrated promising results in enhancing bone regeneration, soft tissue maturation and wound healing [1,5-7].

Platelet concentrations could be divided into various categories based on their contents of leukocyte and fibrin; they are P-PRP (pure platelet rich plasma), L-PRP (Leucocyte and platelet rich plasma), pure platelet-rich fibrin (P-PRF) and leukocyte- and platelet-rich fibrin (L-PRF) [8]. L-PRF contains many more leucocytes than other concentrations. To obtain P-PRP, venous blood is collected in numerous small tubes and then centrifugation process is applied. After the PPGF (platelet poor in growth factors) has been removed, the left over PRGF (platelet rich in growth factors) is collected, and fibrin polymerization is triggered with 10% calcium chloride. The aim of this application is to avoid collecting the leucocytes. In contrast, to obtain L-PRF, venous blood is collected in dry glass tubes without anticoagulants and centrifugation process was applied at low speed. In this way, both platelets and leucocytes can be obtained with great efficiency [8].

In addition to all these properties, the antimicrobial properties of platelet concentrations have recently been addressed. The mechanisms behind the antibacterial characteristics of platelet concentrates are still not fully understood and remain to be explored [9]. The antimicrobial potential of platelet blood concentrations is based on the roles in host response of platelets and leukocytes. Platelets which are essential for tissue healing possess receptors that are critical in recognizing the viruses, bacteria and parasites, also affect bacteria through a mechanism that involves direct contact. Activated platelets release some bactericidal structures such as defensins, kinocidins, platelet factor 4 and synthesize hydrogen peroxide and reactive oxygen species which are toxic to bacteria [9]. Leukocytes, like platelets, exhibit antimicrobial activity in circulation. White blood cells maintain these properties through phagocytic activity and antimicrobial molecules demonstrate phagocytic activity; these constitute a rich source of anti-microbial substances including defensins, cathelicidins, lysozyme, myeloperoxidase [9].

There are many studies in literature with particular focus on the antibacterial effects of PRP [10-16]. However, few studies [11,13,17] have investigated the antibacterial effects of L-PRF. Postoperative infection is among the most critical and primary causes of disruption to wound healing and may have an adverse effect on surgical success. Hence, the prevention of bacterial contamination is a prerequisite for the success of the surgery. Although Escherichia coli (E. Coli) and Enterococcus faecalis (E. Faecalis) have an important place in postoperative infection, none of the previous studies focused on the antibacterial effects of L-PRF against E. coli and E. faecalis. For all these reasons, we aimed to investigate the antibacterial effects of L-PRF, which was prepared according to the Choukroun's technique, against standard strains of E. coli and E. faecalis.

METHODOLOGY

Study population

Blood specimens were obtained from 21 patients, in which 10 were female and 11 were male. The age range of the patients was 21–32 years. They were admitted to the Department of Periodontology of the Baskent University Faculty of Dentistry for periodontal treatment and they volunteered to participate in this study. All the study subjects were healthy (ASA 1–2). Smokers, patients who had taken antibiotics during the last six months or patients who underwent anticoagulant or immunosuppressive therapy, were excluded from this study. The written informed consents were obtained from all patients who were enrolled to

the study, which was conducted in accordance with the guidelines of the Helsinki Declaration for biomedical research involving human subjects and approved by the Ethics Board of Baskent University (approval no:15/12).

Blood collection and production of L-PRF

The blood specimens were handled and treated in accordance with the PRF protocol described previously using a PC-02 table centrifuge and kits (Process, Nice, France) for collection [2]. Venous blood was collected in 10 mL tubes lacking anticoagulants and centrifuged immediately at 2700 rpm for 12 minutes. Following centrifugation, PRF was separated from red corpuscle base utilizing tweezers and scissors. Then it was transferred to a PRF BOX (Process, Nice, France), and PRF membranes were obtained. Two membranes were obtained from each volunteer (Figure-1.

Bacterial strains

Standard strains of E. faecalis ATCC (American Type Culture Collection) 29212 and E. coli ATCC 25922 were used to evaluate the antibacterial effects of PRF.

Determination of antimicrobial activity

The antimicrobial activity for L-PRF was performed in a medical microbiology laboratory. The profiles of antimicrobial susceptibility were detected using the disc diffusion method, meeting the criteria of Clinical and Laboratory Standards Institute (CLSI). The isolates were stored at -20 °C until further usage. Prior to the test, the isolates were subcultured on blood agar and MacConkey agar in order to determine any possible contamination. The standard strains with 0.5 McFarland turbidity were plated on both Blood Agar Medium (BD, USA) and Mueller Hinton Agar (BD, USA). Mueller Hinton agar and blood agar were then used for in-vitro testing. An ampicillin disc (10 µg) was also used as a quality control. Following 24 and 48 hours of incubation at 37 oC, the diameter of the zones was measured visually and evaluated according to CLSI standards.

RESULTS

The zone of inhibition of the control disc was concordant with CLSI standards. At both 24 and 48 hours of incubation, no inhibition zones were reported against the E. faecalis ATCC 29212 or E. coli ATCC 25922 standard strains (Figure-1 and Figure-2), (Table 1).

Table 1: Demographic characteristics of patients and antimicrobial activity of L-PRF

	Age	Gender	Antimicrobial activity of L-PRF against the E. faecalis	Antimicrobial activity of L-PRF against the E. coli
P1	30	Male	No	No
P2	26	Male	No	No
P3	21	Female	No	No
P4	22	Male	No	No
P5	24	Male	No	No
P6	21	Female	No	No
P7	22	Female	No	No
P8	22	Female	No	No
P9	24	Female	No	No
P10	26	Male	No	No
P11	24	Male	No	No
P12	25	Male	No	No
P13	21	Female	No	No
P14	26	Male	No	No
P15	22	Male	No	No
P16	22	Male	No	No
P17	22	Male	No	No
P18	25	Female	No	No
P19	24	Female	No	No
P20	32	Female	No	No
P21	30	Female	No	No

P, patient; E. faecalis, Enterococcus faecalis; E. coli, Escherichia coli.

DISCUSSION

Platelet concentrates have been used in the medical field [18] and in oral surgery with promising results. There is growing evidence that suggests that platelet concentrates not only slow bleeding but also contribute to protecting against infection [10]. The current research was conducted to investigate the antibacterial characteristics of L-PRF against E. faecalis and E. coli, which have been shown to be responsible for the postoperative infection [19,20], and the findings of this research indicated that L-PRF did not demonstrate any antibacterial activity against the selected bacteria.

The studies that have evaluated the antimicrobial

activities of different platelet concentrations have reported conflicting results [11,13]. For example, Badade et al. [13] scrutinized the antimicrobial activities of platelet-rich fibrin (PRF) and PRP against Porphyromonas gingivalis (P. gingivalis) and Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans) and reported that PRF demonstrated no antibacterial activity, whereas PRP was capable of inhibiting P. gingivalis at three–four days of incubation and A. actinomycetemcomitans at 48 h of incubation. When obtaining PRP, intravenous blood was procured in a blood collection tube coated with an anticoagulant, and 10% calcium chloride was used to activate PRP. It was concluded that the calcium chloride used for PRP activation may be responsible for the antibacterial activity of PRP.

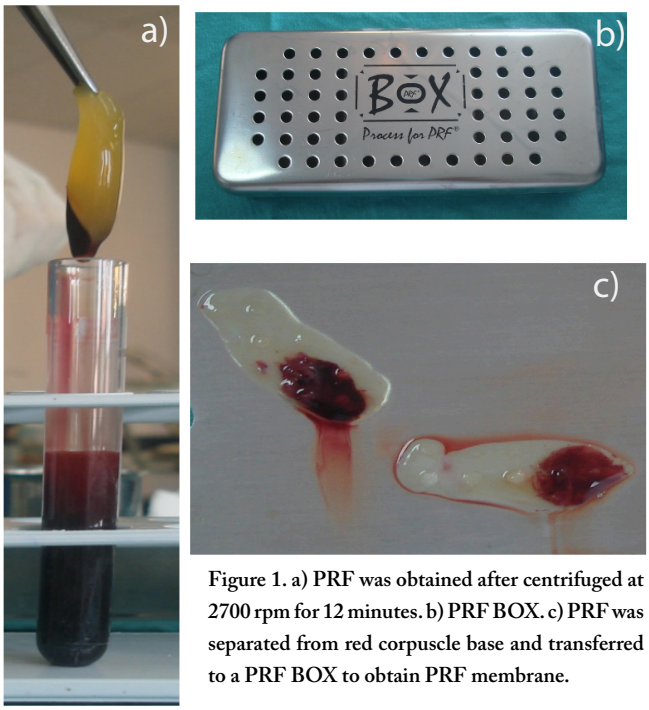

Figure 1. a) PRF was obtained after centrifuged at 2700 rpm for 12 minutes. b) PRF BOX. c) PRF was separated from red corpuscle base and transferred to a PRF BOX to obtain PRF membrane.

In another study Yang et al. tested four distinct plasma fractions (PRF, PPP, PDP, PRP) in terms of their antibacterial characteristics against P.gingivalis, A. actinomycetemcomitans and Fusobacterium nucleatum [11]. The authors found that all plasma concentrations, including PRF, inhibited bacterial growth, but when a comparison was made between the platelet concentrates, PRP was found to be the most effective antibacterial agent. Since Yang et al. used a different preparation protocol from our study to obtain PRF, a direct comparison with the present study was not possible. They obtained PRF from a fraction of PRP that was activated by calcium chloride, whereas in the present study, L-PRF was prepared by a protocol that is in conformity with the method proposed by Choukroun et al. [2].

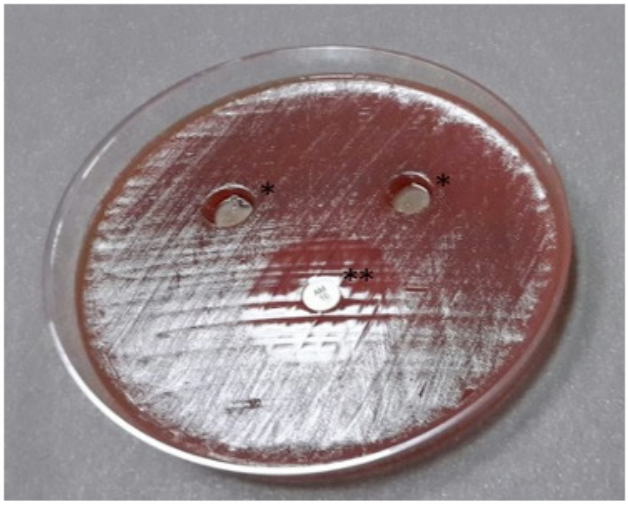

Figure 2. *Agar diffusion antibacterial testing of L-PRF against the E. faecalis, ** Agar diffusion antibacterial testing of control disc (ampicillin disc) for E. faecalis. (Blood Agar)

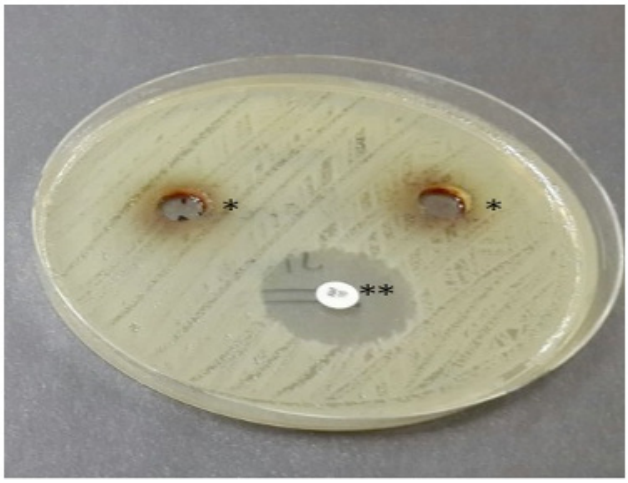

Figure 3. *Agar diffusion antibacterial testing of LPRF against the E. coli, ** Agar diffusion antibacterial testing of control (ampicillin disc) against the E. coli. (Mueller Hinton Agar)

Different results have been reported in a few studies [11,13,17] evaluating the antimicrobial effect of L-PRF. Among these, one study concluded that L-PRF had no antimicrobial properties [13], whereas in another, L-PRF showed the precise opposite [11,17]. In addition, there have been no study that have analyzed the antimicrobial effects of L-PRF on E. faecalis and E. coli. Therefore, the antibacterial effects of L-PRF against these were investigated in the present study to address the issue of the proven roles of these bacteria in postoperative infection

[19,20]. Although the literature does not address the antimicrobial action of PRF against E. coli and E. faecalis, the antimicrobial activities of other platelet concentrations against these have been evaluated in previous studies, with contradictory results [10,11,12,16,21,22,23]. Drago et al. [10], reported an antibacterial effect of PRP, whereas Bielecki et al. [21] and Li et al. [23], did not find out any antimicrobial effects of PRP against these bacteria.

Some authors have argued that antimicrobial activities of leukocytes increase the anti-inflammatory activity of platelet concentrations, although the antimicrobial effects of leucocytes that are removed from circulation when applied directly to the surgical field, are still open to question [9]. It has even been stated that leukocytes should be completely removed from their plasma concentrations, with suggestions that they may increase inflammatory response [24]. The reason for the lack of antimicrobial activity of L-PRF in this study may be a result of the large quantity of leucocytes in the contents and its tight fibrin structure, which may cause the trapping of several antimicrobial effect products. Further research is required to identify the antimicrobial efficacy of platelet concentrations.

Limitations: In-vitro antibacterial characteristics of leukocyte and platelet-rich fibrin (L-PRF) against Escherichia coli (E. Coli) and Enterococcus faecalis (E. Faecalis) was investigated in the present study. Evaluation of only these two bacteria can be considered as a limitation of this study.

Conclusion: In conclusion, L-PRF showed no inhibition zone against E. coli and E. faecalis.

Conflict of Interest: The author has no conflict of interest related to this article.

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Ethics Committee Approval: This study approved by the Ethics Board of Baskent University (approval no:15/12).

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Author/ORCID	Authorship Contrubition
Mehtap Bilgin Çetin 0000-0003-3164-6886	Concept, Design, Materials, data collection, literature search, manuscript writing, final approval, critical review
Yasemin Sezgin 0000-0002-4732-805X	Concept, Design, Materials, data collection, literature search, manuscript writing, final approval, critical review
Ebru Evren 0000-0001-7615-0521	Concept, Design, Materials, data collection, manuscript writing, final approval, critical review
Şule Bulut 0000-0002-8808-0185	Concept, Design, Materials, final approval, manuscript writing, critical review