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# Effect of Er:YAG Laser on Scaling and Root Planing and Usage of i-Platelet Rich Fibrin as a Biomodifier

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**ABSTRACT**

**Objectives:** The purpose of this study was to compare scaling and root planing (SRP) with Er:YAG laser and Gracey curettes and the effectiveness of using injectable platelet-rich fibrin (i-PRF) as a biomodifier was also investigated.

**Materials and Methods:** There were 4 groups of extracted human teeth: Gracey Group (n=9): SRP with Gracey curettes; Gracey + i-PRF Group (n=9): SRP with Gracey curettes followed by application of i-PRF to the root surface; Er:YAG Group (n=9): SRP with Er:YAG laser; Er:YAG + i-PRF Group (n=9): SRP with Er:YAG laser followed by application of i-PRF to the root surface. The width of dentin tubules and the presence/absence of smear layer were examined using scanning electron microscopy (SEM).

**Results:** There was significantly less smear layer in the Er:YAG group compared to the Gracey group (p=0.001). The width of dentin tubules was found to be significantly higher in the Er:YAG and Er:YAG+i-PRF groups compared to the Gracey group (respectively;p=0.015;p<0.001). The width of dentin tubules in the Er:YAG+i-PRF group was profoundly higher than in the Gracey+i-PRF group (p=0.026).

**Conclusions:** Er:YAG laser was found to be more effective than Gracey curettes, which are the gold standard in root surface cleaning. Especially when combined with Er:YAG laser, i-PRF resulted in wider dentin tubules.

***Keywords:*** Er:YAG laser, i-PRF, In vitro, Root Biomodification.

# Er:YAG Lazerin Diş Yüzeyi Temizliği ve Kök Yüzeyi Düzleştirme Üzerindeki Etkisi ve Biyomodifiye Edici Olarak e-Trombositten Zengin Fibrinin Kullanımı

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**ÖZET**

**Amaç:** Bu çalışmanın amacı, Er:YAG lazer ve Gracey küretleri ile diş yüzeyi temizliği ve kök yüzeyi düzleştirme (KYD) prosedürünü karşılaştırmak ve biyomodifiye edici olarak enjekte edilebilir-trombositten zengin fibrin (e-TZF) kullanımının etkinliğini araştırmaktır.

**Gereç ve Yöntemler:** Çekilen insan dişleri 4 gruba ayrıldı: Gracey Grubu (n=9): Gracey küretleri ile KYD; Gracey + e-TZF Grubu (n=9): Gracey küretleri ile KYD ve ardından kök yüzeyine e-TZF uygulanması; Er:YAG Grubu (n=9): Er:YAG lazeri ile KYD; Er:YAG + e-TZF Grubu (n=9): Er:YAG lazeri ile KYD ve ardından kök yüzeyine e-TZF uygulanması. Dentin tübüllerinin genişliği ve smear tabakasının varlığı/yokluğu taramalı elektron mikroskobu kullanılarak incelendi.

**Bulgular:** Er:YAG grubunda Gracey grubuna kıyasla önemli ölçüde daha az smear tabakası vardı (p=0,001). Dentin tübüllerinin genişliğinin Er:YAG ve Er:YAG+e-TZF gruplarında Gracey grubuna kıyasla önemli ölçüde daha yüksek olduğu bulundu (sırasıyla;p=0,015; p<0,001). Er:YAG+e-TZF grubundaki dentin tübüllerinin genişliği Gracey+e-TZF grubuna kıyasla belirgin şekilde daha yüksekti (p=0,026).

**Sonuçlar:** Er:YAG lazerin, diş yüzeyi temizliğinde altın standart olan Gracey küretlerinden daha etkili olduğu bulundu. Özellikle Er:YAG lazerle birleştirildiğinde, e-TZF daha geniş dentin tübülleriyle sonuçlandı.



***Anahtar Kelimeler:*** Er:YAG lazer, e-TZF, in vitro, kök biyomodifikasyonu.

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## Introduction

Regenerating the lost periodontium and transforming the root surface into a biologically suitable substrate for the attachment of epithelial and connective tissue cells is one of the goals of periodontal treatment.[1] The mechanical debridement of root surfaces affected by periodontitis focuses on eliminating the etiological factors of periodontal disease and facilitating the attachment of periodontal tissues to restore biological compatibility.[2] The mechanical debridement of deposits on the root surface is considered the gold standard in the treatment of this disease.[3]

Root biomodification refers to procedures aimed at detoxifying, decontaminating, and demineralizing the root surface in order to remove the smear layer and expose the collagen matrix of the dentin and cementum.[4] Various agents are used for root surface biomodification, including mechanical agents (hand instruments/ultrasonics and lasers), chemical agents, and growth factors (biostimulants).[5]

The hemostatic effects, selective calculus ablation, and bactericidal activity are among the specific characteristics of lasers that are worth considering. In this way, it can be observed that appropriate laser application may provide an alternative to mechanical or modified root debridement.[6] The mechanism of action of laser application is based on thermomechanical ablation, which relies on the high radiation absorption by surface water and hydroxyapatite groups.[7] Meanwhile, working at the surface level does not cause thermal damage to the underlying tissues. Additionally, it has reported that high-intensity lasers have a significant bactericidal effect on periodontal pathogenic bacteria.[8] The reason for this is thought to be that the laser radiation promotes the evaporation of water in the bacterial cell cytoplasm, leading to cell rupture,[9] or it may directly melt or coagulate the bacterial cells.[8]

Er:YAG lasers are lasers that pulse freely while operating and can be used on both soft and hard tissues without causing any damage. The most important feature of this type of laser is that they have very good water absorption. Due to this feature, they can be used safely on both soft and hard tissues. Due to the good ablation feature of Er:YAG lasers, they can be used safely in periodontics; soft tissue surgery, scaling and root planing (SRP), disinfection and detoxification applications.[10] It has been shown that Er:YAG lasers have high bactericidal effects on periodontopathogenic bacteria at low energy levels and have a detoxifying effect on toxins such as lipopolysaccharides diffused to the root surface.[10,11] In addition, Er:YAG laser does not cause denaturation in periodontal tissues and positively affects the adhesion and proliferation of fibroblasts.[12]

Autogenous platelet-derived products obtained from the patient's own blood have increasingly been used in regenerative applications in recent years, yielding positive results both clinically and histologically. Clot formation and stabilization play a crucial role in regenerative healing during the healing process. Platelets are the major cells of the coagulation cascade and contain signaling molecules essential for healing.[13] Considering these characteristics, platelet-rich concentrations have become the preferred blood products for inducing regeneration in dentistry for over thirty years. Research has reported that platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) stimulate cell proliferation and differentiation, thereby supporting tissue regeneration. Another distinguishing feature that separates PRF from PRP is its production without the use of anticoagulants. Reported modified advantages include faster wound healing, quicker angiogenesis, and lower cost.[14] Researchers have periodically made various protocol modifications to evaluate the developable properties of PRF.[15] By using low centrifugation speeds and shorter centrifugation times, a liquid form of PRF has been obtained, and this product is referred to as injectable PRF (i-PRF). Injectable PRF, which has the capacity to act as a biomodifier on root surfaces, possesses noteworthy properties due to its enhancing effects on gingival fibroblast attachment and wound healing, making it worthy of further investigation. The liquid fibrin, as described by J. Choukroun, contains no anticoagulants or any additives and is used in its liquid form.[16]

In light of all this information, the aim of this study is to compare Er:YAG laser, SRP with Gracey curettes, and to evaluate the effectiveness of i-PRF as a biomodifier. The hypothesis of the study is that surfaces treated with Er:YAG laser and i-PRF will exhibit better surface roughness, leave less smear layer, and have wider dentinal tubules compared to the control groups.

## Materials and Methods

This study was approved by Kırıkkale University Clinical Research Ethics Committee (No: 17/01 Date: 14.10.2021).

The study included single-rooted teeth of patients referred to the Department of Periodontology at Kırıkkale University Faculty of Dentistry with indications for extraction. Teeth included in the study were defined as follows: i) teeth with hopeless prognosis, ii) teeth with probing depths greater than 8 mm, iii) teeth without root canal treatment, iv) teeth with the presence of dental calculus on the root surfaces, and v) teeth that had not undergone SRP procedures within the last 6 months. Teeth with caries or restorations under the enamel-cementum junction, teeth with shape/size anomalies and teeth with root fractures were excluded from the study. The teeth were stored in phosphate-buffered sterile saline at 4°C until the procedures were performed.

The included teeth were divided into four groups according to the following criteria:

1. Gracey Group: SRP performed with Gracey curettes
2. Gracey + i-PRF Group: SRP performed with Gracey curettes followed by application of i-PRF to the root surface
3. Er:YAG Group: SRP performed with Er:YAG laser
4. Er:YAG + i-PRF Group: SRP performed with Er:YAG laser followed by application of i-PRF to the root surface

The surfaces where the procedures would be applied were marked with a marker pen, 1 mm apical to the enamel-cementum junction coronally and 3 mm coronal

to the root apex apically. To obtain i-PRF, blood was collected from one volunteer aged 18 or older, non-tobacco-user, without any systemic diseases and who had not taken any medication affecting coagulation in the last 3 months. Two tubes of blood were centrifuged at room temperature for 3 minutes at 700 rpm using a Duo centrifuge device (Process for PRF, Nice, France).

In the Gracey group, SRP was performed with only Gracey 3-4 curettes (Hu-Friedy, Frankfurt, Germany) until a flat and smooth surface was obtained. In the Gracey + i-PRF group, after SRP with Gracey curettes, i-PRF was applied to the root surfaces immediately after it was obtained for 5 minutes. In the Er:YAG group, SRP was performed using a chisel tip R14 tip (R14 Perio Tip, Fidelis, Fotona, Slovenia) at approximately 20°-30° angle from the tooth surface, 2 mm away, at settings of 120 mJ and 10 Hz, with 70% air and 30% distilled water cooling. In the Er:YAG + i-PRF group, after SRP with Er:YAG laser, i-PRF was applied to the root surfaces immediately after it was obtained for 5 minutes. Subsequently, blocks in disk shape were obtained from the marked areas using trephine burs with a diameter of 4 mm under water cooling and prepared for scanning electron microscopy (SEM) examination. All procedures were performed by a single researcher (NFG).

### *SEM Procedures*

SEM (JSM 5600 LV; JEOL, Tokyo, Japan) was used to examine the surface morphology of the obtained samples. To eliminate possible external contaminations, the tooth samples were washed with ethanol before SEM examination. After the washing process, a 30-minute air drying process at room temperature was carried out to remove any remaining moisture. To enhance the conductivity of the samples during analysis, as well as to obtain higher resolution images, their surfaces were coated with a thin layer of Gold/Palladium using a gold plating device (Polaron SC7620, Kent, UK). SEM images were obtained at magnifications of 1000x and 5000x. A blinded investigator graded the images and assigned a score in accordance with the Sampaio index.[17]

The criteria for this index are as follows:

Score 1: The initial score is attributed to the presence of a root surface devoid of smear layer, with the dentinal tubules exhibiting complete openness and an absence of smear layer within them.

Score 2: The presence of a smear layer at the entrance of the dentin tubules was observed, whilst the root surface was found to be devoid of such a layer. The dentin tubules were found to be fully open.

Score 3: The root surface exhibited partially open dentin tubules, devoid of a smear layer.

Score 4: The root surface is characterized by the presence of partially open dentin tubules, which are covered by a uniform smear layer.

Score 5: The root surface was found to be covered with a uniform smear layer, and no open tubules were observed in the dentin.

Score 6: The root surface was found to be covered with an irregular smear layer, and there was evidence of grooves and/or scattered debris.

Additionally, the diameters of dentin tubules seen at 5000x magnification were measured.

### *Statistical Analysis*

To achieve 80% power (effect size, f = 0.3) and detect differences among groups, 36 extracted teeth were required. Statistical analyses of the data from our study were performed with the use of the SPSS software package. (Version 22.0, SPSS Inc., Chicago, IL, USA). Descriptive statistics of numerical data were reported as median (minimum-maximum) and mean ± standard deviation (SD) depending on the normal distribution of the data. Normal distribution of the data obtained was assessed using the Shapiro-Wilk test and homogeneity of variances using the Levene test. The Kruskal-Wallis test was used to compare data that did not follow a normal distribution. After this test, the Dunn-Bonferroni post-hoc test was used for pairwise comparisons to identify the groups responsible for the statistically significant differences. A p-value of $<0.05$ was considered to be statistically significant.

## Results

Statistical findings regarding the comparison of scores between groups are presented in Table 1. The scores among groups were statistically significantly different ($p < 0.001$). It was found that the scores of the Gracey group were significantly higher than those of the Er:YAG group ($p = 0.001$). There was no statistically significant difference to be found between the scores of the other groups. ($p > 0.05$).

Statistical findings regarding the comparison of the width of dentin tubules among research groups are presented in Table 2. The widths of dentin tubules among groups were statistically significantly different ($p < 0.001$). It was determined that the widths of dentin tubules in the Er:YAG and Er:YAG + i-PRF groups were significantly higher than those in the Gracey group ($p = 0.015$; $p < 0.001$, respectively). Additionally, it was found that the width of dentin tubules in the Er:YAG + i-PRF group was significantly higher than that in the Gracey + i-PRF group ($p = 0.026$). There was no indicated difference which is significant found in the width of dentin tubules among the other groups ($p > 0.05$).

***Table 1.*** *Smear Layer Scores According to Sampaio Index in Groups*

| Groups | n | Median (min-max) | Mean ± SD | *P* value | Post hoc *P* value |
|---|---|---|---|---|---|
| **Gracey** | 9 | 4.5 (4 - 6) | 4.67 ± 0.82 | **0.002**[*] | 1-2: 0.430[**] |
| **Gracey+i-PRF** | 9 | 3.5 (3 - 4) | 3.50 ± 0.55 | | **1-3: 0.001**[**] |
| **Er:YAG** | 9 | 2.5 (2 - 3) | 2.50 ± 0.55 | | 1-4: 0.087[**] |
| | | | | | 2-3: 0.322[**] |
| **Er:YAG+i-PRF** | 9 | 3 (2 - 4) | 3.17 ± 0.75 | | 2-4: 1.000[**] |
| | | | | | 3-4: 1.000[**] |

[*] *Kruskal-Wallis test,* [**] *Dunn-Bonferroni post-hoc test*

***Table 2****. Dentinal Tubule Diameters and Widths in Groups*

| Groups | n | Median (min-max) | Mean ± SD | *P* value | Post hoc *P* value |
|---|---|---|---|---|---|
| **Gracey** | 9 | 1.25 (1.10 – 1.62) | 1.29 ± 0.18 | **<0.001**[*] | 1-2: 0.991[**] |
| **Gracey+i-PRF** | 9 | 2.01 (1.61 – 2.06) | 1.95 ± 0.17 | | **1-3: 0.015**[**] |
| **Er:YAG** | 9 | 2.72 (2.29 – 2.82) | 2.62 ± 0.24 | | **1-4: <0.001**[**] |
| **Er:YAG+i-PRF** | 9 | 3.15 (2.70 – 3.23) | 3.04 ± 0.22 | | 2-3: 0.615[**] |
| | | | | | **2-4: 0.026**[**] |
| | | | | | 3-4: 1.000[**] |

** Kruskal-Wallis test, ** Dunn-Bonferroni post-hoc test*

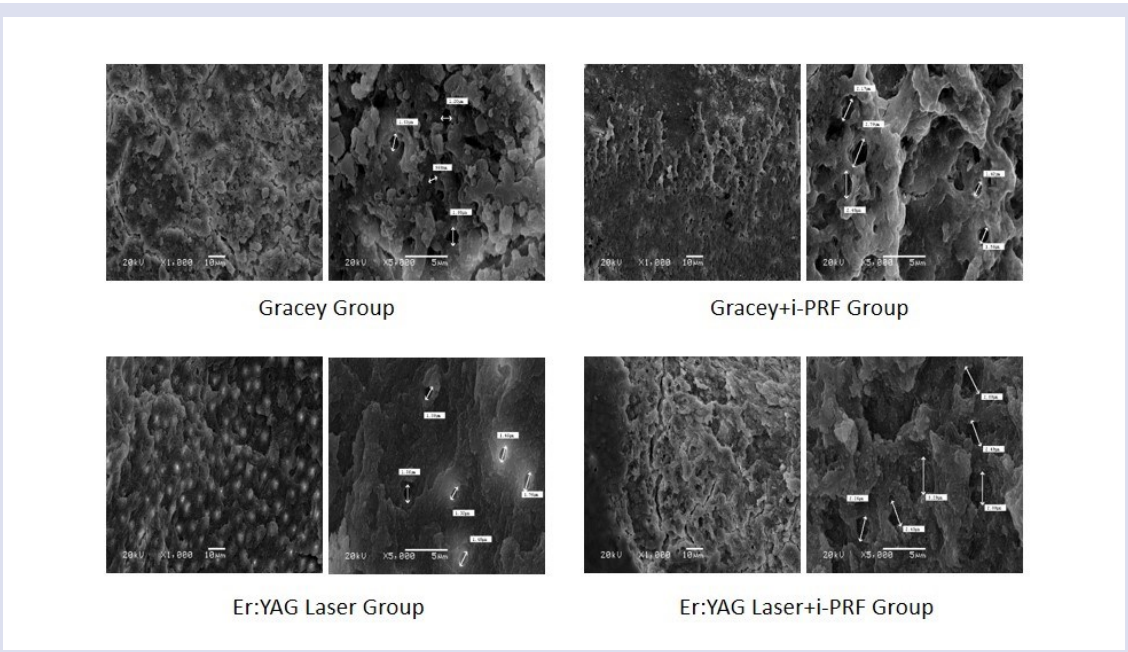


***Figure 1.*** *SEM images in four groups*

### *SEM Results*

*Gracey Group:* In the SEM micrographs of the group where only SRP was applied, a typical smear layer appearance was observed at 1000x magnification. Dentin tubules were partially open. The images obtained at 5000x magnification showed fewer and rather narrow dentin tubules compared to the other groups (Figure 1).

*Gracey + i-PRF Group:* Compared to the gracey group, it was observed that the oral parts of dentin tubules were more prominently opened at 1000x magnification. In the images obtained at 5000x magnification in this group, wider and more distinct dentin tubules were observed compared to the first group (Figure 1).

*Er:YAG Group:* In the 1000x micrographs of this group, a higher number and relatively prominent dentin tubules were observed compared to the two groups where SRP was performed with a gracey curette. Smear layers were very rarely observed at tubule openings, and the smear layer on the root surface was also recorded to be less than in the gracey groups. At 5000x magnification, fewer smear layers were observed, and wider dentin tubules were seen (Figure 1).

*Er:YAG + i-PRF Group:* Similar images to the Er:YAG laser group were recorded in terms of the visibility of dentin tubules and the amount of smear layer. Less amount of smear layer and more prominent dentin tubules were observed compared to the gracey groups. In the images obtained at 5000x magnification where dentin tubule diameters were measured, it was determined that no smear layer was observed at the tubule openings. Larger tubules were seen in terms of their width compared to the gracey groups (Figure 1).

## Discussion

The primary goal in periodontal therapy is based on the removal of bacteria and calcified structures such as dental calculus within the biofilm, disruption of the mechanical integrity of the biofilm structure on the cementum surface, and removal of contaminated cementum and endotoxins. The elimination of the smear layer formed on the contaminated root surface, detoxification of the root surface, exposure of collagen fibers, and obtaining a root surface close to the original for the adhesion of the clot, which is the first step in wound healing, are being investigated for alternative methods. For this purpose, the effectiveness of many chemical and physical agents such as citric acid, tetracycline, ethylene diamine tetra acetic acid (EDTA), platelet-derived growth factor (PDGF), fibronectin, and lasers (Nd:YAG, CO2, Er:YAG) on the root surface has been evaluated.[18-20] The lack of an optimised protocol for root surface modification and the lack of complete knowledge of the effectiveness of the Er:YAG laser, which has been increasingly used in recent years to overcome the disadvantages of the conventional SRP method used as the gold standard treatment, highlight the need for new studies.

If employed at low energy levels, Er:YAG laser does not cause protein denaturation on root surfaces and other tissues, instead, it exposes collagen fibrils or amino acids, creating a chemotactic effect for fibroblasts. Thus, it has been shown to positively affect fibroblast adhesion and proliferation.[12,21-23] Rossa *et al*.[24] reported that root surfaces treated with conventional SRP alone did not tend to adhere and proliferate. In contrast, samples treated with the Er:YAG laser showed predominantly flat cells on their surfaces. This was independent of the energy level or pulse rate. Laser-treated root surfaces showed more spindle-shaped cells compared to samples after mechanical SRP. In their study, Karthikeyan *et al*.[25] examined the morphological and chemical changes on the root surface following Er:YAG and Nd:YAG laser applications using SEM and infrared spectroscopy. Their findings from infrared spectroscopy analysis reported no changes in the inorganic substances on the root surface with Er:YAG laser. In our study, samples treated with laser-assisted SRP significantly produced less smear layer compared to conventional SRP samples. The ability to expose wider dentinal tubules and leave less smear layer behind carries the potential for clinical effectiveness. It is believed that after cleaning the infected root surface with this method, fibroblasts' adhesion to the root surface will be positively influenced. Additionally, the well-known stimulating effect of lasers on healing could manifest in the surrounding soft tissues during periodontal treatment.

The particular advantage of i-PRF is its longer-term release of growth factors such as transforming growth factor-beta (TGF-β), PDGF and vascular endothelial growth factor (VEGF), stimulating local angiogenesis, increasing adhesion of stem cells, modulating the immune system of the area, and enhancing epithelial mitogenesis.[16] A study examining the effect of i-PRF on the proliferation and osteogenic differentiation of gingival mesenchymal stem cells reported that a 5% i-PRF culture significantly increased cell proliferation after 7 days, while a 10% i-PRF culture significantly decreased cell proliferation. In the same study, it was also reported that the expression of all osteogenic genes for gingival mesenchymal stem cells decreased in i-PRF cultures.[26] In studies evaluating the effect of i-PRF on human periodontal ligament cells, it was reported that cell proliferation, cell migration, biological differentiation and mineralization increased after biomodification with i-PRF.[27,28] Another study compared the effects of i-PRF and PRP on human gingival fibroblasts cultured in vitro on titanium implant surfaces.[29] Given studies reported that compared to PRP, i-PRF could significantly influence the proliferation, differentiation, and migration of human osteoblasts, affecting osteoblast behavior more prominently. i-PRF exhibited significantly higher mRNA, PDGF, TGF-β, fibronectin, and type 1 collagen compared to PRP. An in vitro study by Okuda *et al*.[30] evaluated the efficacy of topical application of PRP on gingival fibroblasts. It was reported that PDGF and TGF-β are growth factors found in high concentrations and that platelet-rich blood products may be a source of these factors. In addition, it has been noted that PRP induces cell proliferation in a cell type-specific manner and that its ability to suppress epithelial cell proliferation is beneficial for regeneration. In light of all this information, it was thought that the addition of i-PRF, known to contain TGF-β, to the root surface affected by periodontitis and the external supplementation of this growth factor could be effective in this study. Aydınyurt *et al*.[31] compared the results of SRP, SRP + i-PRF and i-PRF-only applications in experimental periodontitis treatment in rats. The findings suggested that i-PRF might significantly contribute to bone mineralization by influencing osteoblast behavior. It was reported that root surface biomodification with i-PRF supported root coverage and increased the formation of new gingival tissue in a study investigating the effect of i-PRF on root coverage in free gingival grafting.[32] In another study, the effect of the use of i-PRF in combination with a connective tissue graft in gingival recession surgery on root closure results was investigated and it has been shown that the incorporation of i-PRF into the graft material results in a significant reduction in the depth of recession and an increase in the height of keratinized tissue in comparison to a connective tissue graft alone. However, no significant difference was found between the two treatment procedures after six months in terms of pocket depths, clinical attachment levels, recession width, gingival thickness, mean and complete root coverage.[33] Albonni *et al*.[34] evaluated the clinical effectiveness of i-PRF as a subgingival irrigation adjunct to SRP in 15 periodontitis patients with pockets (>5mm) in at least two teeth bilaterally. In their study, it was reported that the use of i-PRF in addition to SRP did not show a significant difference in clinical periodontal parameter values over a three-month period.

In conclusion, there is still no consensus on the effect of i-PRF on periodontal parameters evaluated in vivo, although in vitro studies have shown promising results for the periodontal regenerative potential of i-PRF. Based on the results of our study, i-PRF did not show a significant effect in removing the smear layer. i-PRF appears to be effective at the cellular level but ineffective at the morphological level. The emergence of wider dentin tubules may serve as evidence for its cellular-level effects. This could be attributed to a mechanism where the growth factors contained in i-PRF penetrate more easily into the increased amount of collagen structure and wider dentin tubules exposed after laser application.

## Conclusions

In conclusion, the application of Er:YAG laser appears to induce beneficial changes at both the cellular and morphological levels compared to conventional methods. Specifically, the combination of Er:YAG laser with i-PRF has yielded significant morphological results. Further animal studies and clinical trials are needed to explore the combined use of Er:YAG and i-PRF. The applications of Er:YAG laser and i-PRF hold promising evidence for clinical practice in facilitating periodontal regeneration, potentially eliminating the need for exogenous materials with evolving and advancing technology.

## Author contributions

NFG, MKH, EO participated in designing the study. NFG participated in generating the data for the study. NFG, EO participated in gathering the data for the study. NFG and EO participated in the analysis of the data. NFG, MKH, EO participated in writing the paper. NFG, MKH, EO has had access to all raw data of the study. EO has reviewed the pertinent raw data on which the results and conclusions of this study are based. NFG, MKH, EO have approved the final version of this paper. MKH guarantees that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

## Conflict of Interest Statement

The authors declared that they have no conflict of interest.

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