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Comparative Evaluation of Healing Following Gingival Laser Depigmentation Procedure with and Without Use of Injectable Platelet Rich Fibrin: A Randomized Controlled Split-Mouth Clinical Trial

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ABSTRACT

AIM - *To compare healing following gingival laser depigmentation with and without the application of injectable Platelet-Rich Fibrin (i-PRF).*

MATERIALS AND METHODS - *A randomized controlled split-mouth clinical trial was conducted on systemically healthy patients with gingival hyperpigmentation. Laser depigmentation was performed using a diode laser (980 nm). On the test side, i-PRF was prepared from venous blood and injected into the depigmented area; on the control side, no i-PRF was used. Healing was assessed using Landry's Healing Index, epithelization with 5% hydrogen peroxide, and recurrence with Dummett Gupta Oral Pigmentation Index.*

RESULTS - *Sites treated with i-PRF showed better healing scores, faster epithelization, and reduced recurrence of pigmentation compared to control sites.*

CONCLUSION - *Adjunctive use of injectable PRF after gingival laser depigmentation enhances wound healing and reduces recurrence.*

KEYWORDS - *Laser depigmentation, Injectable platelet-rich fibrin, Gingival pigmentation, Healing index, Split-mouth trial*

INTRODUCTION

Aesthetic demand in dentistry has risen considerably, with gingival appearance playing a crucial role in smile harmony. While coral-pink gingiva indicates health, excessive melanin deposition in the oral epithelium may cause gingival hyperpigmentation, often perceived as unesthetic despite being physiological. Although primarily benign, hyperpigmentation can also result from systemic factors, medications, heavy metal exposure, smoking, or pathology, underscoring the need for accurate diagnosis. Several depigmentation methods exist, including scalpel surgery, electrosurgery, cryosurgery, and lasers. Among these, lasers are preferred for their precision, haemostasis, and patient comfort, though delayed healing due to thermal damage remains a limitation. To address this, adjunctive therapies such as low-level laser therapy, ozonated oil, and platelet concentrates have been explored.

Injectable Platelet-Rich Fibrin (i-PRF), a novel autologous platelet concentrate rich in growth factors, promotes angiogenesis, fibroblast proliferation, and collagen synthesis. Its injectable form allows sustained release of regenerative mediators, making it minimally invasive and clinically advantageous. While PRF membranes have shown benefits in periodontal healing, evidence on i-PRF following laser-assisted gingival depigmentation is lacking.

This study aims to evaluate the effect of intramucosal i-PRF on gingival wound healing post-laser depigmentation, hypothesizing that i-PRF enhances healing and patient satisfaction compared to untreated sites.

AIM

Compare the healing of gingival laser depigmentation procedure with and without application of Injectable Platelet-Rich Fibrin.

OBJECTIVES

1. The healing at the surgical site using the healing index by Landry et al, 1988.
2. To assess

a. Epithelization at the surgical site using 5% hydrogen peroxide.
b. Recurrence- using Dummett Gupta oral pigmentation index (DOPI) (Dummett & Gupta, 1964)

MATERIALS AND METHODS

This study compares the effect of intramucosal injection of injectable platelet-rich fibrin following laser gingival depigmentation.

CLINICAL STUDY

Study design:

A clinical randomized controlled split-mouth trial

PATIENT SELECTION

The study was approved by the institutional research and ethical committee of DY Patil Deemed to be University School of Dentistry, Navi Mumbai.

IREB REFERENCE NO.- IREB/2025/PERIO/13

Place of research:

Department of Periodontology and Oral Implantology, D Y Patil deemed to be university, School of Dentistry, Nerul, Navi Mumbai

CRITERIA FOR SAMPLE SELECTION

Inclusion Criteria

1. Systemically healthy individuals aged between 18 to 50 years.
2. Gingival hyperpigmentation in esthetic zone- Dummet score 2.
3. Thick gingival phenotype
4. Good oral hygiene

Exclusion Criteria

1. Pregnancy
2. Lactating females
3. Smokers
4. Caries
5. Patients with severe crowding, gingival recession, caries, endodontic restorations, or fixed prosthesis on the examined teeth/anterior maxilla.
6. Patients with clinical signs of gingival inflammation or a history of surgery in the region of interest.
7. Patients taking any medications that can affect the periodontium.
8. Patients with periodontal disease.
9. Patients under orthodontic treatment or the presence of previous orthodontic treatment.
10. Poor oral hygiene, noncompliance with treatment, or persistence of gingival inflammation following phase I therapy
11. Previous treatment of gingival hyperpigmentation (surgical or non-surgical)

Written consent was obtained from all participants before data collection.

PROCEDURE

After taking a detailed case history, patients who met the selection criteria were enrolled for the study. Following phase 1 therapy, clinical parameters were re-evaluated after 2 weeks. Each patient was verbally informed about the procedure, and informed consent was obtained. Each patient was given post-operative

instructions after the surgical procedure.

PRE-OPERATIVE PREPARATION

1. Detailed case history and blood investigations
2. Phase 1 therapy – scaling and root planing
3. Indices (Dummet pigmentation index)
4. Evaluation of biotype
5. Informed Consent

MATERIALS AND METHODS (Figure 1)

1. Diode laser (Biolase Epic-980 nm)
2. #15 k file
3. Plain plastic tube
4. Insulin syringe
5. Centrifuge machine (eitek TC 650 D) (Figure 2)
6. 5% hydrogen peroxide

SURGICAL PROCEDURE

Local anesthesia was achieved using an infiltration technique on the buccal aspect of the anterior sextant. Gingival depigmentation was performed with a diode laser (EpicX Diode Laser, Biolase, CA, USA) operating at a wavelength of 980 nm. The procedure was carried out with the following parameters: 400-µm initiated tip, average power of 2 W, continuous mode, duty cycle of 100%, power density of 796.17 W/cm^2 at the fiber tip, and without water or air spray.

The laser tip was positioned in contact with the gingival surface at an angle of approximately 30° to minimize thermal damage. Interdental papillae with hyperpigmentation were also included. A saline-soaked gauze was intermittently used to remove epithelial remnants, hydrate tissues, and enhance visualization. The procedure was repeated until a thin layer of carbonized tissue was visible over a fresh, non-bleeding surface.

For i-PRF preparation, 9 mL of venous blood was collected in plain plastic and centrifuged immediately at 700 rpm for 3 minutes at room temperature using a standardized centrifuge. The upper plasma layer (1 mL) was aspirated using a 29-gauge, 5/16″, 1 cc (0.33 × 8 mm) needle for injection.

- **Group 1 (G1 – Test):** i-PRF was injected into the depigmented region at 1 mm depth, parallel to the epithelial–connective tissue junction, using a beveled upward technique. Injections were placed 2–3 mm apart, with 0.1 mL administered at each point.
- **Group 2 (G2 – Control):** Laser-depigmented sites were left to heal without the use of periodontal dressings.

Postoperative Instructions

- Avoid hot and spicy foods.
- Do not touch the surgical site with fingers or tongue.
- Avoid brushing and flossing at the surgical site for 7 days.
- Maintain plaque control by rinsing with 10 mL of 0.2% chlorhexidine gluconate twice daily for 1 week.

Follow-up Schedule

Patients were evaluated postoperatively on:

- Day 4
- Day 7
- 1 month
- 3 months

CLINICAL INDICES

A. ASSESSMENT OF GINGIVAL PIGMENTATION

Dummett–Gupta oral pigmentation index (DOPI) (Dummett & Gupta, 1964)

a. Subjective visual assessment of the gingival pigmentation was assessed using DOPI. The DOPI assessment was carried out using a standardized frontal-retracted lips photograph of the participant.

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b. The criteria of DOPI were based on the Scale of 0–3. Briefly

i. Scale 0 - pink tissue with no clinical pigmentation
ii. Scale 1 - mild clinical pigmentation (light brown tissue)
iii. Scale 2 - moderate pigmentation (medium brown or mixed
iv. pink and brown coloration)
v. Scale 3 - heavy clinical pigmentation (deep brown or blue–black tissue)

B. ASSESSMENT OF HEALING

COLOUR PALETTE

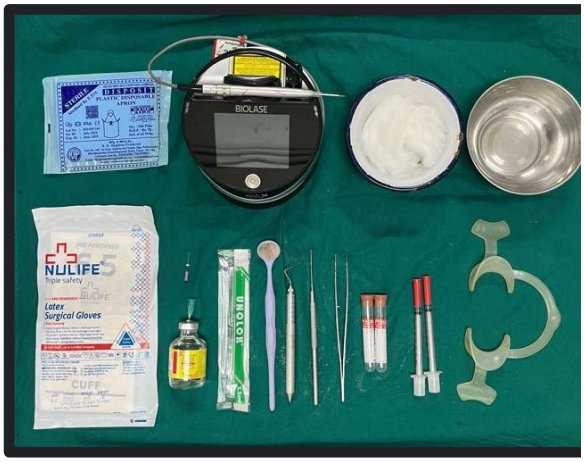

Figure 1 - Armamentarium

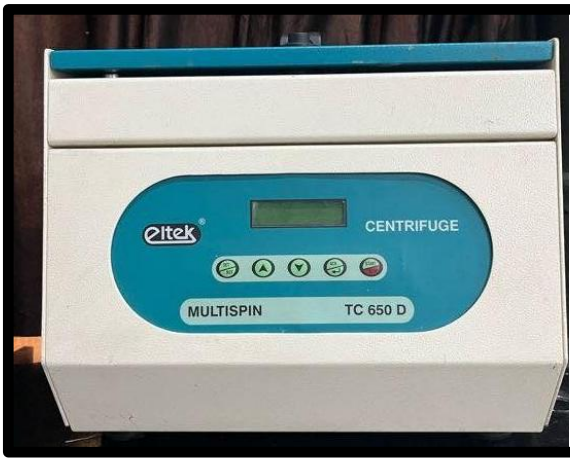

Figure 2 - Centrifuge Machine

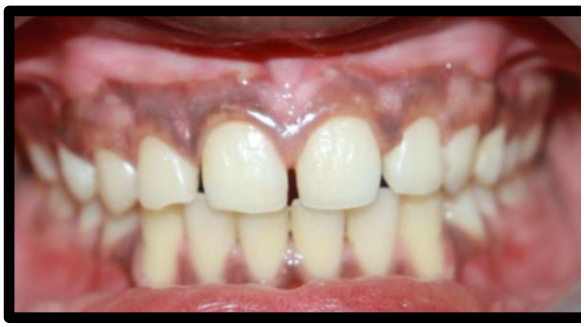

Figure 3 - Pre-Operative Image

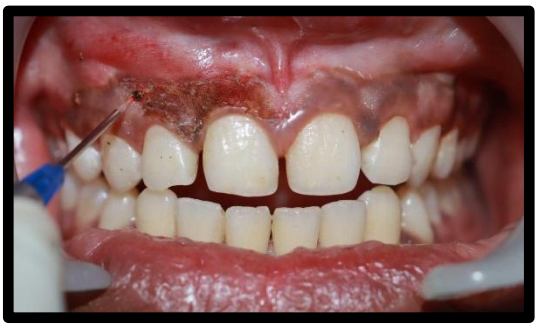

Figure 4 - During Diode Laser Depigmentation

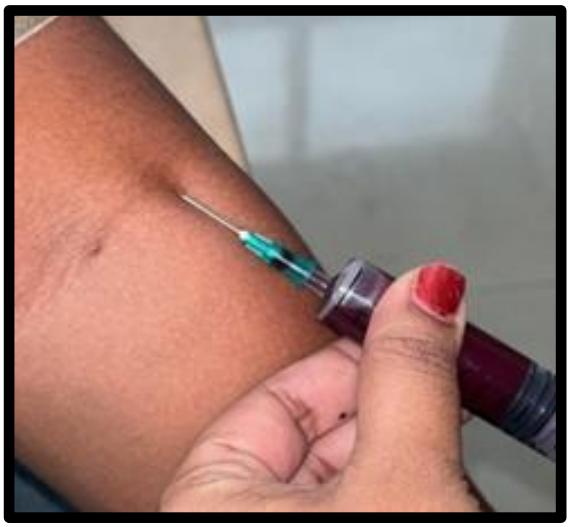

Figure 5 - Withdrawing blood

Figure 6 - Withdrawn blood placed in a centrifuge machine

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Figure 7- Injectable prf

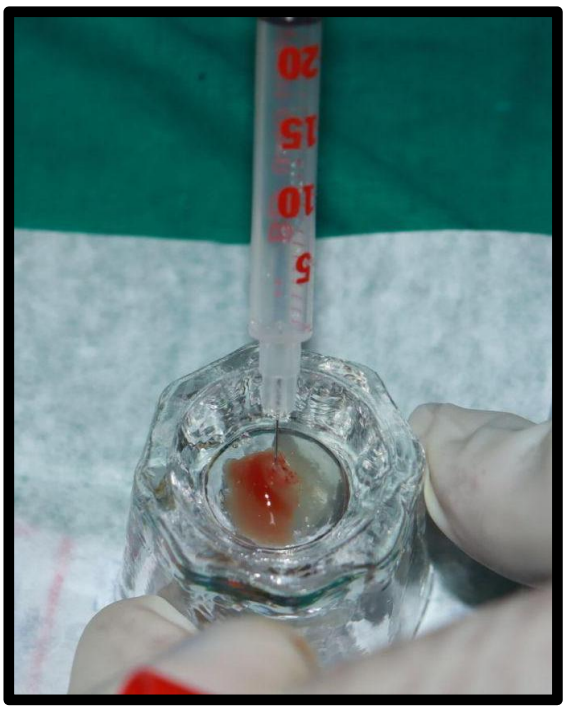

Figure 8 - Iprf withdrawn in the insulin syringe

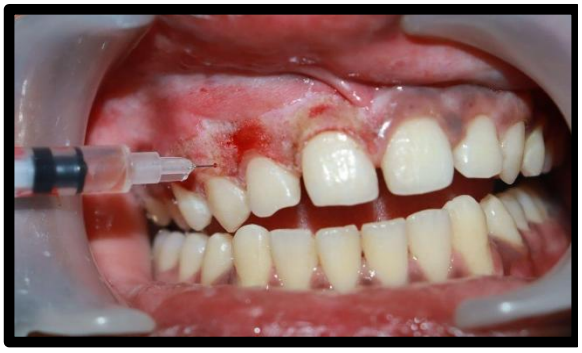

Figure 9 - Injecting Iprf on the right side

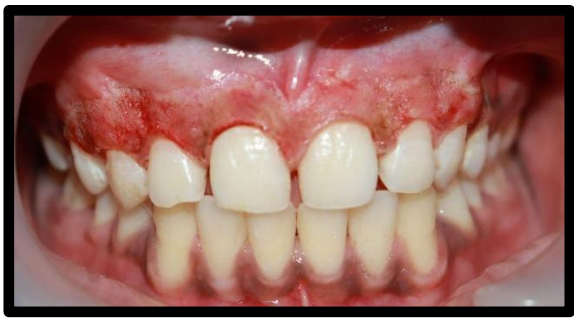

Figure 10 - Post-operative image

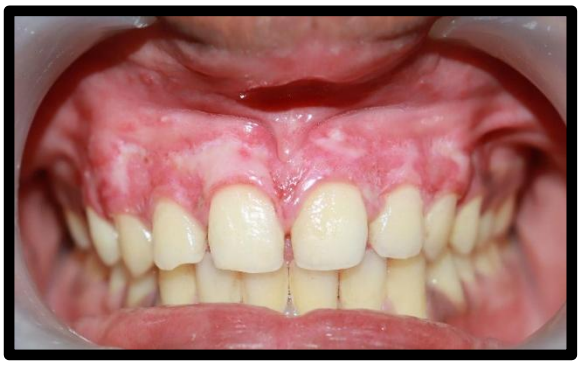

Figure 11 - Follow up at day 3

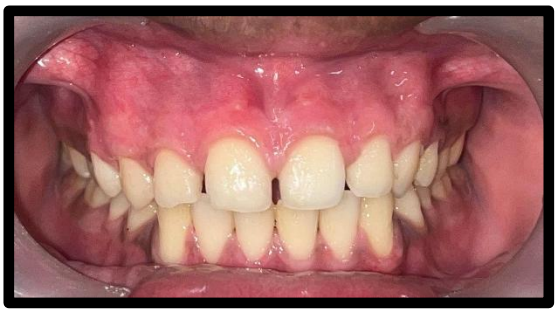

Figure 12 - Follow up at day 7

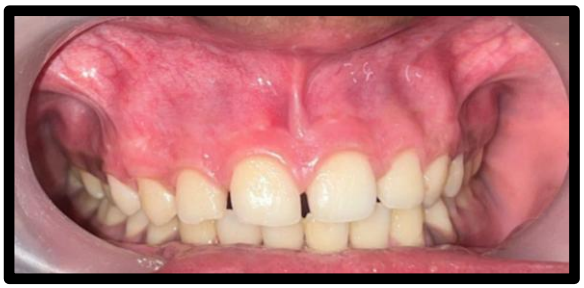
Figure 13 - Follow up at 1 month

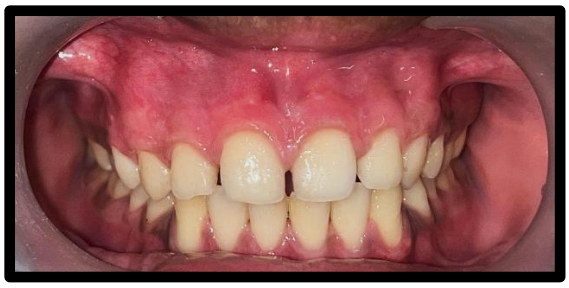
Figure 14 - Follow up at 3 months

STATISTICAL ANALYSIS

Descriptive and analytical statistics were performed. The Shapiro–Wilk test was applied to assess the normality of the data. For normally distributed variables, parametric tests were used, including one-way ANOVA with Tukey's post hoc test for multiple group comparisons, and the unpaired t-test for intergroup comparisons at specific time intervals. For non-normally distributed data, non-parametric tests were applied, including the Kruskal–Wallis test with Dunn's post hoc test for intragroup comparisons and the Mann–Whitney U test for intergroup comparisons.

Categorical variables such as epithelialization and recurrence were analyzed using the Chi-square test or Fisher's exact test, as appropriate. Intragroup comparisons across different time points were further assessed using repeated-measures ANOVA (parametric) or Friedman test (non-parametric), with post hoc tests applied when significant. McNemar's test with Bonferroni correction was used for paired nominal data.

Descriptive statistics, including mean, median, mode, standard deviation (SD), mean difference, and proportions, were calculated for all clinical parameters. A p-value ≤ 0.05 was considered statistically significant. Statistical analyses were performed using [insert software, e.g., SPSS version XX (IBM Corp., Armonk, NY, USA)].

Statistical Summary:

- **Normality**: Use Shapiro-Wilk.
- **Parametric**: Repeated Measures ANOVA with Tukey Post Hoc.
- **Non-Parametric**: Kruskal-Wallis ANOVA with Dunn's Post Hoc.
- **Nominal data** (like epithelialization or Recurrence): Use Chi-Square.

RESULTS

All participants (n = 20) completed the study without any adverse events or dropouts. The mean age was 28 ± 6.4 years (range: 18–38 years), comprising 12 males (60%) and 8 females (40%).

Healing Outcomes

The mean wound healing score (WHS) at day 4 was 2.7 ± 0.47 in the test group and 2.5 ± 0.51 in the control group, indicating partial loss of epithelium. By day 7, scores improved significantly in both groups (test: 4.5 ± 0.51; control: 4.05 ± 0.76; $p < 0.05$), and complete re-epithelialization was observed by 1 month and maintained at 3 months.

Intragroup analysis revealed statistically significant improvement in WHS across time points in both groups ($p < 0.05$). Intergroup comparison showed no significant difference at any follow-up interval ($p > 0.05$).

Epithelialization

At day 4, positive epithelialization was noted in 90% of test sites and 100% of control sites, with no significant intergroup difference ($p = 0.118$) By day 7, positive epithelialization increased to 93% in both groups ($p = 1.0$). Complete epithelialization was achieved in all participants at 1 and 3 months.

Intragroup analysis showed a statistically significant trend of increasing epithelialization in the test group ($p = 0.037$), while no significant difference was observed in the control group ($p = 1.0$).

Recurrence

Recurrence of pigmentation was not observed at 1 month in either group. By 3 months, recurrence was noted in 3 participants (15%) in the control group and none in the test group, showing a statistically significant intergroup difference ($p = 0.023$). Intragroup comparison did not reveal significant changes across time.

According to the Dummett Oral Pigmentation Index (DOPI), gingival tissues in both groups showed spreading of pinkish areas by 1 and 3 months postoperatively. However, recurrence was significantly higher in the control group at 3 months.

DISCUSSION

This randomized controlled split-mouth clinical study is among the first to evaluate the role of injectable platelet-rich fibrin (i-PRF) in enhancing healing outcomes following laser-assisted gingival depigmentation. The study introduces intramucosal injection of i-PRF as a minimally invasive and biologically driven approach to improve tissue regeneration and postoperative recovery. Unlike conventional platelet concentrates such as PRP or PRF membranes, i-PRF remains in a liquid state for a clinically useful period, allowing direct injection into target tissues. This property ensures uniform distribution while avoiding additives or anticoagulants, making it a natural source of growth factors, leukocytes, and fibrin matrix components essential for wound healing.

Enhanced Wound Healing

Our findings revealed significantly greater improvements in Wound Healing Scores (WHS) in the i-PRF group compared with controls, particularly at one week postoperatively. Statistically significant differences were also observed intragroup at day 4, day 7, 1 month, and 3 months. The enhanced healing effect is likely mediated by the fibrin scaffold, which facilitates cellular migration, angiogenesis, and controlled release of bioactive molecules such as PDGF, VEGF, and bFGF. These factors are known to stimulate fibroblast proliferation, neovascularization, and extracellular matrix remodeling, all of which accelerate tissue repair.

Comparable studies have demonstrated similar benefits. Bozkurt and Uslu reported improved LTH index scores following i-PRF application in gingivectomy and gingivoplasty patients, while Debnath and Chatterjee found superior outcomes with PRF membranes compared to periodontal dressings. Importantly, i-PRF offers the additional advantage of a less invasive mode of delivery, shorter chairside preparation, and better patient compliance.

Epithelialization and Patient Comfort

Epithelialization plays a critical role in wound closure and esthetic stability. In the i-PRF group, faster epithelial coverage was noted, with significant intragroup differences across all time intervals and intergroup significance on day 4. Growth factors such as EGF and TGF-β1 in i-PRF promote keratinocyte migration and proliferation, thereby expediting epithelialization. Patients treated with i-PRF also reported lower postoperative pain, possibly due to its leukocyte-mediated anti-inflammatory effects and the protective fibrin matrix.

These results align with Ibrahim et al., who observed greater epithelial thickness and vascularity in i-PRF–treated sites after diode laser depigmentation. Similarly, Bozkurt and Uslu demonstrated improved epithelial wound healing using both LTH and MMS indices, while Kızıltoprak and Uslu found that autologous fibrin glue (AFG) provided superior epithelialization compared with i-PRF. Collectively, these findings highlight the role of i-PRF in enhancing patient comfort and clinical outcomes in mucogingival procedures.

Pigmentation Recurrence

Although both groups exhibited reduced pigmentation at 3 months, the i-PRF group demonstrated slightly greater reductions, which may be related to modulation of melanogenesis by growth factors such as TGF-β1 and EGF. TGF-β1 downregulates melanogenesis by inhibiting ERK phosphorylation, while EGF suppresses prostaglandin-E2 and tyrosinase activity, potentially lowering recurrence rates.

Ibrahim et al. reported comparable findings, with both i-PRF and control groups showing significant DOPI score reductions, though differences between groups were not statistically significant. Similarly, Esfandiary et al. compared diode laser depigmentation with cryosurgery and highlighted the role of treatment modality in influencing recurrence rates. Moreover, dermatological studies have reported

promising outcomes when platelet concentrates were used adjunctively with lasers to treat hyperpigmentation, further supporting the potential of i-PRF as a natural depigmentation adjunct.

CONCLUSION

After analysing the results of present study, we may conclude that

- The effect of immediate intramucosal injection of injectable platelet-rich fibrin (i-PRF) following laser-assisted gingival depigmentation demonstrated a beneficial role in promoting early soft tissue healing.
- The i-PRF-treated sites were associated with reduced postoperative discomfort, including pain and irritation, thereby improving overall patient satisfaction. The minimally invasive nature of i-PRF application, combined with its ease of use and biological compatibility, highlights its practicality in clinical settings, particularly in esthetic periodontal procedures.
- The i-PRF group demonstrated a trend toward better cosmetic outcomes, statistically significant improvement in recurrence of pigmentation was observed over the follow-up period of three months. This suggests that while i-PRF may enhance early healing and patient comfort, its influence on delayed recurrence of pigmentation.

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