



CLINICAL RESEARCH:

The Effect of Platelet-Rich Fibrin and Low-Level Laser Therapy Associated with Simplified Papilla Preservation Flaps in the Management of Supra-Alveolar Periodontal Defects
El efecto de la terapia con láser de baja intensidad y fibrina rica en plaquetas asociada con colgajos de preservación de papila simplificados en el tratamiento de defectos periodontales supraalveolares

Sangeetha Subramanian¹ <https://orcid.org/0000-0003-3720-1095>; Prakash PSG¹ <https://orcid.org/0000-0003-4243-5865>
Vijayanth Singh¹ <https://orcid.org/0000-0002-7894-6125>; Devapriya Appukuttan¹ <https://orcid.org/0009-0009-2534-0443>
Dhayanand John Victor¹ <https://orcid.org/0000-0002-1631-6427>; Devi Bala RT¹ <https://orcid.org/0000-0002-0969-5887>
Thodur Madapusi Balaji² <https://orcid.org/0009-0007-2606-8651>; Rodolfo Reda³ <https://orcid.org/0000-0003-1532-6524>
Luca Testarelli³ <https://orcid.org/0000-0003-3904-3000>; Shankargouda Patil⁴ <https://orcid.org/0000-0003-0783-8263>

¹SRM Dental College, Bharathi Salai, Chennai 600089, India.

²Department of Dentistry, Bharathirajaa Hospital, and Research Institute, Chennai-600017, India.

³Department of Oral and Maxillofacial Sciences, Sapienza University of Rome, 00161 Rome, Italy.

⁴College of Dental Medicine, Roseman University of Health Sciences, South Jordan, UTAH-84095, USA.

Correspondence to: Luca Testarelli - luca.testarelli@uniroma1.it
Prakash PSG - akashpsg@gmail.com

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ABSTRACT: The current study aimed to evaluate and compare the effect of Platelet Rich Fibrin (PRF) and Low-Level Laser Therapy (LLLT) on the clinical outcomes when supraalveolar defects were accessed with Simplified Papilla Preservation Flap (SPPF). Forty-five patients presenting with supra-alveolar defects were allocated into three study groups with 15 patients in each group. SPPF approach was performed in each group with the addition of PRF and LLLT in the test groups. In all the study groups, a significant reduction in pocket depth was observed ($P < 0.001$) at 6 months after therapy. The Relative Attachment Gain was significantly higher in the PRF Group ($P < 0.001$) and in the Control Group ($P = 0.02$) at six months compared to baseline. The study groups failed to show significant differences between them when clinical parameters were compared at six months.

KEYWORDS: Platelet-rich fibrin; PRF; Low-level laser therapy; Periodontal defects; Papilla preservation flaps.



RESUMEN: El presente estudio tuvo como objetivo evaluar y comparar el efecto de la fibrina rica en plaquetas (PRF) y la terapia láser de baja intensidad (LLLT) en los resultados clínicos cuando se accedió a los defectos supraalveolares con colgajo de preservación de papila simplificado (SPPF). Cuarenta y cinco pacientes que presentaban defectos supraalveolares fueron asignados a tres grupos de estudio con 15 pacientes en cada grupo. Se realizó un abordaje SPPF en cada grupo con la adición de PRF y LLLT en los grupos de prueba. En todos los grupos de estudio, se observó una reducción significativa en la profundidad de la bolsa ($P < 0,001$) a los 6 meses después de la terapia. La ganancia de inserción relativa fue significativamente mayor en el grupo PRF ($P < 0,001$) y en el grupo de control ($P = 0,02$) a los seis meses en comparación con el inicio. Los grupos de estudio no mostraron diferencias significativas entre ellos cuando se compararon los parámetros clínicos a los seis meses.

PALABRAS CLAVE: Fibrina rica en plaquetas; PRF; Terapia con láser de baja intensidad; Defectos periodontales; Colgajos de preservación de papilas.

INTRODUCTION

Supra-alveolar defects are common and are highly prevalent in patients with periodontitis and yet represent the least predictable periodontal defects. Currently, therapeutic protocols that ensure complete preservation of the interdental soft tissues using surgical incisions and primary wound closure gain paramount importance. Cortellini developed minimally invasive surgical procedures like simplified and modified papilla preservation techniques that preserve the integrity of the interdental papilla (1-4). SPPF when utilized with barrier membranes in intrabony defects demonstrated consistent improvements in probing attachment gains (5-7). The advantage of the simplified papilla preservation technique is that it ensures primary wound closure that preserves and protects the clot in the operated area. The application of regenerative materials like growth factors supports the natural process of periodontal healing.

In that regard, Choukron's Platelet Rich Fibrin (PRF), an autologous second-generation platelet concentrates acts as a natural matrix that comprises of viable platelets that liberate diverse growth factors like Platelet Derived Growth Factor, Insulin-like Growth Factor, Transforming growth

factor, Vascular Endothelial Growth Factor, basic Fibroblast Growth Factor, into the wound area while preventing epithelial down growth (8).

PRF also enhances the extracellular matrix synthesis and promotes angiogenesis, all of which are fundamental to periodontal wound healing.

Several *in vitro* and *in vivo* studies have established that stimulation with LLLT resulted in accelerated healing of bone defects (9-11). Biostimulation by lasers promoted beneficial intracellular metabolic changes, leading to faster cell division, proliferation, and migration of fibroblasts as well as prompt matrix production (1,10). This perhaps accelerates the wound healing response of the defect.

Considering all these facts and the relevant factors, the most appropriate and reliable treatment needs to be devised for the supra-alveolar defects. Generally, clinical gain in these types of defects is usually a result of epithelial and connective attachment to the root surface. Hence the clinical outcomes of these defects are usually determined by the surrogate markers such as probing depth and attachment gain, which gives us an indication of the extent of soft tissue healing (12).

Although the prevalence of supra-alveolar defects is very common in periodontitis, there are very limited clinical trials available concerning the management of these defects. There are no studies till date in the literature that compared the effect of PRF and LLLT on the healing of supra-alveolar defects with minimally invasive surgical procedures. The study hypothesizes that the application of PRF and LLLT to SPPF improves wound healing favorably compared to SPPF alone in supra-bony defects. Therefore, the present study aimed to evaluate and compare the efficacy of PRF and LLLT on the clinical outcomes when supra-alveolar defects were accessed with SPPF.

MATERIALS AND METHODS

The study protocol of this original research was approved by the Institutional Review Board SRM Dental College (Approval Number: SRMDC/IRB/2015/MDS/No.507). The Sample size for the current study was calculated based on the mean of clinical attachment level / periodontal attachment level obtained from articles (13-15) using the formula Hypothesis testing for three means (equal variances). The Standard deviation in the I group S1, II group S2, III group S3 were 0.89, 1.76, and 1.13 respectively. The effect size is 0.4965084. The type II error β was set at 80% and the type I error α was set at 5%. Based on this, the required sample size estimated for the study was a total of 45 patients with 15 patients in each of the three groups. The study was conducted at the outpatient clinics of the Department of Periodontics and Oral Implantology in accordance with the 1975 Helsinki Declaration Guidelines as revised in 2013. Patients with suprabony defects were provisionally recruited into the study. The study protocol, nature of the bony defects, treatment plan, and their possible complications were explained in detail to the participants and a written informed consent was signed by them. Initially, clinical models, blood investigations, and intraoral periapical radiographs were taken. All the clinical parameters were measured

by a single calibrated examiner. Final recruitment was done when the patient fulfilled the selection criteria after phase I therapy. Patients from both genders of age group 30-65 years diagnosed with Chronic Periodontitis presenting with at least two adjacent sites with a probing pocket depth (PPD) of ≥ 5 mm or clinical attachment loss (CAL) of ≥ 3 mm associated with a horizontal bone defect after completion of phase I therapy were recruited for the study. Full-mouth plaque scores (FMPS) and bleeding scores (FMBS) $<20\%$ were taken for surgery. Patients with medical conditions that contraindicate periodontal surgery, Patients requiring antibiotic prophylaxis, current smokers, pan chewers, pregnancy and lactating women, and non-vital tooth or teeth with furcation involvement were excluded from the study. The following are the control and the test groups of the study, Group A: (Control Group): Supra-alveolar defects treated with Simplified Papilla Preservation Flap (SPPF). Group B: (Test Group): Supra-alveolar defects treated with Simplified Papilla Preservation Flap and Platelet-Rich Fibrin (SPPF with PRF) Group C: (Test Group): Supra-alveolar defects treated with Simplified Papilla Preservation Flap and Low-Level Laser Therapy (SPPF with LLLT). Initially over 50 patients were recruited for the study. Some of them did not comply with oral hygiene instructions and recall visits after phase I therapy. Hence 45 patients were recruited for the study with 15 patients in each group. The clinical evaluation of subjects was done where all the patients were reviewed after nonsurgical therapy to evaluate their oral hygiene and the clinical parameters like FMPS, FMBS, Probing Pocket Depth (PPD), Relative Attachment Level (RAL), Relative Position of Gingival Margin (PGM) were assessed at the surgical site. A customized resin template was prepared using light cure composite (REVOTEC LC) to fit over the selected teeth. Vertical grooves were made for the reproducibility of the probing angulations. Forty-five sealed envelopes were prepared with a paper inside in which Group A, Group B, or Group C were mentioned. The envelopes were

chosen at random and opened by the operator at the time of surgery. The surgical technique used in this study is the Simplified Papilla Preservation Flap. Following the administration of 2% local anaesthesia, the simplified Papilla Preservation technique was used to raise the mucoperiosteal flap associated with the defect. A buccal intracrevicular incision is made to extend obliquely across the papilla of the involved teeth from the gingival margin at the buccal line angle to the mid-interproximal portion of the papilla below the contact point of the adjacent tooth. The incision was then continued intrasulcularly along the buccal aspect of the neighboring teeth. Palatally, the incision

is intrasulcular that is limited to the mid-palatal aspect of the tooth. A full-thickness flap was elevated on the buccal aspect and the interdental papillary tissues at the defect site were gently elevated along with the lingual /palatal flap to fully expose the interdental defect. Root and defect debridement was performed with manual curettes and ultrasonic instruments. In the Control Group, (Group A) following SPPF access, the root surface debridement was meticulously performed. In the Test Group B an occlusive PRF matrix was grafted onto the suprabony defects (Figure 1) and in Test Group C biostimulation with LLLT was done for the suprabony defects (Figure 2).

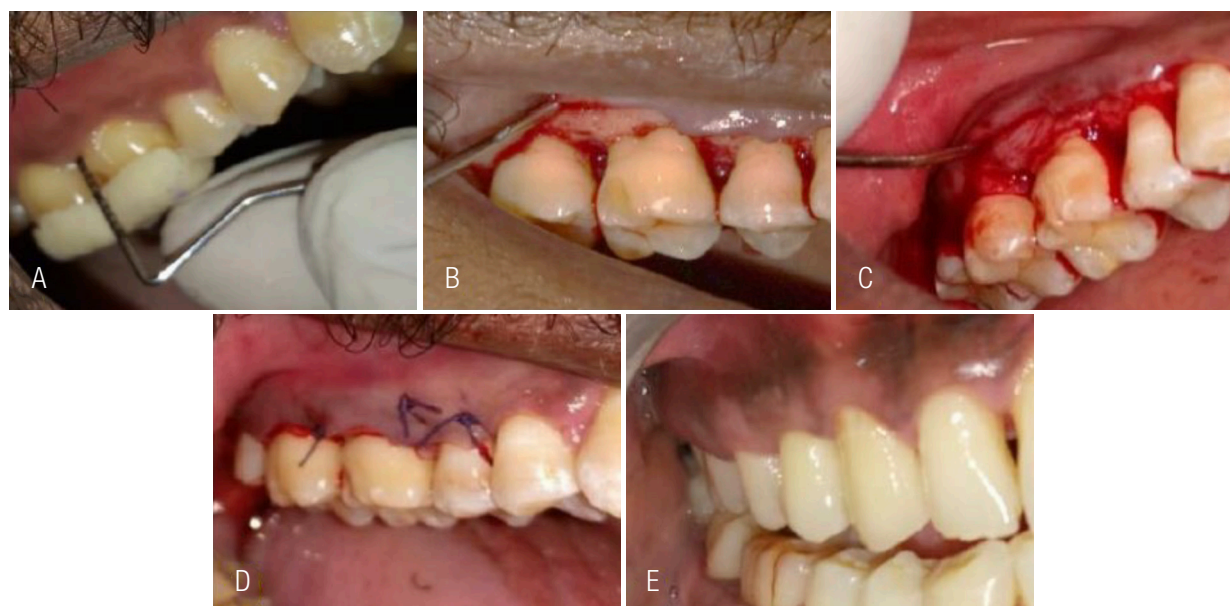


Figure 1. Surgical protocol of PRF Group A) Pre-operative image of surgical site in relation to 15,16,17 B) Simplified Papilla Preservation Flap performed in relation to 15,16,17 C) Platelet Rich Fibrin placement done in relation to 15,16,17 D) Internal Vertical mattress suturing done in relation to 15,16,17 E) Post-operative image after six months.

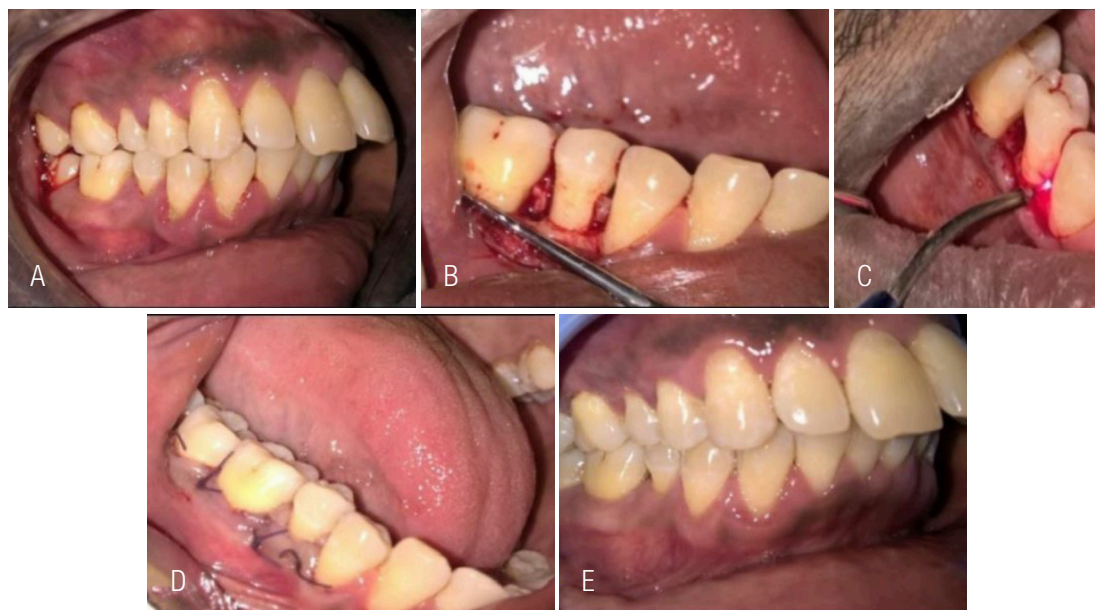


Figure 2. Surgical protocol of LLLT group A) Pre-operative image of surgical site in relation to 44,45,46 B) Simplified Papilla Preservation Flap performed in relation to 44,45,46 C) Low Level Laser Therapy stimulation done in 44,45,46 D) Internal Vertical mattress suturing done in 44,45,46 E) Post-operative image after six months.

The buccal incision was intracrevicular with an oblique incision across the papilla starting from the gingival margin at the buccal line angle of a tooth to the mid-interproximal portion of the papilla below the contact point of the adjacent tooth. A buccal intracrevicular incision is made to extend obliquely across the papilla of the involved teeth from the gingival margin at the buccal line angle to the mid-interproximal portion of the papilla below the contact point of the adjacent tooth.

Wound closure was obtained using vertical internal mattress sutures with vicryl 4.0 and periodontal dressing (COE-PAK) was placed. The PRF was prepared by collecting ten ml of intravenous blood in a sterile glass tube without anticoagulant which was centrifuged at 3000 rpm for 13 minutes. A structured fibrin matrix, PRF is formed in the middle of the tube between the red corpus-

cles at the bottom and acellular plasma at the top. The PRF was then separated from red corpuscles base using sterile tweezers and scissors. In Test Group C, the defects were irradiated with a diode laser at 0.5 W power with a 0.6 mm optical fiber tip in continuous non-contact mode for 20 seconds and then retracted for eight seconds. The biostimulation was repeated three times such that the defects were lased for about 60 seconds. The patients were instructed not to perform mechanical oral hygiene procedures in the treated site and to use Chlorhexidine mouth rinse at 0.2% concentration for two weeks. The Early Wound Healing Index (16), was assessed in all groups in the first and second weeks. Each recall visit included reinforcement of oral hygiene procedure, removal of supra-gingival plaque, and calculus if necessary. After suture removal in the 2nd week, patients were reviewed for a monthly maintenance regime for up

to six months. Clinical parameters were reassessed at six months. The data were collected and subjected to statistical analysis.

STATISTICAL ANALYSIS

The data was analyzed statistically using SPSS software version 22 to find the mean, standard deviation, and test of significance. Except for the variable age, other variables do not follow normal distribution according to the Normality tests Kolmogorov-Smirnov and Shapiro-Wilks. Both Parametric and Non-parametric methods were hence applied to analyze the data. One-way ANOVA was applied to compare the variable mean age between the study groups. Chi-Square test was applied to compare proportions between groups and if any expected cell frequency is less than five then Fisher's exact test was used. Kruskal Wallis test was used for variables that do not follow Normal distribution. To compare variables between two-time points Wilcoxon Signed Rank test was used.

RESULTS

Table 1 represents baseline characteristics of patients' age, gender, and clinical parameters.

The Early wound healing index of the study groups does not show any difference between the groups (Table 2).

A Comparison of various clinical parameters like Probing Pocket Depth, Position of Gingival Margin, Relative Attachment Level, and Sulcular bleeding index at baseline and six months between the study groups was given in Table 3.

All the clinical parameters show considerable improvement at six months within the study groups except the position of the gingival margin.

However, none of the parameters showed significant differences between them when compared between the groups.

Table 1. Descriptive Statistics of patients at baseline.

Groups	SPPF	SPPF+PRF	SPPF+LLLT	P-value
Age (Years)	37.80± 7.06	38.40±8.90	38.13 ± 9.19	0.98
Male %	40%	33.3%	60%	0.3
Female %	60%	66.7%	40%	
FMPS	16.67± 1.83	16.27±1.83	16.40 ± 1.12	0.8
FMBS	16.93± 1.62	16.00±1.06	16.20 ± 1.37	0.2
PPD (mm)	6.80 ± 1.42	6.53 ± 1.24	7.07 ± 1.38	0.5
PGM (mm)	1.67 ± 0.61	2.20 ± 0.77	1.80 ± 0.56	0.1
RAL (mm)	8.47 ± 1.40	8.73 ± 1.33	8.87 ± 1.45	0.6

Data were presented as mean ± standard deviation for age PPD, RAL and PGM. Gender, FMPS and FMBS were expressed as percentages. P Value of ≤0.05 % is considered significant.

*SPPF- Simplified Papilla Preservation Flap, PRF- Platelet Rich Fibrin, LLLT- Low Level Laser Therapy, FMPS- Full Mouth Plaque Score, FMBS- Full Mouth Bleeding Score, PPD- Probing Pocket Depth, PGM-Position of Gingival Margin, RAL- Relative Attachment level, mm-millimeters.

Table 2. Early Wound Healing index.

Wound healing	Study Groups		
	SPPF	SPPF+PRF	SPPF+LLLT
I Week Mean \pm SD	1 \pm 0.00	1 \pm 0.00	1 \pm 0.00
II Week Mean \pm SD	1 \pm 0.00	1 \pm 0.00	1 \pm 0.00

Table 3. Comparison of clinical parameters at baseline and six months between the study group.

Variables	SPPF	SPPF+PRF	SPPF+LLLT	P value (Kruskal wallis)
PPD Baseline	6.80 \pm 1.42	6.53 \pm 1.24	7.07 \pm 1.38	0.5
Six months	4.07 \pm 0.59	3.93 \pm 0.25	4.40 \pm 0.73	0.087
P value [¶]	0.001	0.001	0.001	
RAL Baseline	8.47 \pm 1.40	8.73 \pm 1.33	8.87 \pm 1.45	0.6
Six months	6.93 \pm 1.48	6.60 \pm .82	7.80 \pm 1.97	0.370
P value [¶]	0.020	0.001	0.205	
PGM Baseline	1.67 \pm 0.61	2.20 \pm 0.77	1.80 \pm 0.56	0.1
Six months	2.87 \pm 0.99	2.67 \pm 0.72	3.40 \pm 1.40	0.486
P value [¶]	0.004	0.138	0.007	
GBI Baseline	16.93 \pm 1.62	16.00 \pm 1.06	16.20 \pm 1.37	0.213
Six months	13.73 \pm 2.98	14.80 \pm 3.70	14.87 \pm 2.47	0.662
P value [¶]	0.007	0.247	0.131	

[¶] Wilcoxon Rank Test.

P Value of ≤ 0.05 % is considered significant.

*SPPF- Simplified Papilla Preservation Flap, PRF- Platelet Rich Fibrin, LLLT- Low Level Laser Therapy, PPD- Probing Pocket Depth, PGM-Position of Gingival Margin, RAL- Relative Attachment level, GBI – Gingival Bleeding Index.

DISCUSSION

The present study evaluated and compared the efficacy of PRF and LLLT in suprabony defects accessed with SPPF. Early wound healing assessment done in the first and second weeks showed that all the patients in the study groups had complete flap closure and no fibrin line in the interproximal area. Full mouth plaque scores

and bleeding scores evaluated within each group at different time points showed that there was a reduction of both the scores from baseline to six months in each group.

The SPPF technique was preferred as it minimizes the surgical trauma while preserving the soft tissues interdentally. The primary closure achieved with this flap helps to isolate the wound area

from the oral environment and further, it reduces the postsurgical discomfort (6). Graziani 2012 in his meta-analysis demonstrated that compared to Open Flap Debridement (OFD), Simplified and Modified papilla preservation flaps had better clinical outcomes in intrabony defects (17).

All three study groups showed highly significant improvements in probing depth reductions at six months from baseline. The probing depth reductions were 2.73 mm, 2.6 mm, and 2.67 mm in the control group, PRF group, LLLT group respectively at six months. When analyzed statistically, the probing depth does not show significant differences between any of the groups at six months (Table 2).

RAL has traditionally been the reliable surrogate marker for evaluating clinical outcomes. The observed changes in RAL between baseline and six months were gains of 1.54 mm and 1.07 mm for the control group and LLLT group respectively. When the PRF group was analyzed, RAL gain of about 2.13 mm was noted which was also highly significant at six months. Intergroup analysis showed that the study groups did not demonstrate statistically significant differences between them at six months ($P=0.37$) (Table 2).

Periodontal surgeries performed for treating pockets usually results in gingival recession which is an unfavorable outcome affecting the patient's esthetics and function. However, the effect could be limited by using bioactive materials which could reduce the probing depths and increase the attachment gain. In the control group, the position of the gingival margin was altered indicating 1.2 mm of gingival recession at six months. Similarly, in the PRF and LLLT groups, the amount of recession estimated was about 0.47 mm and 1.6mm respectively at six months. This observation explains that there were significantly increased attachment gains in the PRF group compared to the control and the LLLT groups. However, when the

study groups were subjected to intergroup statistical analysis concerning the position of gingival margin, significant differences were not demonstrated at six months.

In the present study, the PRF group revealed an additional clinical attachment gain of 1.5 mm compared to the other study groups. This positive outcome in the PRF group in terms of increased RAL gains emphasizes the fact that space provision effect in a wound is very vital for periodontal regeneration. The reservoirs of growth factors and cytokines present in the PRF would have enhanced the feasibility of soft tissue wound healing.

The SPPF control group in the present study exhibited 1.5mm of CAL gain and 2.7 mm of PPD reduction, which is far more significant than those obtained with Open Flap Debridement studies (18,19). This can be attributed to a very conservative surgical approach with precise incision with regard to SPPF which aided in good interproximal healing. The SPPF offers a stable and closed environment, allowing proper adhesion and maturation of the clot in the wound area. This, in turn, influences the supra-crestal tissue remodeling which results in greater clinical advantage.

The disadvantage of PRF is that it lacks rigidity and hence tends to collapse over the defect. This may compromise the space-making effect of PRF leading to the loss of the clot. The faster biodegradability of the PRF membrane (one to two weeks) is also a significant problem as it is insufficient for the regeneration of hard and soft tissues. Therefore, we need to look at the factors that enhance the linkage of the fibrin clot to the root/defect surface for sufficient time so that it matures into new connective tissue.

The surgical application of lasers in clinical trials is very limited. Furthermore, the use of specific lasers such as diode lasers in periodontal surgeries is sparse thus making the comparison of

our results with previous reports difficult. Ozcelik *et al.* and Sanz-Moliner *et al.* in their study has reported that LLLT with diode laser significantly modulated wound healing in periodontal surgeries favourably while Masse *et al.* and Damante *et al.* did not find any additional benefits of LLLT in periodontal surgeries (14, 20-22). A meta-analysis by Fabrizio Sgolastra also confirmed that the adjunctive use of diode lasers did not offer superior clinical advantage (23, 24). In the present study, LLLT was applied only once during the therapy. Repeated applications in the following week on the buccal and lingual surfaces would have improved the clinical benefits.

The present study was undertaken based on the working hypothesis that PRF and LLLT could positively modulate and improve the healing of suprabony defects when accessed with SPPT. Clinically, there is improved attachment gain and PPD reduction in PRF group compared to other groups. However, there is a lack of statistical significance between the study groups at six months which rejects the hypothesis. Based on the present study, the SPPT technique itself can be considered a stand-alone protocol to treat suprabony defects because of its wound-stabilizing properties. The addition of bioactive factors did not facilitate or improve wound healing.

The findings of the present study are further supported by the trials conducted by Cortellini *et al.*, Trombelli *et al.*, and Shan Liu *et al.* with minimally invasive surgical therapy on intrabony

defects. They also reported that minimally invasive surgical techniques alone yielded better clinical outcomes and that the addition of regenerative materials to the technique did not offer any significant advantage (8, 25, 26).

From the results of the study, it is observed that all three study groups have shown improvements in clinical parameters from baseline to six months within each group. However, when the clinical outcomes were compared between the groups at six months, none of the groups exhibited significant differences (27). Therefore, the addition of bioactive factors to SPPF did not yield additional clinical benefits for supra-alveolar defects.

The limitations of the current study are that the follow-up period after surgery is only 6 months. In addition, only the clinical parameters of supra-bony defects were evaluated and radiographic parameters were not considered as an outcome measure. Future trials on the wound healing response of suprabony defects with the application of different types of platelets concentrates and other biomatrices in larger sample sizes are required to be conducted.

CONCLUSIONS

The results of the study showed that the application of bioactive factors like Platelet-rich fibrin and low-level laser therapy to Simplified papilla preservation flaps did not yield additional clinical benefits for supra-alveolar defects.

AUTHOR CONTRIBUTION STATEMENT

Conceptualization: S.S., P.P.S.G., V.S., R.R. and L.T.
Methodology: D.A., D.J.V., D.B.R.T., T.M.B. and S.P.
Formal analysis and investigation: P.P.S.G., V.S., D.J.V. and S.P.

Resources: S.S., D.A., T.M.B. and L.T.

Data curation: P.P.S.G., V.S. and D.B.R.T.

Roles-writing-original draft: P.P.S.G., V.S., S.P. and S.P.

Writing-review & editing: D.A., D.J.V., R.R., T.M.B. and D.B.R.T.

Supervision: S.S., P.P.S.G., TMB and S.P.

Project administration: P.P.S.G., D.A., D.J.V. and L.T.

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INSTITUTIONAL REVIEW BOARD STATEMENT

Institutional Review Board SRM Dental College (Approval Number: SRMDC/IRB/2015/MDS/No.507).

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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