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Microsurgical management of gingival recession using pouch and tunnel technique with or without 20 % propolis irrigation: A randomized controlled trial

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ABSTRACT

Aim: To evaluate the adjunctive effect of 20 % propolis irrigation with platelet-rich fibrin (PRF) in the microsurgical Pouch and Tunnel technique for Miller's Class I and II gingival recession defects.

Materials and methods: Eighteen patients with Miller's Class I and II recession defects were randomly assigned to two groups: Group A (test, n = 16 sites) received 20 % propolis irrigation followed by the Pouch and Tunnel technique with PRF under a surgical microscope, while Group B (control, n = 13 sites) received the same procedure without propolis. Clinical parameters included Plaque Index (PI), Gingival Index (GI), Probing Depth (PD), Relative Clinical Attachment Level (RCAL), Recession Depth (RD), and Width of Keratinized Tissue (WKT) which were recorded at baseline and 3 months.

Results: Both groups showed significant intragroup improvement in GI, PI, RCAL, RD and WKT from baseline to 3 months. Intergroup comparison revealed significantly greater reduction in RD and gain in WKT in the propolis group ($p < 0.05$).

Conclusion: Within the study's limitations, adjunctive use of 20 % propolis irrigation with PRF enhanced root coverage outcomes and increased keratinized tissue width compared to PRF alone. These preliminary findings suggest that propolis may serve as a beneficial adjunct in minimally invasive periodontal plastic surgery.

1. Introduction

Gingival recession refers to the downward migration of the gingival margin beyond the cementoenamel junction (CEJ), leading to exposure of root surface. The prevalence of gingival recession increases with age and is associated with multiple etiological factors including traumatic tooth brushing, thin periodontal phenotype, malocclusion, orthodontic movement and periodontal disease.[1]

Periodontal plastic surgery aims to correct mucogingival deformities and achieve stable long-term soft tissue coverage. Among the available surgical techniques, the pouch and tunnel technique has gained significant clinical acceptance as it preserves the papillae and maintains vascular integrity at the surgical site, ensuring adequate support for the grafts.[2,3] The introduction of platelet-rich fibrin (PRF), a concentrated platelet derivative, further enhances soft-tissue healing by releasing autologous growth factors creating more aesthetically pleasing results.[4]

The interest in natural bioactive agents as adjuncts to mucogingival surgery has led to the exploration of propolis, a resinous bee product rich in flavonoids, phenolic acids, and esters.[5,6] Propolis exhibits antimicrobial, anti-inflammatory, and antioxidant properties and has demonstrated significant potential in improving wound healing.[7,8]

For enhanced esthetics and patient morbidity, present day periodontal plastic surgery uses magnification in the form of loupes or surgical microscopes. A magnification-assisted periodontal surgical approach ensures gentle tissue handling, improves the surgeon's skill, boosts visual sharpness, delivers ergonomic benefits, minimizes patient morbidity, ensures consistent outcomes, accelerates healing, and enhances patient experience.[9]

However, clinical evidence on the use of propolis as an adjunct in periodontal plastic surgery remains scarce, particularly in combination with modern minimally invasive microsurgical approaches. Hence, this study was conducted to compare the minimally invasive Pouch and Tunnel technique followed by PRF membrane placement in Miller's class I & II recession under a surgical microscope with or without Propolis irrigation.

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2. Materials and methods

2.1. Study design and ethical approval

A randomized controlled clinical trial was conducted after receiving clearance from the Institutional Ethical Committee. The CTRI number is CTRI/2025/03/083661. Participants were chosen from individuals undergoing routine oral examinations. Written informed consent was obtained from all participants prior to enrolment.

2.2. Randomization

Eligible sites were randomly assigned into two groups using Random Allocation Software 2.0. Allocation concealment was ensured using the Sequentially Numbered Opaque Sealed Envelope (SNOSE) method, opened only at the time of surgery by an independent assistant not involved in the study. Patient allocation and the CONSORT flowchart are depicted in Fig. 1.

2.3. Blinding protocol

Outcome assessors and participants were blinded to group allocation to reduce bias and ensure an objective assessment of clinical outcomes.

2.4. Study population

A total of 18 patients (29 recession sites) were included in the study based on the following criteria:

Inclusion Criteria:

1. Age 20–50 years
2. Miller's Class I and II gingival recession defects
3. Good oral hygiene
4. Single or multiple adjacent recession defects
5. Non-smokers

Exclusion Criteria:

1. Systemic diseases affecting healing (e.g., diabetes mellitus)
2. Pregnant or lactating women

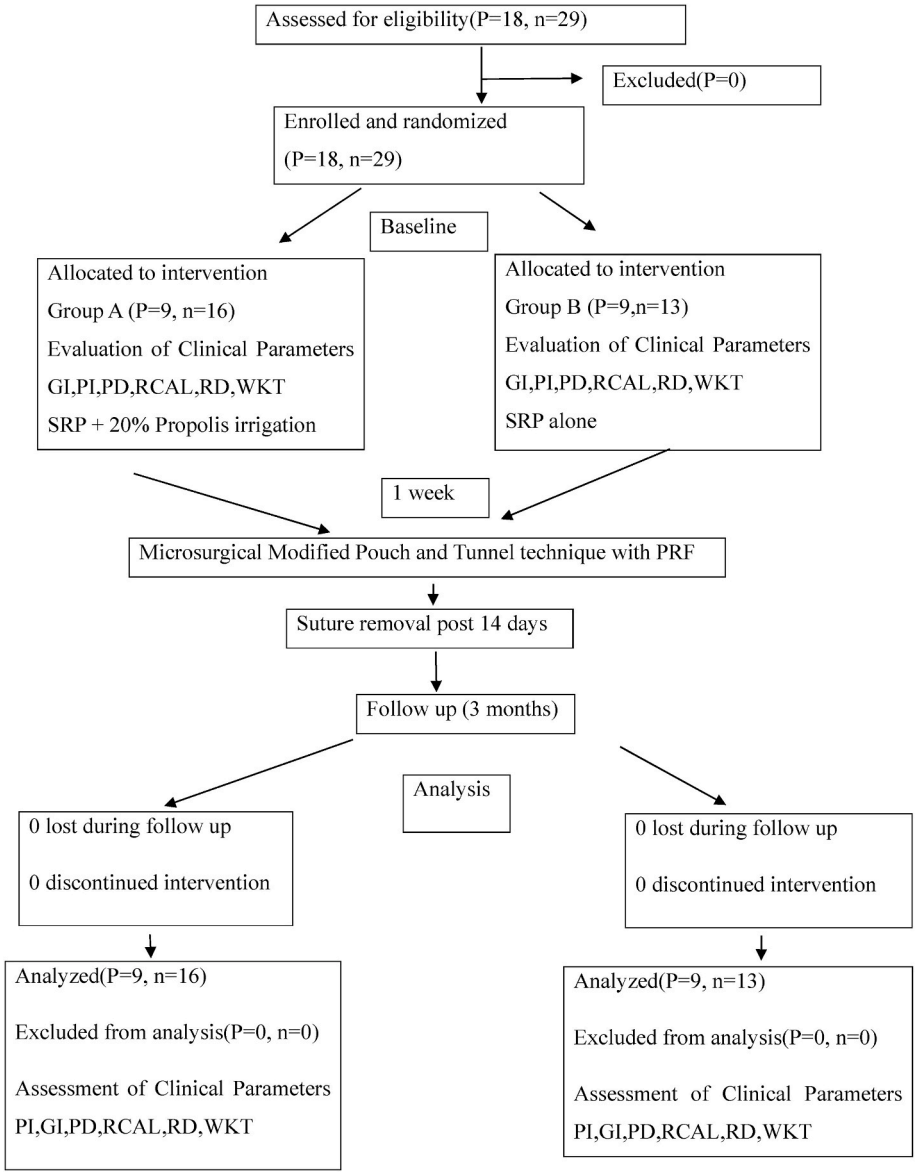


Fig. 1. CONSORT flow chart of the studied patients. (P=Number of Patients, n = Number of Sites).

3. Allergies to bee products or propolis
4. Previous mucogingival surgery in target sites
5. Tooth mobility > Grade I

The interventions in the two groups were as follows:

Group A (Control): 9 individuals (16 sites) received treatment with Pouch and tunnel technique + PRF.

Group B (Test): 9 individuals (13 sites) received treatment with Pouch and tunnel technique + PRF+ 20 % propolis irrigation.

The demographic details of the study participants are summarized in Table 1.

2.5. Clinical parameters

The clinical measurements were recorded with a UNC-15 periodontal probe. To maintain uniform probe positioning, specially fabricated self-cured acrylic stents with occluso-apical grooves corresponding to the mid-buccal area were utilized as fixed reference points.

The clinical parameters i.e. Gingival Index (GI),[10] Plaque Index (PI),[11] Pocket Probing depth (PPD) and Relative Clinical Attachment Level (RCAL) and also the recession related clinical parameters which include Recession depth (RD) and Width of keratinized tissue (WKT) were recorded prior to scaling and root planing and at 3 months in both groups.

Intra-examiner calibration was assessed using Cohen's Kappa, resulting in a value of 0.87, indicating excellent reliability.

2.6. Pre-surgical phase

All patients received supra and sub-gingival scaling and root planing prior to surgery, and were instructed on proper oral hygiene practices. FOR GROUP A, irrigation was done with 20 % Propolis at the recession sites post scaling and root planing 1 week prior to the surgical procedure. After one week, patients were assessed for oral hygiene status. Only those who maintained optimal oral hygiene were selected to participate in the study.

2.7. Preparation of 20 % propolis irrigant

A 20 % propolis irrigation solution was formulated in collaboration with ITS Pharmacy College, Ghaziabad. 1 g of 'Superbee' propolis powder (Hi-tech Natural Product India Limited) was combined with 3 mL of 99.8 % ethanol and incubated for one week which was centrifuged, filtered, vacuum-dried, and re-dissolved in ethanol. The extract was stored in brown glass bottles at room temperature which retains the stability and antibacterial activity for up to six months.[12,13]

2.8. Preparation of L-PRF

Preparation of L-PRF was carried out as per the method described by Choukroun et al.[4] Prior to the surgery, 10 mL of venous blood was taken from each patient by venipuncture of the antecubital vein and collected in two sterile glass test tubes, each with 5 mL of blood, without any anticoagulant. The blood was quickly collected, and the tubes were immediately centrifuged. For L-PRF, the tubes were centrifuged at 2700 rpm for 12 min.

Table 1
Demographic characteristics of the research participants in the study groups.

Variable	Category	Group A (9)	Group B (9)	p-value
Age		37.6250 ± 7.35640	40.1538 ± 9.76257	0.433
Gender	Male	13 (81.2 %)	11 (84.6 %)	0.057
	Female	3 (18.8 %)	2 (15.4 %)	
Site	Anterior	13 (81.2 %)	11 (84.6 %)	0.057
	Posterior	3 (18.8 %)	2 (15.4 %)	
Arch wise distribution	Maxillary	6 (37.5 %)	8 (61.5 %)	1.6
	Mandibular	10 (62.5 %)	5 (38.5 %)	

2.9. Surgical procedure

Group A (Pouch and Tunnel with PRF and Propolis):

Local anesthesia (2 % lignocaine with adrenaline 1:80000) was administered. Under aseptic conditions and 6× magnification, surgery began with a labial crevicular incision (excluding interdental papilla) using a No. 15c BP blade. Tunneling was performed beyond the mucogingival junction and around adjacent teeth to ensure flap mobility. A PRF membrane was positioned into the tunnel, and the flap was coronally advanced and secured with 5-0 sutures and composite.

Group B (Pouch and Tunnel with PRF Alone):

The same procedure was followed as in Group A.

2.10. Post-treatment and follow-up

Patients received Amoxicillin 500 mg and Paracetamol 325 mg thrice daily for five days. Brushing was avoided at the surgical site until suture removal; rinsing was recommended. Patients were instructed to avoid hot food for 24 h and not to touch the area. Follow-up was scheduled at 14 days for suture removal and at 3 months. The primary outcome measures were reduction in RD and gain in WKT. Secondary outcome measures included improvements in GI, PI, PPD and RCAL, .

2.11. Sample size and statistical analysis

Based on literature, group A & group B had standard deviation of 0.59 and 0.61 respectively, with a mean difference of 0.89. The power analysis was done by Software G*Power version 3.1.9.7. The actual power and the effect size were 83.95 % and 1.48 respectively and the calculated sample size was 9 per group, totaling 18 participants.[14]

Data were tabulated in MS Excel and analysis was performed using SPSS version 16.0. Shapiro-Wilk test was used for normality assessment. Descriptive statistics (Mean and SD) were computed. Intergroup and intragroup comparisons were made using paired and unpaired t-tests, respectively. For non-normal data, Mann-Whitney U and Wilcoxon signed-rank tests were applied. Significance was set at $p < 0.05$ with a 95 % confidence interval.

3. Results

A total of 18 patients (29 recession sites) completed the 3-month follow-up. No postoperative complications such as membrane exposure, flap necrosis or allergic reactions to propolis were observed. All patients demonstrated good compliance throughout the study period.

Intragroup comparison showed significant improvements in both groups from baseline to 3 months in GI, RCAL, RD and WKT. Group A additionally demonstrated a significant reduction in PI, while PD changes in both groups were not statistically significant (Table 2).

Intergroup comparison revealed significantly greater improvement in recession depth reduction and increase in keratinized tissue width in the propolis group indicating superior clinical outcomes (Table 2 & Graph 1 and 2).

4. Discussion

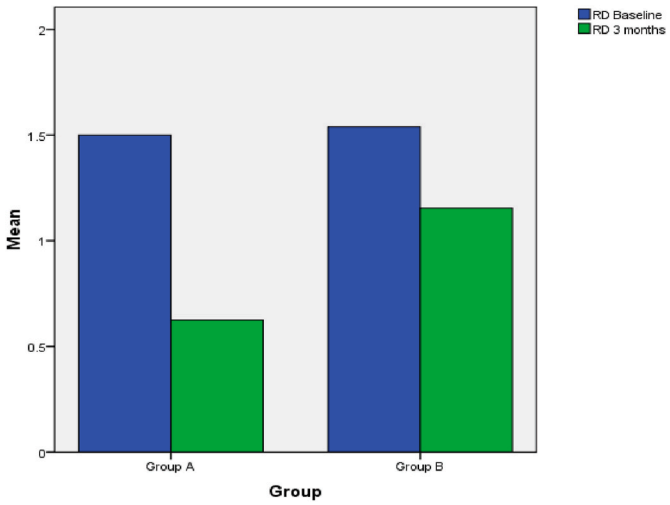
4.1. Summary of main findings

The present randomized controlled clinical trial evaluated the adjunctive effect of 20 % propolis irrigation combined with platelet-rich fibrin (PRF) in the microsurgical management of Miller's Class I and II gingival recession using the pouch and tunnel technique. The clinical parameters were measured at baseline and 3 months using UNC-15

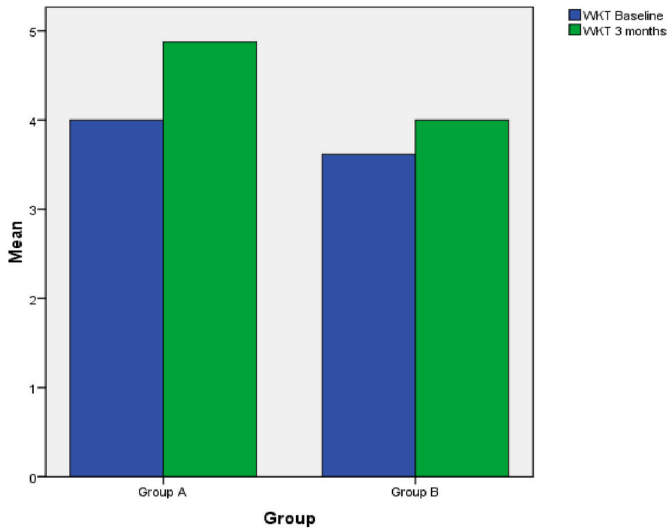
Table 2
Comparison of different clinical parameters at baseline and 3 months.

Variable		Baseline			3 months			p-value
		Mean ± S.d.	95 % Confidence Interval		Mean ± S.d.	95 % Confidence Interval		
			Lower	Upper		Lower	Upper	
GI	Group A	0.5978 ± 0.25213	0.4040	0.7916	0.382 ± 0.2806	0.167	0.598	0.007*
	Group B	0.7178 ± 0.26902	0.5110	0.9246	0.519 ± 0.2703	0.311	0.727	0.008*
	p-value	0.353			0.325			
PI	Group A	1.1956 ± 0.16195	1.0711	1.3200	1.04 ± 0.119	0.95	1.13	0.008*
	Group B	1.1211 ± 0.18503	0.9789	1.2633	1.08 ± 0.148	0.96	1.19	0.292
	p-value	0.479			0.891			
PD	Group A	1.06 ± 0.250	0.93	1.20	1.00 ± 0.000	1.00	1.00	0.317
	Group B	1.31 ± 0.480	1.08	1.69	1.15 ± 0.376	0.93	1.38	0.157
	p-value	0.088			0.110			
RCAL	Group A	11.25 ± 1.528	10.44	12.06	10.38 ± 1.310	9.68	11.07	0.000*
	Group B	10.54 ± 1.050	9.90	11.17	10.15 ± 1.281	9.38	10.93	0.025*
	p-value	0.204			0.751			
RD	Group A	1.50 ± 0.516	1.22	1.78	0.62 ± 0.500	0.36	0.89	0.000*
	Group B	1.54 ± 0.519	1.22	1.85	1.15 ± 0.555	0.82	1.49	0.025*
	p-value	0.839			0.016*			
WKT	Group A	4.00 ± 0.730	3.61	4.39	4.88 ± 0.719	4.49	5.26	0.000*
	Group B	3.62 ± 0.961	3.03	4.20	4.00 ± 0.913	3.45	4.55	0.025*
	p-value	0.300			0.009*			

Wilcoxon signed rank test; * indicates significant difference at $p \leq 0.05$.



Graph 1. Mean Recession Depth (RD).



Graph 2. Mean Width of Keratinized tissue (WKT).

probe and standardized acrylic stents to ensure reproducibility of probe positioning.

Both groups demonstrated a statistically significant reduction in the Gingival Index (GI) from baseline to 3 months, indicating improved gingival health. However, intergroup differences were not significant. The Plaque Index (PI) showed a significant intragroup reduction in Group A, whereas Group B exhibited a non-significant change. Probing Depth (PD) remained stable throughout the study period, showing no significant intra- or intergroup variation. Both groups revealed significant gains in Relative Clinical Attachment Level (RCAL) and significant reductions in Recession Depth (RD), with greater improvement in Group A. The Width of Keratinized Tissue (WKT) increased significantly in both groups. Collectively, these outcomes suggest that 20 % propolis irrigation, when used as an adjunct to PRF and microsurgical techniques, may enhance periodontal healing and soft-tissue outcomes.

4.2. Comparison with previous studies

The improvement in GI parallels the findings of Chandra et al.,[15] who reported similar intragroup reductions following the pouch and tunnel technique. The significant decrease in PI in Group A aligns with Seth et al.,[16] although the absence of intergroup differences is consistent with Chandra et al.[15] PD values remained unchanged, corroborating the observations of Azaripour et al.[17] using the Modified Microsurgical Tunnel Technique. However, this contrasts with El-Shennawi et al.,[18] who found significant PD reductions following propolis irrigation. The gain in RCAL in both groups concurs with Chandra et al.[15] The reduction in RD observed in both groups, particularly in Group A, is in accordance with Bali et al.[19] The increase in WKT aligns with Salem et al.[20] but diverges from Patel et al.,[21] who reported no significant change.

The clinical improvements may be attributed to the biologic properties of propolis. Previous investigations by Devitaningtyas et al.[22] and Mali et al.[13] demonstrated that propolis at 10–20 % concentrations effectively inhibits *Porphyromonas gingivalis*. The anti-inflammatory potential of propolis arises from its inhibition of cyclooxygenase and lipoxygenase enzymes, reducing prostaglandin and leukotriene synthesis.[23] Its antimicrobial efficacy is mediated through the synergistic action of phenolic and flavonoid compounds such as pinocembrin, galangin, and pinobanksin.[24,25] Moreover, propolis has been shown to enhance wound healing and modulate inflammation in various periodontal applications (Filho & Carvalho; Nakao et al.; Zohery et al.),[26–28]

possibly through upregulation of TGF-β expression (Martinotti & Ranzato).[29]

The inclusion of PRF in this study aligns with evidence that platelet concentrates promote wound healing by releasing growth factors such as PDGF, VEGF, and IGF (Choukroun et al.).[4] Studies have demonstrated positive outcomes with PRF in Class I and II recession defects.[30]

The application of magnification represents another important factor contributing to the success of this approach. Kang et al.[31] and Zuhr et al.[32] demonstrated predictable root coverage and excellent soft-tissue outcomes using microsurgical tunnel approaches, consistent with the present results.

4.3. Strengths of the study

The strengths of this study include its randomized design, use of a minimally invasive microsurgical pouch and tunnel approach, and the application of both PRF and 20 % propolis as biologically active adjuncts. The use of customized acrylic stents ensured reproducibility of measurements, while the employment of surgical magnification optimized precision and postoperative outcomes.

4.4. Limitations

Despite the promising results, it is essential to acknowledge some limitations of the study. Although the findings were statistically significant, future studies with larger sample sizes and longer follow-up periods are necessary to validate and strengthen these outcomes. The inclusion of both maxillary and mandibular sites may have introduced variability that could have influenced the results. In the present design, PRF was utilized in both groups, with propolis serving as an adjunct only in the test group to assess its additional benefits. Incorporating an additional group treated with propolis alone could have provided clearer insights into its independent effects, considering the inherent regenerative potential of PRF. Moreover, the pouch and tunnel technique is most suitable for shallow recession defects in thick gingival biotypes, thereby limiting its generalizability to all clinical scenarios. Finally, as all procedures were performed by a single operator, the possibility of operator bias cannot be completely excluded, despite efforts to ensure consistency through standardized protocols.

4.5. Clinical implications

The findings suggest that adjunctive 20 % propolis irrigation can enhance soft tissue healing and improve clinical outcomes when used in conjunction with PRF and the microsurgical pouch and tunnel technique. Propolis, being a natural, cost-effective, and biocompatible agent, may thus represent a promising alternative to synthetic chemical root conditioners in periodontal regenerative therapy.

4.6. Future directions

Future investigations should aim to include larger sample sizes, longer follow-up periods, and standardized defect sites to validate these findings. Comparative studies assessing propolis alone versus PRF or other biologic membranes would help delineate its independent contribution. Additionally, research exploring different concentrations, formulations, and delivery systems of propolis could further optimize its therapeutic efficacy.

5. Conclusion

Within the limitations of this study, the adjunctive use of 20 % propolis irrigation with the Pouch and Tunnel technique and PRF showed significant improvements in recession depth reduction and gain in keratinized tissue width in Miller's Class I and II gingival recessions. Both groups demonstrated clinical improvement, with propolis providing additional benefit in root coverage outcomes. However, as these are preliminary findings based on a limited sample and short follow-up, they should be interpreted with caution. Larger, long-term controlled studies are warranted to validate the adjunctive potential of propolis in periodontal regenerative therapy.

Data availability declaration

All data supporting the findings of this study are available within the paper and its Supplementary Information.

Ethics approval

The study was conducted after obtaining approval from the Institutional Ethical Committee (ITSCDSR/IIEC/LD/PERIO/2022-25/001).

Consent for publication

Informed consent was obtained from all participants in this study.

Informed consent form

Dr. AKASH ALOK BORA has informed me to my full satisfaction, in the language 1 understand about the purpose, nature of the study and various investigations to be carried out for the study. I have been informed about the duration and possible complications caused by the study. I give full consent for being enrolled in the above study and reserve my rights to withdraw from the same, whenever I wish, without prejudice to my right to undergo further treatment at ITS-CDSR, Gaziabad.

I have understood all the aspects included in this study and I have agreed to it willfully.

Sources of funding

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Declaration of competing interest

The authors declare no competing interests.

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