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Original Article

Dentistry Section

Efficacy of Hyaluronic Acid and Injectable Platelet-rich Fibrin in the Treatment of Gingival Recession using Coronally Advanced Flap Technique: A Split Mouth Randomised Clinical Trial

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ABSTRACT

Introduction: Injectable Platelet-Rich Fibrin (I-PRF) provides a promising substitute for the conventional Connective Tissue Grafts (CTG), in gingival recession management cases. Being a bioactive material, it has the ability to promote tissue regeneration and healing. Hyaluronic Acid (HA) is synthesised in the plasma membrane and contributes in periodontal regeneration by supporting the cellular activity like cell migration, proliferation and tissue hydrodynamics, thereby improving tissue healing.

Aim: To evaluate the efficacy of I-PRF and HA along with Coronally Advanced Flap (CAF) technique in the management of isolated Class I gingival recession.

Materials and Methods: The present prospective, split-mouth, randomised clinical trial was conducted at the Department of Periodontology, Vivekanandha Dental College for Women, Namakkal, Tamil Nadu, India from January to March, 2024. A total of 23 participants who had Class I recession bilaterally were recruited and grouped as: Control site (treated with CAF+IPRF) and the test site (treated with CAF+I-PRF+ HA) and non-eugenol periodontal pack was given which was removed after 14 days. The baseline value was recorded for the clinical parameters {Recession Depth (RD), Keratinised Tissue Width (KTW), Recession Width (RW), Keratinised Tissue Thickness (KTT) and Mean Root Coverage (MRC)}. These parameters were then recorded at one month and three months. Analysis of Variance (ANOVA) with Bonferroni post-hoc correction was applied for intragroup comparisons and paired t-test for intergroup comparison. A p-value≤0.05 was considered statistically significant.

Results: A significant reduction in RW and RD as well as enhancement in KTT and KTW by the end of three months was observed for all the parameters recorded. The test site (HA+I-PRF) showed better performance in comparison to the control group (p<0.001). The test protocol achieved MRC of 72.62%, significantly outperforming the control group (p<0.001) while securing superior gains in both tissue width and thickness.

Conclusion: According to the results of the present study HA and I-PRF provided successful coverage of the gingival recession and was also beneficial in increasing the gingival phenotype thickness.

Keywords: Fibronectin, Growth factors, Mean root coverage, Mucogingival defects, Recession depth and width

INTRODUCTION

The movement or migration of gingival margin to the apical direction, resulting in the exposures of tooth root beyond the cementoenamel junction is gingival recession [1]. Though gingival recession is primarily caused by inflammation, several anatomic and lifestyle factors like mispositioned tooth, traumatic brushing habits, thin biotype, improper attachment of frenum, smoking, Class II division 2 malocclusion and periodontal disease can also contribute [2-4]. The mucogingival defects associated with exposed root surface needs to be managed by surgical procedures like CTG that is often regarded as the benchmark for periodontal root coverage therapies [3,5,6]. In case of gingival recessions involving one tooth or extending to several teeth, CAF technique is suitable. Although ideal, the CTG approach requires the need for a second surgical site, especially palate region when used for root coverage, which is the main disadvantage of this treatment modality [3]. So, to avoid these complications, an alternative minimally invasive procedure like I-PRF can be used instead of CTG [7,8].

I-PRF is a bioactive material with tissue regeneration and healing property, obtained by low-speed centrifugation, in the liquid form [8]. As compared to other PRF formulations, I-PRF has more regenerative cells with higher Growth Factor (GF) concentrations and fibroblast migration as well as higher expression of type I collagen and the GF like PDGF - platelet derived and TGF - transforming GF [8]. Fibronectin is another component found in I-PRF. Fibronectin application onto the exposed root surfaces prior to the root coverage procedure has shown improvement in proliferation of cells towards the supracrestal parts from the periodontal ligament [9].

The HA is synthesised by a membrane-bound protein in the plasma membrane. It improves healing properties of the tissue and leads to periodontal regeneration by contributing towards the hydrodynamics of the tissue as well as the migration and proliferation of the cells [10]. An in-vitro study was published recently, demonstrating the effects of PRP combined with HA in the regeneration of cartilage [11]. Another research reported that the addition of HA showed enhancement in the GF release by PRP [10]. There are many studies in which I-PRF alone or HA alone were used in the gingival recession therapies but their use together has not been researched before, especially in treating gingival recession. Both HA and I-PRF demonstrate the capacity to stimulate periodontal regeneration, enhanced collagen synthesis, and promotion of wound healing as well as HA has shown interdental papilla augmentation. These properties suggest a potential synergistic effect, where the combined application of HA and I-PRF may amplify their individual regenerative capabilities, leading to improved clinical outcomes in root coverage procedures

[12]. Thus, the present study evaluated the efficacy of I-PRF +HA in Class I gingival recession using CAF technique.

MATERIALS AND METHODS

The present prospective, randomised clinical trial with split mouth design, was conducted in the Department of Periodontology, Vivekanandha dental college for Women, Namakkal, Tamil Nadu, India. from January to March, 2024. The Institution's Ethical Committee approval (approval no. VDCW/IEC/337/2023) was obtained and performed in accordance with the Helsinki Declaration of 1975, as revised in 2013. Prior to participation, the nature of the study was explained, and written informed consent was obtained from all the recruited participants.

Sample size calculation: Sample size was determined using G*Power software (version 3.1.9.7) based on an effect size of 0.8, chosen based on a clinically meaningful difference observed in prior studies evaluating root coverage outcomes with similar biomaterials(6), α=0.05, and power=0.80 for a paired t-test, yielding a required sample of 23 patients (46 sites).

Inclusion and Exclusion criteria: Patients aged 20-40 years, systemically healthy, and presenting with bilateral Miller's Class-I gingival recessions were included [13]. Inclusion criteria required the presence of a thick gingival biotype, and adequate width of keratinised tissue. Smokers, pregnant/lactating women, patients with systemic diseases or drug allergies, and teeth with restorations or malocclusion were excluded.

Randomisation was performed using a computer-generated random sequence into Group-A (Control: I-PRF+CAF) and Group-B (Test: I-PRF+HA+CAF). Allocation concealment was achieved using opaque, sealed envelopes opened only by the operator at the time of surgery. While the operator could not be blinded due to the nature of the biomaterials, the clinical examiner was blinded to the treatment assignments to ensure unbiased outcome assessment.

Study Procedure

A single, calibrated examiner (other than the operator) recorded all clinical parameters at baseline, one month, and three months. Intra-examiner reliability was assessed using the Intraclass Correlation Coefficient (ICC), with a value of 0.92, indicating high reproducibility. Parameters measured included RD, RW, KTW, KTT- measured via transgingival probing (bone sounding) using a standardised endodontic spreader with a silicone stopper and MRC - calculated by MRC (%)=(Baseline RD) - (Postoperative RD) ÷ (Baseline RD)×100.

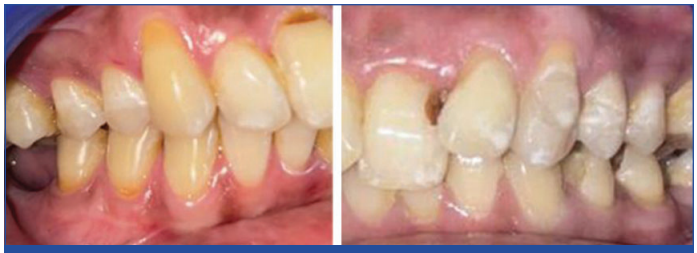
I-PRF and HA Preparation

I-PRF Protocol: The I-PRF was prepared according to the Low-Speed Centrifugation Concept (LSCC). Briefly, 10 mL of whole venous blood was collected in plain plastic vacuum tubes (without anticoagulants) and centrifuged at 700 rpm (approx. 60 g) for three minutes [7]. The upper orange-coloured liquid layer (approx. 1 mL) was aspirated.

I-PRF+HA Combination: For the test site, 1mL of I-PRF was mixed with 1mL of commercially available non-crosslinked HA (2 mg/mL) (manufactured by Meditech, Mumbai) in a 1:1 ratio [14]. This was performed immediately prior to injection to ensure a homogeneous liquid state.

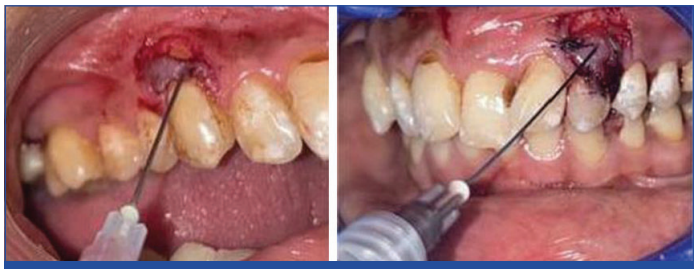
Surgical intervention and biomodification: Before proceeding with the surgical procedure, thorough scaling and root planning were carried out for all the 23 participants. The participants were regularly recalled once in two weeks for a period of 3 months and their oral hygiene status was monitored. Baseline [Table/Fig-1,2] clinical parameters were evaluated before the surgery.

A semilunar incision was made apical to the recession following the root contour using a No. 15 blade. A split-thickness flap was reflected to allow coronal displacement. The exposed root surfaces



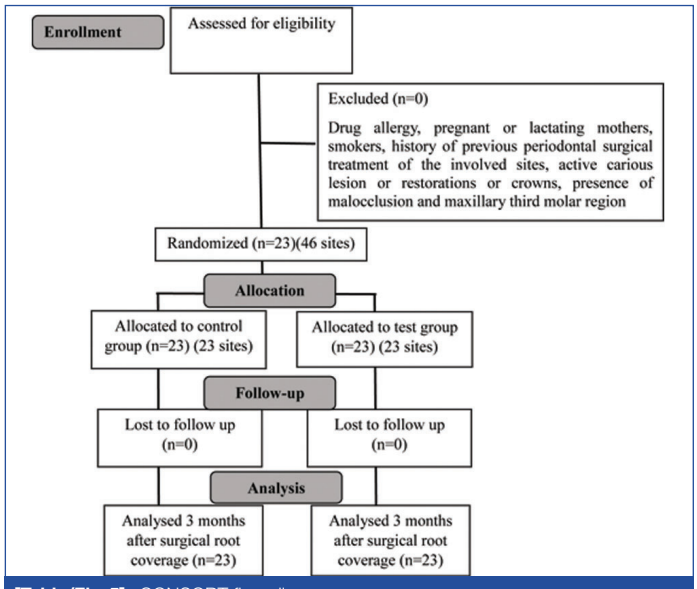
[Table/Fig-1]: Pre-op view of control site.
[Table/Fig-2]: Pre-op view of test site. (Images from left to right)

were treated with I-PRF using sterile cotton pellets for 2-4 minutes. This was intended to enhance the root surface's biocompatibility by depositing a thin fibrin film before flap repositioning. At the standard treatment/control site, 0.5 mL of I-PRF was injected into the mesial and distal aspects of the attached gingiva (3 mm apical to the margin) using a 30G needle [Table/Fig-3] [7]. 1 mL of the I-PRF+HA mixture was injected similarly at test site [Table/Fig- 4] [14].



[Table/Fig-3]: Injecting I_PRF in the control site.
[Table/Fig-4]: Injection of I-PRF+HA. (Images from left to right)

Injections were halted once slight mucosal blanching was observed, indicating tissue saturation. The flap was coronally advanced, held with moist gauze for two minutes to stabilise the initial clot, and secured using 3.0 black silk sutures (interrupted technique). A non-eugenol periodontal pack (GC COE PAK™ periodontal dressing, GC America, USA) was applied. The flow of participants in different phases of the randomised controlled trial has been illustrated in the CONSORT flow diagram [Table/Fig-5].

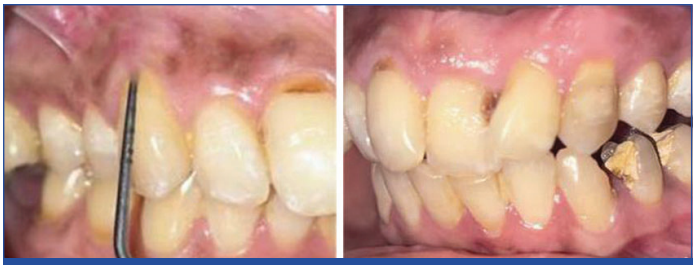


[Table/Fig-5]: CONSORT flow diagram.

Patients were prescribed analgesics and instructed to use a 0.12% chlorhexidine rinse. Sutures and packs were removed after 14 days. Clinical measurements were formally recorded at one and three months [Table/Fig-6,7]. Any adverse events, such as excessive swelling or pain, were monitored throughout the healing period.

STATISTICAL ANALYSIS

Data were analysed using Statistical Package for Social Sciences (SPSS) version 26.0. Intragroup comparisons were performed using



[Table/Fig-6]: Post-op view of control site after 3 months.
[Table/Fig-7]: Post-op view of test site after three months. (Images from left to right)

repeated measures ANOVA with Bonferroni post-hoc correction. Intergroup comparisons between test and control sites within the same patient were performed using independent t-tests, and mean differences are reported with 95% Confidence Intervals (CIs). Level of significance was set under 0.05.

RESULTS

[Table/Fig-8] demonstrates that while both surgical protocols yielded statistically significant improvements (p<0.001), the test group achieved markedly superior clinical outcomes by the three-month follow-up. Intragroup analysis confirmed that both interventions effectively reduced recession dimensions (RW and RD) and enhanced the keratinised tissue profile (KTW and KTT).

Parameters	Site	Baseline	1 Month	3 Months	p (Intra)*	p (Inter)†	MD (95% CI)‡
RW (mm)	Control	3.05±1.02	1.05±0.87	1.57±0.68	<0.001	--	--
	Test	3.11±1.06	0.58±0.59	0.86±0.61	<0.001	0.003	0.71 (0.28–1.14)
RD (mm)	Control	3.20±1.05	1.54±0.82	1.93±0.79	<0.001	--	--
	Test	3.25±1.08	0.92±0.57	0.89±0.52	<0.001	<0.001	1.04 (0.58–1.50)
KTW (mm)	Control	3.40±1.32	4.48±1.44	4.19±1.39	<0.001	--	--
	Test	3.36±1.38	5.18±1.29	5.11±1.24	<0.001	0.015	-0.92 (-1.66 to -0.18)
KTT (mm)	Control	0.77±0.33	1.22±0.28	1.12±0.31	<0.001	--	--
	Test	0.81±0.34	1.84±0.24	1.78±0.26	<0.001	<0.001	-0.66 (-0.88 to -0.44)

[Table/Fig-8]: Intergroup and intragroup comparison of clinical parameters (Mean±SD).
*Intragroup p-value from repeated measures ANOVA with Bonferroni post-hoc correction.
†Intergroup p-value from independent t-test at 3 months.
‡MD: Mean Difference (Test - Control) at 3 months with 95% CI.

The interval-wise analysis of the control group [Table/Fig-9] showed significant changes in RW, RD, and KTW across all time points (p<0.001). Both RW and RD demonstrated marked reductions from baseline to one month and from baseline to three months, with a slight but significant increase between one and three months. KTW showed a significant increase from baseline to one month and baseline to three months, followed by a modest but significant reduction between one and three months. KTT increased significantly from baseline to one month and baseline to three months; however, the change between one and three months was not statistically significant (p=0.284).

Parameters	Between intervals	Mean difference	p-value
RW	Baseline-1 month	2.035	0.0001
	Baseline-3 months	1.480	0.0001
	1 month-3 months	-0.555	0.0001
RD	Baseline-1 month	1.660	0.0001
	Baseline-3 months	1.270	0.0001
	1 month-3 months	-0.390	0.0001
KTW	Baseline-1 month	-1.055	0.0001
	Baseline-3 months	-0.760	0.0001
	1 month-3 months	0.295	0.0001
KTT	Baseline-1 month	-0.450	0.0001
	Baseline-3 months	-0.350	0.0001
	1 month-3 months	0.100	0.284

[Table/Fig-9]: Control group - pair-wise comparison between intervals using post-hoc Bonferroni test.
**highly statistically significant difference

In [Table/Fig-10], the pair-wise comparison of test group revealed highly significant improvements in RW, RD, KTW, and KTT from baseline to both one month and three months (p<0.001). RW showed a small but statistically significant increase between one and three months (p=0.001), indicating a minor rebound after initial healing. RD and KTW remained stable between one and three months, with no significant interval change. KTT demonstrated a slight yet statistically significant increase between one and three months (p=0.016).

[Table/Fig-11] demonstrates a statistically significant intergroup difference in MRC at three months. The test group achieved a substantially higher MRC (72.62±9.87%) compared with the control group (58.12±12.45%), with a mean difference of 14.50% (95% CI: 10.24-18.76; p<0.001). No adverse events were observed during the healing period, in the present study.

DISCUSSION

One of the frequently encountered periodontal conditions is the root surface being exposed due to gingival recession [15]. Managing and correcting gingival recession with mucogingival surgical procedures remains a significant concern in periodontal practice. Although it does not cause tooth loss, it is considered as an aesthetic problem [16]. Gingival recession is most often seen in the vestibular surface

Parameters	Between intervals	Mean difference	p-value
RW	Baseline-1 month	2.530	0.0001
	Baseline-3 months	2.250	0.0001
	1 month-3 months	-0.280	0.001**
RD	Baseline-1 month	2.330	0.0001
	Baseline-3 months	2.360	0.0001
	1 month-3 months	0.030	0.353
KTW	Baseline-1 month	-1.820	0.0001
	Baseline-3 months	-1.750	0.0001
	1 month-3 months	0.070	0.353
KTT	Baseline-1 month	-1.030	0.0001
	Baseline-3 months	-0.970	0.0001
	1 month-3 months	0.060	0.016*

[Table/Fig-10]: Test group-pair-wise comparison between intervals using post-hoc Bonferroni test.
**highly statistically significant difference
- statistically significant

Site	MRC (%) (Mean±SD)	Mean Difference (Test–Control)	95% CI	p-value (Paired t-test)
Control	58.12±12.45	--	--	--
Test	72.62±9.87	14.50	10.24 to 18.76	<0.001

[Table/Fig-11]: Intergroup comparison of Mean Root Coverage (MRC) at 3 months.

of the maxillary anterior and premolars and mandibular anterior region [17]. Various treatment modalities especially surgical procedures have been proposed for the management of the defects in the mucogingival area of the root surface being exposed due to recession. In the present study, the efficacy of I-PRF+HA along with CAF technique was evaluated in the Class I gingival recession cases.

An improvement in the depth and width of recession as well as the parameters of gingival tissue were observed in both the groups over three months, which was significant with the test group showing better performance. Greater reduction in RW and RD along with higher gain in the gingival parameters like width and thickness of gingiva was demonstrated by the test site, compared to the control. Furthermore, an intergroup comparison confirmed this with superior coverage of root in the test site, indicating its clinical efficacy.

I-PRF maintains a liquid consistency that allows for a higher concentration of regenerative cells, including leukocytes and mesenchymal stem cells. Its ability to slowly release essential GFs like PDGF and TGF-3 beta over an extended period promotes fibroblast migration and collagen synthesis, which are critical for the long-term stability of the gingival margin [7]. Complementing this, HA serves as more than just a lubricant. It is a key constituent of the extracellular matrix in the periodontium. HA enhances tissue hydrodynamics and serves as a signalling molecule that facilitates cell proliferation and angiogenesis. Evidence suggests that HA can modulate the inflammatory response, potentially reducing postoperative discomfort and accelerating the early phases of wound healing [10].

Since there are no investigations published yet on the effect of CAF+I-PRF and CAF+I-PRF+HA in gingival recession cases, this study cannot be directly compared with other studies. In prior research conducted by Potey AM et al., he effect of PRF in gingival recession was compared and they demonstrated significant enhancement in the gingival thickness and KTW in CAF+PRF over six months compared to the other group, where CAF alone without PRF was employed [6]. However, solid PRF acts as a membrane, whereas the use of I-PRF (liquid form) allows for better adaptation to the root surface and easy mixing with HA. The combination of CAF+I-PRF showed significant result in this study when used for three months. Another research published recently studied the effects of I-PRF via semi-surgical technique in 53 Class I recession cases, which was applied as a session of four times at 10 days interval. It was concluded that I-PRF injection or application in the gingival recession areas of the tooth, improved the coverage and heightened the KW as well as the thickness of gingiva, when compared for six months [18]. Similarly, in the present study both the groups showed enhancement in gingival width and thickness by the 3rd month.

At present, HA serves as a valuable chemotherapeutic material contributing to technical advancement in dental practice. HA forms the natural carbohydrate element of the extracellular matrix and has biological effects that varies depending on the molecular weight, which ranges from 0.4 to 4.0 kDa [19]. HA was proven as an efficient material in promoting the healing of wounds in the periodontal area as well as regeneration of the tissues of those areas by decreasing the depth of probing, enhancing gain in Clinical Attachment Level (CAL) and enabling complete coverage of the tooth affected by gingival recession [19]. In a prior in vitro study on osteoarthritis patients, the use of HA+PRP injection in the intra-articular region strongly improved the tear of meniscus as well as cartilage breakdown, which resulted in a decrease in the OA-related immune cells. They concluded that cartilage regeneration and inhibition of OA inflammation can be achieved by utilising the HA+PRP combination [11]. These findings align with that of the present study, in which the enhancement of gingival thickness and reduction in the RD and width were observed in the test site. Saxena A et al., conducted a comparative study with CAF+HA and CAF alone for 90 days to treat the gingival recessions (both Class I and II) [20]. HA showed significant difference in CAL and reduction in gingival RD. By adding I-PRF to HA, the test group achieved not just recession coverage, but a qualitative improvement in the gingival biotype (thickness), which is often a more reliable predictor of long-term stability than coverage alone.

The CTG is often associated with high patient morbidity due to the secondary donor site [7]. By utilising a semilunar flap (a variation of CAF) combined with these bioactive agents, the authors achieved successful root coverage and phenotype conversion without the need for palatal harvesting. The integration of HA with I-PRF appears to offer a potent biological boost to standard surgical techniques. This protocol not only achieves significant MRC but also addresses the structural quality of the gingiva, making it a viable, patient-centric alternative in contemporary periodontal plastic surgery.

Limitation(s)

Although the present study utilises minimally invasive procedures like I-PRF and HA, certain limitations are inherent. The duration of follow-up was short The success of the procedure may vary depending on handling of I-PRF and HA and the flap design as well as the operator's skill. The operator blinding was not possible due to the visibility of biomaterial during the procedure but outcome assessment blinding helped in mitigating the detection bias. Patient dependent variability in PRF composition (influenced by age, systemic health and haematocrit levels) may also affect the outcome of the study. Future studies should focus on long-term evaluation for around 12-24 months or more to assess tissue maturation, relapse and secondary outcomes like postoperative pain and aesthetic satisfaction. In addition, comparison of I-PRF and HA with CTG to establish their predictability and stability in gingival recession treatment. Involvement of advanced defects, combination therapies, histological outcomes, and patient-centred measures will enhance their effectiveness in clinical applicability.

CONCLUSION(S)

The data obtained reveal that both I-PRF and HA proved efficacious in the treatment of gingival recession (Class I), with the test group (I-PRF+HA combination) showing enhanced treatment outcomes in terms of reduction in RD and width, as well as gains in gingival tissue thickness and width, thereby providing successful coverage of the gingival recession as well as benefits in increasing the thickness of the gingival phenotype. Further well-designed studies with larger populations, longer observation periods, and comparisons with CTG are required to validate their long-term stability and clinical reliability.

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