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ORIGINAL PAPER

Clinical Evaluation of Gingival Augmentation Using Injectable Platelet-Rich Fibrin and Microneedling in Thin Periodontal Phenotype

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ABSTRACT

Background: *In periodontology, gingival phenotype is important for both therapeutic and cosmetic reasons. Drug delivery is improved for people with a thin gingival phenotype by using DERMAPEN microneedling to introduce medications through tiny gingival microinjuries. Injectable platelet-rich fibrin (i-PRF) is a useful tool in regenerative periodontal therapy because it offers a rich source of autologous growth factors when paired with microneedling (MN). This study aimed to determine the influence of i-PRF alone and in combination with MN on gingival thickness (GT) and keratinized tissue width (KTW) in individuals with thin gingival biotypes.*

Materials and method: *A split-mouth study with 30 participants aged 16-20 compared the effects of i-PRF alone* versus *i-PRF combined with MN on GT and KTW. Assessments were made at baseline and one month. Group 1 received i-PRF with MN and group 2 only i-PRF.*

Results: *At baseline, there were no significant differences in GT and KTW scores between the two groups. After one month, the MN + i-PRF group had significantly higher GT and KTW than the i-PRF group. Both groups showed statistically significant reductions in the mean scores of gingival index, plaque index, clinical attachment level and probing pocket depth from baseline to one month.*

Conclusion: *Microneedling and i-PRF could serve as an effective non-surgical approach to enhance GT and KTW in individuals with thin periodontal phenotypes. Both i-PRF alone and in combination with MN have the potential to improve these parameters.*

Keywords: gingiva, phenotype, augmentation, wound healing, microneedling, keratinized tissue.

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INTRODUCTION

The gingival phenotype is crucial for periodontal health, affecting treatment response and disease susceptibility (1). Muller and Eager note that thick gingival tissue, which is characterized by a broad keratinized zone, correlates with strong bone structure and higher resistance to stress and inflammation (2). In contrast, thin gingival tissue, with a narrow-keratinized zone and scalloped shape, is more prone to recession and pocket formation under stress. Gingival thickness greater than 1.5 mm is considered a thick biotype, while less than 1.5 mm is a thin biotype (3). Traditional methods for gingival augmentation included connective tissue and pedicle grafts, but recent advancements have introduced collagen matrices, acellular dermal matrices, amnion allografts, enamel matrix derivatives, platelet-rich plasma (PRP), platelet-rich fibrin (PRF) and platelet-derived growth factors (4). In 2000, Choukroun *et al* (5) described PRF as a chemical-free autologous platelet concentrate that promotes wound healing and leverages the body's regenerative potential. Injectable PRF (i-PRF), rich in white blood cells, enhances vascularization, collagen mRNA and growth factor expression, showing promise as an effective autologous therapy.

Injectable platelet-rich fibrin releases higher levels of growth factors such as epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1) and platelet-derived growth factor (PDGF) after 10 days compared to PRP, enhancing fibroblast and osteoblast migration and collagen production. Microneedling (MN), by creating micro-injuries, stimulates wound repair, growth factor release and natural collagen production, maintaining gingival tissue integrity. Gingival recession is often linked to a thin biotype, reduced keratinized tissue and probing depths beyond the mucogingival interface (6).

The aim of this present study was to evaluate the effect of gingival thickness (GT) and keratinized tissue width (KTW) using i-PRF alone and with MN in individuals with thin gingival phenotypes. ❑

MATERIALS AND METHODS

Source of data

This clinical study was conducted at the Department of Periodontology, Rama Dental College Hospital & Research Centre in, Mandhana, Kanpur, India. The study included 30 subjects aged 16 to 20 years, who were matched by age

FIGURE 1. Group receiving injectable platelet-rich fibrin (i-PRF) combined with microneedling (MN):
(A) measurement of keratinized tissue width using Williams probe and stopper; (B) measurement of gingival thickness using endodontic spreader and stopper; (C) measurement using Vernier caliper;
(D) application of i-PRF; (E) MN procedure using Dermapen

FIGURE 2. Group 2 receiving only injectable platelet-rich fibrin (i-PRF): (A) measurement of keratinized tissue width using Williams probe and stopper; (B) measurement of gingival thickness using endodontic spreader and stopper; (C) application of i-PRF; (D) measurement of gingival thickness using Vernier caliper

and gender based on specific inclusion and exclusion criteria. Participants were informed about the study and provided their written consent. Ethical approval was granted by the institutional ethics committee.

Study method

All 30 included patients received comprehensive oral hygiene instructions and were taught proper brushing techniques. They were then randomly assigned to two equal groups of 15 each, with Group 1 receiving i-PRF in combination with MN (Figure 1) and Group 2 receiving only i-PRF (Figure 2).

Subjects meeting the following inclusion criteria were selected for the study: age ≥ 18 years, free of systemic disease, with a thin gingival phenotype; full mouth plaque index (PI) and full-mouth bleeding on probing (BOP) score of ≤ 15% (2); no malocclusion, crowding, fillings, or missing/supernumerary mandibular anterior teeth; no blood-borne conditions. Exclusion criteria comprised: undergoing active orthodontic treatment; pregnancy or breastfeeding; previous periodontal surgery; smoking; use of blood thinners; and use of any drugs that may have caused gingival enlargement.

Periodontal examination

Using a mouth mirror and a UNC-15 periodontal probe, the study assessed the following indices and clinical parameters: plaque index (Silness and Loe, 1964) (7), gingival bleeding index (Ainamo and Bay, 1975) (8) and Pocket Depth (Grant *et al*, 1965) (9). The probe was passed along the gingival sulcus around each tooth. Clinical attachment level (CAL) was measured from the cemento-enamel junction to the pocket base with the UNC-15 probe (7). Six measurements per tooth were recorded: three on the buccal (mesiobuccal, midbuccal, distobuccal) and three on the lingual aspect (mesiolingual, midlingual, distolingual). Transgingival probing using an endodontic spreader and veneer caliper was conducted to check KTW and GT.

Techniques for blood sample collection and preparation of injectable platelet-rich fibrin (i-PRF)

A venous blood sample was drawn from each patient using a 20-mL syringe and divided into two 10-mL i-PRF tubes, both without anticoagulant. These tubes were centrifuged at room temperature for three minutes at 700 rpm. The resulting i-PRF was then transferred into 2.5 cc dental injectors and injected using an insulin needle (Figure 3).

FIGURE 3. Preparation of i-PRF using centrifugation at 700 rpm for three minutes: (A) blood drawing; (B) blood collection; (C) centrifugation machine; (D) i-PRF test tube in centrifugation machine; (E) i-PRF test tube; (F) formation of i-PRF

FIGURE 4. Dermapen used for microneedling procedure

Procedure for performing microneedling

Micro-needling was performed using a DermaPen device equipped with 12-pin needle cartridge tips. The procedure targeted the keratinized gingiva from the mesial line of the central tooth to the distal part of the canine tooth (Figure 4).

Application methods for i-PRF and microneedling in the study group

In this clinical study, one side of the mandibular anterior region received only i-PRF treatment, while the other side was given a combined treatment of MN and i-PRF. Initially, a lidocaine topical aerosol spray was applied as anesthesia be-

fore each GT measurement, which was conducted using a vernier caliper.

For the MN + i-PRF group, MN was performed on the keratinized tissue using a MyM Dermapen with a 12-pin needle cartridge, followed by the injection of i-PRF into the apical region of the mucogingival margin in the alveolar mucosa. In the i-PRF group, the procedure involved only the injection of i-PRF into the same region. Bleeding control was managed by placing a saline-soaked sponge between the lip and gingiva, which was removed after 15 minutes.

Both MN + i-PRF and i-PRF treatments were administered at 10-day intervals over 30 days. Pain relief medication was provided and patients were scheduled for follow-up appointments to monitor progress and ensure optimal healing and tissue regeneration following the one-month interventions.

Statistical analysis

Data analysis was conducted using IBM SPSS Statistics 20.0. Statistical methods included one-way ANOVA, Tukey's multiple post hoc procedures for pairwise comparisons and independent t-tests. For all groups, mean, standard deviation, standard error and percentage of the mean were calculated. One-way ANOVA compared test and control groups, and if significant, Tukey's post hoc tests assessed pairwise differences. Gender comparisons within groups were performed using repeated measures independent t-tests. To achieve 85% power and detect significant differences with an effect size of 0.47 ($P \leq 0.05$), each group required 15 participants, as determined by G*Power version 3.0.1. ❑

RESULTS

Demographic characteristics of study participants

Participants' mean age was 30.00 ± 4.39 years in the MN + i-PRF group and 31.73 ± 3.22 years in the i-PRF group. Each group consisted of 10 males and five females.

Intragroup comparison of variables between baseline and one-month interval in the MN + i-PRF group

Table 1 summarizes the mean scores for each variable at baseline and after one month in the MN + i-PRF group. Baseline scores were 0.62 ± 0.10 mm, 0.60 ± 0.08 mm, 0.70 ± 0.09 mm, 2.27 ± 0.59 mm and 1.67 ± 0.49 mm, respectively. After one month, the scores were 0.45 ± 0.08 mm, 0.45 ± 0.07 mm, 1.73 ± 0.46 mm and 0.97 ± 0.48 mm, respectively. The mean differences in baseline and one-month scores for gingival index (GI), plaque index (PI), clinical attachment level (CAL) and probing pocket depth (PPD) were statistically significant ($p \leq 0.05$), showing a significant reduction after one month. Additionally, the baseline scores for GT and KTW were 0.70 ± 0.09 mm and 0.64 ± 0.07 mm, respectively, while one-month scores were 1.52 ± 0.29 mm and 1.51 ± 0.30 mm, respectively. These differences were also statistically significant ($p \leq 0.05$), indicating a significant increase after one month.

TABLE 1. Intragroup comparison of variables between baseline and one-month interval in the MN + i-PRF group

Variable	Baseline		After one month		Difference	Critical value	p-value
	Mean	SD	Mean	SD			
GI#	0.62	0.10	0.45	0.08	0.18	7.361	<0.001*
PI#	0.60	0.08	0.45	0.07	0.16	10.476	<0.001*
GT#	0.70	0.09	1.52	0.29	-0.83	-11.658	<0.001*
KTW#	0.64	0.07	1.51	0.30	-0.88	-11.704	<0.001*
CAL^	2.27	0.59	1.73	0.46	0.54	-2.309	0.021*
PPD^	1.67	0.49	0.97	0.48	0.76	-2.994	0.003*

Statistically significant ($P<0.05$); #paired t test; ^Wilcoxon signed rank test;
*a significant difference at $p \leq 0.05$ (negative value for GT and KTW indicates the gain in the gingival thickness and keratinized). GI=gingival index; PI=periodontal index; KTW=keratinized tissue width; CAL=clinical attachment loss; PPD=probing pocket depth; SD=standard deviation.

Intragroup comparison of variables between baseline and one-month interval in the i-PRF group

Table 2 compares the mean scores of each variable at baseline (0.63 ± 0.09 mm, 0.61 ± 0.07 mm, 2.27 ± 0.46 mm, 1.73 ± 0.46 mm) and at one month (0.47 ± 0.09 mm, 0.46 ± 0.06 mm, 1.67 ± 0.49 mm, 0.93 ± 0.37 mm) in the i-PRF group. In the i-PRF group, the difference in mean baseline and one-month scores of GI, PI, CAL and PPD were statistically significant ($p \leq 0.05$), with all these variables showing a significant reduction after one month. Also, the difference in mean scores of GT (0.69 ± 0.07 mm, 0.63 ± 0.07 mm at baseline) and KTW (1.01 ± 0.20 mm, 1.03 ± 0.20 mm after one-month) were statistically significant ($p \leq 0.05$),

TABLE 2. Intragroup comparison of variables between baseline and one-month interval in the i-PRF group

Variable	Baseline		After one month		Difference	Critical value	p-value
	Mean	SD	Mean	SD			
GI#	0.63	0.09	0.47	0.09	0.15	5.098	<0.001*
PI#	0.61	0.07	0.46	0.06	0.14	5.271	<0.001*
GT#	0.69	0.07	1.01	0.20	-0.31	-5.643	<0.001*
KTW#	0.63	0.07	1.03	0.20	-0.39	-7.158	<0.001*
CAL^	2.27	0.46	1.67	0.49	0.60	-2.714	0.007*
PPD^	1.73	0.46	0.93	0.37	0.74	-3.025	0.002*

#Paired t test; ^Wilcoxon signed rank test; *a significant difference at p≤0.05 (negative value for GT and KTW indicates the gain in the gingival thickness and keratinized; GI=gingival index; PI=periodontal index; KTW=keratinized tissue width; CAL=clinical attachment loss; PPD=probing pocket depth; SD=standard deviation.

TABLE 3. Intergroup comparison of variables between MN + i-PRF and i-PRF groups at baseline

Variable	MN + i-PRF		i-PRF		Difference	Critical value	p-value
	Mean	SD	Mean	SD			
GI#	0.62	0.10	0.63	0.09	-0.01	-0.336	0.739
PI#	0.60	0.08	0.61	0.07	-0.01	-0.244	0.809
GT#	0.70	0.09	0.69	0.07	0.01	0.535	0.597
KTW#	0.64	0.07	0.63	0.07	0.01	0.595	0.557
CAL^	2.27	0.59	2.27	0.46	0.00	-0.101	0.920
PPD^	1.67	0.49	1.73	0.46	-0.06	-0.392	0.695

#Independent t test; ^Mann-Whitney test; GI=gingival index; PI=periodontal index; KTW=keratinized tissue width; CAL=clinical attachment loss; PPD=probing pocket depth; SD=standard deviation.

TABLE 4. Intergroup comparison of variables between MN + i-PRF and i-PRF groups after one month

Variable	MN + i-PRF		i-PRF		Difference	Critical value	p-value
	Mean	SD	Mean	SD			
GI#	0.45	0.08	0.47	0.09	-0.02	-0.722	0.476
PI#	0.45	0.07	0.46	0.06	-0.01	-0.365	0.718
GT#	1.52	0.29	1.01	0.20	0.51	5.743	<0.001*
KTW#	1.51	0.30	1.03	0.20	0.48	5.244	<0.001*
CAL^	1.73	0.46	1.67	0.49	0.06	-0.392	0.695
PPD^	0.97	0.48	0.93	0.37	0.04	-0.072	0.943

#Independent t test; Mann-Whitney test; *a significant difference at p≤0.05. GI=gingival index; PI=periodontal index; KTW=keratinized tissue width; CAL=clinical attachment loss; PPD=probing pocket depth; SD=standard deviation.

TABLE 5. Intergroup comparison of changes in scores (baseline to one month) between MN + i-PRF and i-PRF groups

Variable	MN + i-PRF		i-PRF		Difference	Critical value	p-value
	Mean	SD	Mean	SD			
GI#	0.18	0.09	0.15	0.12	-0.02	0.291	0.773
PI#	0.16	0.06	0.14	0.11	-0.01	0.062	0.951
GT#	-0.83	0.27	-0.31	0.22	0.51	-5.563	<0.001*
KTW#	-0.88	0.29	-0.39	0.22	0.48	-5.085	<0.001*
CAL^	0.54	0.74	0.60	0.63	0.06	-0.161	0.872
PPD^	0.76	0.53	0.74	0.56	0.04	-0.570	0.568

#Independent t test; ^Mann-Whitney test; *a significant difference at p≤0.05 (negative value for GT and KTW indicates the gain in the gingival thickness and keratinized). GI=gingival index; PI=periodontal index; KTW=keratinized tissue width; CAL=clinical attachment loss; PPD=probing pocket depth; SD=standard deviation.

with these two variables showing a significant increase after one month.

Intergroup comparison of variables between MN + i-PRF and i-PRF groups at baseline

Table 3 compares the mean baseline scores of each variable between the MN + i-PRF group (0.62 ± 0.10 mm, 0.60 ± 0.08 mm, 0.70 ± 0.09 mm, 0.64 ± 0.07 mm, 2.27 ± 0.59 mm, 1.67 ± 0.49 mm) and the i- PRF group 0.63 ± 0.09 mm, 0.61 ± 0.07 mm, 0.69 ± 0.07 mm, 0.63 ± 0.07 mm, 2.27 ± 0.46 mm, 1.73 ± 0.46 mm. At baseline, the difference in mean scores of GI, PI, GT, KTW, CAL and PPD of the two groups were statistically non-significant (p≥0.05).

Intergroup comparison of variables between MN + i-PRF and i-PRF groups after one month

Table 4 compares the one-month mean scores of various variables between the MN + i-PRF group and the i-PRF group. The MN + i-PRF group had scores of 0.45 ± 0.08 mm, 0.45 ± 0.07 mm, 1.73 ± 0.46 mm and 0.97 ± 0.48 mm, while the i-PRF group had scores of 0.47 ± 0.09 mm, 0.46 ± 0.06 mm, 1.67 ± 0.49 mm, and 0.93 ± 0.37 mm. The differences in mean scores for GI, PI, CAL and PPD between the two groups were not statistically significant (p ≥ 0.05) after one month. However, there was a significant difference (p ≤ 0.05) in mean GT (1.52 ± 0.29 mm) and KTW (1.51 ± 0.30 mm) scores, with the MN + i-PRF group showing significantly greater increases compared to the i-PRF group.

Intergroup comparison of changes in scores (baseline to one month) between MN + i-PRF and i-PRF groups

Table 5 compares the changes in scores of various variables from baseline to one month between the MN + i-PRF group and the i-PRF group. The MN + i-PRF group showed changes of 0.18 ± 0.09 mm, 0.16 ± 0.06 mm, 0.54 ± 0.74 mm and 0.76 ± 0.53 mm, while the i-PRF group showed changes of 0.15 ± 0.12 mm, 0.14 ± 0.11 mm, 0.60 ± 0.63 mm and 0.74 ± 0.56 mm. Changes in GI, PI, CAL and PPD were similar between the two groups, with no statistically significant differences (p ≥ 0.05). However, GT increased by 0.83 ± 0.27 mm and

KTW by -0.88 ± 0.29 mm in both groups, with the MN + i-PRF group showing a significantly greater increase in GT (-0.31 ± 0.22 mm) and KTW (-0.39 ± 0.22 mm) compared to the i-PRF group. ❑

DISCUSSION

This study is the first to examine the combined use of MN and i-PRF, revealing significant improvements in collagen production, tissue regeneration and gingival augmentation. The MN + i-PRF group showed significantly lower mean scores for GI, PI, CAL and PPD at baseline and one month, consistent with the findings reported by Fotani S. *et al* in 2019 (10). Positive outcomes are likely due to enhanced oral hygiene and patient compliance. However, in 2012, Collins JR *et al* noted differences between thin and thick gingival phenotypes, though both showed favourable clinical results with increased root coverage, gingival tissue thickness and KTW (11).

The combination of MN and i-PRF has been investigated for its potential benefits in periodontal therapy. Microneedling enhances the absorption of i-PRF into the gingiva, improving the delivery and efficacy of growth factors and platelets, thereby accelerating tissue regeneration (12). Our study observed significant increases in GT and KTW after one month, aligning with the findings reported by Ozsagir ZH *et al* in 2020 (13). The positive effects on neo-angiogenesis and neo-collagenases suggest MN and i-PRF can effectively control gum disease and enhance dental health. In 2023, Soundarajan S *et al* (14) also reported greater increases in GT with the combined MN and i-PRF treatment compared to i-PRF alone.

The present study examined the effects of combined MN and i-PRF treatment *versus* i-PRF alone, focusing on tissue regeneration and repair in periodontology. Injectable platelet-rich fibrin, a variation of PRP, was evaluated for its impact on gingival health. Baseline and one-month measures in the i-PRF group showed significant reductions in GI, PI, CAL and PPD, which was consistent with the findings reported by Patra L *et al* in 2020 (15). Additionally, the present study observed significant increases in GT and KTW after one month, highlighting the effectiveness of i-PRF in improving these parameters (Figures 5 and 6).

FIGURE 5. Group 1 one month after surgery: (A) microneedling and i-PRF one month postoperatively; (B) measurement of gingival thickness using endodontic spreader and stopper; (C) measurement using Vernier caliper

FIGURE 6. Group 2 one month after surgery: (A) i-PRF one month postoperatively; (B) measurement of gingival thickness using endodontic spreader and stopper; (C) measurement using Vernier caliper

The investigation revealed no significant differences in baseline values for GI, PI, GT, KTW, CAL and PPD between the groups. However, the MN + i-PRF combination resulted in a significant increase in GT and KTW after one month, which was in agreement with the findings reported by both Fotani S. *et al* in 2019 (10) and Ozsagir ZH *et al* in 2020 (13). The study underscores the benefits of MN in enhancing neo-angiogenesis and neo-collagenases, leading to increased GT levels (16). Furthermore, no new gingival recessions or worsening of existing conditions were observed during our study, supporting Matter & Cimasoni *et al*'s findings on the absence of creeping attachment within a short time frame (17).

Within the limitations of the present study, the approach of combining i-PRF with MN shows significant benefits for augmenting gingival tissue in individuals with thin phenotypes. The results validate the clinical use of this combination, providing a more comfortable experience with reduced pain and trauma. ❑

CONCLUSIONS

The combination of i-PRF and MN significantly improves periodontal health by increasing gingival thickness and keratinized tissue width. This method enhances cellular renewal and collagen synthesis, making gingival augmentation more achievable for individuals with thin periodontal phenotypes. It also improves the aesthetic appearance and strengthens periodontal health, representing a major advancement in non-surgical periodontal treatments. ❑

Patients' written informed consent: Written informed consent was obtained from all participants prior to inclusion in the present study.

Conflicts of interest: none declared.

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