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Comparative Evaluation of Microneedling with I-Prf and I-Prf Alone Following Non-Surgical Periodontal Therapy in Chronic Periodontitis Patients: A Split Mouth Clinical Trial

K. Rudhra, K. Vennil, Elanchezhiyan, Anitha Balaji, J. Angelin Fiona and S. Mohanasatheesh

[1,4,6]*Senior lecturer Sree Balaji dental college and hospital, India*
[2]*Reader Vivekanandha dental college and women, India*
[3]*Professor Principal and HOD of JKKN dental college and hospital, India*
[5]*HOD and Professor Sree Balaji dental college and hospital, India*

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Corresponding Author
K. Rudhra,
Senior lecturer Sree Balaji dental college and hospital, India
rudhrakannan97@gmail.com

Author Designation
[1-5]MDS Periodontics

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ABSTRACT
New techniques based on non-invasive approaches have been presented to maximize tissue preservation and reduce patient's morbidity. A novel technique termed Microneedling has been used for soft tissue volumizing and regeneration. This therapy in combination with injectable platelet rich fibrin can increase the periodontal phenotype by growth factors activity. The aim of the study is to compare and evaluate the effect of Microneedling with I-PRF and I-PRF alone in periodontitis patients following non-surgical periodontal therapy through clinical parameters. This is a randomized split-mouth clinical study comparing, Microneedling + I-PRF and I-PRF alone in the treatment of attachment loss in chronic periodontitis patients. A total of 120 sites were selected with stage II periodontitis. After split mouth randomisation, test sites were administered with Microneedling followed by I-PRF (n = 60) and contralateral sites were treated with I-PRF (n = 60) alone respectively. demonstrated the mean pocket depth was observed to decrease in both the groups after the first and third month to 3.388±0.42 and 3.65±0.57 respectively in MN+I-PRF group. Mean relative attachment loss decreased from 4.91±0.77 to 3.66±0.65 and 3.87±0.69 with statistically significant results. Periodontal phenotype had significantly improved with gain in gingival thickness of 0.6±0.31 mm and Keratinized tissue width gain of 0.8 mm at 3 month in test group with p-value (<0.001). Microneedling and I-PRF proves to be an excellent option for improving phenotype as it utilizes the platelet concentrates in a unique way to enhance periodontal wound healing.

INTRODUCTION

Periodontal disease is an inflammatory condition that leads to degradation of periodontal tissues, causing tooth movement and eventually tooth loss. Both non-surgical and surgical therapies attempt to minimize symptoms and prevent disease progression, but cannot restore the original periodontal tissues attachment[1].

Non-surgical periodontal therapy (NSPT) remains the "gold standard" for treatment of periodontal disease. One of the critical factors in periodontium which affects the outcome of any periodontal therapy is periodontal phenotype[2-4]. Gingival augmentation techniques improves the keratinized tissue width and thickness to enhance esthetics and support the dentogingival unit. Since, these techniques are technique sensitive, currently minimally invasive surgical approaches are gaining importance, one such product is platelet rich fibrin for periodontal regeneration[5,6].

A new injectable form of PRF (termed I-PRF) formulated using low centrifugation concept has platelets, growth factors and leucocytes and maintains its liquid viscosity for about 15 min to be used in more compatible form. It forms a gel by increasing the expression of TGF-beta and collagen-1 synthesis for cellular migration of fibroblasts, osteoblasts and various periodontal cells[7-10]. One currently studied therapy is Microneedling (MN) is used to enhance PRF regenerative potential and local availability. It is also known as "percutaneous collagen induction therapy" a novel therapeutic concept of multiple punctures in body tissues. Its original conception was given by Orentreich and Orentreich (1995) to induce wound healing in depressed cutaneous scars. Microchannels or injuries releases a cascade of wound healing factors and due to minimal disruption of epithelial barrier a scarless wound healing occurs. Regenerative potential is further enhanced by neocollagenesis and neoangiogenesis.

Microneedling has been a widely studied in dermatological literatures for dermal scars and diseases. Also, various literature reviews have highlighted the efficacy of microneedling for its role in various fields of dentistry. In line with this Ozsagir *et al*.[11-14] first demonstrated the clinical benefits of microneedling in combination with I-PRF for improving gingival thickness in thin phenotype orthodontic patients.

With the highlights of these ideas of MN uses in periodontal phenotype, this split mouth clinical study demonstrated the clinical significance of Microneedling and I-PRF in enhancing width of keratinized gingiva, gingival thickness along with improvement in clinical attachment level in chronic periodontitis patients as an adjunctive to non-surgical periodontal therapy.

MATERIALS AND METHODS

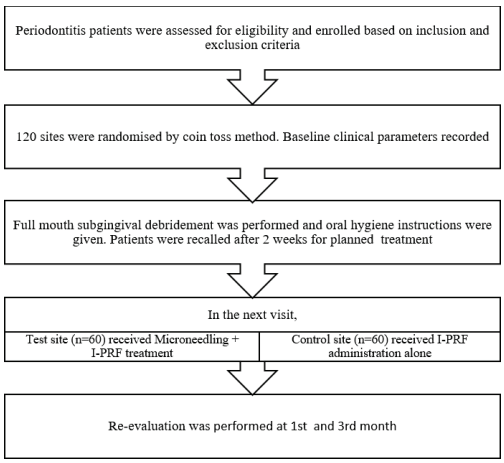
This is a randomized split-mouth clinical study comparing the effect of Microneedling + I-PRF and Injectable platelet rich fibrin (I-PRF) alone in periodontitis patients. This study was assessed and accepted by an institutional ethical committee. Written informed consent was procured among voluntary participants from department of periodontics in Vivekanandha dental college for women.

Inclusion criteria: Systemically healthy individuals; stage I and II and grade A and B periodontitis.

- Percentage of bone loss / age = 0.25-1.0
- Non-smoker/smoker <10 Cigarettes/day
- Interdental CAL <4 mm
- Radiographic horizontal bone loss upto coronal third <30%
- Maximum probing depth ≤5 mm

Exclusion criteria: Periodontal surgical therapy within the last 12 months, Drugs affecting periodontium, Pregnancy or nursing, any tobacco use, Blood borne conditions.

The parameters taken are probing pocket depth (PPD)-measured in all 6 sites of the using UNC 15 probe, Relative attachment level (RAL) measured from the base of the stent to gingival margin + PPD, Plaque index Silness J and Loe [15] Gingival index (Loe and Silness[16] Keratinized tissue width (KTW)[17]. Distance from free gingival margin (lower end of silicon disc) to mucogingival junction using a periodontal probe with a silicon disc (Fig. 7) Gingival thickness (GT)[18]. No 15 endodontic spreaders was held tight in center of a 3-mm-diameter silicone disc inserted perpendicularly



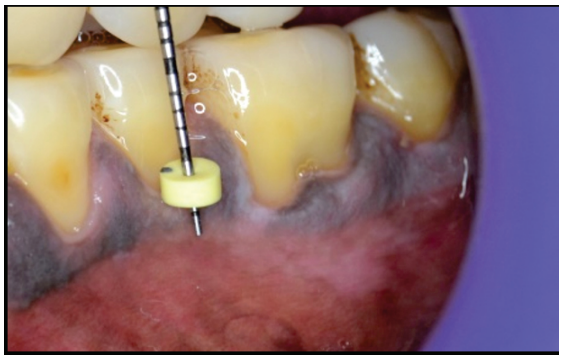


Fig. 1: Pre-operative KTW

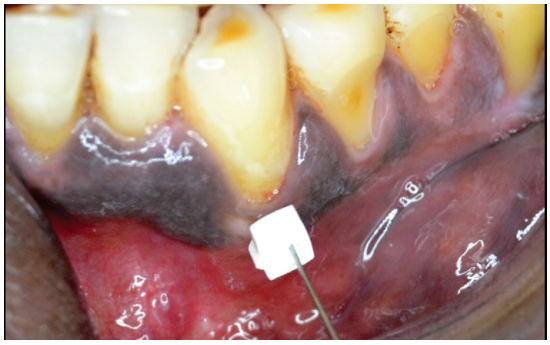


Fig. 2: Pre-operative GT

at 1.5 mm apical of the gingival margin, until a hard surface felt. The penetration depth between the silicone disc and the spreader tip was measured (Fig. 2) Selected patients were thoroughly informed on self-performed plaque control activities. After randomization, subgingival debridement was performed in all diseased sites with or without local anesthesia in a single appointment using ultrasonic tips and curettes.

In the next visit, venous blood was collected in two 5 mL sterile test tubes without anticoagulant and centrifuged at 700 rpm (60 g force) for 3 min. The upper liquid component was collected as injectable PRF (I-PRF) and injected into periodontal pocket using insulin syringe injectors in control site subgingivally into four regions (buccal, lingual, mesial and distal sites) and superficially on mucogingival surfaces until there was evident intensification of liquid component of platelet concentrate[19]. In the test site, Microneedles of size 0.18* 7 mm (Sujok needles) was interposed into the demarcated areas of gingival tissues perpendicular to long axis of tooth until the needle hits the periosteum. Concomitantly, several micro injuries were generated between the gingival margin and base of the pocket in both buccal/labial and lingual/palatal mucosa depending on tooth surface area. The procedure is repeated till there is an evident pin point bleeding areas which was an implication for

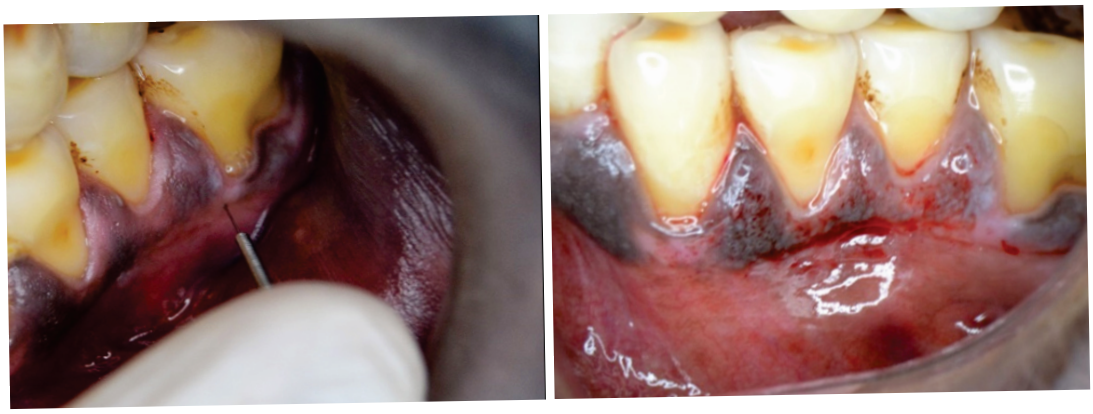


Fig. 3: Micro needling technique

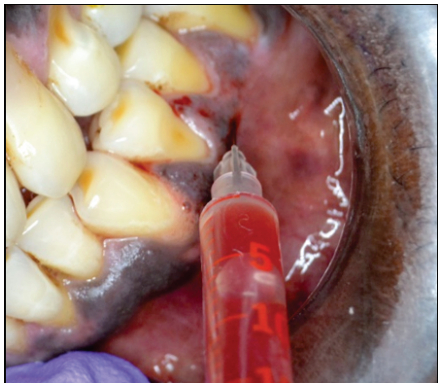


Fig. 4: I-PRF administration

accomplishment of microneedling procedure. Subsequently, I PRF was injected subgingivally corresponding to the control site procedure after microneedling injuries. (Fig. 3 and 4). Post-operative instructions were given and follow up was planned at 1st month and 3rd month for re-evaluation.

RESULTS

The statistical analysis was done by using SPSS software Version 25.0. The Friedman test was used to compare Plaque index, Gingival index, Keratinized tissue width, Gingival thickness, Pocket depth and Clinical attachment loss and Repeated measures ANOVA and independent t-test was used for

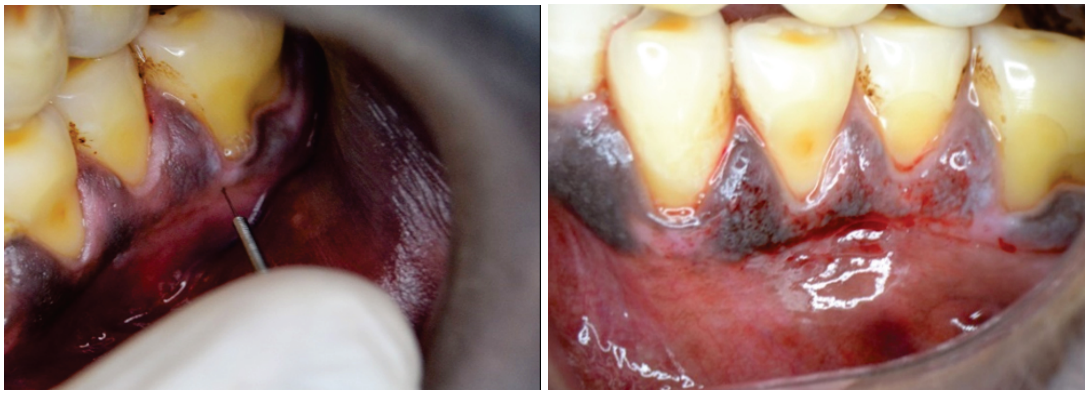


Fig. 5,3: Month post-OP

Table 1: Intergroup comparison of the mean of the plaque and gingival index score between baseline, 1month and after 3 months

Plaque Index	N	Mean	Gingival Index	Mean	p-value
MN+PRF					
Baseline	30	2.067	Baseline	2.187	0.000*
1month	30	1.27	1 month	1.207	
3months	30	1.55	3 months	1.427	
PRF					
Baseline	30	2.047	Baseline	2.167	0.000*
1 month	30	1.323	1 month	1.247	
3months	30	1.58	3 months	1.46	

*Significant, SD- Standard deviation

Table 2 Intragroup comparison of the mean of the Keratinized Tissue Width and Gingival thickness

Keratinized Tissue Width	No	Mean	SD	Gingival thickness	Mean	SD	p-value
Micro needling + I-PRF							
Baseline	30	1.807	0.6634	Baseline	1.84	0.7255	0.000
1 month	30	2.865	0.5689	1 month	2.717	0.4026	
3 months	30	2.663	0.5249	3 months	2.41	0.4397	
I-PRF							
Baseline	30	1.868	0.6052	Baseline	1.744	0.6509	0.000
1 month	30	2.647	0.5178	1 month	2.5	0.4194	
3 months	30	2.333	0.5074	3 months	2.197	0.4657	

*Significant, SD- Standard deviation

Table 3: Intragroup comparison of the mean of the pocket depth and RAL between baseline, 1month and after 3 months

Pocket depth	No	Mean	Std. deviation	Relative attachment level	MeanStd. deviation	p-value
Micro needling +I-PRF						
Baseline	30	4.628	0.8053	Baseline	4.9050.7713	0.000
1 month	30	3.388	0.4147	1 month	3.657	0.6537
3 months	30	3.65	0.5716	3 months	3.867	0.6935
I-PRF						
Baseline	30	4.617	0.7779	Baseline	4.715	0.5316 0.000
1 month	30	3.615	0.5517	1 month	3.81	0.6402
3 months	30	4.08	0.6365	3 months	4.118	0.6064

*Significant

Table 4 Intergroup comparison of the mean of the keratinized tissue width and Gingival thickness at different time intervals

Intergroup comparison		Keratinised tissue width			Gingival thickness		
Time interval	Study groups	Mean	Std. deviation	p-value	Mean	Std. deviation	p-value
Baseline							
	MN+I-PRF	1.807	0.6634	0.44	1.84	0.7255	0.417
	I-PRF	1.868	0.6052		1.744	0.6509	
1 month							
	MN+I-PRF	2.865	0.5689	0.001*	2.717	0.4026	0.014*
	I-PRF	2.647	0.5178		2.5	0.4194	
	MN+I-PRF	2.663	0.5249	0.000*	2.41	0.4397	0.000*
3 months	I-PRF	2.333	0.5074		2.197	0.4657	

Table 5: Intergroup comparison of the mean of the pocket depth and RAL

Intergroup comparison		Probing pocket depth			Relative attachment level		
Time interval	Study groups	Mean	Std. Deviation	p-value	Mean	Std. Deviation	p-value
Baseline	MN +I-PRF	4.628	0.8053	0.000*	4.905	0.7713	0.000*
	I-PRF 4.617	0.7779	4.715	0.5316			
After 1 month	MN +I-PRF	3.388	0.4147	0.009*	3.657	0.6537	0.000*
	I-PRF 3.615	0.5517	3.81	0.6402			
After 3 months	MN +I-PRF	3.65	0.5716	0.001*	3.867	0.6935	0.000*
	I-PRF 4.08	0.6365	4.118	0.6064			

* Significant

intragroup comparison. The statistical significance was kept as p>0.005.

Table 1 shows comparison of the mean plaque index and gingival index at different time intervals for both the groups and observed to be highly significant (p<0.001). The Intragroup comparison of the means of the KTW and GT in Table 2 indicates, mean increased to 2.87±0.57 and 2.66±0.52 after 1 month and 3 months respectively from the baseline. The result found to be statistically significant (p<0.001). for gingival thickness the mean value increased at 1 month and 3 months with 2.717±0.4 and 2.41±0.44 respectively from baseline (1.840±0.73) while in control group it increased to 2.5±0.41 at 1 month and 2.20±0.47 after 3 months. All results were observed to be highly significant (p<0.001).

Table 3 shows Intragroup comparison of the means of the PPD and relative attachment level between different timespan at the end of three months, the mean value of PPD was reduced to 3.65±0.57 in test group and 4.08±0.64 in control group from baseline value 4.628± 0.81. Significant difference was observed in both the groups with increasing duration of time (p<0.001). RAL in the test group, mean attachment loss decreased from 4.91±0.77 to 3.66±0.65 and 3.87±0.69 at 1 month and 3 months respectively. On analysing the means at 3 month there has significant increase from 1-month values although not statistically significant (p<0.005).

Intergroup comparison for KTW and gingival thickness was done using independent t-test. The keratinized tissue width gain was more in test group than control group after 1 month (1 mm) and after 3 months (0.8 mm) respectively. The gingival thickness gain is more in test site than control site after 1 month and 3 months with 0.9 mm and 0.6 mm respectively. (Table 4).

The mean difference of PPD was noteworthy in test group than control group with reduction up to 1.3±0.4 mm at 1 month and up to 1.0±0.2 mm at 3 months from baseline has been demonstrated. The p-value was statistically significant of about 0.009 and 0.001 respectively. On intergroup comparison between the mean of relative attachment level of test group at 1 month 1.3 mm at 1 month and 1.1 mm at 3rd month. (Table 5).

DISCUSSIONS

To the best of our knowledge, no clinical study has been done with micro needling therapy in periodontitis patients. The results of the study have proved that microneedling can improve periodontal phenotype along with attachment level.

The results of mean plaque (1.55) and gingival index (1.427) was comparable to Bhansali *et al*.[20] and Panicker *et al*.[21] studies on NSPT clinical outcomes.

The clinical effect of I-PRF adjunct to SRP was demonstrated by Albonni *et al*.[22] Vučković *et al*.[23]. Results of this study showed that the mean value of CAL reduced from 1.97±0.75 (0.25-3.31) to 1.07±0.44 (0.12-1.78) in the study group, Gingival Margin Level (GML) and PPD also showed statistically significant difference between the groups (p = 0.040 and p = 0.006, respectively). Comparably, Albonni *et al*.[22] showed statistically significant results (p<0.005) in 3 months post-treatment period.

In accordance with the above studies, the clinical parameters of control sites in this study also showed similar statistically significant results with PPD and RAL. The mean value in this study of RAL came down from 4.72±0.53 at baseline to 3.81±0.64 and 4.12±0.61 after 1 month and 3 months respectively and for PPD the mean reduced to 4.08±0.64 at 3 months from baseline 4.617±0.78 with p<0.001.

Kavi *et al*.[24] used I-PRF for increasing gingival thickness and obtained a statistically significant increase in gingival thickness by 0.54 mm±0.0854 (p<0.05). In concordance with this above finding, this study exhibited similar significant values it increased to 0.46 mm after 3 months. Results were also compatible with other histologic studies[25-27].

Out of various literature perspective by Hou *et al*.[28], Singh *et al*.[29] and Iriarte *et al*.[11] the upcoming concept Microneedling can be a best minimally invasive, safer and cheaper alternative for various soft tissue regeneration procedures.

Microneedling therapy efficacy has been best demonstrated in various treatment modality in dermatology as suggested by Ramaut *et al*.[30] Elghblawi *et al*.[31] and Alster *et al*.[32]. The mechanism was controlled dermal wounding and stimulation of the wound healing cascade that enhances collagen production and is likely responsible for the clinical results obtained.

In harmony with these concepts, the clinical improvements obtained from the test site results after periodontal therapy in conjunction with MN with I- PRF were likely to be pertinent with respect to microneedling uses in skin rejuvenation as result of cellular recruitment phenomenon[33-36].

The effectiveness of MN with I-PRF in periodontal therapy was inspired from Ozsagir *et al*.[14] in individuals with thin periodontal phenotypes. Results have shown statistically significant increase in GT within both I-PRF [from 0.43 mm±0.14-0.62 mm±0.11 (p<.001)] and MN with I-PRF [from 0.4 mm±0.14-0.66 mm± 0.12 (p<.001) groups. The results suggested that application of I-PRF and MN may be a first step of non-surgical method for increasing gingival thickness.

The mean KTW was found to be significantly different after 1 month as well as after 3 months. The mean increased to 2.87±0.57 and 2.66±0.52 after 1 month and 3 months respectively. In GT the mean value increased to 2.717±0.71 at 1 month and 2.41±0.44 at 3 months from baseline 1.840±0.73. All results were observed to be highly significant (p<0.001) Although statistically significant results were obtained with all clinical parameters from baseline, it is also noticeable that clinical results have drastically reduced

from 1 month to 3-month time intervals though not statistically significant in both the groups suggesting clinical instability of the treatment modality used. (Chart 1 and 2).

The limitations of the study include less sample size, lack of plaque control standardization, radiographic analysis and longer follow-up investigations needed in context of possible treatment relapse. Accordingly, Microneedling therapy efficacy must be further analyzed for its sole and repeated applications by histological and longitudinal studies.

In conclusion, MN+I-PRF seems to be an excellent option for treating periodontitis patients with following advantages like enhanced periodontal wound healing in non-surgical phase, maintain the integrity and enhancement of periodontal phenotype, gain in clinical attachment level and optimization of platelet concentrates in a more effective way for periodontal management.

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