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Comparative Evaluation of Injectable Platelet-Rich Fibrin Alone and in Combination with Microneedling for Gingival Augmentation in Thin Gingival Phenotype: A Systematic Review and Meta-Analysis

Dr. Renuka Metgud, Professor, Department of Periodontics, KAHER's KLE VK Institute of Dental Sciences, Belagavi - 590010

Dr. Kirti A. Kamat, Postgraduate student, Department of Periodontics, KAHER's KLE VK Institute of Dental Sciences, Belagavi -590010

Corresponding author –

Dr. Renuka Metgud, Professor, Department of Periodontics, KAHER's KLE VK Institute of Dental Sciences, Belagavi - 590010

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KEYWORDS

gingival thickness, injectable platelet-rich fibrin, thin gingival phenotype, microneedling, width of keratinized tissue

ABSTRACT:

Context: In modern dentistry, esthetics alongwith function play an important role with periodontal phenotype having a significant impact on treatment outcomes. The 2017 World Workshop describes periodontal phenotype as combination of gingival phenotype (gingival thickness and width of keratinized tissue) and bone morphotype (buccal bone plate thickness). Individuals with a thin phenotype are more susceptible to gingival recession in presence of trauma or inflammation. While conventional treatment modalities to improve gingival phenotype include surgical procedures such as soft tissue grafting (gold standard), non-surgical treatment options include microneedling (MN) and injectable platelet-rich fibrin (i-PRF). MN also known as percutaneous collagen induction therapy (PCIT), produces microinjuries that cause minor superficial bleeding which leads to a cascade of wound healing that releases numerous growth factors which facilitate neoangiogenesis. Among different types of PRF, injectable platelet-rich fibrin (i-PRF) forms a gel rich in fibrin consisting of leukocytes, platelets and growth factors, enhancing tissue regeneration and offering advantages such as sustained growth factor release and improved cellular migration. Also, i-PRF shows superior biological properties and clinical outcomes with minimal side effects when compared to PRP.

Aim: To assess the clinical efficacy of injectable platelet-rich fibrin alone and in combination with microneedling for gingival augmentation in patients with thin gingival phenotype.

Settings and Design: The eligible studies included randomized controlled trials (RCTs) with split mouth design. Studies comparing clinical efficacy of injectable platelet-rich fibrin alone and in combination with microneedling for gingival augmentation in patients with thin gingival phenotype, which were published in English language from January 2014 till December 2024 were included.

Methods and Material: A systematic search of literature in three databases: PubMed, Google Scholar and Science Direct and a hand search of relevant scientific journals was performed.

Results: Three RCTs that met the eligibility criteria were included in the qualitative analysis. Out of the three included studies, two studies demonstrated a low risk of bias while one study demonstrated high risk of bias. The clinical parameters assessed in all studies were GT and KTW. All the studies showed improvement in all clinical parameters in both groups. A significant improvement in GT was found in the intervention groups of all the three studies whereas in case of

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KTW there was no significant difference found in the intervention and control groups in two of the included studies.

Conclusions: This review indicates that i-PRF when used alongwith MN resulted in significant improvement in gingival thickness, indicating that i-PRF alongwith MN may increase GT without the need for surgical periodontal procedures.

Key Messages: The present systematic review and meta -analysis emphasizes on the use of i-PRF alongwith MN for increasing the gingival thickness thereby avoiding the need for surgical periodontal procedures.

Introduction:

In the modern society, esthetics has a major impact on dental practice in a way that blends form and function[1]. For esthetic restorations to be successful, several factors need to be considered such as gingival biotype, framework of the gingival tissue and morphology of the anterior teeth. Therefore, it is essential to recognise gingival tissue discrepancies during treatment planning[3]. While biotype refers to a set of organs or tissues having same specific genotype, phenotype refers to appearance of an organ or tissue based on combination of multiple factors such as genetic factors alongwith environmental factors[5,6]. Phenotypic traits of the hard and soft tissues that make up the periodontium are referred to as periodontal phenotype[2]. The combination of gingival phenotype and bone morphotype is known as periodontal phenotype. Gingival phenotype comprises of gingival thickness (GT) and keratinized tissue width (KTW) whereas bone morphotype includes the buccal bone plate thickness (BBPT)[4].

Among the factors that may influence the success of dental treatments, gingival biotype is a major concern influencing the outcome of various procedures such as periodontal therapy, root coverage procedures and implant placement[7]. Ochsenbien and Ross (1969) categorised gingival biotype as scalloped and thin gingiva or flat and thick gingiva[8]. Seibert and Lindhe (1989) classified gingival thickness ≥ 2mm as thick biotype and a gingival thickness < 1.5 mm as thin biotype. While thick gingiva is associated with broad zone of keratinized tissue, flat form of gingiva and more resistance to inflammation and trauma, thin gingiva is associated with narrow zone of keratinized tissue, scalloped gingival form and less resistance to inflammation and trauma[3]. The 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and conditions suggested adoption of the term periodontal phenotype[1].

Gingival biotype may be evaluated by several methods such as direct visual method, by evaluating the transparency using a periodontal probe via gingival sulcus i.e. TRAN or by transgingival probing with the help of an endodontic reamer. The transgingival probing method used for evaluating GT is considered very accurate but is invasive in nature. Ultrasonic method and cone beam computed tomography (CBCT) are some of the non-invasive methods available[12].

While individuals with thin phenotype are more susceptible to gingival recession in presence of trauma and inflammation, those with thick phenotype are more likely to develop periodontal pockets. The periodontal phenotype also influences the mucogingival surgical techniques performed for root coverage procedures. Reports suggest that 100% root coverage can be achieved in case of flap thickness of > 0.8 mm whereas partial root coverage occurs in case of flap thickness of < 0.8 mm in coronally advanced flap procedures for Miller class I or class II root coverage. In addition, individuals with thin phenotype are at an increased risk of developing gingival recession in presence of trauma and inflammation[4].

Conventional treatment modalities include surgical procedures such as soft tissue grafting (gold standard) which increase gingival thickness and keratinized tissue width while non-surgical procedures available include microneedling (MN) and injectable platelet-rich fibrin (i-PRF)[8]. MN also known as percutaneous collagen induction therapy (PCIT), produces microinjuries that cause minor superficial bleeding, which leads to a cascade of wound healing that releases a variety of growth factors such as platelet-derived growth factors, transforming growth factors, connective tissue growth factors and fibroblast growth factors which facilitate neoangiogenesis[4].

Platelet concentrates are proven to carry higher levels of peptide growth factors into periodontal tissues. In 1954, a

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platelet concentrate was developed by Kingsley which was later named as platelet-rich plasma (PRP). It was mainly developed to treat patients with thrombocytopenia. PRP preparation takes 30-60 minutes and has two centrifugation processes. The two major drawbacks of PRP are it is expensive and takes longer duration to produce[9]. Also, it is not completely autologous and the bovine thrombin used could spread illness to other patients[11]. By centrifugation of blood collected in glass tubes devoid of anticoagulants and activators, a second-generation platelet concentrate known as platelet-rich fibrin (PRF) was created to overcome these problems. PRF is a special kind of fibrin meshwork that acts as a reservoir for platelet-derived cytokines, growth factors and cells. This network of fibrin may serve as a membrane that breaks down over time. Leucocyte platelet-rich fibrin (L-PRF), titanium platelet-rich fibrin (T-PRF), advanced platelet-rich fibrin" (A-PRF) and injectable platelet-rich fibrin (i-PRF) are among the various forms of PRF that have been produced over time[9].

Amongst different types of PRF, a liquid form of PRF also known as i-PRF represents the most recent and significant advancement. I-PRF was created by centrifuging the blood in plastic tubes for 3 min at 700 rpm. According to reports, i-PRF forms a gel rich in fibrin consisting of platelets and leukocytes that may act as a scaffold and contribute to wound-healing process with increased vascularization. I-PRF shows superior biologic qualities when compared to PRP. The advantages of i-PRF include prolonged release of growth factors, increased cellular migration and bone transplant bonding mechanism which assists in proper adaptation to the affected area. It also induces the release of transforming growth factor-β and collagen-1 mRNA that have been reported to effectively increase the release of growth factors, fibroblasts, osteoblast migration and collagen synthesis[9,11].

Thus, this systematic review is being carried out to evaluate the clinical effectiveness of i-PRF alone and in combination with MN for gingival augmentation in thin gingival phenotype.

Subjects and Methods:

Subjects:

The eligibility criteria were based on PICOS (population, intervention, comparator, outcome and study design) as follows:

Population: Patients with age 18 to 70 years with no significant systemic abnormalities and thin gingival phenotype (< 1.5 mm) were included in the study. Patients undergoing active

orthodontic therapy, taking blood thinners or any medication that may cause gingival hyperplasia, pre-existing mucogingival deformities, bruxism, history of periodontitis or periodontal therapy, pregnant female patients, lactating women, patients with a history of tobacco chewing in any form or smoking were excluded from the study.

Intervention: Microneedling alongwith injectable platelet-rich fibrin administered in patients with thin gingival phenotype.

Comparator: Injectable platelet-rich fibrin in patients with thin gingival phenotype.

Outcome: Baseline and post-follow-up outcomes (Gingival thickness and Keratinized tissue width)

Study design: Randomized controlled clinical trials

Inclusion criteria:

- All randomized controlled clinical trials (RCTs) conducted to assess the efficacy of injectable platelet-rich fibrin (i-PRF) alone and in combination with microneedling (MN) for gingival augmentation in patients with thin phenotype of gingiva as Gingival thickness (GT) and Keratinized tissue width (KTW).
- The articles were restricted to those published up till December 2024 in English language and with full text only.

Exclusion criteria:

Reviews, in-vitro studies, animal studies, cross-sectional studies, retrospective studies and repeated reports of the same study, studies with only abstracts, inadequate data to examine the question of interest and published in language other than English where translation in English is not available were excluded.

Methods:

A comprehensive search of data was performed in PubMed, Google Scholar and Science Direct. While carrying out the search on PubMed following filters were put:

1. Article type- Randomized controlled clinical trials

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2. Publication date- January 2014 till December 2024
3. Species- Humans
4. Best match option

Studies were only excluded due to language. No filters for full text articles were applied. The keywords for search were as follows:

Table No. 1: Table showing details of MeSH and Entry terms from PubMed.

Keywords

Primary keywords	Secondary keywords
Patients with age 18 to 70 years with no significant systemic abnormalities and thin gingival phenotype (< 1.5 mm) (P)	• Patients with thin gingival biotype • Patients with thin periodontal phenotype
Microneedling alongwith injectable platelet-rich fibrin (I)	• Collagen induction therapy
Injectable platelet-rich fibrin (C)	• Liquid PRF • Flowable PRF
Gingival thickness and Keratinized tissue width (O)	• Soft tissue thickness • Mucosal thickness • Width of keratinized gingiva • Keratinized mucosa width

Table No. 2: Table showing details of search strategies used

Sr.no		Strategy	Hits	Final Selected (after exclusion)
1	PubMed	Patients with thin gingival phenotype AND Microneedling	121	2
2	PubMed	Patients with thin gingival phenotype AND Microneedling AND Injectable platelet-rich fibrin	114	0
3	PubMed	Patients with thin gingival phenotype AND Microneedling AND Injectable platelet-rich fibrin AND Gingival thickness and Keratinized tissue width	160	0
4	PubMed	(Patients with thin Gingival biotype) AND (Microneedling OR Collagen induction therapy) AND (Injectable platelet-rich fibrin OR Liquid PRF OR Flowable PRF) AND (Gingival thickness and Keratinized tissue width)	1	0
5	Google scholar	Patients with thin Gingival biotype AND Microneedling AND Collagen induction therapy AND Liquid PRF AND Gingival thickness and Keratinized tissue width	117	1
6	Google scholar	Patients with thin Gingival biotype AND Flowable PRF AND Gingival thickness and Keratinized tissue width	24	0
7	Google scholar	(Patients with thin gingival phenotype OR Patients with thin Gingival biotype) AND (Microneedling OR Collagen induction therapy) AND (Injectable platelet-rich fibrin OR Liquid PRF OR Flowable PRF) AND (Gingival thickness and Keratinized tissue width)	28	0

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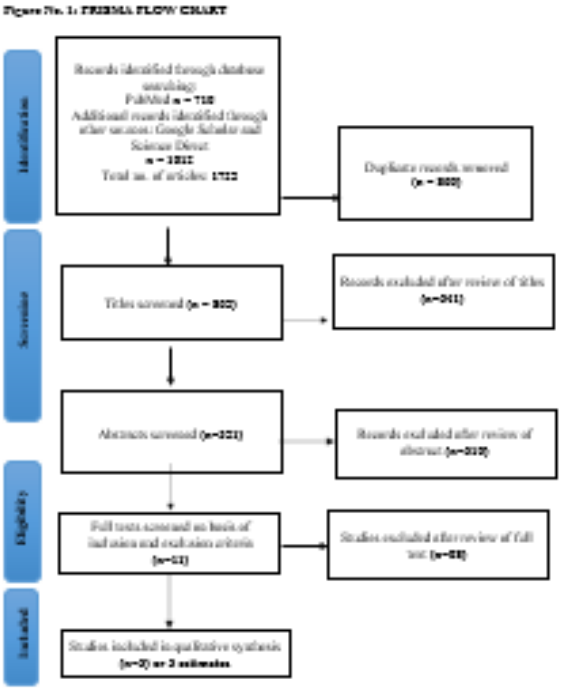
8	Google scholar	(Patients with thin gingival phenotype OR Patients with thin Gingival biotype) AND (Microneedling OR Collagen induction therapy) AND (Injectable platelet-rich fibrin OR Liquid PRF OR Flowable PRF) AND (Gingival thickness and Keratinized tissue width OR Soft tissue thickness OR Mucosal thickness)	3	0
9.	Science Direct	(Patients with thin gingival phenotype OR Patients with thin Gingival biotype) AND (Microneedling OR Collagen induction therapy) AND (Injectable platelet-rich fibrin OR Liquid PRF OR Flowable PRF) AND (Gingival thickness and Keratinized tissue width OR Soft tissue thickness)	102	0
10.	Science Direct	Patients with thin Gingival biotype AND Injectable PRF AND Gingival thickness and Keratinized tissue width	10	0
11.	Hand search	No articles found	0	0
12.	Cross references	No articles found	0	0

Results:

In this process, one reviewer initially evaluated the titles and abstracts identified through the search strategy, considering whether they satisfied the inclusion criteria. Subsequently, the full texts of all the studies meeting these criteria were obtained. The full-text articles were then thoroughly assessed and then decided if they met the inclusion criteria. In cases of uncertainty regarding a study's eligibility, the issue was settled through consultation/discussion with a second author. In case of differences in data extraction, both reviewers reached a point of agreement before making a decision. Rayyan QCRI software was used to eliminate duplicates and MS Excel 2013 was used to store the data. Ultimately, the systematic review included three studies identified through the search process. All the three articles were selected for meta-analysis. The screening process of studies is presented in the form of PRISMA flow-chart (Figure 1). The search yielded 3 articles for inclusion in systematic review. All the excluded studies were recorded with reason for exclusion. (Table 3)



EXCLUDED STUDIES:

8 articles were excluded from this review, after reviewing the full text, because they did not fulfil the inclusion criteria. The reason for exclusion for each of the article is depicted in Table no. 3

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Table No 3: Table showing excluded studies with reason.

Sr. No.	Author Name	Reason for exclusion
1.	Manasa et al[28]	The test group received i-PRF while the control group did not receive anything
2.	Yadav et al[29]	One side received i-PRF while the contralateral side received MN
3.	Sajjad et al[30]	One side received concentrated – platelet-rich fibrin (C-PRF) while the contralateral side received i-PRF
4.	Kavi et al[31]	There were no separate treatment and control groups instead i-PRF was injected into thin gingival tissue following MN
5.	Akolu et al[32]	There was no control group in this study. There was only test group which received i-PRF
6.	Siddharthan et al[5]	One side received MN + i-PRF while the contralateral side received MN
7.	Faour et al[33]	One side received i-PRF while the contralateral side received Hyaluronic acid (HA)
8.	Gottumukkala et al[34]	One group received i-PRF + MN while the other group received free gingival graft (FGG)

Table no. 4: Table showing data extraction sheet of all included studies

Sr No	Author	Year of Publication	Study design	Sample size	Location of study	Year of study
1	Ozsagir et al[4]	2020	RCT	33	Department of Periodontics, Faculty of Dentistry, Bezmialem Vakif University, Istanbul, Turkey	ND
2	Adhikary et al[1]	2023	RCT	32	Department of Periodontology, Shree Bankey Bihari Dental College and Research Centre, Ghaziabad, Uttar Pradesh, India	ND
3	Soundarajan et al[8]	2023	RCT	36	Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India	October 2020 to February 2021

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Sr No.	Author	Sex (M/F)	Age (Years)	Mean Age
1	Ozsagir et al[4]	28 females 5 males	18-34	22.2
2	Adhikary et al[1]	ND	18-34	ND
3	Soundarajan et al[8]	ND	20-45	ND

Sr No.	Author	Test group	Participants in test group	Control group	Participants in control group
1	Ozsagir et al[3]	MN + i-PRF	33	i-PRF	33
2	Adhikary et al[1]	MN + i-PRF	32	i-PRF	32
3	Soundarajan et al[8]	MN + i-PRF	36	i-PRF	36

Table No 5: Summary of primary outcomes

S.NO	AUTHOR AND YEAR OF PUBLICATION	PRIMARY OUTCOME				
		GINGIVAL THICKNESS (GT)				
			TEST		CONTROL	
			Mean	SD	Mean	SD
1.	Ozsagir et al (2020)[4]	Baseline	0.40	0.14	0.43	0.14
		1 month	0.62	0.14	0.61	0.14
		2 months	0.63	0.12	0.60	0.13
		3 months	0.65	0.13	0.62	0.13
		4 months	0.64	0.11	0.62	0.13

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		5 months 6 months	0.66 0.66	0.11 0.12	0.63 0.62	0.12 0.11
2.	Adhikary et al (2023)[1]	Baseline 3 months 6 months	0.62 0.74 0.87	0.21 0.23 0.22	0.57 0.64 0.69	0.19 0.20 0.20
3	Soundarajan et al (2023)[8]	Baseline 1 month 2 months 3 months	0.40 0.63 0.65 0.66	0.16 0.12 0.11 0.13	0.46 0.61 0.62 0.62	0.16 0.13 0.17 0.11

S.NO	**AUTHOR AND YEAR OF PUBLICATION**	**PRIMARY OUTCOME**				
		KERATINISED TISSUE WIDTH (KTW)				
			TEST		**CONTROL**	
			Mean	**SD**	**Mean**	**SD**
1.	Ozsagir et al (2020)[4]	Baseline 1 month 2 months 3 months 4 months 5 months 6 months	2.94 2.95 2.96 2.97 2.98 2.98 2.99	1.21 1.22 1.22 1.22 1.22 1.22 1.22	2.98 2.99 2.99 2.99 2.99 2.99 2.99	1.1 1.09 1.09 1.09 1.09 1.09 1.09
2.	Adhikary et al (2023)[1]	Baseline 3 months 6 months	3.29 3.96 4.03	0.59 0.87 0.98	3.26 3.89 3.97	0.63 0.78 0.72

The risk of bias was assessed by Risk-of-bias VISualization tool (ROBVIS) tool. The risk of bias of the included studies is presented as a Traffic Light Plot of individual studies and a summary diagram. RoB2 tool for randomized controlled studies were used in the ROBVIS.

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Any disagreement was discussed until consensus was achieved. Studies were categorized into high, unclear and low risk.

Low risk of bias: If all quality criteria were judged as present.

Some concerns: If one or more key domains were not described and

High risk of bias: if one or more key domains were not present.

Figure 2a: Risk of bias traffic light plot using RoB-2 tool

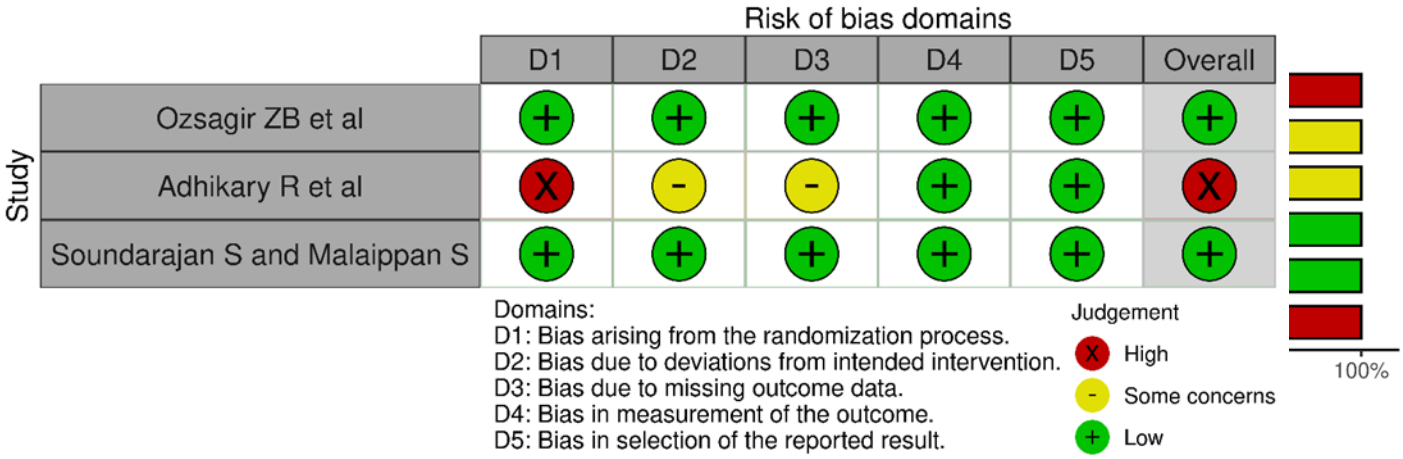

Figure 2b: RoB2 "Summary Plot" distribution of risk of bias among the studies

Three studies were assessed via the RoB2 tool for randomized controlled studies. The RoB2 tool revealed that two of the included studies had low risk of bias; followed by one study which had some concerns and had high risk of bias with reference to domains: D1, D2 and D3. The summary plot showed that more than 25% high risk bias was noted for randomization domain and more than 25% showed some concerns for deviation from intended interventions and missing outcome data. Overall, more than 50% studies showed low risk of overall bias (Figure 2a and Figure 2b).

RESULTS OF SYSTEMATIC REVIEW

The characteristics of the studies included in the systematic review are presented in the below tables (Table 6 and 7)

Table no. 6: Details of the studies included in the systematic review

Study Id	Author	Year	Study design	Sample size
1	Ozsagir ZB et al[4]	2020	A 6-month follow-up, single-blind, prospective, split-mouth randomized controlled clinical trial	n= 198 sites in mandibular anterior teeth from 33 patients
2	Adhikary R et al[1]	2023	A prospective randomized Split mouth single-blinded clinical trial	n= 64 sites in lower anterior teeth from 32 patients
3	Soundarajan et al[8]	2023	A 3-months follow-up, split mouth, single blind, prospective randomized controlled trial	n= 216 sites in lower front teeth from 36 patients

Table 4 to 7 represent study characteristics with respect to age group, population, intervention, comparator, outcomes and results of the included studies. The age of the patients in the included studies ranged from 18 years to 45 years. Systemically

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healthy patients with thin gingival and periodontal phenotypes (< 1.5 mm) were included in the studies. All the 3 studies had the Intervention/treatment (Test group) group as MN + i-PRF and the Comparator (Control group) as i-PRF. In majority of the studies the follow-up period ranged from 3 months to 6 months. The primary outcome in all of the included studies was GT and KTW. The results of all the three studies depicted that after evaluation of GT between the groups, a statistically significant difference was found between i-PRF and MN + i-PRF group at the end of three and six months. Moreover, there was a statistically significant increase in the GT in the test group (MN + i-PRF) at the end of three and six months. In case of KTW, two studies which evaluated it, showed no statistically significant difference between both the groups at the end of six months. Thus, all the study results concluded that application of i-PRF alongwith MN resulted in significant improvement in GT, indicating that i-PRF alongwith MN may increase GT without the need for surgical periodontal procedures.

Table 7- Details of the study participants, intervention, and comparator of the studies included in the systematic review

Sr. no	Author	Age group	Population	Interventions /treatments used (Test group)	Exposure/ Comparator (Control group)	Follow-up period	Primary outcomes	Secondary outcome, If any	Results	Conclusion
1	Ozsagir ZB et al	18–34 years	33 systemically healthy patients with thin periodontal phenotypes	Microneedling (MN) alongwith injectable platelet-rich fibrin (i-PRF)	injectable platelet-rich fibrin (i-PRF)	Before the treatment and every month for six months after the final injection.	Gingival Thickness (GT) and Keratinized Tissue Width (KTW)	PI, RD, PD, BOP and CAL parameters	After the evaluation of GT between the groups, a statistically significant increase was found in MN + i-PRF group at the sixth month on inter-group comparison. On intra-group comparison, there were no statistically significant differences in GT observed within both i-PRF and i-PRF+MN	In individuals with thin periodontal phenotype, i-PRF alone and i-PRF + MN may have an influence in increasing GT. The results suggest that MN may have an additional effect on increasing gingival thickness.

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Sr. no	Author	Age group	Population	Interventions /treatments used (Test group)	Exposure/ Comparator (Control group)	Follow-up period	Primary outcomes	Secondary outcome, If any	Results	Conclusion
									groups at the sixth month.	
2	Adhikary R et al	18-34 years	32 systemically healthy patients between 18-34 years with thin gingival biotype (< 0.8 mm)	Microneedling (MN) alongwith Injectable platelet-rich fibrin (i-PRF)	injectable platelet-rich fibrin (i-PRF)	baseline, three months, and six months	Gingival Thickness (GT) and Keratinized Tissue Width (KTW)	NR	The findings revealed that on comparison Group A which received just i-PRF to Group B which received i-PRF + MN, there was a statistically significant increase in GT in Group B.	This is a unique method to expand the gingiva without the need of invasive procedures such as surgical innervations. Although neoangiogenesis caused by MN contributed to thicker gingiva, i-PRF showed better release of growth factors.

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Sr. no	Author	Age group	Population	Interventions /treatments used (Test group)	Exposure/ Comparator (Control group)	Follow-up period	Primary outcomes	Secondary outcome, If any	Results	Conclusion
3	Soundarajan et al	20–45 years.	36 patients between 20-45 years who were diagnosed with thin periodontal phenotype	Microneedling (MN) alongwith injectable platelet-rich fibrin (i-PRF)	injectable platelet-rich fibrin (i-PRF)	At baseline and 3 months following therapy	Gingival Thickness (GT)	NR	The study results showed a significantly greater increase in GT at sites where both MN + i-PRF injection were used together compared to i-PRF alone with a p value of .04	Both treatment modalities showed improvement in GT, being a minimally invasive alternative for mucogingival surgery. Adjunctive MN was found to be more beneficial than i-PRF alone. However, further research should be carried out to address the drawbacks of the study and for a better understanding of this novel approach

NR- Not reported

RESULTS OF META-ANALYSIS

The quantitative synthesis via meta-analysis was performed for two outcomes assessed studies; Gingival thickness (GT) and Keratinized Tissue Width (KTW). All three included studies were taken into consideration for meta-analysis since their results depicted Mean and SD values of GT and two included studies were taken into consideration for meta-analysis since their results depicted Mean and SD values of KTW.

Table 8 represents the outcome GT in the included studies. The mean and SD values for GT have been presented for each study. It can be found that the mean GT is higher across

intervention group in all the studies on comparison to control group in all the included studies.

Table 9 represents the outcome; KTW in the included studies. The mean and SD values of KTW have been presented for each study. It can be found that the mean KTW is almost similar and comparable in both the intervention and control group in all the included studies.

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Table 8: Quantitative data of the outcome, GT in all included studies

Sr. no	Included studies	MN + i-PRF (Test group)			i-PRF (Comparator/control)		
		Mean	SD	Total	Mean	SD	Total
1	Ozsagir ZB et al[4]	0.65	0.13	33	0.62	0.13	33
2	Adhikary R et al[1]	0.74	0.23	32	0.64	0.20	32
3	Soundarajan et al[8]	0.66	0.13	36	0.62	0.11	36

Table 9: Quantitative data of the outcome, KTW in all included studies

Sr. no	Included studies	MN + i-PRF (Test group)			i-PRF (Comparator/control)		
		Mean	SD	Total	Mean	SD	Total
1	Ozsagir ZB et al[4]	2.97	1.22	33	2.99	1.09	33
2	Adhikary R et al[1]	3.96	0.87	32	3.89	0.78	32

Figure 3a represents the forest plot distribution of GT across the studies. It can be seen that the pooled estimate denotes that the outcome favours test group as compared to intervention group. The heterogeneity across the studies was low i.e. 0%. The results across the studies are consistent. The mean difference between the groups was 0.34 (95% confidence interval: 0.06 to 0.61). The pooled estimate showed a statistically significant difference (p value = 0.02) in the GT between the test (MN + i-PRF) and control group (i-PRF). Thus, it can be interpreted that the test group (MN + i-PRF) showed better results statistically with no heterogeneity across the three studies.

Figure 3b represents the forest plot distribution of KTW across the studies. It can be seen that the pooled estimate denotes that the outcome is equally affected in control group as well as test group. Thus, both the groups show similar efficacy. The heterogeneity across the studies was low, i.e. 0%. The results across the studies were consistent. The mean difference between the groups was 0.03 (95% confidence interval: -0.31 to 0.38). The pooled estimate for KTW showed no statistically significant difference (p value =0.85) between the intervention (MN + i-PRF) and control groups (i-PRF). Thus, it can be interpreted that both intervention and control groups showed similar results statistically.

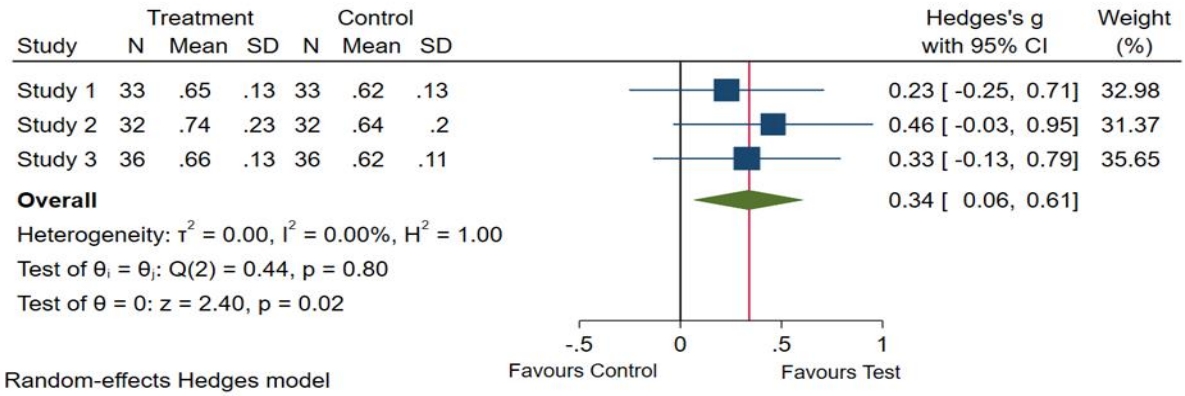

Figure 3a: Forest Plot Distribution for GT outcome

Study 1- Ozsagir et al; Study 2 – Adhikary et al; Study 3 – Soundarajan et al

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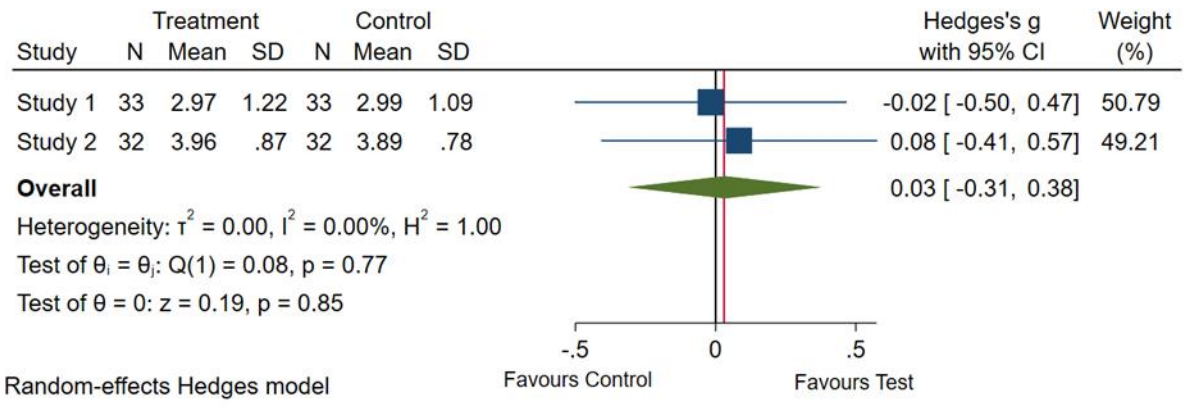

Figure 3b: Forest Plot Distribution for KTW outcome

Study 1- Ozsagir et al; Study 2 – Adhikary et al

Discussion:

In modern dentistry, the success of esthetic restorations mainly depends on the gingival biotype, morphology of gingival tissue and the shape of the anterior teeth. Amongst these factors, gingival biotype plays an important role in determining the outcome of esthetic and regenerative procedures[1,3]. Biotype denotes a group of organs or tissues with same specific genotype whereas phenotype refers to appearance of an organ or tissue based on combination of several factors such as genetic and environmental factors [5,6]. Thick gingival biotype also known as flat-thick gingiva has been found to be associated with squared crown form whereas thin gingival biotype also known as scalloped thin gingiva has been found to be associated with tapered crown form[16]. While individuals with thin phenotype are more prone to gingival recession in presence of trauma and inflammation, those with thick phenotype have been reported to develop periodontal pockets[4]. The association between GT and gender has been controversial. Sonmez et al reported that there is a higher prevalence of thin phenotype among females when compared to males[13]. However, Kolte et al reported that gender has no effect on GT[14].

Thus, it is important to take into consideration phenotype while treatment planning since individuals with thin gingival phenotype are more susceptible to gingival recession[15]. Although surgical procedures such as subepithelial connective tissue grafting (gold standard), modified roll flap and acellular dermal matrix have been used for increasing the GT, there are minimally invasive procedures such as i-PRF and MN that have recently taken over surgical procedures in the field of periodontics[8,26,27].

In this context, several developments have been made in the field of periodontics mainly focusing on making PRF injectable devoid of anticoagulants and fibrin matrix, resulting in increased concentration of regeneration cells and growth factors. The advantages of i-PRF over PRF include increased release of growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and insulin like growth factor-1 (IGF-1) at the end of ten days[17]. MN also referred to as PCIT, produces microinjuries that cause minor superficial bleeding, which leads to a cascade of wound healing that releases a variety of growth factors such as platelet-derived growth factors, transforming growth factors, connective tissue growth factors and fibroblast growth factors which facilitate neoangiogenesis. One of the advantages of MN is that it enhances epidermis permeability and blood flow. This procedure allows topical drugs and growth factors to pass through the stratum corneum and facilitate regeneration of elastin and collagen[1,4].

Among the three studies included in this systematic review, two studies by Ozsagir et al and Adhikary et al reported a statistically significant improvement in GT in the MN + i-PRF group at the end of six months, while the study by Soundarajan et al demonstrated a significant improvement in GT at the end of three months. This gain is typically evident within few weeks post injection and may continue to improve over several months. The

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mechanism underlying this improvement is likely due to sustained release of growth factors that stimulate local tissue remodelling and new connective tissue formation. Additionally, i-PRF is autologous, biocompatible, cost-effective, and minimally invasive, making it highly acceptable for patients, especially those reluctant to surgical interventions such as connective tissue grafting. Unlike grafts, i-PRF does not require a donor site, thereby minimising postoperative pain and complications. MN has added benefit in increasing GT as it helps to enhance the production of elastin and collagen fibers causing neo-collagenases and also enhances epidermis permeability and blood flow causing neoangiogenesis[1,4,5,8].

Esfahrood et al in a review reported that gingival biotype considerably affects the outcome of periodontal therapy, root coverage procedures and implant placement[7]. In a study conducted by Peixoto et al it was found that there exists a positive relationship between keratinized tissue width, probing depth and clinical attachment level[18]. Anderegg et al reported that for GT > 1mm there was less post-operative gingival recession than for GT < 1mm[19]. Baldi et al stated that thick gingival tissue was associated with a more favourable root coverage than thin gingival tissue[20]. According to Hakkinen et al the overall collagen synthesis increased within 7-14 days of healing process[21]. Miron et al reported that PRF resorption occurred within 7-11 days while i-PRF showed sustained release of growth factors for a period of ten days[22]. Thus, four sessions of i-PRF and MN + i-PRF were administered at an interval of 10 days in two of the included studies while three sessions were administered in one of the included studies [1,4,8].

In a study conducted by Aust et al, it was stated that when MN was performed as an adjunct to topical vitamin A and vitamin C administration, the epidermis thickness increased to about 140% when compared to topical vitamin A and C administration alone which was about 22%[23]. In an eight-month follow up study conducted by Fabbrocini et al, it was reported that with two sessions of MN for treating wrinkles at the cervical region, tissue thickness improved by 0.45 mm at eighth month after treatment[24]. Also, in patients with alveolar bone dehiscence as a result orthodontic movements, it was found that they were more vulnerable to gingival recession especially those with thin gingival phenotype. Therefore, MN + i-PRF application in orthodontic patients with thin phenotype may reduce the probability of gingival recession in these patients[25]. Based on the current data, it is evident that the administration of both i-PRF and MN + i-PRF led to clinical improvement in GT and KTW in patients with thin gingival phenotype. However, administration of MN + i-PRF led to significant clinical improvement in GT in patients with thin gingival phenotype when compared to i-PRF alone.

However, this systematic review also presents certain limitations. Primary limitation is lack of assessment of patient-related outcome measures (PROM) such as pain, discomfort, esthetic satisfaction and overall acceptance of the procedure. Other limitations included limited number of randomized controlled trials (RCTs), small sample size, short term follow up. In addition, i-PRF was injected only in the apical mucogingival junction due to the tight architecture of the keratinized tissue and there was a lack of standardized protocol for MN in terms of depth, frequency and number of sessions. Also, position of teeth in relation to the dental arch was not assessed. Future studies should focus on well-designed RCTs with large sample size and duration, longer follow up, standardization of microneedling protocol (needle depth and frequency), histological assessment of tissue changes post-treatment, evaluation of patient-related outcome measures and comparative studies with other non-surgical and surgical augmentation techniques. Although the use of i-PRF alongwith MN may be beneficial particularly in preventive periodontal therapy, orthodontic treatment and esthetic zone management, clinicians should interpret the findings with caution due to the drawbacks mentioned and the need for standardized treatment protocol.

CONCLUSION

The current systematic review indicates that in patients with thin gingival phenotype, both i-PRF alone and in combination with MN showcase promising results for gingival augmentation. However, the combination therapy seems to produce better outcomes in terms of GT and overall soft tissue improvement, likely due to the combined effects of increased growth factor release and microneedling-induced localised tissue remodelling. However, to establish clear treatment protocol and to get a deeper understanding of the molecular mechanisms behind the observed outcomes, further high-quality, randomised controlled trials with standardised guidelines and long-term assessment are necessary. In conclusion, while i-PRF alone is effective, adjunctive use of MN appears to improve its regeneration capacity, making the combination a more successful minimally invasive approach for treating thin gingival phenotype.

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