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Evaluation of Gingival Thickness with Microneedling and Vitamin C Versus I-PRF (Injectable Platelet Rich Fibrin) in Thin Gingival Phenotype: A Randomized Controlled Clinical Trial

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Abstract

Thin gingival phenotype, marked by delicate, translucent tissue with minimal keratinized gingiva and narrow attached gingiva, increases susceptibility to recession, trauma, and inflammation, posing challenges across periodontal, implant, and restorative therapy. Conventional correction via free gingival grafts or connective tissue grafts requires a second surgical site and carries added patient morbidity, prompting interest in non-surgical alternatives. Microneedling induces controlled micro-injuries that trigger fibroblast activation, neoangiogenesis, and collagen/elastin synthesis, while injectable Vitamin C (i-Vit. C) supports collagen maturation and matrix metalloproteinase inhibition, and injectable platelet-rich fibrin (i-PRF) delivers concentrated growth factors to promote tissue regeneration. No prior randomized controlled trial has compared i-Vit. C with i-PRF as adjuncts to

microneedling for augmenting gingival thickness (GT) and attached gingiva width (AGW) in thin phenotype cases.

Keywords: Thin gingival phenotype, Microneedling, Injectable Vitamin C, Injectable platelet-rich fibrin, Gingival thickness, Attached gingiva width, Gingival augmentation

Introduction

In modern periodontal and esthetic dentistry, understanding and managing gingival phenotypes, especially the thin gingival phenotype has become crucial for the success of both surgical and non-surgical treatments. A thin gingival phenotype is characterized by a delicate, translucent gingiva with minimal keratinized tissue, shallow vestibules, and narrow attached gingiva.[1] Such tissue is more prone to recession, trauma, and inflammation, making it a clinical challenge in periodontal therapy, implantology, and restorative treatments.[2] The identification and management of thin phenotype is therefore essential to prevent undesirable treatment outcomes and achieve long time success.

Various methods given by different authors for thickness of gingiva:

1. Visibility of periodontal probe: A periodontal probe is inserted into the gingival sulcus till its base. If it is visible, it is considered to be thin gingiva; if it is not, it is considered to be thick gingiva.[3]
2. Measurement with endodontic file instrument: An endodontic reamer or endodontic file with a silicon disc is inserted perpendicular to tooth surface 1.5 mm apical to the zenith of gingival margin till the hard surface of tooth is felt and the distance is measured by transferring the readings on the vernier caliper (VC).[3]

Various values given by different authors for thickness of gingiva (GT):

1. Fischer et al (2016)[4]- thin: 0.43mm and thick: 0.83mm
2. Baldi et al. (1999)[5] - thin: < 0.8mm
3. Eger et al. (1996) and Seibert et al. (1989)[6]- thick: > 2mm and thin: < 1.5mm

Various values given by different authors for width of attached gingiva (AGW):

1. Bhatia et al. (2015)[7]: a) Maxillary teeth 31 to 45 years-3.11mm

b) Mandibular teeth 15 to 45 years- 2.1mm

2. Bowers (1963)[7]: a) Maxillary incisors-4.5mm

b) Mandibular incisors-3.3mm

3. Ainamo and Loe (1966)[7]: a minimum of two mm of keratinized gingiva including one mm of attached gingiva is sufficient to maintain gingival health.
4. Lang and Loe (1972)[7]: a) Maxillary incisors- 4.5mm

b) Mandibular incisors- 3.3mm

5. Lindhe and Nyman (1980)[8]: Even if attached gingiva is < 1 mm, but can resist dental plaque from invading into deeper periodontal tissues, resist frictional forces from surrounding musculature and masticatory forces from occlusion, it is adequate.

Conventionally, methods like free gingival grafts (FGG) and connective tissue grafts have been employed to address the issue of thin phenotype and inadequate width of attached gingiva, but these methods require two surgical sites and these surgical procedures are invasive, technique-sensitive, and often associated with patient morbidity.[9,10] So, the authors of this literature were concerned for exploring non-invasive methods.

Microneedling, also known as percutaneous collagen induction therapy, involves the creation of controlled micro-injuries in the tissue using fine needles. These micro-channels stimulate the body's wound healing response, which includes the release of growth factors, fibroblast activation, and enhanced production of

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collagen and elastin.[11] Originally used in dermatology for treating scars, fine lines, and alopecia, this technique has shown promising results.[12]

When used in the oral mucosa, microneedling promotes neoangiogenesis and fibroblast proliferation, which are crucial for the thickening of gingival tissue.[13] A study by Orsini et al. reported improved healing and enhanced tissue tone in patients undergoing mucogingival surgeries when microneedling was applied adjunctively.[14] These outcomes are particularly significant in individuals with thin gingival phenotypes, who require increased soft tissue volume to withstand mechanical and microbial insults.

Apart from its regenerative benefits, this therapy is relatively safe, cost-effective, and well-tolerated by patients. The minimally invasive nature of microneedling makes it a viable option for patients unwilling or unable to undergo conventional grafting procedures.[15] It can also be easily integrated into routine periodontal maintenance visits or as a preparatory phase for more advanced regenerative or prosthetic procedures.[16]

Another game changer in increasing thickness of gingiva is injectable platelet rich fibrin (i-PRF), which is rich in growth factors, platelets and leukocytes. It promotes regeneration process at low-speed configuration. The blood is centrifuged in plastic tubes at 700 rpm for 3 min.[17,18] This has led to a growing interest in minimally invasive therapies such as microneedling and i-PRF to stimulate endogenous regenerative processes in the gingival tissues.[3,17,18]

Chaudhary et al.[19] (2023) employed injectable Vitamin C (i-Vit. C)/ mesotherapy for gingival depigmentation and achieved increase in gingival thickness in their subjects.

Moreover, a case study done by Dawar et al.[20](2025) on Vitamin C Mesotherapy with Microneedling for Gingival Depigmentation on skin suggested that though the primary focus was on reducing melanin pigmentation, it was also observed that there is a potential increase in gingival thickness, suggesting that this combination may serve dual purposes in enhancing gingival aesthetics and health.

According to Swarna et al.[21] (2024) the microneedling group shows improved wound healing and a more uniform depigmentation effect, indicating the potential benefits of combining microneedling with Vitamin C in gingival treatments. It also showed esthetically gratifying outcomes when compared to conventional surgical technique.

The addition of bioactive topical agents further amplifies the effects of microneedling. Among various agents studied, Vitamin C (ascorbic acid) stands out for its role in collagen synthesis and antioxidant defense. Vitamin C is a water-soluble antioxidant that supports epithelial barrier function, modulates inflammatory responses, and facilitates collagen maturation through its role as a cofactor for prolyl and lysyl hydroxylases: enzymes critical in stabilizing the collagen triple helix.[22] In dermatological literature, topical Vitamin C has been shown to improve skin texture, elasticity, and density.[23]

When microneedling is used in combination with Vitamin C, the induced micro-channels allow for deeper penetration and absorption of the Vitamin C into the connective tissue layer. This synergistic interaction results in enhanced fibroblast activity and extracellular matrix remodeling.[24] In the context of periodontal soft tissues, this translates into increased gingival thickness, improved vascularity, and potentially greater resistance to trauma and disease progression.[25]

Furthermore, Vitamin C has been shown to modulate gene expression related to collagen and matrix metalloproteinases (MMPs), which play a role in tissue remodeling and degradation.[26] By inhibiting MMP

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activity, Vitamin C helps preserve the structural integrity of newly formed connective tissues, supporting long-term tissue augmentation. This aspect is particularly relevant for thin gingival phenotypes, where preserving volume is as critical as gaining it.[27]

So, microneedling combined with topical Vitamin C offers a promising, non-surgical approach for augmenting thin gingival tissues. By leveraging the body's natural healing mechanisms and the biochemical properties of ascorbic acid, clinicians can achieve improved gingival thickness, vascularity, and resilience, ultimately contributing to better periodontal and aesthetic outcomes.[20]

As there are no published randomized controlled clinical trials aiming at exploring role of i-Vit. C as an adjunct to microneedling to increase the thickness and width of attached gingiva; the authors of present case reports took up the research to comparatively evaluate the differences in GT, AGW, color and texture of gingiva with microneedling and i-Vit. C versus microneedling and i-PRF in thin gingival phenotype between baseline and six months post-operatively.

Material and Methods

This longitudinal study consisted of 34 systemically healthy participants. The study was designed to comparatively evaluate GT, AGW, color and texture with microneedling and i-Vit. C versus microneedling and i-PRF in thin gingival phenotype, performed with following eligibility criteria:

Young individuals with thin gingival phenotype, having good oral hygiene and concerned about esthetics with willingness for microneedling procedures were included in the study.

Individuals excluded were those having any contraindication for surgery, acute infection, medically compromised/ non-compliant patients, subjects with abnormal bleeding/clotting times, presence of any pathology causing facial bony plate dehiscence, history of Irradiation in Head and Neck and uncontrolled diabetic patients.

Methods of Measurement:

Gingival thickness (GT): To measure GT from the apical 1.5 mm of the gingival margin, a No: 10 K file was placed in the centre of a 3 mm diameter silicone disc. The file was inserted perpendicularly from the facial zenith at 1.5 mm apical of the gingival margin, through the soft tissues until a hard surface was felt. The silicone disc was laid in tight contact with the soft tissue surface. The depth of penetration was measured between the silicone disc and the file.[28]

Attached gingiva width (AGW): To measure adequacy of AGW, the lower lip was retracted in outward, forward and downward direction. Movement of marginal gingiva away from the tooth surface depicted its inadequacy. Furthermore, numerical distance from the free gingival groove to mucogingival junction was measured using a periodontal probe.

Vernier caliper readings: Main scale reading + (sequential vernier scale marking x Least count)**. Least count**: smallest division on main scale/ number of divisions on main scale.

Soft-tissue color[29]: Post-operative color (healthy pink) vs. baseline values of lower six anterior teeth.

Score 0 – No difference

Score 1 - Moderate difference

Score 2 - Obvious difference

Soft-tissue texture[30]: Post-operative texture (presence of stippling) vs. baseline values of lower six anterior teeth.

Score 0 - no difference

Score 1 - Moderate difference

Score 2 - Obvious difference

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Operative procedure will be done under strictly aseptic conditions. Patients will be anesthetized at the site by the appropriate method using (2 % lidocaine, 1:200000 epinephrine).

Method of injecting i-PRF[17,18]:

A venous blood sample was taken once for each patient using a 20 ml injector and was separated into two i-PRF tubes of 10 ml each containing no anticoagulant and centrifuged at room temperature for 2 min at 2000 rpm with Choukroun PRF Duo centrifuge. The i-PRFs obtained were placed in 2.5 ml dental injectors. The 27-guage dental injector needles were used for injection of i-PRF. 0.1-0.2 ml was injected at epithelial connective tissue interface into the gingiva till the tissue blanched. This was done in attached gingiva as well as vestibular area.

Method of injecting Vitamin C[19]:

Vitamin C ampoule of 2 ml about 0.1-0.2 ml (200-300mg Vitamin C) was injected at approximately epithelial connective tissue interface into the attached gingiva until the tissue blanched, 2-3 mm apart using an insulin syringe (needle-31 gauge and 8mm length). This was done in vestibular area also. The insulin syringe was introduced into the respective gingival tissues with bevel facing in upward direction.

Figures 1A to 1M: Microneedling and injectable Vitamin C (4 appointments microneedling and 2 appointments injectable Vitamin C)

1. **Vitamin C**

Firt To Fourth Appointment of Microneedling:

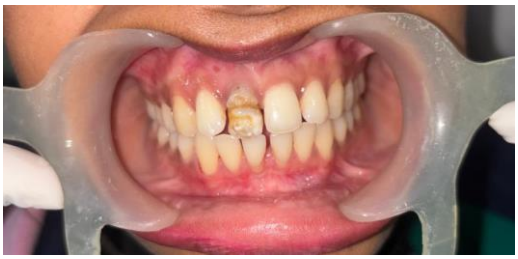

Fig. 1A): Preoperative view

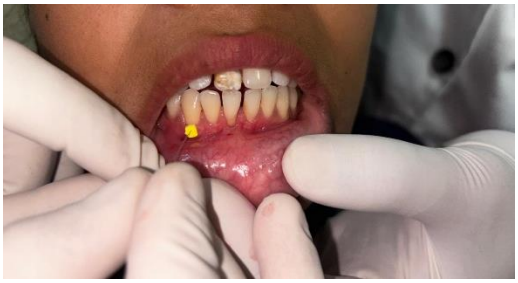

Fig. 1B): Measurement of GT

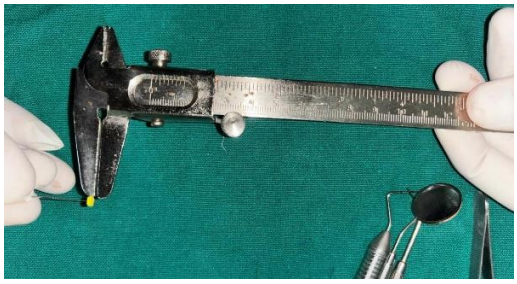

Fig. 1C): Readings transferred to VC

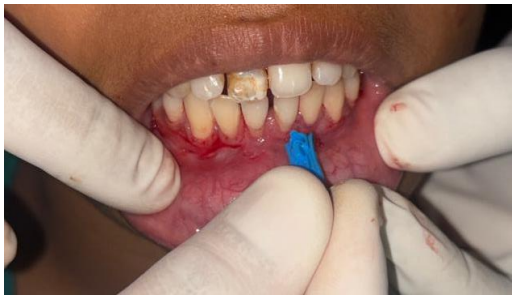

Fig. 1D): Microneedling with lancet

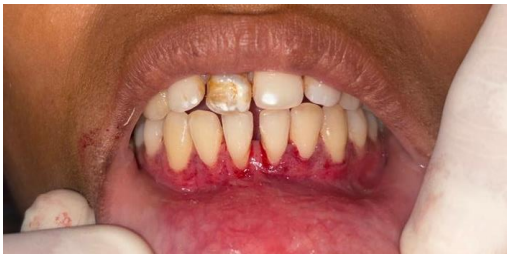

Fig. 1E): Post-microneedling

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Table 1a: Thickness of Gingiva: Baseline Values

Thickness	0.5 mm	0.5 mm	0.1 mm	0.3 mm	0.4 mm	0.2 mm
Tooth No.	43	42	41	31	32	33

Table 1b: Width of Keratinized Gingiva: Baseline Values

AGW	2 mm	3 mm	0 mm	2 mm	3 mm	1 mm
Tooth No.	43	42	41	31	32	33

Fifth and Sixth Appointment of Vitamin C

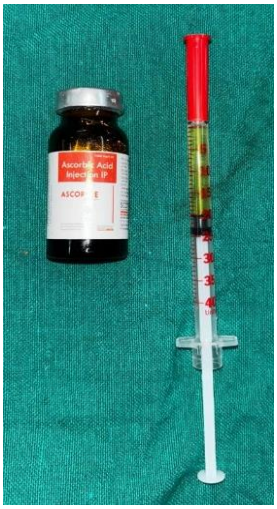

Fig. 1F): i-Vit. C

Fig. 1G): Vitamin C injected at epithelial connective tissue interface

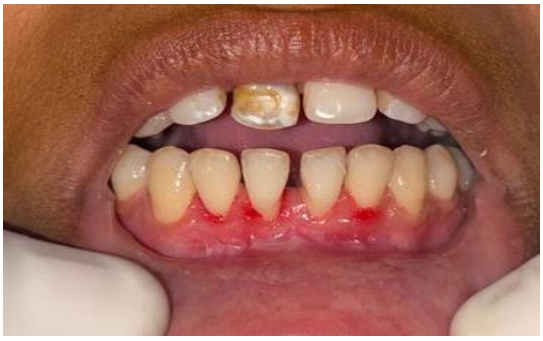

Fig. 1H): Immediate postop

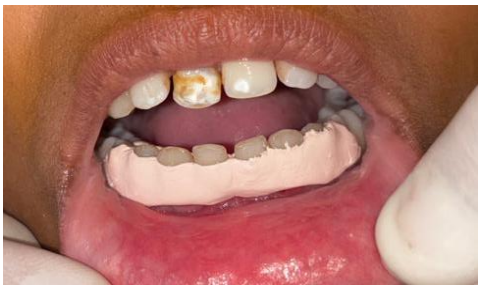

Fig. 1I): Coe-Pak given

Seventh Appointment: Post six months

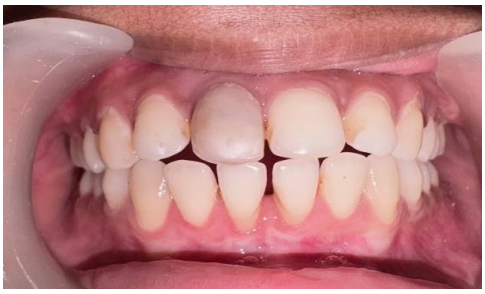

Fig. 1J): Healthy Gingival colour and texture

Fig. 1K): Measurement of AGW

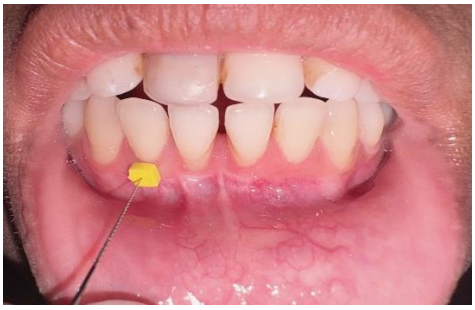

Fig 1L): Measurement of GT

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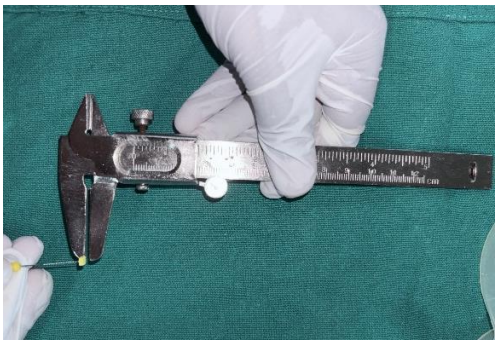

Fig. 1M): Readings transferred to VC

Table 1c: Thickness of Gingiva: Six-Month Post Operative Values

Thickness	1.1 mm	1.0 mm	0.7 mm	0.8 mm	0.9 mm	0.9 mm
Tooth No.	43	42	41	31	32	33

Table 1d: Width of Keratinized Gingiva: Six-Month Post Operative Values

AGW	4 mm	4 mm	2 mm	3 mm	4 mm	2 mm
Tooth No.	43	42	41	31	32	33

2. i-PRF:

Figures 2A to 2M: Microneedling and i-PRF (4 appointments microneedling and 2 appointments i-PRF)

First To Fourth Appointment of Microneedling:

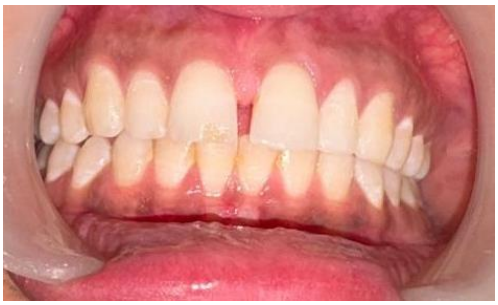

Fig. 2A): Preoperative view

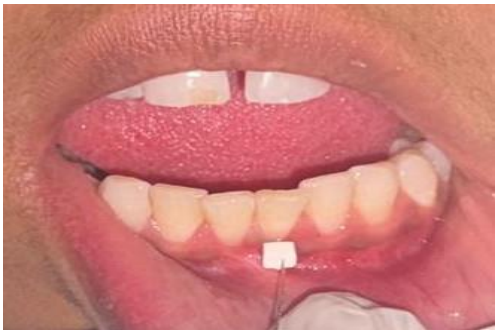

Fig. 2B): Measurement of GT

Fig. 2C): Microneedling with lancet

Fig. 2D): Post-microneedling

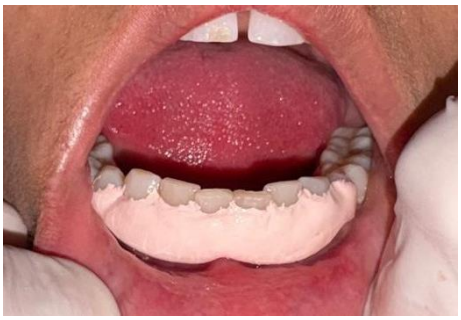

Fig. 2E): Coe pak given

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Table 2a: Thickness of Gingiva: Baseline Values

Thickness	1.0 mm	0.9 mm	0.7 mm	0.8 mm	1.0 mm	0.9 mm
Tooth No.	43	42	41	31	32	33

Table 2b: Width of Keratinized Gingiva: Baseline Values

AGW	4 mm	4 mm	2 mm	3 mm	4 mm	3 mm
Tooth No.	43	42	41	31	32	33

Fifth And Sixth Appointment of i-PRF:

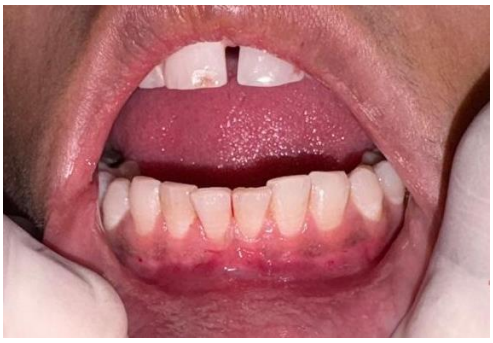

Fig. 2F): Preoperative view

Fig. 2G): Venipuncture

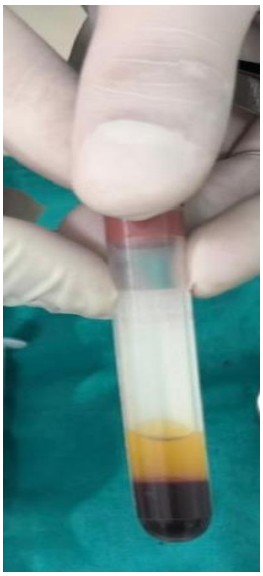

Fig. 2H): i-PRF

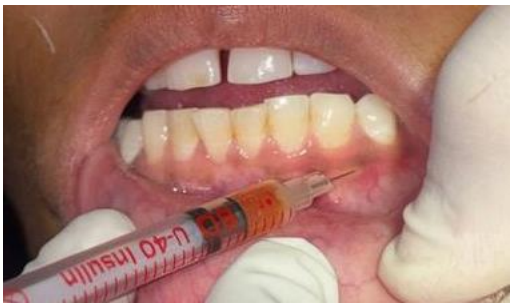

Fig 2I): Injecting i-PRF

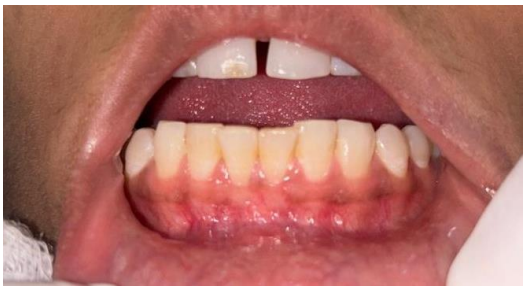

Fig. 2J): Immediate Post-operative view

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Seventh Appointment: Post six months

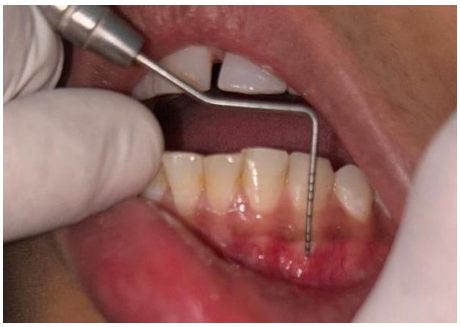

Fig. 2K): Measurement of AGW

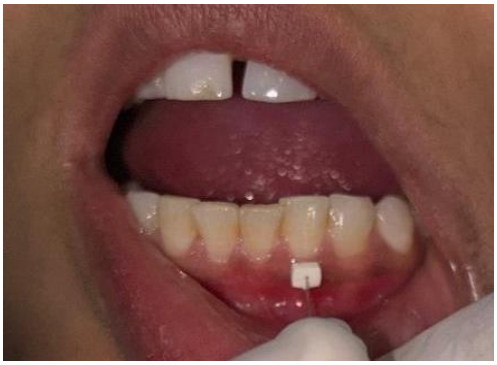

Fig. 2L): Measurement of GT

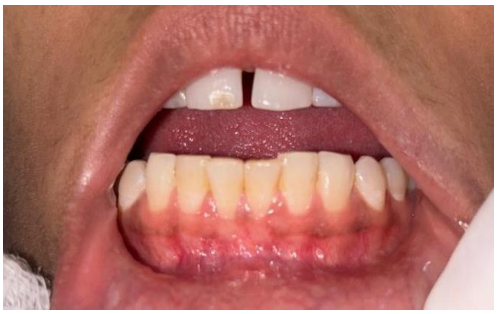

Fig. 2M): Healthy Gingival colour and texture

Table 2c: Thickness of Gingiva: Six-Month Post Operative Values

Thickness	0.8 mm	1.0 mm	0.8 mm	0.8 mm	1.0 mm	0.9 mm
Tooth No.	43	42	41	31	32	33

Table 2d: Width of Keratinized Gingiva: Six-Month Post Operative Values

AGW	7 mm	4 mm	3 mm	3 mm	4 mm	5 mm
Tooth No.	43	42	41	31	32	33

Results

Table 3: Normality assessment

Variable	Group	Statistics	df	p-value
Width of attached gingiva	Control	0.440	17	<0.001
	Test	0.254	17	0.005
Thickness of gingiva	Control	0.420	17	<0.001
	Test	0.141	17	0.200

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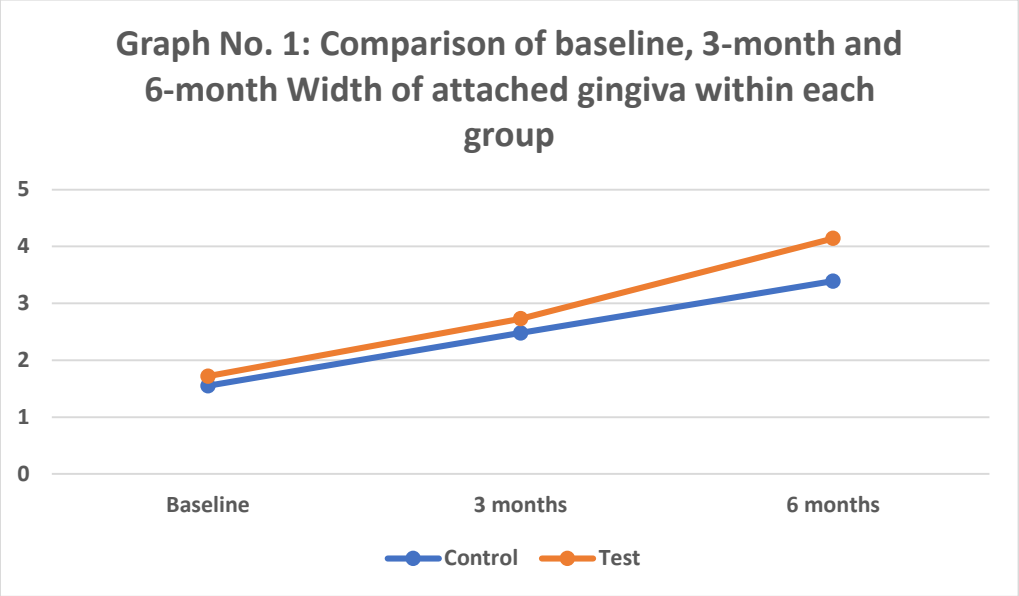
Kolmogorov Smirnov test

Normality of the study variables was assessed using the Kolmogorov–Smirnov test (Table no. 3). The width of attached gingiva showed a non-normal distribution in both; the control group (statistic = 0.440, df = 17, p < 0.001) and the test group (statistic = 0.254, df = 17, p = 0.005). Gingival thickness demonstrated a non-normal distribution in the control group (statistic = 0.420, df = 17, p < 0.001), while the test group showed a normal distribution (statistic = 0.141, df = 17, p = 0.200).

Table 4: Comparison of baseline, three-month and six-month Width of attached gingiva within each group

Group	Interval	Mean	SD	χ^2 value	p-value
Control	Baseline	1.55	0.08	34.00	<0.001*
	3 months	2.48	0.31		
	6 months	3.39	0.33		
Test	Baseline	1.72	0.27	31.12	<0.001*
	3 months	2.73	0.66		
	6 months	4.14	0.43		

Friedman's test; * indicates a significant difference at p≤0.05



Within-group comparison of the width of attached gingiva across baseline, three months, and six months showed a statistically significant increase in both groups by Friedman's test, p < 0.001(Table No.4 and Graph No. 1). In the control group, mean values increased from 1.55 ± 0.08 mm at baseline to 2.48 ± 0.31 mm at 3 months and 3.39 ± 0.33 mm at 6 months (χ^2 = 34.00). Similarly, in the test group, the width increased from 1.72 ± 0.27 mm at baseline to 2.73 ± 0.66 mm at 3 months and 4.14 ± 0.43 mm at 6 months (χ^2 = 31.12).

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Table 5: Pairwise comparison of Width of attached gingiva within each group

Group	Interval	Mean difference	p-value
Control	Baseline vs 3 months	-0.93	0.011*
	Baseline vs 6 months	-1.84	<0.001*
	3 months vs 6 months	-0.91	0.011*
Test	Baseline vs 3 months	-1.01	0.039*
	Baseline vs 6 months	-2.42	<0.001*
	3 months vs 6 months	-1.42	0.008*

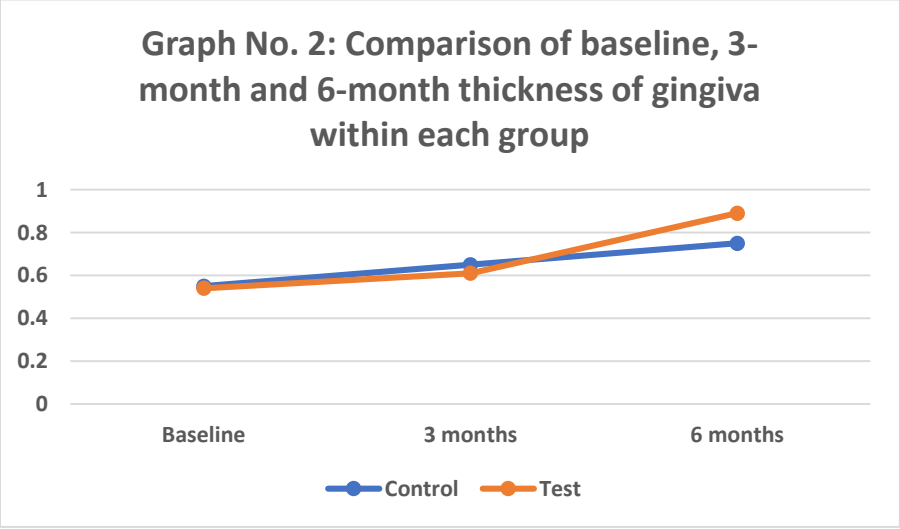
Post hoc Bonferroni test; * indicates a significant difference at p≤0.05

Post hoc Bonferroni analysis revealed statistically significant differences between all time intervals within both groups (Table No. 5). In the control group, significant differences were observed between baseline and 3 months (mean difference = −0.93 mm, p = 0.011), baseline and 6 months (mean difference = −1.84 mm, p < 0.001), and 3 months and 6 months (mean difference = −0.91 mm, p = 0.011). In the test group, significant differences were also noted between baseline and 3 months (mean difference = −1.01 mm, p = 0.039), baseline and 6 months (mean difference = −2.42 mm, p < 0.001), and 3 months and 6 months (mean difference = −1.42 mm, p = 0.008).

Table 6: Comparison of baseline, three-months and six-months thickness of gingiva within each group

Group	Interval	Mean	SD	χ^2 value/F-value	p-value
Control[#]	Baseline	0.55	0.03	33.522	<0.001*
	3 months	0.65	0.05		
	6 months	0.75	0.05		
Test[¥]	Baseline	0.54	0.04	201.512	<0.001*
	3 months	0.61	0.07		
	6 months	0.89	0.05		

[#]Friedman's test; [¥]Repeated measures ANOVA test; * indicates a significant difference at p≤0.05



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Comparison of gingival thickness at baseline, three months and six months demonstrated a statistically significant increase in both groups with p value < 0.001 (Table No. 6 and Graph No. 2). In the control group, Friedman's test showed an increase from 0.55 ± 0.03 mm at baseline to 0.65 ± 0.05 mm at 3 months and 0.75 ± 0.05 mm at 6 months (χ^2 = 33.522). In the test group, repeated measures ANOVA revealed a significant increase from 0.54 ± 0.04 mm at baseline to 0.61 ± 0.07 mm at 3 months and 0.89 ± 0.05 mm at 6 months (F = 201.512).

Table 7: Pairwise comparison of thickness of gingiva within each group

Group	Interval	Mean difference	p-value
Control	Baseline vs 3 months	-0.10	0.018*
	Baseline vs 6 months	-0.20	<0.001*
	3 months vs 6 months	-0.10	0.008*
Test	Baseline vs 3 months	-0.07	0.025*
	Baseline vs 6 months	-0.35	<0.001*
	3 months vs 6 months	-0.28	<0.001*

Post hoc Bonferroni test; * indicates a significant difference at p≤0.05

Bonferroni-adjusted pairwise comparisons showed statistically significant differences between all evaluation intervals in both groups (Table No. 7). In the control group, significant differences were observed between baseline and 3 months (mean difference = −0.10 mm, p = 0.018), baseline and 6 months (mean difference = −0.20 mm, p < 0.001), and 3 months and 6 months (mean difference = −0.10 mm, p = 0.008). In the test group, significant differences were found between baseline and 3 months (mean difference = −0.07 mm, p = 0.025), baseline and 6 months (mean difference = −0.35 mm, p < 0.001), and 3 months and 6 months (mean difference = −0.28 mm, p < 0.001).

Table 8: Intergroup comparison of Width of attached gingiva at baseline, three months and six months

Interval	Control		Test		z-value	p-value
	Mean	SD	Mean	SD		
Baseline	1.55	0.08	1.72	0.27	-1.938	0.062
3 months	2.48	0.31	2.73	0.66	-0.821	0.433
6 months	3.39	0.33	4.14	0.43	-4.134	<0.001*

Mann Whitney test; * indicates a significant difference at p≤0.05

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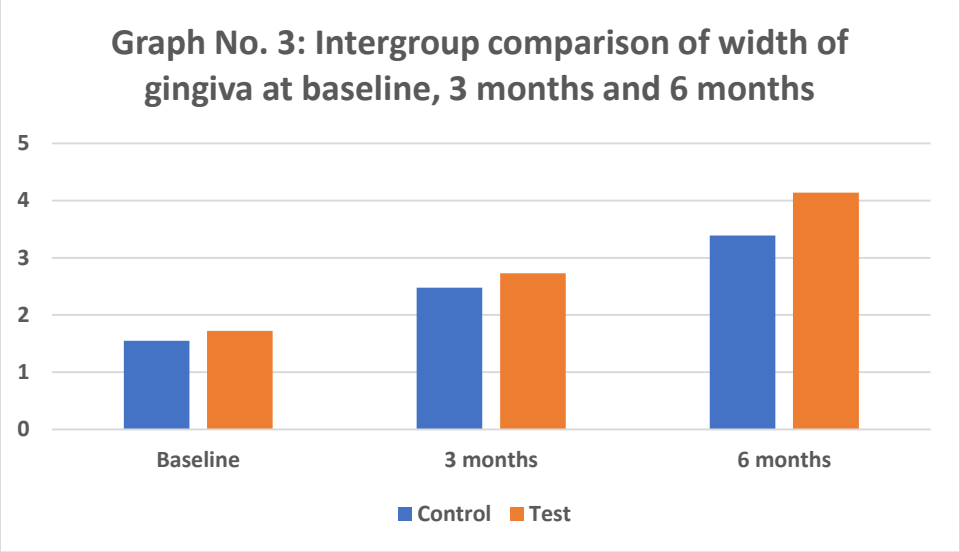
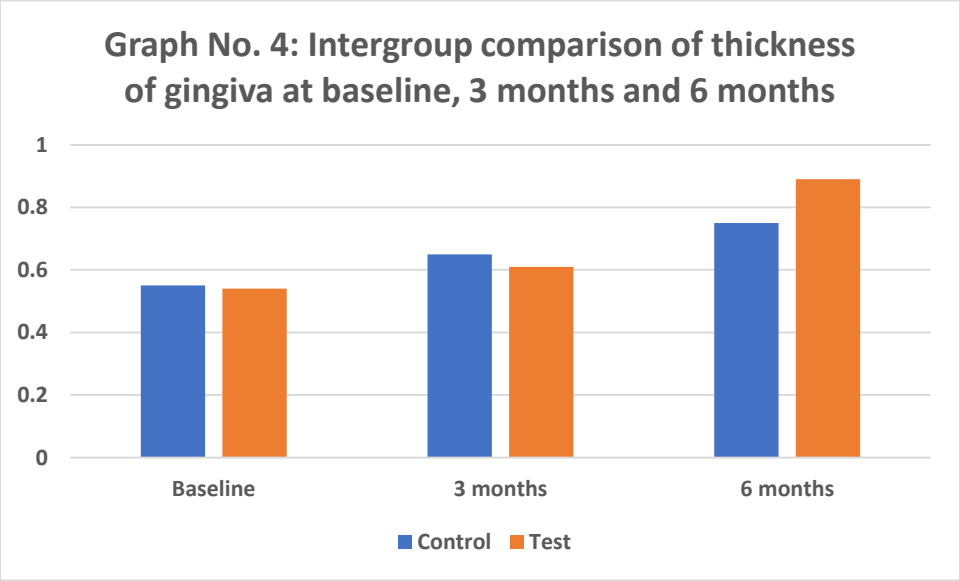


Intergroup comparison using the Mann–Whitney U test showed no statistically significant difference in the width of attached gingiva between the control and test groups at baseline (p = 0.062) and at three months with p value = 0.433 (Table No. 8 and Graph No. 3). At six months, however, the test group demonstrated a significantly greater width of attached gingiva compared to the control group (4.14 ± 0.43 mm vs. 3.39 ± 0.33 mm; p < 0.001).

Table 9: Intergroup comparison of thickness of gingiva at baseline, three months and six months

Interval	Control		Test		z-value	p-value
	Mean	SD	Mean	SD		
Baseline	0.55	0.03	0.54	0.04	-1.140	0.274
3 months	0.65	0.05	0.61	0.07	-1.667	0.106
6 months	0.75	0.05	0.89	0.05	-4.703	<0.001*

Mann Whitney test; * indicates a significant difference at p≤0.05



Mann–Whitney U test analysis showed no statistically significant intergroup differences in gingival thickness at baseline (p = 0.274) and at three months with p value = 0.106 (Table No. 9 and Graph No. 4). At six months, the

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test group exhibited a significantly higher gingival thickness than the control group (0.89 ± 0.05 mm vs. 0.75 ± 0.05 mm; $p < 0.001$).

Discussion

Thin gingival phenotype poses greater risk of gingival recession, poor wound healing and susceptibility to mechanical trauma.[2] Micro-needling stimulates the body's wound healing response by release of growth factors, fibroblast activation, and enhanced production of collagen and elastin.[11] This procedure, combined with Vitamin C presents a novel, minimally invasive alternative that leverages the body's natural healing processes to enhance collagen production and improve gingival tissue quality.[20] i-PRF is rich in platelets, leukocytes, insulin derived growth factors, transforming growth factors, platelet derived growth factors and vascular endothelial growth factors. It promotes regeneration process by neoangiogenesis and neocollagenesis.[17,18]

Chaudhary et al. (2023)[19] and Dawar et al. (2025)[20] have employed Vitamin C for gingival depigmentation without and with microneedling respectively. They found Vit. C efficient in reducing hyperpigmentation of gingiva. However, Dawar et al.[20] also found increased thickness of gingiva in the study subjects, though the main objective of their trial was to treat gingival hyperpigmentation. Furthermore, the authors did not use any standard materials and methods to evaluate gingival thickness.

Swarna et al. (2024)[21] also found promising results indicating that collaborative efforts of combining microneedling with Vitamin C portrayed improved wound healing and uniform depigmentation effect.

Our trial is focused to explore GT and AGW changes after application of i-Vit. C versus i-PRF post-microneedling in thin gingival phenotype.[2] Results of the cases portrayed increase in GT and AGW. The statistical analysis of the completed study has been done, title of which is approved by the Ethical committee of the institution (DCA/IEC/83/2025) and the project is registered with Clinical Trial Registry of India (CTRI/2025/05/087132); and as of now ours is the first trial to evaluate effects of Microneedling and i-Vit. C on thin gingival phenotype (gingival thickness and width of attached gingiva). We have received Copyright for this article from Copyright Office, Government of India; application No.: LD-31336/2025-CO. We have compared the results with already proven agent for increasing GT i.e. Microneedling and i-PRF. i-PRF is capable of increasing GT only and it involves an additional traumatic procedure of venipuncture.

Conclusion

Microneedling combined with i-Vit. C offers an innovative, minimally invasive approach to increase GT and AGW in individuals with thin gingival phenotype. Gingival inflammatory changes can be reversed to health preventing plaque retention, gingival recession and trauma. This technique offers a more patient friendly alternative to surgical interventions/venipuncture improving both periodontal health and esthetics.

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