

ENHANCING GINGIVAL AESTHETICS: MICRO-NEEDLING WITH TOPICAL VITAMIN C FOR DEPIGMENTATION -A IN VIVO CLINICAL STUDY

¹Dr. Pradnya Gaikwad, ^{2*}Dr. Yogesh Doshi, ³Dr. Mona Shah, ⁴Dr. Rasika Painter, ⁵Dr. Vishnu Maske

¹Postgraduate Student, Pandit Deendayal Upadhyay Dental College and Hospital, Kegaon, Solapur- 413002.

²Head of Department, Department of Periodontics and Oral Implantology, Pandit Deendayal Upadhyay Dental College and Hospital, Kegaon, Solapur- 413002.

³Professor, Department of Periodontics and Oral Implantology, Pandit Deendayal Upadhyay Dental College and Hospital, Kegaon, Solapur- 413002.

^{4,5}Reader, Department of Periodontics and Oral Implantology, Pandit Deendayal Upadhyay Dental College and Hospital, Kegaon, Solapur- 413002.



***Corresponding Author: Dr. Yogesh Doshi**

Head of Department, Department of Periodontics and Oral Implantology, Pandit Deendayal Upadhyay Dental College and Hospital, Kegaon, Solapur- 413002.

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ABSTRACT

Background: Aesthetics is vital in dental treatment, emphasizing cosmetic outcomes alongside functionality. Healthy gums are crucial for an appealing smile, but conditions like gingival melanin hyperpigmentation can negatively affect aesthetics, prompting interest in cosmetic procedures. While various depigmentation methods are available, they often have drawbacks such as pain and recurrence. Newer techniques like micro-needling and vitamin C mesotherapy show promise for reducing pigmentation and enhancing tissue quality, thereby improving patient satisfaction. The study aims to assess the effectiveness of micro-needling for treating gingival hyperpigmentation. **Materials and methods:** In a study involving 30 patients with gingival depigmentation, a pre-operative phase included thorough medical histories, clinical exams, periodontal treatment, and baseline measurements. Key clinical characteristics evaluated were gingival pigmentation, discomfort, and wound healing. Each patient underwent scaling and root planning, followed by a surgical procedure with micro-needling and ascorbic acid paste application. Follow-up assessments at baseline, 2, 4, and 12 weeks measured pigmentation, discomfort, wound healing, and included clinical photography. **Results:** Results showed a significant reduction in pigmentation scores over 12 weeks when analyzed using Friedman's test with statistically significant differences in DOPI and WHI across multiple time intervals, attributed to micro-needling enhancing vitamin C absorption and its anti-melanogenic properties. Postoperative discomfort was minimal, and most patients reported high satisfaction with the results. **Conclusion:** The study highlights that the positive results can be linked to the combined effects of micro-needling and topical vitamin C, positioning this treatment as a minimally invasive, cost-effective, safe, and clinically effective option for gingival depigmentation.

KEYWORDS: Gingival aesthetics, Micro-needling, Depigmentation, Vitamin C, Gingival wound healing.

INTRODUCTION

In Dentistry, Aesthetics of teeth as well as gingiva is prime concern while considering the treatment. Now a days focus has been shifted towards aesthetics rather than functional rehabilitation.^[1] A pleasing smile is determined by the alignment, morphology, and color of teeth along with the harmony of the surrounding gingival

tissues. Gingival appearance, health and consistency considered as essential component of attractive smile.^[2] Gingival colour is termed as Coral pink and aesthetics in regarding this termed as Pink aesthetics.^[3] Apart from this feature change in colour of gingiva is caused by many factors amongst which most common is gingival melanin hyperpigmentation that raise aesthetic concern.

Clinically, the disorder appears as widespread or patchy brown, dark brown, or bluish-black gingival discoloration caused by excessive melanin deposition. While it is usually physiological and doesn't suggest a medical problem, it can be unappealing, especially in people with a high smile line or considerable gingival display.^[4]

Melanocytes create melanin, which is then deposited in the gingival epithelium to provide gingival coloration. Tyrosine is the starting point of the process, which is called melanogenesis. Tyrosinase catalyzes a number of metabolic stages. Genetics, skin tone, hormone levels, and lifestyle choices all have an impact on pigmentation.^[5,6] Although there are methods to lessen pigmentation, such as scalpel surgery, bur abrasion, electrosurgery, cryosurgery, laser depigmentation, and chemical cauterization, they frequently encounter problems like recurrence because of melanocyte migration.^[7] A significant limitation of these approaches is the repigmentation of gingival tissues following treatment. Repigmentation occurs when active melanocytes migrate from untreated areas to treated areas and leftover melanocytes reactivate within the tissue.^[8]

Emerging less invasive treatments, such as micro-needling, disturb those melanocytes while encouraging healing and regeneration. Micro-needling involves creating controlled micro-injuries in tissues using fine needles to stimulate wound healing, collagen synthesis, and tissue remodeling. Dermapen devices utilize fine needles within cartridges to penetrate tissues at controlled depths and speeds, particularly for gingival procedures where the depth ranges from 0.5 mm to 1.5 mm based on the gingiva's thickness and biotype. This targeted penetration injures the basal epithelial layer that houses melanocytes, resulting in pinpoint bleeding. The procedure triggers the release of crucial growth factors, such as platelet-derived growth factor and transforming growth factor-beta, which collectively facilitate tissue regeneration.^[9] It has been widely utilized for its simplicity, cost-effectiveness, good tolerance, and ability to provide both cosmetic and therapeutic benefits.^[10]

Furthermore, Vitamin C also known as ascorbic acid, it is known for its potent antioxidant properties and noteworthy anti-melanogenic effects. It works by inhibiting the enzyme tyrosinase, which is essential in the melanin synthesis pathway, and thereby reducing melanin production by preventing the enzymatic conversion of tyrosine into melanin. Furthermore, vitamin C promotes collagen formation, which is essential for skin health, and speeds up wound healing. When administered to gingival tissues, vitamin C reduces pigmentation and improves tissue quality, making it useful for both dermatological and dental health purposes.^[6,11] Synergistic action of micro-needling and Vitamin C enhances the effectiveness of treatment by deeper penetration of agent through micro-channels that

allows vitamin c to reach basal epithelial layer and melanocytes that explains superior depigmenting effect and reduced reoccurrence.^[12,13]

Despite these advancements reoccurrence of pigmentation is a great challenge. Therefore, there is a need for a minimally invasive, effective, and patient-friendly treatment modality that can provide predictable aesthetic outcomes with minimal recurrence. so, the aim of study is to evaluate effectiveness of micro-needling technique using Derma-pen with topical ascorbic acid paste on gingival depigmentation.

METHODOLOGY

The primary goal of study was to evaluate the effectiveness of micro-needling for treating physiologic gingival hyperpigmentation along with measurement of pain experienced during and after treatment sessions, as well as assessing the healing rate and recovery time after ascorbic therapy. In-vivo clinical Study was conducted in department of Periodontology and Oral Implantology at the institution after obtaining approval from the Ethical Committee.

Inclusion Criteria

- Patients aged 18–40 years.
- Presence of physiologic gingival hyperpigmentation.
- Dummett–Gupta Oral Pigmentation Index (DOPI) score ≥ 1 .
- High smile line.
- Good general health.

Exclusion Criteria

- Smokers.
- Patients with a low smile line.
- Presence of periodontal disease.
- Pregnant or lactating women.
- Patients with systemic diseases, active systemic infections, or any medical condition that could affect wound healing or the treatment outcome.
- Patients with scarring history

Total 30 Patients were selected based on the criteria's and clinical examination along with proper dental/medical history using convenient sampling technique. all the patients were undergone with the phase 1 therapy that is scaling and root planning, followed by oral hygiene instructions. Various clinical parameters assessed Gingival pigmentation using Dummett-Gupta Oral Pigmentation Index - DOPI,^[14] Pain using Visual Analog Scale (VAS),^[15] wound healing using Wound Healing Index (WHI)^[16] and Huang LH index (2005)^[16] at baseline(before treatment) and various time intervals (after the treatment) along with clinical photographs.

After recording, baseline measurements [Figure 1] and completion of phase 1 therapy, surgical procedure was carried out. The treatment area underwent cleaning with

an antiseptic solution, followed by the administration of local anesthesia using 2% lignocaine with adrenaline through an infiltration technique. Micro-needling was conducted [Figure 2] with the Dermapen M8 device, equipped with a sterile cartridge of 12 micro-needles, adjusting the needle penetration depth between 0.5 mm and 1.5 mm based on gingival thickness. The device was operated at speed mode 6, applying it in a sweeping motion across pigmented gingival areas for around 30–40 seconds per tooth, continuing until pinpoint bleeding was noted. The mucosa was then irrigated with sterile saline and gently dried with sterile gauze. Then Ascorbic

acid powder (1000 mg/ml) was combined with normal saline to create a paste-like mixture, which was then applied to the micro-needled gingival surface [Figure 3]. The treated area was left uncovered, without a periodontal dressing. The micro-needling procedure was repeated after two weeks as part of the treatment protocol. Patients were showered with post operative instructions and to record clinical parameters immediately after treatment [Figure 4], at two weeks, four weeks and 12 weeks [Figure 5]. All this data were collected and subjected for analysis.



Fig. 1: Preoperative intraoral view showing physiologic gingival hyperpigmentation at baseline.



Fig. 2: Micro needling procedure performed using Dermapen over pigmented gingival tissues



Fig. 3: Topical application of vitamin C following micro-needling procedure.



Fig. 4: Immediate postoperative clinical appearance following micro needling-assisted gingival depigmentation.



Fig. 5: Clinical view at 2 weeks, 4 weeks and 12 weeks postoperatively showing initial reduction in gingival pigmentation.

STATISTICAL ANALYSIS

All participants completed a treatment protocol consisting of micro-needling, achieving a 100% completion rate. Baseline assessments revealed that 73.33% showed Grade 3 pigmentation and 26.67% Grade 2. Statistical analysis utilized SPSS, with normality checks for clinical data, Friedman's test for intra-group comparisons, Tukey's post-hoc test for specific time points, and Wilcoxon Signed Rank test for pain analysis. A significance level of $p < 0.05$ was set for statistical relevance.

RESULTS

The study evaluated the effectiveness of micro-needling combined with topical ascorbic acid for reducing pigmentation, achieving high statistical significance ($p < 0.001$). The mean DOPI (Degree of Oral Pigmentation Index) score started at 2.7333 ± 0.44978 at baseline. Significant reductions were observed at all follow-up intervals: 1 week (1.7333 ± 0.44978), 4 weeks (0.7333 ± 0.52083), and at the 12-week mark (0.1667 ± 0.37905), indicating a consistent decline in pigmentation. [Table 1]

Friedman's test confirmed a significant difference in DOPI scores over time ($F = 187.463$, $p = 0.000$), with Tukey's post-hoc analysis validating the significance of reductions between each time interval (Baseline to 1 week, 1 week to 4 weeks, and 4 weeks to 12 weeks) with $p = 0.000$. [Table 2 and Graph 1]. Ultimately, 83.33% of participants achieved complete clinical depigmentation (Grade 0 status of pink gingiva) by the 12-week follow-up.

Pain perception was assessed immediately after a procedure and again one week later using a 100-cm Visual Analog Scale (VAS). The mean VAS score at baseline was 1.6333 ± 0.61495 , indicating slight pain. By the one-week follow-up, the mean pain score significantly decreased to 0.4000 ± 0.49827 . Statistical analysis via the Wilcoxon Signed Rank test revealed a

mean difference of 1.23333, which was highly significant ($t = 13.403$, $p = 0.000$). [Table 3 and Graph 2]. These findings suggest that given treatment is a minimally invasive technique associated with low patient morbidity, with most subjects reporting no pain after one week.

Gingival wound healing was assessed over a 12-week period using the Wound Healing Index (WHI) at intervals of 2, 4, and 12 weeks. The observed healing scores indicated a progressive improvement in tissue recovery, with mean WHI scores of 1.8333 ± 0.46113 at 2 weeks, 2.5667 ± 0.50401 at 4 weeks, and 2.8667 ± 0.34575 at 12 weeks using Friedman's test. [Table 4 and Graph 3] Analysis demonstrated a highly significant improvement in healing quality ($F = 43.386$, $p = 0.000$) across these intervals. Further analysis via Tukey's post-hoc test revealed highly significant differences between the scores at 2 weeks and both 4 weeks ($p = 0.000$) and 12 weeks ($p = 0.000$). [Table 5]. Although the transition from 4 weeks to 12 weeks showed statistical significance as well ($p = 0.027$), suggesting initial healing occurs early while tissue maturation persists throughout the entire three-month evaluation period.

Throughout a 12-week study involving 30 participants, micro-needling combined with topical ascorbic acid demonstrated safety and efficacy in enhancing gingival aesthetics. Healing was uneventful, with no significant postoperative complications reported. Clinical photographs showed a transition from deep-brown pigmentation to a healthy, uniform pink gingival color by the final follow-up.

TABLES

Table 1: Difference in DOPI at different time intervals using Friedman's test.

Time interval	Mean	Standard Deviation	95% Confidence Interval for the Mean		F	p
			Lower	Upper		
Baseline	2.7333	.44978	2.5654	2.9013	187.463	.000 (HS)
2 Week	1.7333	.44978	1.5654	1.9013		
4 Weeks	.7333	.52083	.5389	.9278		
12 Weeks	.1667	.37905	.0251	.3082		

Table 2: Multiple comparison for table 2a using Tukey's post-hoc test.

Groups		Mean Difference	p	95% Confidence Interval for Mean Difference	
				Lower	Upper
Baseline	2 Week	1.00000*	.000 (HS)	.6954	1.3046
	4 Weeks	2.00000*	.000 (HS)	1.6954	2.3046
	12 Weeks	2.56667*	.000 (HS)	2.2620	2.8713
2 Week	Baseline	-1.00000*	.000 (HS)	-1.3046	-.6954
	4 Weeks	1.00000*	.000 (HS)	.6954	1.3046
	12 Weeks	1.56667*	.000 (HS)	1.2620	1.8713
4 Weeks	Baseline	-2.00000*	.000 (HS)	-2.3046	-1.6954
	2 Week	-1.00000*	.000 (HS)	-1.3046	-.6954
	12 Weeks	.56667*	.000 (HS)	.2620	.8713
12 Weeks	Baseline	-2.56667*	.000 (HS)	-2.8713	-2.2620
	2 Week	-1.56667*	.000 (HS)	-1.8713	-1.2620
	4 Weeks	-.56667*	.000 (HS)	-.8713	-.2620

Tukey's post-hoc test shows that there was highly significant difference in DOPI between each time interval.

Table 3: Difference in pain perception at different time intervals between using Wilcoxon Signed Rank test.

Time interval	Mean	Standard Deviation	Mean Difference	95% Confidence Interval for the Mean Difference		t	p
				Lower	Upper		
Baseline	1.6333	.61495	1.23333	1.04513	1.42153	13.403	.000 (HS)
1 Week	.4000	.49827					

HS = Highly Significant (p<0.001)

Table 4: Difference in WHI at different time intervals using Friedman's test.

Time interval	Mean	Standard Deviation	95% Confidence Interval for the Mean		F	p
			Lower	Upper		
2 Weeks	1.8333	.46113	1.6611	2.0055	43.386	.000 (HS)
4 Weeks	2.5667	.50401	2.3785	2.7549		
12 Weeks	2.8667	.34575	2.7376	2.9958		

HS = Highly Significant (p<0.001)

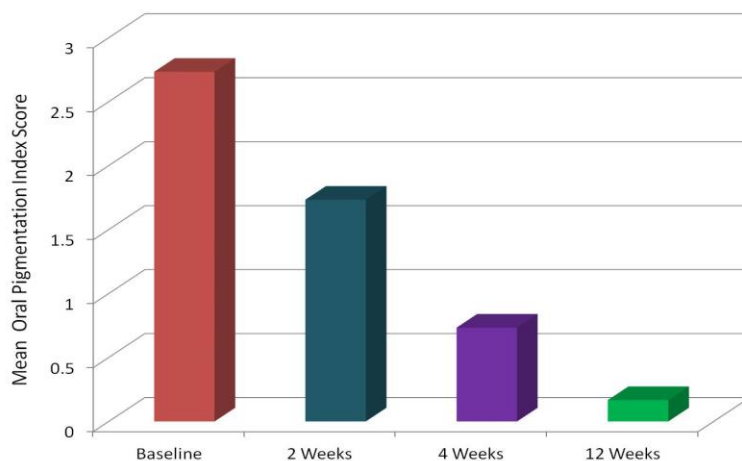
Table 5: Multiple comparison for table 4a using Tukey's post-hoc test.

Groups		Mean Difference	p	95% Confidence Interval for Mean Difference	
				Lower	Upper
2 Week	4 Week	-.73333*	.000 (HS)	-1.0055	-.4612
	12 Weeks	-1.03333*	.000 (HS)	-1.3055	-.7612
4 Week	2 Weeks	.73333*	.000 (HS)	.4612	1.0055
	12 Weeks	-.30000*	.027 (S)	-.5722	-.0278
12 Weeks	2 Weeks	1.03333*	.000 (HS)	.7612	1.3055
	4 Weeks	.30000*	.027 (S)	.0278	.5722

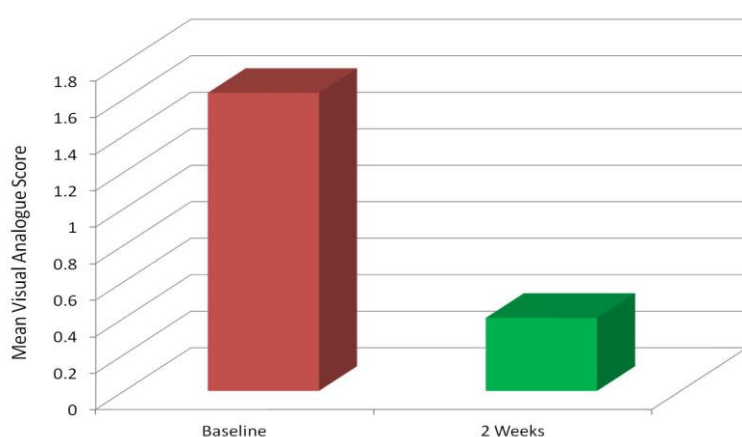
S = Significant (p<0.05)

Tukey's post-hoc test shows that there was highly significant difference in WHI between each time interval.

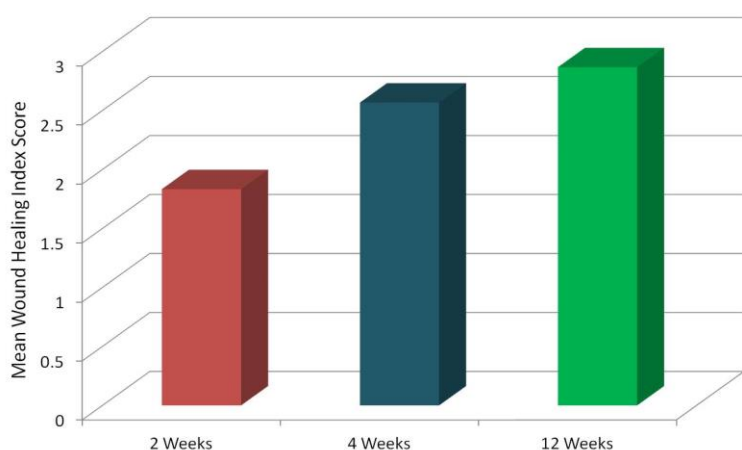
GRAPHS



Graph 1: Difference in DOPI at different time intervals between Baseline, 2 Weeks, 4 Weeks and 12 Weeks using Friedman's test.



Graph 2: Difference in Pain Perception at different time intervals between Baseline and 2 Weeks using Wilcoxon Signed Rank test.



Graph 3: Difference in Wound Healing Index (WHI) at different time intervals between 2 Weeks, 4 Weeks and 12 Weeks using Friedman's test.

DISCUSSION

This In- vivo prospective clinical study evaluated the effectiveness of micro-needling with Dermapen combined with topical vitamin C for managing physiologic gingival hyperpigmentation. Gingival

architecture and color are crucial for smile attractiveness, as highlighted by Blatz et al. (2019).^[17] Although physiologic, gingival hyperpigmentation often leads patients to seek cosmetic correction for improved confidence and social acceptance.^[4] Melanin

pigmentation results from increased synthesis in the epithelial layers, influenced by factors like race, genetics, and environmental stimuli. This pigmentation is genetically based, particularly in darker-skinned individuals, underscoring its importance in periodontal aesthetics.^[8]

For removal of this pigmentation various treatment options evolved till this date including scalpel surgery, bur abrasion, electrosurgery, cryosurgery, gingival grafting, chemical cauterization, and laser therapy.^[5] All these treatment modalities significantly lowered pigmentation but poses limitations and disadvantages such as intra-operative bleeding, Post operative pain, delayed healing, patient discomfort and requirement of periodontal dressing.^[7] Amongst which laser offered improved precision, excellent hemostasis, and minimal bleeding, the high costs, need for technical expertise, and sophisticated equipment limit the routine use of these techniques in periodontal practice.^[5,18] One of the challenging part of treating gingival depigmentation is its reoccurrence. Repigmentation is thought to result from the migration of active melanocytes from untreated areas or reactivation within the treated site. Alasmari (2018) reviewed depigmentation techniques, noting recurrence periods of 6 months to 3 years based on treatment methods and patient factors. This has led to increased interest in minimally invasive procedures that not only eliminate pigmentation but also suppress melanocytic activity and reduce recurrence i.e., microneedling.^[5] Micro-needling, or collagen induction therapy, is a minimally invasive dermatological procedure that creates controlled micro-injuries using fine needles. This technique stimulates wound healing, collagen synthesis, neovascularization, and tissue remodelling.^[19]

Micro-needling is an effective, minimally invasive regenerative procedure that boosts collagen production while maintaining epithelial integrity. McCrudden et al. (2015) showed that micro-needling enhances trans-epithelial drug delivery by forming micro-channels, allowing deeper therapeutic penetration.^[20]

This study utilized micro-needling to improve topical vitamin C delivery into gingival tissues. Vitamin C mesotherapy is gaining recognition in dermatology for managing hyperpigmentation due to its anti-melanogenic, antioxidant, and collagen-stimulating properties. Sanadi and Deshmukh (2020) highlighted vitamin C's effectiveness in reducing melanin pigmentation by inhibiting melanogenesis and promoting tissue repair.^[6] Vitamin C reduces tissue pigmentation by converting oxidized melanin into lighter forms and neutralizing reactive oxygen species, thus decreasing melanocyte stimulation. It also enhances collagen biosynthesis, leading to better wound healing and tissue quality. Studies indicate significant reduction in gingival melanin pigmentation following ascorbic acid application, reaffirming its value in periodontal esthetics.^[9] However, its hydrophilic nature limits

penetration through epithelial barriers, prompting the development of mesotherapeutic delivery systems.^[21] These systems enhance diffusion of vitamin C into melanocyte-rich layers via micro-needling, resulting in clinical improvements in pigmentation while being minimally invasive. Mostafa et al. (2023)^[12] further demonstrated significant reduction in gingival pigmentation scores following micro needling-assisted vitamin C therapy, thereby supporting the effectiveness of this minimally invasive approach.

In this study, gingival pigmentation was evaluated using the Dummett Oral Pigmentation Index (DOPI), revealing a statistically significant reduction in DOPI scores over time. Mean DOPI scores decreased at 2, 4, and 12 weeks, indicating a sustained reduction in pigmentation. These results align with previous research by Mostafa et al. (2023), and Mansuri et al. (2025), which also noted significant decreases in pigmentation following vitamin C-assisted depigmentation procedures.^[12,13] The study indicates that a reduction in pigmentation is due to the combined effects of micro-needling and topical vitamin C mesotherapy. Micro-needling creates micro-channels in the gingival epithelium, allowing deeper penetration of vitamin C to target melanocytes. The depth of needle penetration varied from 0.5 mm to 1.5 mm, influenced by tissue thickness and pigmentation severity. This method is more effective than standard topical application in delivering vitamin C to melanocyte-rich areas. The vitamin's anti-melanogenic properties inhibit tyrosinase activity and melanin production, leading to depigmentation. Repeated sessions may further enhance tissue remodeling and aesthetic results.

Pain perception was assessed using the Visual Analog Scale (VAS), with results indicating mild postoperative discomfort that decreased significantly over time. Micro-needling emerged as a minimally invasive procedure compared to conventional methods, which often involve substantial postoperative pain and trauma. The micro-needling technique induces controlled micro-injuries, promoting patient comfort and minimal tissue damage. The study reported favorable wound healing outcomes, attributed to the stimulation of growth factors such as PDGF, TGF- β , and FGF, enhancing angiogenesis and collagen synthesis. Additionally, Vitamin C aided in collagen maturation, resulting in improved gingival texture and tissue quality during follow-ups.

Patient satisfaction is crucial in esthetic procedures, significantly affecting treatment success. In this study, most patients reported high satisfaction due to minimal invasiveness, reduced discomfort, favorable healing, and improved gingival appearance.^[5,12] Similar high acceptance was noted in other studies on vitamin C mesotherapy and minimally invasive procedures. Minimal pigmentation recurrence was observed during a 12-week follow-up, with results surpassing conventional techniques like scalpel surgery, which often see recurrence within 6 to 18 months. This success may be

due to the removal of pigmented cells and the suppressive effects of vitamin C on melanocytic activity, aided by its antioxidant and anti-inflammatory properties.^[5,7,8]

An important observation in the study noted that depigmentation in the interdental papillary regions was sometimes ineffective with Dermapen micro-needling due to inadequate penetration in narrow spaces. Consequently, manual micro-needling using a lancet needle improved results by enhancing precision and accessibility. This approach facilitated better vitamin C delivery and uniform depigmentation, suggesting that manual techniques may be beneficial when automated methods prove insufficient, as highlighted by Dawar *et al.* (2022).^[22] Micro-needling therapy showed improved gingival tissue quality and thickness due to collagen induction and tissue remodeling from controlled micro-trauma. Bhatsange and Kulkarni (2025)^[23] reported significant enhancements in gingival phenotype and thickness, which may enhance tissue resilience and periodontal esthetics.

However, the study's short follow-up period limits the evaluation of long-term outcomes like recurrence and stability of depigmentation. Further longitudinal studies with larger populations and extended follow-up are suggested to confirm the long-term efficacy and predictability of micro needling-assisted vitamin C mesotherapy for gingival depigmentation.

CONCLUSION

Current study investigated the use of micro-needling with a Dermapen, combined with topical vitamin C, to treat physiological gingival hyperpigmentation. The approach used resulted in a significant decrease of the pigmentation after 12 weeks with minimal postoperative pain and a satisfactory wound healing leading to a high patient satisfaction and a low recurrence rate at the follow-up. The best clinical results were achieved with the synergistic effect of micro-needling and vitamin C penetration. In hard to reach areas the residual in interdental papillary areas was treated with a sterile diabetic lancet needle for aesthetic reasons. This technique is proposed as a simple, inexpensive and less painful alternative to the existing techniques of gingival depigmentation.

Limitations of the study

The results are limited by the small sample size and limited follow-up duration, which calls for more randomized controlled trials to confirm the long-term efficacy, stability and clinical utility of this treatment approach.

Future Scope

Future research will encompass the use of several additional infusing solutions or gels with a stronger anti-melanogenic impact and less post-operative patient discomfort. One can also utilize a controlled and

stabilized diffusion instrument or apply formulations with clinical trials over a longer length of time.

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