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EVALUATION OF MICRONEEDLING AS A TREATMENT OPTION FOR GINGIVAL HYPERPIGMENTATION: A RANDOMIZED CONTROLLED CLINICAL TRIAL

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ABSTRACT

Background: In individuals who have an excessive gingival display, gingival hyperpigmentation is an aesthetic issue. The goal of this study was to examine the effectiveness of the minimally invasive microneedling (MN) approach in the treatment of gingival hyperpigmentation.

Patients and methods: A total of 40 people with gingival hyperpigmentation were split into two groups at random**.** Group I: was managed with the surgical scalpel method, whereas group II was managed with MN approach. At the time of the procedures, the bleeding was compared. On the first and seventh days after the depigmentation procedures, wound healing and pain severity were assessed while at 1 month and 6 months following the treatments, the oral pigmentation indices were assessed.

Results: When compared to baseline data, both groups demonstrated a substantial reduction in oral pigmentation indices at 1 and 6 months, while group I demonstrated a statistically significant ($P \leq 0.05$) improvement compared to group II. There was a significant decrease ($P \leq 0.001$) in the bleeding index and pain severity and improvement in wound healing in MN treated group compared to the surgically-treated group.

Conclusion: MN successfully reduces gingival hyperpigmentation and might be regarded as an alternative minimally invasive treatment option.

KEYWORDS: Hyperpigmentation, microneedling, surgical treatment

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INTRODUCTION

The number and melanogenic activity of melanocytes, the thickness of the keratinized epithelium, and the degree of vascularization all influence the color of the oral mucosa, which differs from person to person. One of the gingival features that affect soft tissue aesthetics and the overall appearance of a smile is the gingival color. [1-3]

The most common natural pigment that contributes to the endogenous pigmentation of the gingiva is melanin, a brown pigment.[4] Melanin is synthesized in the stratum basal by melanocytes. Between 30 and 40 keratinocytes can be pigmented by a single melanocyte. Melanocytes cytologically transmit melanosomes (melanin transporters) into keratinocytes in the prickle and basal cell layers through dendritic tentacles. The melanosome is 'injected' into the keratinocyte through the constricted opening of the melanocyte dendrite.[5]

Treatment options for gingival hyperpigmentation may be divided into two headings: techniques aimed at eliminating pigments and others that hide the pigments. Pigment elimination can be accomplished surgically, non-surgically, or chemically.[6,7] Scalpel surgery, bur abrasion, laser ablation, cryosurgery, electrocautery, and radiosurgery are some of the most common surgical techniques. Chemical cauterization is the most common nonsurgical approach. While the use of free gingival autograft operations and the acellular dermal matrix allograft are examples of ways of masking gingival colors. All of these therapy options have their own set of benefits and drawbacks. [8,9,10,11,12]

Microneedling (MN) also recognized as "percutaneous collagen induction therapy" has indeed been described as a successful treatment approach for a wide range of dermatological disorders, including rejuvenation of the skin and medication delivery after the MN.[13,14] Furthermore, MN has lately earned a lot of interest for its usage in the treatment of pigmentations like melasma and periorbital hypermelanosis.[15-17] MN is a technique that necessitates puncturing the skin with sterilized microneedles on a regular basis. Physical trauma is the theoretical basis of MN, the needles pierce the skin and generate very tiny pores known as micro-conduits, these micro-injuries cause minor surface bleeding and initiation of a wound-healing pathway that includes inflammation, proliferation, and remodeling.[18]

Based on the concept of using microneedling in the treatment of hyperpigmented skin. This research is a clinical trial aimed to investigate the effectiveness of microneedling in the treatment of gingival hyperpigmentation

MATERIALS AND METHODS

The research was carried out with the approval of the Suez Canal University, Faculty of Dentistry's Ethical Committee. The current study followed the World Medical Association's ethical principles for investigations involving human participants (Declaration of Helsinki, 1978, as updated in 2008). Before taking part in the study, all individuals completed an informed comprehensive consent form in which the advantages, stages, and adverse effects of the treatment regimen were completely outlined. The current clinical study comprised healthy individuals whose primary issue was to treat the dark-brown to black gingival hyperpigmentation.

Sample size calculation

For sample size estimation, the effect size was 0.65 using an alpha (α) level of 0.05 and a beta (β) level of 0.05, i.e., power = 80%; the minimum sample size (n) estimation was a total of 40 samples for 2 groups.

Screening procedures

To evaluate patients' eligibility for the research, an initial evaluation was performed, which included a medical and dental history and clinical and radiographic examination. Forty participants were chosen for the study who fit the following criteria: 1)

were above the age of 18; 2) were systemically free, and 3) had physiologic gingival hyperpigmentation in the aesthetic zone. All factors that may cause an inflammatory response were ruled out, including 1) systemic disorders (especially auto-immune diseases or bleeding disorders); 2) patients under chemotherapy; 3) pregnant and breastfeeding women; 4) smokers, and 5) periodontal diseases (plaque and non-plaque induced gingivitis or periodontitis).

Patients grouping

The participants were randomized into two groups (20 patients each). Group 1 (control group) consists of 20 patients and the depigmentation procedure was accomplished by conventional scalpel surgery and group 2 (test group) consisted of 20 patients and the depigmentation was performed by MN technique. A randomization table with the use of a computer-generated randomization list (SPSS v23.0; IBM corp., Armonk, NY, USA) was used to conduct the randomization with an allocation ratio of 1:1.

Clinical Assessments

Patients were examined and diagnosed, and their pigmentation was evaluated using two indices**:** the first index was the Dummett oral pigmentation index (DOPI)[19] for the intensity of pigmentation which was graded as follows: as 0 = gingiva is in pink color; 1 = mild light brown gingival color; 2 = medium brown gingival color or mixed brown and pink color; or 3 = deep brown/ blue-black gingival color. Each gingival unit in both maxilla and mandible consisted of an interdental papilla with half of the marginal gingiva on either side of it and the accompanying attached gingiva was measured. The second index is the Hedin melanin index [20] for distribution of the pigmentation in the gingiva. It was scored as 0 = no gingival pigmentation; 1 = one or two solitary units of interdental papillae are pigmented; 2 = more than 3 separate units of interdental papillae are pigmented; 3 = 1 short continuous band of pigmented gingiva; or 4 = one wideband continuous pigmented gingiva between the canines. The total scores of (DOPI & Hedin index) of the mandible and maxilla were utilized in the study, which were obtained at baseline, one month, and six months after the depigmentation procedures.

The severity of bleeding during procedures, pain, and gingival wound healing was all assessed. The severity of bleeding during the procedures was recorded based on a four scale index [21] as follows: 0= no bleeding, 1= mild bleeding, 2= moderate bleeding, 3= severe bleeding. While on the first and seventh postoperative days, the pain was measured using a Visual Analogue Scale (VAS) [22] of 1–10. Gingival wound healing was estimated and scored [23] as follows: 0 = presence of necrotic gingival tissue, 1 = presence of ulcer in the gingiva, 2 = incomplete gingival epithelialization, and 3 = complete gingival epithelialization, total scores of mandible and maxilla were used for analysis, measured on 1st & 7 th day postoperatively.

Depigmentation procedures

Scalpel procedure

Infiltration local anesthetic (2 percent Lidocaine with Adrenaline 1:2,00,000) was used in the proximity of the operative site. Using Bard Parker blades number 15, the gingival epithelium of the pigmented area was extirpated extending from right to left first premolar and from the marginal gingiva to the mucogingival junction, placing the blade approximately parallel to the teeth's long axis where the epithelium was completely removed in one piece keeping the underlying bone from being bare. The exposed connective tissue surface was then carefully examined, and any residual tissue tags were excised using surgical scissors. A pressure pack was applied to control the bleeding, and after hemostasis was attained, the periodontal dressing was applied over the wound for a week.

Microneedling procedure

For the MN procedure, local infiltration anesthesia was administered before the procedure and Dermapen (Dr. pen ™ Auto Microneedle System, ULTIMA-A1, CHINA) was used and supplied with disposable microneedle cartridges. The depth of penetration of the microneedle can be adjusted from the device that has penetration depth (0.2 mm, 0.5 mm, 0.75mm, 1 mm, 2 mm, 3 mm) according to the gingival thickness.

The gingival tissue thickness was measured starting at 1.5 mm apical to the marginal gingiva using an endodontic spreader number 15 with a rubber stopper that was inserted perpendicularly into the soft tissues until a hard surface was felt and the rubber stopper rest in close contact with the gingival surface. A digital calliper was used to measure the length between the silicone disc and the spreader tip.[24] According to the measured gingival thickness, the penetration depth of microneedles was adjusted. Dermapen was laid perpendicular to the gingival surface and the MN was carried out in horizontal, vertical, and diagonal directions about four to five times for the whole hyperpigmented gingival surface until mild micro bleeding and mild erythema was clearly visible.[25] Individuals received a total of four sessions of the MN treatment, spaced out by 14 days

Post operative instructions:

Participants in the surgical depigmentation group were asked not to use mechanical mouth hygiene for the first week following surgery to minimize mechanical damage to the treated areas. Patients were given an anti-inflammatory mouthwash (Orovex- H mouth wash, Marco pharmaceutics, Egypt) and analgesic (Brufen 400 mg tablets) to use as needed. While in MN group, Patients were instructed to refrain from mechanical oral hygiene practices in regard to the target region for the day of the operation following each visit. If discomfort or itching was reported on the first day, analgesics were prescribed.

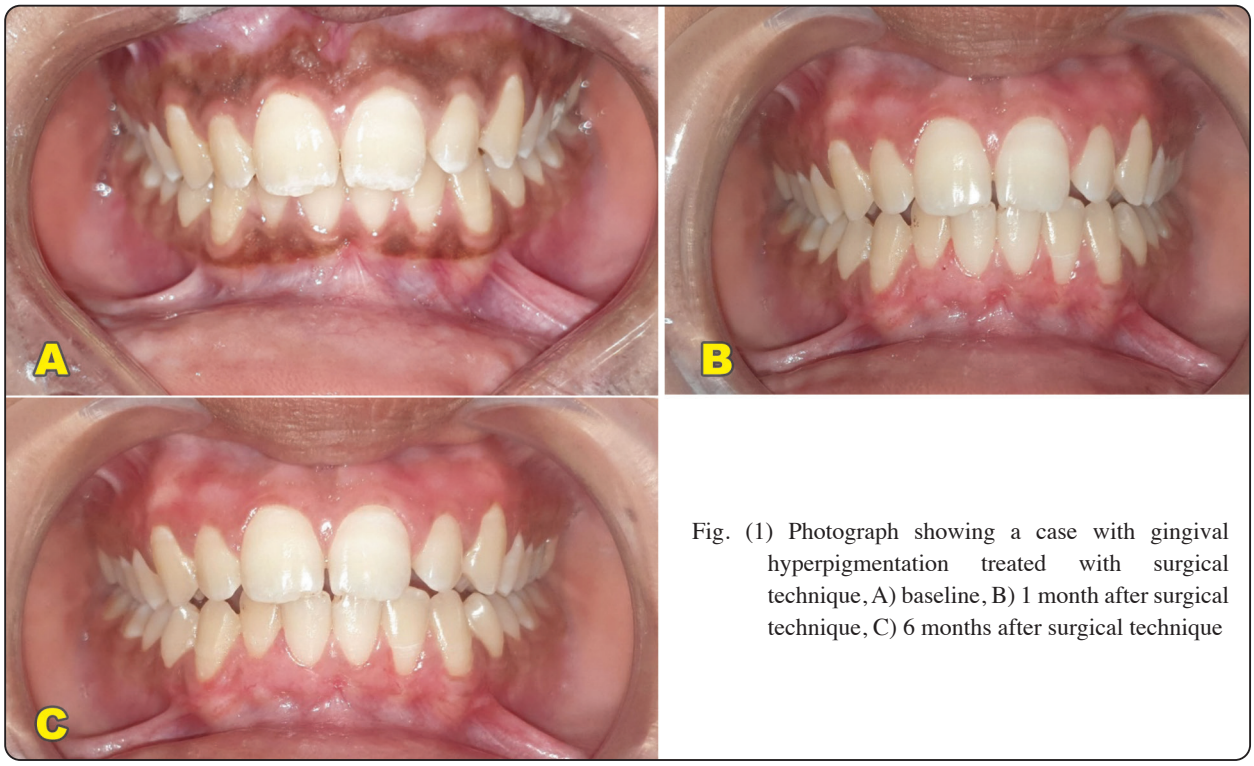

Fig. (1) Photograph showing a case with gingival hyperpigmentation treated with surgical technique, A) baseline, B) 1 month after surgical technique, C) 6 months after surgical technique

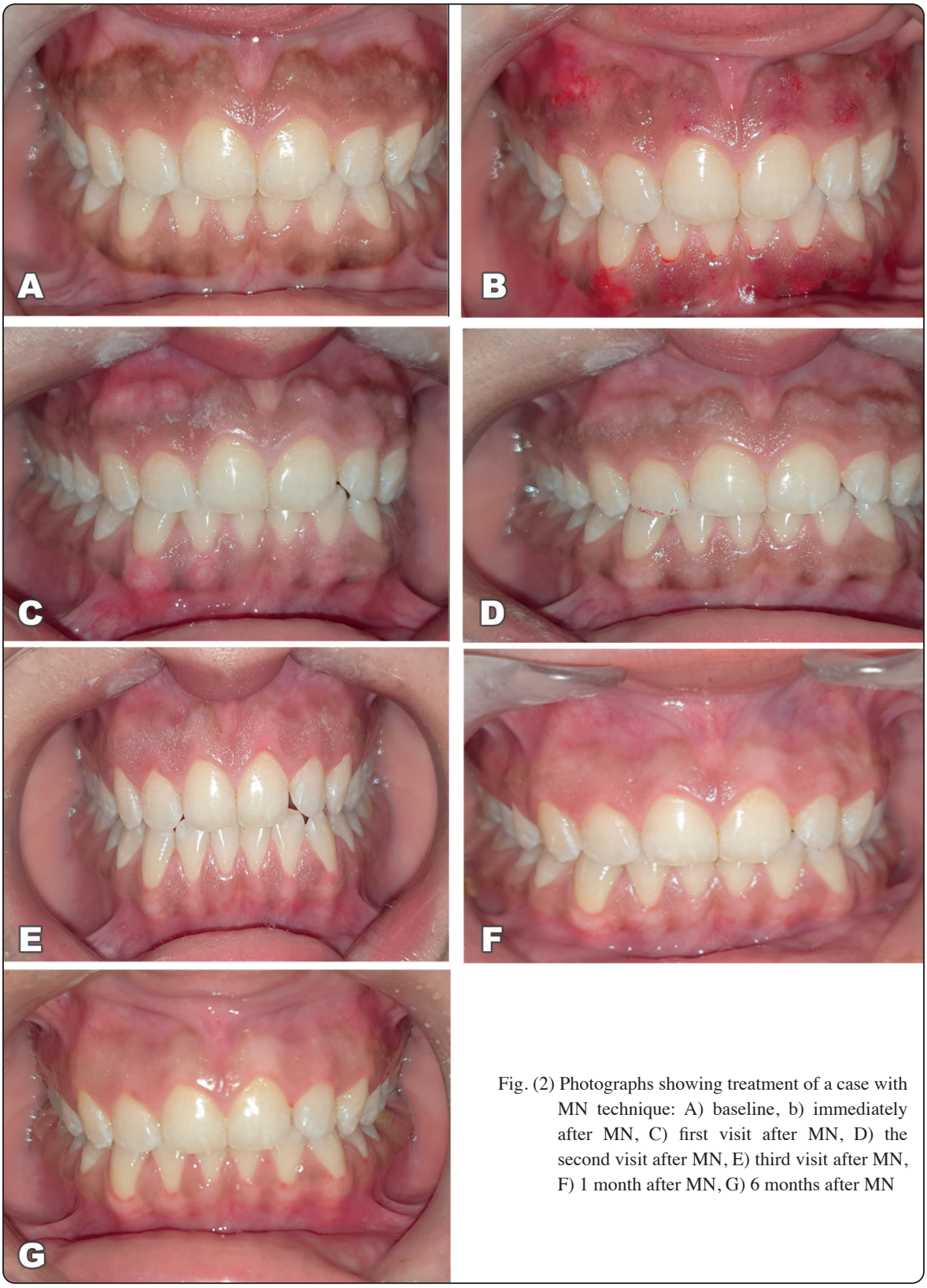

Fig. (2) Photographs showing treatment of a case with MN technique: A) baseline, b) immediately after MN, C) first visit after MN, D) the second visit after MN, E) third visit after MN, F) 1 month after MN, G) 6 months after MN

Statistical analysis

Mean and standard deviations of the score were computed. For Bleeding, VAS and wound healing an unpaired t-test was used to estimate the difference between groups and paired t-test was used to estimate the difference between time intervals in the same group. For DOPI and Hedin indices, One- way ANOVA was used for the time intervals comparison (baseline, 1st month and 6th months) followed by Tukey's post hoc test for pairwise comparisons. A $P < 0.05$ was supposed to be statistically significant. Statistical analysis was established using the SPSS software version 26.

RESULTS

This study involved 40 patients who needed gingival depigmentation in both the mandible and maxilla. No patients dropped out during the study. Gingival depigmentation techniques performed in this study were the conventional surgical technique (group I) and MN technique (group II).

The results of the present study showed that the MN procedure displayed a statistically significant decrease ($P \leq 0.001$) of bleeding at the time of the procedure compared to the conventional technique. (table 1) Regarding pain, the MN procedure showed a statistically significant ($P \leq 0.001$) decrease in VAS on the 1st and 7th day postoperative compared to the conventional technique. (table 2) Most of the cases treated with conventional procedure showed incomplete healing and ulceration on the 1st and 7th day after the procedure, which resulted in a statistically significant ($P \leq 0.001$) decrease in the score of wound healing compared to the MN technique. (Table 3)

Regarding DOPI (table 4) and Hedin index (table 5), both treated groups showed no statistically significant difference at baseline ($P > 0.05$), and both treated groups showed statistically significant ($P \leq 0.001$) improvement at 1 month and 6 months compared to the baseline data. (Figures 1 and 2) While the conventional technique showed more improvement in treating the gingival hyperpigmentation than the MN treated group, this resulted in a statistically significant decrease ($P \leq 0.05$) in DOPI and Hedin index compared to the MN treated group.

TABLE (1) Showed the mean and standard deviation of bleeding at the time of the depigmentation procedure.

Study groups	Group I		Group II		*T-test*	*P-value*
	Mean	SD	Mean	SD		
	2.050	0.605	0.875	0.222	8.156	<0.001**

Test used: independent T-test at P<0.05 **; significant

TABLE (2) A comparison of the mean and standard deviation of VAS between treated groups

Table 1. VAS

Study groups	Group I		Group II		*T-test*	*P-value*
	Mean	SD	Mean	SD		
1St day	2.900	1.119	0.200	0.410	10.129	<0.001**
7th Days	0.750	0.444	0.00	0.00	10.129	<0.001**
Mean difference	2.15	0.269	0.200	0.092		
T test	7.98		2.179			
P value	<0.001**		0.036**			

Test used: independent T-test at P<0.05 - **; significant

TABLE (3) A comparison of the means and standard deviation of wound healing scores between treated groups

Study groups	Group I		Group II		*T-test*	*P-value*
	Mean	**SD**	**Mean**	**SD**		
1st day post	1.425	0.466	2.625	0.559	7.37	<0.001**
7th Days	1.650	0.462	2.900	0.307	10.08	<0.001**
Mean difference	0.225	0.146	0.275	0.143		
T test	1.53		1.927			
P value	0.134		0.061			

Test used: independent T-test at P<0.05 **; significant

TABLE (4) A comparison the means and standard deviation of DOPI between the studied groups

Study groups	Group I		Group II		*T-test*	*P-value*
	Mean	**SD**	**Mean**	**SD**		
bas	2.80	0.410	2.750	0.444	0.370	0.714
1 M	0.150	0.366	0.450	0.510	2.13	0.039*
6 M	0.380	0.455	0.750	0.444	1.983	0.01*
ANOVA(F)	224.36		143.15			
P value	<0.001**		<0.001**			
Pair wise comparison by Tukey`s Post hoc						
Bas Vs 1M	P<0.001**		P<0.001**			
Bas Vs 6M	P<0.001**		P<0.001**			
1.M Vs 6 M	P=0.204		P=0.114			

Test used: ANOVA test at P<0.05 & independent T-test **; significant

TABLE (5) A comparison of Hedin index means and standard deviation between treated groups

Study groups	Group I		Group II		*T-test*	*P-value*
	Mean	**SD**	**Mean**	**SD**		
bas	3.400	0.502	3.350	0.489	0.319	0.752
1 M	0.100	0.262	0.375	0.483	2.238	0.031**
6 M	0.450	0.510	0.825	0.591	2.25	0.038**
ANOVA(F)	338.99		187.61			
P value	<0.001		<0.001			
			Pair wise comparison by Tukey`s Post hoc			
Bas Vs 1M	P<0.001**		P<0.001**			
Bas Vs 6M	P<0.001**		P<0.001**			
IM Vs 6 M	P=0.039**		P=0.023**			

Test used: ANOVA test at P<0.05 & independent T-test **; significant

DISCUSSION

Gingival hyperpigmentation is an aesthetic issue that many people are concerned about. Because of the high aesthetic standards, the recommended approach should be simple and efficient. The surgical intervention is a gold standard procedure and is most commonly used for the management of gingival hyperpigmentation. It is based on the excision of the entire thickness of the epithelial and the papillary connective tissue layer and the secondary intended healing of the denuded connective tissue. [26, 27] Surgical depigmentation continues to be a source of apprehension for people seeking enhanced aesthetics, despite its multiple advantages. Bleeding together with a large postoperative wound, pain, and recurrence are the most common drawbacks reported by patients.[27] Moreover, in the area of thin gingiva the surgical technique may be associated with denuded alveolar bone. [28] As a consequence, alternative less invasive treatment methods for gingival depigmentation are required. The objective of the present clinical trial was to examine the effectiveness of the MN technique as a treatment option for gingival hyperpigmentation.

The present study revealed that treatment with MN significantly reduced pain and bleeding compared to surgical technique and all cases showed complete epithelialization on the 7th day after the procedure. Both techniques statistically significantly decrease the pigmentation intensity and distribution that was evaluated by DOPI and Hedin index respectively at 1 month and 6 months after the applied procedures compared to baseline data. While the conventional technique showed more improvement regarding the DOPI scores and Hedin index compared to MN treated group.

To the author's knowledge, no previous research has investigated the impact of MN technique on the treatment of gingival hyperpigmentation. The MN technique was originally applied for the treatment of cutaneous hyperpigmentation diseases, it is utilized alone or in conjunction with topical application of medication to improve transdermal drug delivery.[29, 30] After two to four sessions, MN alone successfully diminishes the melanin intensity of melasma lesions [31] with at least a four-week interval between sessions (or, less commonly, a two-week interval) until the required results were obtained.[32]

Microneedle pens are uses small needles coupled to an automated instrument to generate thousands of tiny holes in the gingiva it creating stamper-like motions. This instrument is made up of disposable portable cartridges composed of 12 sterilized microneedle. Every patient has its own cartridge that was fitted into the tip of the pen. It's a motorized device with customizable speed and depth. It should be employed perpendicular to the tissue surface where the needles oscillate rapidly and stamp it uniformly, the device provides a moderate yet consistent velocity of automated motions that penetrate the tissue more efficiently and reliably without causing deepithelialization.[33]

It was documented that MN helps to reverse hyperpigmentation and normalize cellular activity and signaling between keratinocytes and melanocytes in more than one way including the improvement of keratinocyte function so the transfer of melanosomes between keratinocytes and melanocytes becomes more regulated. [34, 35] It also caused mild epithelial hyperplasia, fibroblast proliferation, subepithelial formation of glycosaminoglycans and fibrin, and a rise in Ki67-positive keratinocytes, implying that MN induced preliminary alteration in the epithelium and underlying connective tissue, causing some structural changes in the hyperpigmented tissue. Furthermore, melanocytes are less likely to come into contact with melanogenic stimulating factors including stem cell factor, endothelin1, and hepatocyte growth factor in this altered environment.[36]

The significance of the basal membrane and fibroblasts in the etiology of hyperpigmentation is regarded to be crucial.[37] MN damages the basal membrane, allowing melanocytes and melanin granules

to migrate down into the connective tissue beneath. [38] According to a histological study of melanocytes following MN, it leads to proliferation of fibroblast with the enhanced deposition of extracellular matrix components as well as the remodeling of the basal membrane, a reduction in pendulum melanocytes. Furthermore, accelerated keratinocyte turnover enhances epithelial melanin clearance.[39] Its possible role in treating hyperpigmentation is through the interaction between keratinocyte, melanocyte, and fibroblast leading to remodeling of most of the epithelium, basement membrane, and connective tissue.[25]

Furthermore, the microscopic wounds created by MN trigger regenerative wound repair. [40] Multiple growth factors required in the healing mechanism such as fibroblast growth factor, platelet-derived growth factor, and transforming growth factor are released ultimately leading to neovascularization, neocollagenesis, and elastin formation at the epithelium/connective tissue junction with thickening of the stratum spinosum all of which contributes to the lightning of hyperpigmented tissues.[16,32,39,41,42,43] These regulatory growth factors trigger the chemotaxis of fibroblasts and subsequent collagen synthesis and deposition of 'scarless collagen' which is stimulated when the fractional channels penetrate into the epithelium and underlying connective tissues. [35]

The current study found that the MN technique for gingival depigmentation is a successful procedure and most participants were satisfied with the results produced from the MN technique. Moreover, MN has several advantages, including the minimally invasive procedure for patients who are afraid of surgery, minimal bleeding, and painless procedure for patients who did not require analgesics after the procedure. Moreover, the gingiva heals faster than other surgical procedures because the epithelium remains relatively intact. However, MN has a few drawbacks, including the inability to treat severe pigmentation, a delayed depigmentation process, and the requirement for several patient visits. Overall, MN's effectiveness and safety, as well as its simplicity of application, make it a promising treatment option. More research is needed to determine the efficacy of this approach in treating gingival hyperpigmentation in a larger number of participants with a more extended time for follow-up. Moreover, additional investigations may be performed to estimate the effectiveness of MN combined with local drug delivery for treating gingival hyperpigmentation.

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