Microneedling with Platelet Rich Plasma Versus Microneedling Alone For Gingival Depigmentation: A Randomized Controlled Clinical Trial.

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Protocol Submitted for partial fulfillment of the master requirements in Faculty of Dentistry Cairo University

By

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Code

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Name	**Signature**	**Date**
1.		
2.		
Research plan committee		
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1.		
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I. Administrative information:

1. Title:

Microneedling with Platelet Rich Plasma Versus Microneedling Alone for Gingival Depigmentation: A Randomized Controlled Clinical Trial.

2. Protocol Registration:

Protocol will be registered on ClinicalTrials.gov.

3. Protocol version:

First version

4. Funding:

Self-funding.

5. Roles and responsibilities:

Main supervisor

Prof. Dr Enji Ahmed Mahmoud

- Professor of Oral Medicine and Periodontology-Cairo University.

Role:

- Initiating the idea of the clinical trial.
- Randomization of cases.
- Supervising the manuscript.
- Revising & interpretation of collected data.

• Co-supervisor

Dr. Manar Taher Elzanaty

- Lecturer of Oral Medicine and Periodontology- Cairo University.

Role:

- Supervising the manuscript.
- Revising & interpretation of collected data.

• Principle Investigator

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Examination of patients.

- Measuring and recording the outcomes of the study
- Data collection and data management.
- Writing the manuscript.

Trial responsibility:

Department of Oral Medicine and Periodontology, Faculty of Dentistry Cairo University.

Committees:

• Research plan committee
• Periodontology department council
• Evidence based dentistry committee
• Faculty Council

• Ethics committee

II. Introduction:

6. Background and rationale:

Research question:

Will the usage of microneedling with platelet rich plasma as a depigmentation method differ in comparison to microneedling alone for pigment intensity reduction?

Statement of the problem:

The skin and oral mucous membrane normally have a brown pigment called melanin, which is not generated from hemoglobin. The melanocytes found in the basal and supra-basal epithelium deposit too much melanin, which leads to gingival hyperpigmentation(Javali, Tapashetti et al. 2011). Although physiologic gingival hyperpigmentation is not a pathologic condition, it is considered one of the main esthetic problems in dentistry. It was found that the attached gingiva is the most frequently pigmented intraoral tissues followed by the papillary gingiva and the alveolar mucosa(Javali, Tapashetti et al. 2011). Several treatments, such as gingivectomy, mucosal removal with a scalpel, abrasion techniques, free gingival grafts, chemical approaches involving caustic chemicals, electrosurgery, cryotherapy, and newly developed lasers, have been used to achieve pigmentation-free gingiva(Grover, Dadlani et al. 2014).The scalpel technique, which involves eliminating the gingival epithelium and a layer of connective tissue .Subsequently, this will allow the stripped connective tissue to heal by secondary intention which is more painful(Gupta, Kumar et al. 2014). Bur abrasion is a straightforward, inexpensive, and efficient technique for gingival depigmentation but it causes uncomfortable bleeding both during and after the surgery and has the potential to damage bone surface when done with rotary tools(Kathariya and Pradeep 2011).Cryosurgery can lead to tissue inflammation and damage Moreover, electrosurgery that has some drawbacks that include heat accumulation, tissue destruction, and it is a technique sensitive method(Yadav, Kumar et al. 2022).

Rationale for conducting the research:

The term Platelet-Rich Plasma (PRP) is a generic term used to describe a plasma suspension obtained from whole blood, prepared so as to contain platelet concentrations higher than those normally found in circulating blood. Platelets contain granules consisting of various substances, which are released upon activation of the platelets(Smyth, McEver et al. 2009). Among the main substances that are released from these granules is growth factors (GF)(Mazzucco, Balbo et al. 2009). These factors have a well-known role in the process of tissue repair. Thus, the concentration of these substances in injured tissues could be beneficial to provide more agility to the regeneration processes(Sommeling, Heyneman et al. 2013).

Some of the bioactive substances present in the α-granules include platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β1, 2, epidermal growth factor (EGF), and mitogenic growth factors such as platelet-derived angiogenesis factor and fibrinogen(Eppley, Pietrzak et al. 2006). only TGF-β1 and EGF have been investigated about their relation with melanogenesis.

Kim et al. investigated the effects of TGF-β1 on melanogenesis by using a spontaneously immortalized mouse melanocyte cell line, and asserted that TGF-β1 significantly inhibits melanin synthesis in a concentration dependent manner. They declared that TGF-β1 decreases melanogenesis via delayed extracellular signal-regulated kinase activation(Kim, Park et al. 2004).While Yun et al. studied the effect of EGF on melanogenesis by using mouse-derived immortalized melanocytes from a laser-treated keratinocyte-conditioned culture media. They proposed that treatment with EGF lowers melanin production in melanocytes by inhibiting prostaglandin-E2 (PGE2) expression and tyrosinase enzyme activity. They offered that treatment with EGF can be used in cosmetics to whiten the skin and for the prevention of post inflammatory hyperpigmentation(Yun, Bang et al. 2013).
PRP is a useful treatment for periorbital hyperpigmentation and further comparative studies of other treatment modalities are highly recommended (Salah Hashim Al-Shami 2014).

Microneedling 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(Cardinali, Bolasco et al. 2008).
the MN technique for gingival depigmentation is a successful procedure and most participants were satisfied with the results produced from the MN technique. It successfully reduces gingival hyperpigmentation and might be regarded as an alternative minimally invasive treatment option. additional investigations may be performed to estimate the effectiveness of MN combined with local drug delivery for treating gingival hyperpigmentation(Morsy 2022).

Review of literature:

.Normal appearance of the gingiva is pink to light red, with stippling “orange peel texture.” , normal color depends on melanogenesis and distribution of melanin pigment, keratinization depth of epithelization, and vascularity of the gingiva(Feller, Masilana et al. 2014). Gingival color plays a crucial part in facial aesthetics and contributes to the entire appearance of an ideal smile(Altayeb, Hamadah et al. 2021).

Melanin hyperpigmentation has been linked to a number of etiological and pathological causes, including smoking, heavy metal toxicity, heredity, endocrine disorders, UV exposure, inflammation, benign and malignant lesions, and intentional cultural tattooing. In order to determine whether the reason is physiological or pathological, a thorough medical history of the patient is essential(Holmstrup, Plemons et al. 2018).

With the introduction of gingival depigmentation as a periodontal surgical procedure, the hyperpigmented gingival tissues are removed utilizing a variety of surgical treatment methods, such as scalpel excision, bur abrasion, free gingival grafts, ADMA, lasers, cryosurgery, electro surgery(Farid, Shinwari et al. 2017). Pigmentation of melanin can also be treated by numerous means that contain chemical methods using alcohol, ascorbic acid, phenol(Shimada, Tai et al. 2009)

The most popular surgical approaches are associated by anxiety, bleeding, a significant postoperative wound, and recurrence (Patil, Joshi et al. 2015). Those methods could also have negative consequences include chemical burns, delayed healing, extreme pain and suffering, bone loss, and trouble controlling the depth of de-epithelization(Padayatty and Levine 2016).

Needle-based delivery systems are designed to deliver medications by rupturing the skin or mucosal barrier, making the medication easily accessible to the targeted tissues (Batra, Dawar et al. 2020).The collagen induction therapy method known as microneedling approach involves puncturing the skin repeatedly. Microneedling has been widely used in dermatology recently since it is a method that is efficient, straightforward, affordable, well-tolerated, and advantageous from both a cosmetic and therapeutic standpoint(Singh and Yadav 2016).

Instead of cutting through the cells, the used microneedles create micro-conduits that increase the skin's permeability and blood flow into the epidermis. This procedure makes it easier for topical drugs to cross the stratum corneum layer. Additionally, growth factors that support the regeneration of collagen and elastin are created(Iriarte, Awosika et al. 2017).

The microneedling principle is suggested to be used in the treatment of gingival hyperpigmentation, compared to the currently employed techniques such as scalpel surgery, laser ablation, bur abrasion, and electrocautery which frequently result in complications, microneedling could be a promising minimally invasive, straightforward, painless, and cost-effective treatment modality for gingival depigmentation(Batra, Dawar et al. 2020). Subsequent randomized controlled clinical trials are required to validate the findings for the case report that mentioned the use of Dermapen microneedling with vitamin C topical paste as a novel technique resulting in complete gingival depigmentation that lasted for six months (Mostafa and Alotaibi 2022).

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(Morsy 2022).

The combination of microneedling with PRP therapy emerges as a promising and effective solution for pigmentation reduction. Overall, microneedling with PRP holds great promise as a non-invasive and effective approach for revitalizing the skin and reducing pigmentation, offering individuals an opportunity to achieve a more even and rejuvenated complexion(Shahraki, Khazaei et al.). This therapy presents a valuable option for individuals seeking to address pigmentation issues while simultaneously improving overall skin health. Its ability to promote collagen production, minimize downtime, and offer long-lasting results makes it a compelling choice in the field of aesthetic dermatology .Further research and clinical trials are still needed to explore the optimal treatment protocols, long-term efficacy, and potential combination therapies for enhancing the outcomes of microneedling with PRP in pigmentation reduction (Mohamed, Attwa et al. 2023) (Wu, Zhang et al. 2022).

In order to treat Periorbital Hyperpigmentation, patients receive intradermal injection of PRP on their face for three sessions at a 3-week interval. To prepare PRP, 10mL of the patient's venous blood sample was taken manually with a sterile 10mL syringe and then centrifuged with 1800G/6min, buffy coat and plasma were then extracted and re-centrifuged with 2500G/15min. The injection of 1mL of PRP was immediately initiated intradermally into the infraorbital area on one side of the face with a 1 cc insulin syringe 30G, (0.1mL in each point of injection) (Iranmanesh, Rastaghi et al.).

Explanation for choice of comparators:
Microneedling has been widely used in dermatology due to its ability to create micro-conduits that increase the skin's permeability and blood flow into the epidermis (Iriarte, Awosika et al. 2017).
MN technique for gingival depigmentation is a successful procedure and most participants were satisfied with the results produced from the MN technique. additional investigations may be performed to estimate the effectiveness of MN combined with local drug delivery for treating gingival hyperpigmentation(Morsy 2022).

7. Objectives:

The aim of this study is to evaluate efficacy of the microneedling technique with Platelet Rich Plasma application as a depigmentation method in comparison to microneedling Alone for pigment intensity reduction.

Hypothesis:
Null hypothesis: there is no significant difference between both groups

Primary objective:
Oral Pigmentation intensity score (DOPI) which will be measured at baseline, 3 months and 6 months after gingival depigmentation procedures.

Secondary objectives
Pain
Gingival thickness
Wound healing
Patient Satisfaction

8. Trial design:

The trial will be a parallel-group, two-arm, superiority, randomized controlled clinical trial with allocation ratio of 1:1.

III. Methods

A) Participants, interventions & outcomes

9. Study settings:

Study is to be conducted in the Oral Medicine and Periodontology department, Faculty of Dentistry – Cairo University, Egypt.

 Patients are to be selected from the outpatient clinic of the department of Oral Medicine and Periodontology-Cairo University.

10. Eligibility criteria:

Inclusion criteria: (Mostafa and Alotaibi 2022)

- Patients exhibiting melanin hyperpigmentation in the anterior region of the upper or lower gingiva.
- Patients should be free from any systemic diseases according to modified Cornell Medical index (Abramson 1966).
- Non- smokers.

Exclusion criteria:(Mostafa and Alotaibi 2022)

- Fully edentulous patients.
- Patients with endocrine disorders causing hyperpigmentation or drug induced gingival pigmentation. (Sreeja, Ramakrishnan et al. 2015)
- Pregnant or lactating females.

11. Interventions

Pre-operative preparation phase includes ultrasonic scaling and oral hygiene instructions for all patients. Eligible patients will be randomly allocated into two groups.

Clinical photographs: Clinical photographs will be taken at baseline, 1 and 6 months postoperatively.

- **Intervention Group (Group A)**: Microneedling will be done with application of Platelet Rich Plasma for depigmentation.
- **Control Group (Group B)**: microneedling will be done alone for depigmentation.

In both groups:

Local anesthesia (Articaine with 1:100,000 Epinephrine) will be administered by infiltration technique.

- A Dermapen device will be used to microneedle the gingival tissue (model M8) with 24 microneedles arranged in rows, which is adjusted according to the gingival thickness at the 6th mode speed of 700 cycles/min(Mostafa and Alotaibi 2022).
- It will be laid perpendicular to gingival surface and MN will be carried out in horizontal, vertical and diagonal directions about four to five times for the whole hyperpigmented gingiva until mild microbleeding and mild erythema was clearly

visible.

- Once bleeding points are observed on all areas of the pigmented gingiva, it will be irrigated using saline solution and dried using sterile gauze.

Intervention group:

- Patients receive injection of PRP in their Gingiva. To prepare PRP, 10mL of the patient's venous blood sample was taken manually with a sterile 10mL syringe and then centrifuged with 1800G/6min, buffy coat and plasma were then extracted and re-centrifuged with 2500G/15min. The injection of 1mL of PRP was immediately initiated with a 1 cc insulin syringe 30G, (0.1mL in each point of injection) (Iranmanesh, Rastaghi et al.).

Postoperative Care:

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 to minimize mechanical damage to the treated areas. If discomfort or itching was reported on the first day, analgesics were prescribed (Brufen 400 mg tablets). Restricted spicy, acidic, and coloring food. (Yussif, Abdel Rahman et al. 2019, Morsy 2022).

Assessment:

Photos will be taken preoperative, during, postoperative and at follow-up appointments until 6 months. Clinical assessment will be done at baseline, after 3 month and 6 months including the following parameters:

1-**Dummett-Gupta Oral Pigmentation Index (DOPI):** the degree of gingival pigmentation is scored as 0, pink tissue [no clinical pigmentation]; 1 = mild light brown tissue [mild clinical pigmentation]; 2, medium brown or mixed brown and pink tissue [moderate clinical pigmentation]; or 3, deep brown/blue–black tissue [heavy clinical pigmentation] (Dummett and Gupta 1964).

2- Gingival thickness: the gingival tissue thickness was measured starting at 1.5 mm apical to the marginal gingiva using an endodontic spreader number 25 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. The gingival biotype will be considered thin if the measurement is ≤1.0 mm and thick if it measured >1.0 mm (Couso-Queiruga, Tattan et al. 2021).

3- Pain: using Visual analogue score questionnaire. (Becker, Heeschen et al. 2018). The rate of analgesic consumption will be recorded 7 days after the intervention. (Palheiros, Cunha et al. 2021)

4- Patient Satisfaction: questionnaire to score the degree of patient satisfaction with the cosmetic results of the procedure. It will be documented depending on the patient's opinion, measured on a five-point scale 0: Very dissatisfied 1: Dissatisfied 2: Neither satisfied nor dissatisfied 3: Satisfied 4: Very satisfied (Becker, Heeschen et al. 2018).

4- Wound healing: Healing Index by Landry et al.: is used to describe the extent of healing after periodontal surgery (Landry RG, Turnbull RS, Howley T. 1988), Score 1: Very Poor, Score 2: Poor, Score 3: Good, Score 4: Very Good, Score 5: Excellent.

12. Outcomes:

	Recordings	Measuring Device	Measuring Unit
Primary Outcome	Pigmentation Score (Dummett and Gupta, 1964)	Visual	It will be measured using Dummett Oral Pigmentation Index (DOPI 1964) : Score 0: No clinical pigmentation (pink gingival) Score 1: Mild clinical pigmentation (mild light brown color) Score 2: Moderate clinical pigmentation (medium brown or mixed pink and brown) Score 3: Heavy clinical pigmentation (deep brown or bluish black).
Secondary Outcomes	Pain (Becker et al., 2018)	Questionnaire	Visual Analogue Scale (VAS)
	Gingival thickness (Couso-Queiruga, Tattan et al. 2021)	Standard no. 25 endodontic finger spreader with a rubber stop	The gingival biotype will be considered thin if the measurement is ≤1.0 mm and thick if it measured >1.0 mm.
	Wound healing	Photographic clinical pictures	Landry healing index

	Pain (Palheiros, Cunha et al. 2021)	Questionnaire	Rate of analgesic consumption
	Patient Satisfaction(Taher Agha and Polenik 2020)	Questionnaire	It will be documented depending on the patient's opinion, measured on a five-point scale 0: Very dissatisfied 1: Dissatisfied 2: Neither satisfied nor dissatisfied 3: Satisfied 4: Very satisfied

13. Participant timeline

After accepting to be enrolled in this study, all patients will receive proper oral hygiene instructions and will be motivated to stick to these instructions.

 After receiving the treatment, patients will be followed-up for 6 months.

Time point	T_0	T_1	T_2 **2 weeks (post intervention)**	T_3 **4 weeks** (post intervention)	T_4 **3 months** (post intervention)	T_5 **6 months** (post intervention)
Enrollment	X					
Eligibility screen	X					
Informed consent	X					
Initial phase (Oral hygiene measures)	X					
Allocation		X				
Intervention		X	X	X		
Pigmentation Score	X			X	X	X
Wound healing		X	X			X
Gingival thickness		X				X
Pain		X	X			
Patient satisfaction					X	X

14. Sample size:

We are planning a study of a continuous response variable from independent control and experimental subjects with (1) control per experimental subject. In a previous study the response within each subject group was normally distributed with standard deviation 0.444. if the true difference in the experimental and control means is 0.5, we will need to study 13 experimental subjects and 13 control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.8. The type I error probability associated with this test of this null hypothesis is 0.05. To compensate for drop outs, 3 subjects will be added to each group to become 16 subjects, with a total of 32. Calculations were conducted using PS Power and Sample Size Calculations version 3.1.2. (Vanderbilt University, Nashville, Tennessee, USA).

15. Recruitment:

Patients will be selected from the outpatient clinic of the Oral Medicine and Periodontology Department, Faculty of Dentistry-Cairo University.

Screening of patients will continue until the target sample is achieved.

Identifying and recruiting potential subjects will be achieved through patient database

B) Assignment of interventions

16. Allocation:

16a-Random sequence generation:

Methods of generating randomization:

Patients will be randomly assigned to either test or control group using computer generated randomization (www.randomizer.org) which will be performed by the supervisor. The patients will be allocated to either test or control group

.

16b. Allocation concealment mechanism:

The two groups will be equally prepared for both procedures. Then the decision of which group will receive (microneedling with application of platelet rich plasma) and which will receive (microneedling alone) will be taken according to the randomized numbers placed in opaque sealed envelopes. The number will be picked by the supervisor.

16c. Implementation

- All patients who provide an informed consent for participation as well as fulfill the inclusion criteria will be randomized.
- The supervisor will generate the allocation sequence as well as assign the participants to the test or control groups. The principle investigator will enroll the participants.

17. Blinding:

Single blinded:

- Blinding of the participants is not applicable.
- Blinding of the operator is not applicable.
- Outcome assessor (primary and secondary outcomes) & biostatistician will be blinded.

C) Data collection, management, and analysis:

18. Data collection methods

All the date of the patients included in the study will be recorded from them or extracted from the patient's file.

- Plans to promote participant retention and complete follow- up

 Telephone numbers of all patients included in the study will be recorded as a part of the written consent.

 All patients will receive a phone call at the time of the pre-determined follow up dates.

19. Data management:

 All data will be entered and saved electronically.

 Patient files are to be stored in numerical order and stored in secured file. Data will be encrypted using a password.

 All data will be maintained after completion of the study.

20. Statistical methods:

Nominal data will be reported as numbers and frequency and will be analyzed using chi-square test or Fisher's exact test. Numerical data will be explored for normality using Kolmogorov-Smirnov test and Shapiro-Wilk test. In the case of normally distributed numerical variables, a comparison between both groups will be done using independent t-test while for non-normally distributed variables, Mann Whiney U test will be utilized. For intragroup analysis in normally distributed data, Paired t-test will be utilized while Wilcoxon signed rank test will be used for non-normally distributed data. All test will be two-tailed and P-values less than 0.05 will be considered statistically significant.

D) Data monitoring:

21. Monitoring

No formal monitoring committee is currently available.

22. Harms

Any temporary or permanent adverse effect will be recorded and documented.

23. Audit

No formal auditing committee is currently available.

IV. Ethics and dissemination

24. Research ethics approval

This protocol and the template informed consent form will be reviewed by the Ethics Committee of Scientific Research -Faculty of Oral and Dental Medicine- Cairo University.

25. Protocol amendments

Any modifications to the protocol which may have an impact on the conduct of the study, potential benefit of the patient or may affect patient safety, including changes of study objectives, study design, sample sizes, study procedures, or significant administrative aspects will require a formal amendment to the protocol. Such amendment will be agreed upon by the Council of Oral Medicine and Periodontology Department and the Faculty of Oral and Dental Medicine, Cairo University.

26. Informed consent

Researcher will introduce the trial to patients and provide full explanation of its aim and benefits in layman language. Patients will then be able to have an informed discussion with the researcher. Researcher will obtain written consent from patients willing to participate in the trial. All consent forms will be translated into Arabic.

27. Confidentiality

All study-related information will be stored securely. All participant information will be stored in locked file cabinets in areas with limited access. All laboratory specimens, reports, data collection, process, and administrative forms will be identified by a coded ID [identification] number only to maintain participant confidentiality. All records that contain names or other personal identifiers will be stored separately from study records identified by code number. All local databases will be secured with password-protected access systems.

28. Declaration of interest

If there will be any conflict of interest it will be declared.

29. Access to data

The principal investigator and the supervisors will have access to the data sets. All data sets will be password protected. To ensure confidentiality, data dispersed to project team members will be blinded of any identifying participant information.

30. Post-trial care

All patients will be followed up for nine months after the clinical trial is over.

31. Dissemination policy

- Study results will be published as partial fulfillment of the requirements for master degree in Periodontology.
- Topics suggested for presentation or publication will be circulated by the authors.

33. Biological specimens

Blood sample collected from intervention group and centrifuged then injected.

V. References

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