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**GINGIVAL DEPIGMENTATION HEALING: A COMPARATIVE STUDY
 BETWEEN LIQUID PLATELET-RICH FIBRIN AND NON-EUGENOL
 PERIODONTAL PACK**

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ABSTRACT

Background: The gingival depigmentation procedure can result in extensive open wounds, patient discomfort, and an increased risk of infection. A Non-eugenol Periodontal Pack (NEP) is a common material to protect wounds from mechanical trauma and increase patient's comfort but does not promote healing. Liquid Platelet-rich Fibrin (PRF) is clinically proven to regenerate tissues, however, its effectiveness following gingival depigmentation remains limited. **Purpose:** This study aims to compare post-gingival depigmentation outcomes after application of liquid PRF and NEP. **Methods:** Clinical trials were conducted on five healthy subjects who met the inclusion and exclusion criteria. Gingival depigmentation was performed in the anterior region using a scalpel, followed by the application of NEP (left side) and PRF (right side). Healing Index (HI), pain perception by Visual Analog Scale (VAS), and Wound Healing Score (WHS) were measured on days 5, 7, and then statistically analyzed with a significance value of $p < 0.05$. **Results:** The HI on day 5 of the liquid PRF group was higher than NEP ($p < 0.05$), however, the HI on day 7, VAS on days 5 and 7, and WHS on days 5 and 7 were not significantly different ($p > 0.05$). **Conclusion:** Liquid PRF accelerates wound healing faster than NEP. Despite this difference, both materials deliver similar results regarding patient comfort and epithelialization.

Keywords: Gingiva hyperpigmentation; Periodontal pack; Platelet-rich fibrin; Wound healing

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INTRODUCTION

Aesthetics and patient comfort are vital considerations in dentistry, especially in periodontal aesthetics. One aesthetic complaint that often interferes with confidence, especially in patients with high smile lines, is the black color of the gingiva, which is diagnosed as gingival hyperpigmentation. The treatment for gingival hyperpigmentation is called gingival depigmentation. This surgical procedure is performed in several ways, including the use of a scalpel, abrasion techniques with a bur and a laser, gingivectomy with gingival grafting, cauterization, and the application of chemicals.¹ Among these treatments, scalpel- and bur-based surgical techniques are considered more straightforward and easier to apply in the clinic. However, they often result in relatively wide-open wounds, thereby increasing the risk of delayed healing, infection, and discomfort. To prevent these issues, periodontal dressing is applied as a post-surgical wound protector.^{2,3}

One of the most used periodontal dressings to date is the non-eugenol dressing. This material exhibits stable physical properties, enabling it to protect wounds during the physiological process of wound healing.⁴ However, it causes fibroblast toxicity and promotes plaque accumulation.⁵ Additionally, the use of NEP can cause patient discomfort because it compromises aesthetics when applied in visible areas. Therefore, other materials that are safe for fibroblasts are needed to optimize wound healing.

On the other hand, the utilization of platelets in the management of open wounds is growing rapidly. Platelet-rich fibrin (PRF) membrane application after gingival depigmentation resulted in better wound healing

compared to a non-eugenol periodontal dressing.⁶ However, the use of PRF has disadvantage including the limited amount obtained, and the membrane must be applied by suturing.

Injectable PRF is often used extensively to induce gingival and bone regeneration. However, no studies have been found demonstrating the effectiveness of liquid PRF in open wounds, especially after gingival depigmentation. This clinical trial aims to compare the results of gingival depigmentation treatment after the application of liquid PRF and NEP. The study's findings are expected to lead to a breakthrough in the management of open wounds after aesthetic periodontal surgery, particularly in cases with gingival depigmentation.

MATERIALS AND METHODS

Material

The material used in this study included diagnostic set, citoject, scalpel (Osung), blade #15 (Onemed), abrasive bur, 3 ml injection syringes (Onemed), plain vacutainers (BD vacutainer), centrifuge, PRF box (Osung), tweezers (Osung) and scissors (Osung). Additionally, the necessary materials included iodine glycerin, a topical anesthetic, microtubes, Pehacaine, saline, distilled water, and Non-eugenol Periodontal Pack. All stages of the study have been approved for ethical feasibility (No. 00587/KKEP/FKG-UGM/EC/2020).

Methods

This study is a clinical trial with five participants, using a split-mouth design. Subjects were chosen based on the following criteria: 20-30 years old, diagnosed with gingival hyperpigmentation, cooperative, and willing to sign an informed consent form. Patients with a history of systemic disease, who are pregnant, taking medication, or under the care, are excluded from clinical trials. Subject selection is carried out in accordance with inclusion and exclusion criteria.

Steps of Procedure

The clinical trial protocol began with an explanation of the study procedure. After the explanation, the subjects were asked to sign the informed consent form. At the first visit before the surgical procedure, initial treatments were performed, including dental health education, plaque control, scaling, and polishing.

At the second visit, a re-evaluation was performed, followed by gingival depigmentation using a scalpel/blade. Subsequently, liquid PRF and NEP were applied to each side. Liquid PRF was prepared by centrifuging 10 mL of autologous blood at 3,000 rpm for 3 minutes, and the result was stored in single-use sterile tube. After the gingival depigmentation, the patient was given postoperative instructions and instructed to store the tube at -20°C. The patient was also advised to apply the PRF to the wound once it reaches room temperature. Postoperative re-evaluation was conducted on the fifth and seventh days.

Wound healing was assessed by two independent observers using the Healing Index. A Visual Analog Scale (VAS) was used to evaluate patient comfort while epithelialization (Wound Healing Score) was assessed by applying Toluidine blue. All data were analyzed by statistical analysis. The healing index was analyzed using the Mann-Whitney test while the VAS score and percentage of epithelialization data were analyzed using an Independent T-test. All results were considered significant if the p value was less than 0.05.

RESULTS

The Healing Index data were obtained from the objective assessment of the post-depigmentation wound area, which was interpreted according to the Healing Index (HI) scale developed by Landry et al. (1988).⁷ The Visual Analog Scale (VAS) was obtained from the patient's subjective assessment reported during the control. The Wound Healing score was obtained by objective assessment of epithelialization in the wound area, with toluidine blue used to measure the percentage of epithelialization. The clinical features are shown in Figure 1.



Figure 1. The clinical features of studied group

Table 1. Healing Index of Liquid PRF and NEP Groups after Gingival Depigmentation.

Day	Group	n	Median (Minimum-Maximum)	p value
5	PRF	5	3.00 (0.00-3.00)	0.014*
	NEP	5	2.00(2.00-3.00)	
7	PRF	5	3.00(3.00-4.00)	0.513
	NEP	5	3.00(3.00-4.00)	

Table 2. Visual Analog Scale of Liquid PRF and NEP Groups after Gingival Depigmentation

Day	Group	n	Mean $\pm$ Standard Deviation	p value
	PRF	5	1.20 $\pm$ 0.83	0.70
	NEP	5	1.60 $\pm$ 2.07	
7	PRF	5	0.40 $\pm$ 0.54	0.22
	NEP	5	0.60 $\pm$ 0.80	

Table 3. Wound Healing Score of Liquid PRF and NEP Groups after Gingival Depigmentation

Day	Group	n	Mean $\pm$ Standard Deviation	p value
5	PRF	5	2.00 $\pm$ 0.70	1.00
	NEP	5	2.00 $\pm$ 0.00	
7	PRF	5	1.60 $\pm$ 0.54	0.513
	NEP	5	1.80 $\pm$ 0.44	

Table 1 demonstrated that the average HI for both groups increased over time. The HI increased on day 7 compared to day 5. On day 5, the PRF group had a higher HI than NEP ($p < 0.05$). On day 7, the PRF group had a greater HI, although there was no significant difference from the NEP group ($p > 0.05$).

Table 2 illustrated the decline in VAS score over time. The VAS score on day 5 was greater than on day 7. The average VAS PRF score on days 5 and 7 was lower than that of NEP, although the difference was not statistically significant ($p > 0.05$).

Table 3 showed that epithelialization increases over time. The Wound Healing Score on the fifth day was higher than on the seventh day. There was no significant difference ($p>0.05$) between the epithelialization of the PRF group and the NEP group on days 5 and 7.

DISCUSSION

Gingival depigmentation is an aesthetic periodontal surgical procedure that aims to remove gingival hyperpigmentation, particularly in the anterior region, to enhance the smile's aesthetic value. Commonly used techniques in this procedure include scalpels and burs for abrasion. Although this approach is simple and quick to use in clinical practice, it creates a wide-open wound.² Such exposed wounds are prone to delayed healing (delayed wound healing), infections, and postoperative discomfort.³

This wound heals in a sequential process known as secondary intention, with healing progressing from the base of the wound to the surface. As a result, wound dressing is essential to protect the wound surface and promote patient comfort.⁶ One important component of the wound-healing process is growth factors. Growth factors are cytokines that stimulate cell proliferation and migration. These growth factors include platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor beta (TGF- β), epidermal growth factor (EGF), and insulin-like growth factor (IGF).⁸

Platelet-rich fibrin (PRF) is an autologous platelet concentrate that contains a variety of growth factors and is widely used in regenerative therapy. The results of this study show that the HI in the PRF group on the fifth day was higher compared to the group using a non-eugenol periodontal dressing (NEP). The results of this study are thought to be related to the high levels of PDGF-AA, PDGF-AB, PDGF-BB, TGF- β , VEGF, EGF, and IGF, with the highest release occurring within the first 24 hours after application.⁹⁻¹²

PRF has a stronger therapeutic impact in the early stages of wound healing than NEP. This is evidenced by the higher HI in the PRF group on the fifth day. However, once the initial epithelialization phase is complete, the penetration of growth factors from PRF into tissues declines, resulting in a minor but significant increase in the healing index from the fifth to the seventh day. In contrast, the NEP group's healing process accelerated once the covering material was removed on the fifth day, as indicated by a higher HI between the fifth and seventh days. The HI in this study was calculated using the criteria proposed by Landry et al. (1988), which evaluated healing based on five parameters: tissue color, bleeding, granulation, incision edge limit, and suppuration.⁷ This observation is applicable for objectively monitoring the healing process and detecting problems early.¹³

In addition to the clinical parameters of wound healing, patient comfort is a crucial factor in postoperative aesthetic periodontal care. Patient comfort after surgery can be measured subjectively using the Visual Analog Scale (VAS). This study demonstrated that VAS scores decreased over time in both groups; however, the PRF group consistently exhibited lower pain scores than the NEP group. This finding is consistent with previous research showing that PRF can reduce pain intensity and the need for postoperative analgesics, as observed in tooth extraction and odontectomy cases.^{14,15} The analgesic effects of PRF are thought to be mediated by growth factors, including TGF- β and PDGF. These two growth factors not only promote tissue healing but also exhibit anti-inflammatory and analgesic properties. Furthermore, PRF, which promotes wound healing, helps to shorten the duration of discomfort. Unlike PRF, NEP simply protects against mechanical injuries and has no biological consequences.⁵

Epithelialization is a key indicator in the postoperative phase of soft tissue regeneration. This process is typically completed within 48 and 72 hours postoperatively. In general, the overall tissue regeneration phase occurs between the third and the eleventh day.¹⁶

The evaluation of epithelialization in this study was conducted using the Wound Healing Score, which employed toluidine blue dye to detect areas of the wound that had not undergone complete epithelialization. The results showed that on the fifth day, there was no significant difference between the liquid PRF and NEP groups. On the seventh day, the liquid PRF group showed greater epithelialization than the NEP group, although the difference was not statistically significant ($p>0.05$). These findings are consistent with those that mention the effect of PRF on epithelialization became apparent on the third day but was no longer significant by the fifth day.⁶ This indicates that PRF is crucial in the early stages of epithelialization, but its long-term effects are comparable to those of traditional techniques.

The findings of this study are consistent with previous study regarding the therapy of gingival depigmentation.¹⁷ A previous case study demonstrates that using Concentrated Growth Factor (CGF) as a

bandage membrane after gingival depigmentation can accelerate healing, reduce inflammatory cell infiltration, and increase patient comfort (as indicated by a higher VAS score). Another study found that liquid PRF can change the gingival phenotype, specifically gingival thickness, in patients with thin biotypes after a few months of observation. However, that study's findings revealed that the width of keratinized tissue and other cosmetic factors did not differ considerably.¹⁸

Within the limitations of subjects in this study, it can be concluded that liquid PRF promotes faster wound healing than NEP. Both liquid PRF and NEP provide comparable levels of comfort and epithelialization following gingival depigmentation with the conventional technique. Further research with a larger sample size is required to generalize the clinical outcomes.

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