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Clinical effect of platelet-rich fibrin layering on peri-implant keratinized tissue

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Peri-implant tissues are very important for the long-term stability of dental implants. Sufficient width of keratinized tissue has been associated with lower levels of plaque formation, and reduced inflammation of the gingiva. Greater thickness of soft tissue has also been associated with less bone resorption. There is evidence that supports the notion that platelet-rich fibrin (PRF) can be used in enhancing the healing and regeneration of soft tissue. The primary objective of this study was to evaluate the effect of different numbers of PRF membrane layers on the thickness and width of keratinized tissue around dental implants during the early healing phase. Fifteen participants per group (N = 45) were sequentially assigned to one of three groups: single PRF membrane group, multiple PRF membrane group, and control group. A standardized two-stage implant placement procedure was performed. The outcomes included the estimation of keratinized tissue width (KTW) and thickness (KTT). The measurements were recorded preoperatively and six and twelve weeks postoperatively. The use of PRF either as a single membrane or in three layers did not cause any significant increase in the keratinized tissue width at the postoperative 6-week or 12-week time points. At the same time, the single and multiple PRF groups had significantly greater increases in the thickness of crestal and buccal soft tissue compared with the control group at 12 weeks. Platelet-rich fibrin had minimal effect on the keratinized tissue width. However, its application, particularly in three layers, significantly enhanced the thickness of buccal and crestal tissues.

Keywords: dental implant; A-PRF; peri-implant tissues; keratinized tissue.

Introduction

The hard and soft tissues adjacent to an implant are very important for the long-term stability, esthetics, and biological seal around dental implants (Monje et al., 2023). Despite the fact that the quality, quantity, and cortical thickness of the bone are important for implant success, soft tissues play important role in the long-term stability (Hassan et al., 2022; Hassan & Al-Radha, 2023). Advancements in implant surface modifications such as laser texturing have demonstrated that tailored surface roughness can enhance the soft tissue integration by promoting cell adhesion and extracellular matrix organization (Hussein et al., 2024). Such tissues include the keratinized mucosa, which improves oral hygiene status and mechanical protection (Kabir et al., 2021). Clinically, keratinized tissue is defined in terms of its width, which is the measurement from the free gingival margin to the mucogingival junction, and thickness, which is the buccolingual measurement from the gingival surface to the bone surface underneath (Wang & Zucchelli, 2019). These two parameters are considered important markers of the health status of peri-implant tissues (Sculean et al., 2014).

Sufficient width of keratinized tissue surrounding dental implants has been associated with lower levels of plaque formation and reduced inflammation of the gums. It also supports enhanced comfort for the patients when performing oral hygiene routines (Ramanauskaite et al., 2022). Similarly, greater thickness of soft tissue has also been associated with less bone resorption at the margins and improved bone stability around the implant. This, as a result, maintains the integrity of the peri-implant seal (Monje et al., 2023). It is important to emphasize that both the width and thickness of keratinized tissue are relevant from the clinical perspective. However, the discussions in the literature have historically placed greater emphasis on the width of keratinized tissue while the thickness has received comparatively less attention. More recently, there is evidence that supports the notion that platelet-rich fibrin (PRF) can be used in enhancing soft tissue healing and regeneration due to the growth factors it contains and composition of the fibrin scaffold (Miron et al., 2017). Nonetheless, there is very limited information available on the changes in KTW and KTT during healing in relation to the amount of PRF used.

The primary objective of this study was to evaluate the effect of different numbers of PRF membrane layers on the thickness and width of keratinized tissue around dental implants during the early healing phase. The null hypothesis was that the application of multiple stacked layers of PRF will not lead to a greater increase in both KTT and KTW compared with the control and single-layer PRF groups. The alternative hypothesis was that the application of multiple layers of PRF will lead to a greater increase in both KTT and KTW compared with the other groups.

Materials and methods

This prospective clinical trial was conducted at the Implantology Unit, College of Dentistry, University of Mosul, Iraq, from December 2024 to May 2025. Ethical approval was obtained from the Scientific Research Committee, Department of Oral Surgery and Periodontology, College of Dentistry, Mustansiriyah University (MUOSU-202131). It was registered at ClinicalTrials.gov under ID: NCT07033351.

Both male and female participants aged between 18 and 65 were consecutively recruited from the teaching clinics at the Implantology Unit, University of Mosul. Eligible participants presented with partially edentulous spaces in the posterior region of the jaws and met the following inclusion criteria: optimal dental health (dental health was defined as gingival bleeding index <10% and O'Leary plaque index <10%; screening was performed by a calibrated examiner through clinical and radiographic examination), no history of bone augmentation procedures at the implant site, and the ability to attend follow-up appointments.

The exclusion criteria included bleeding disorders, immune deficiencies, heavy alcohol or tobacco consumption, poor dental hygiene, diabetes, or inability to comply with follow-up visits. Eligibility was determined via clinical examination, patient history, and radiographic assessment. All the participants provided informed consent after receiving verbal and written information about the study. The patients were aware that they were free to withdraw from the study at any time.

The study was conducted to evaluate the effects of one versus three layers of platelet-rich fibrin (PRF) membranes on the healing of peri-implant soft tissue compared with the control group. A power analysis was conducted using G*Power software (Version 3.1.9.7) to determine the required sample size. A significance level of P = 0.05, a power of 80%, and a medium effect size (f = 0.25) for a three-group repeated measurement design were considered for the analysis. A minimum of twelve participants per group was deemed sufficient, but the sample size was increased to fifteen participants per group (N = 45) to account for potential dropouts. The participants were sequentially assigned to one of the three groups according to a predefined allocation schedule: single PRF membrane group (n = 15), multiple PRF membrane group (n = 15), and control group (n = 15).

The measurements were recorded preoperatively, six weeks postoperatively (only for KTW), and twelve weeks postoperatively.

To reduce variability, a single operator carried out all surgical procedures. A sterile 15c blade was used to make a crestal incision with sulcular incisions on the neighboring teeth after sufficient local anesthetic had been achieved. The implants (CyberMed Core 1, Daejeon, South Korea) were chosen based on the preoperative CBCT ridge width and bone height. Using periosteal elevators, a full-thickness mucoperiosteal flap was reflected, and the osteotomy site was prepared. A standardized two-stage procedure was used. Every patient underwent the same surgical procedure. In the control group, the surgical site was closed with 4/0 nylon sutures with a simple interrupted technique. In the single and triple PRF membrane groups, the respective PRF membranes were adapted to cover the implant before suturing.

The platelet-rich fibrin membranes were prepared following the protocol described by Kobayashi et al., (2016). Blood samples were collected from the median cubital vein using sterile A-PRF glass vacutainer tubes (Process for PRF, Nice, France). The samples were immediately centrifuged at 1,500 rpm for 14 minutes using a laboratory centrifuge (Qingdao Shengzeyun Experimental Equipment Co., Shandong, China). After centrifugation, the tubes were left to rest for three minutes to complete the oxygenation process. The PRF clots were then extracted and placed in a standardized compression box (Jalal Surgical, Sialkot, Pakistan) to ensure a uniform membrane thickness of 1.5 mm. This thickness was standardized across all samples based on a uniform compression technique developed by the authors using metal stoppers (1.5 mm thick) placed inside the PRF box. For the single PRF membrane group, one membrane was placed over the implant site, ensuring passive contact with the surrounding soft tissues. For the triple PRF membrane group, three membranes were stacked and further compressed to produce a condensed 1.5 mm-thick membrane, which was then placed over the implant site.

The method of measuring Keratinized Tissue Width (KTW) involved the following steps and can be seen in Fig. 1:

1. Reference point creation: A prefabricated stent was placed over the site, and a sharp endodontic file was inserted through a hole in the stent to mark a bleeding reference point on the crest of the ridge.

2. Demarcation of mucogingival junction: Lugol's iodine solution was applied to the oral tissues. This solution stains glycogen in the oral mucosa, forming a dark brown or black complex, while keratinized gingiva remains unstained, creating a clear demarcation (Baghele & Bezalwar, 2022).

3. Measurement: A highly flexible 15/03 PLEX-V file (Orodeka, Jining, Shandong, China) with a rubber stopper was used to measure the distance from the mucogingival junction to the reference point. The file tip was placed at the mucogingival junction, and the stopper was positioned at the bleeding point.

4. Recording: A digital vernier caliper was used to measure the distance from the file tip to the stopper to obtain the KTW dimensions.

Measurements were performed preoperatively, six weeks postoperatively, and twelve weeks postoperatively for each implant site.

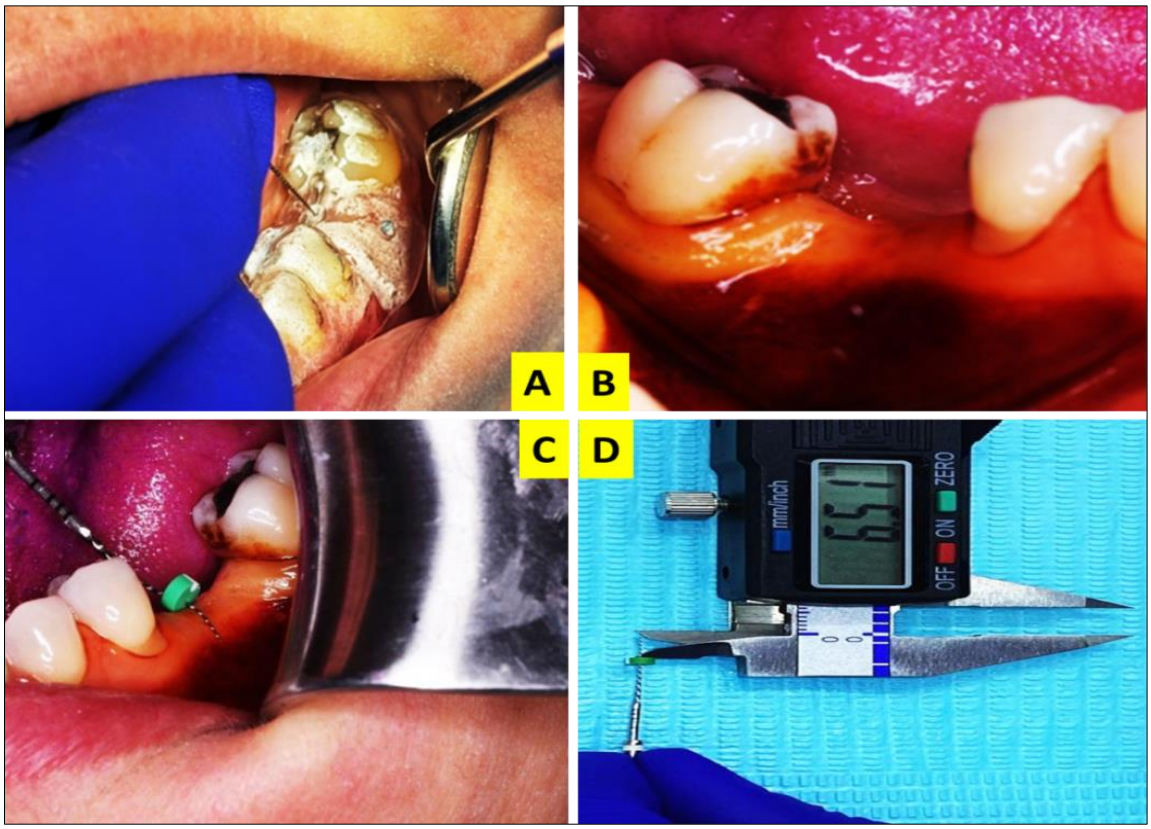

Fig. 1. Measuring the keratinized tissue width (KTW): *A* – marking the tissue with the stent is in place, *B* – demarcation of the mucogingival junction using Lugol's iodine solution, *C* – making the measurement of the keratinized tissue width, *D* – reading the measurement using digital vernier caliper

The assessment method for keratinized tissue thickness (KTT) involved the following steps (Fig. 2):

1. Preparation: A prefabricated stent was created with perforations at three reference points (crest, buccal, and lingual) over the implant site. The stent ensured consistent measurement locations.

2. Reference point creation: The stent was placed intraorally, and an endodontic file was inserted perpendicularly through the holes in the stent to mark the reference points on the gingival tissue.

3. Measurement: A UNC-15 periodontal probe (Hu-Friedy, Chicago, Illinois, USA) with a rubber stopper of uniform thickness (1.30 mm) was inserted into each reference point until it contacted the bone. The stopper was adjusted until it contacted the gingival surface.

4. Recording: The distance from the probe tip to the stopper was measured using a digital vernier caliper.

Measurements were performed preoperatively and twelve weeks postoperatively at the crest, buccal, and lingual reference points.

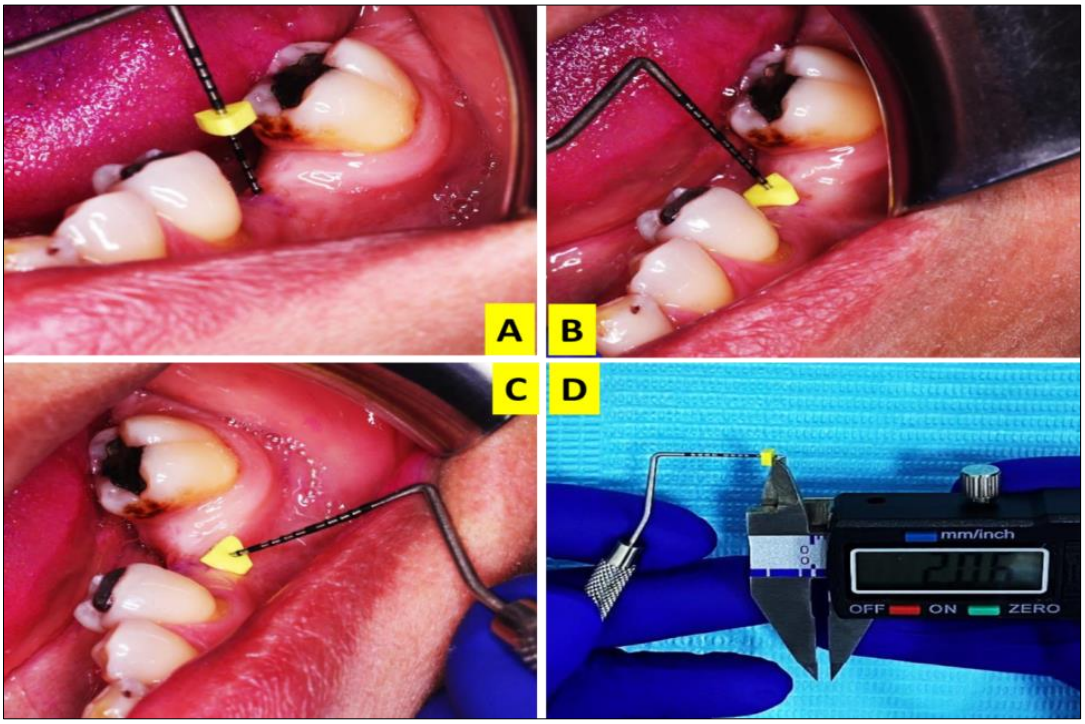

Fig. 2. Measuring the keratinized tissue thickness: *A* – insertion of the probe, *B* – the stopper is brought into contact with the tissue surface, *C* – measurement at the buccal side, *D* – reading the measurement using digital vernier caliper

The statistical analysis was performed using IBM SPSS 26.0. The normality of these data was tested by the Shapiro-Wilk analysis, and the parametric tests were chosen. Frequencies and means, in addition to standard deviations, were calculated. The Chi-square (χ^2) test was used for the nominal data, and the Freeman-Halton Exact test was used instead of Chi when any cell had an expected value less than 5 in tables larger than 2 by 2. The numerical data were compared by one-way ANOVA for the mean differences among the multiple groups (more than 2 groups) under the study, with the post hoc test to find the honestly significant difference among the significant results of one-way ANOVA, and by the paired t-test performed for the numerical data of two paired groups. The general linear model for repeated measurements was used to find the differences among the paired data of more than two groups. Significance was determined at a p-value threshold of ≤ 0.05.

Results

In each group, the participants received the intended treatment and were analyzed for the primary outcomes. Recruitment began in December 2024 and was completed in February 2025. The follow-up assessment for the last enrolled participants was completed in May 2025.

The comparison of the sociodemographic characteristics among the studied groups is demonstrated in Figure 3. The differences concerning age, gender, and location were not statistically significant.

Table 1 shows a comparison of the changes in the keratinized tissue width among the groups at different time intervals. Although the PRF groups showed a greater increase in the tissue width, no significant statistical differences were found among the groups or along the time intervals. Figure 4 shows how these changes took place at the different time intervals of the measurement.

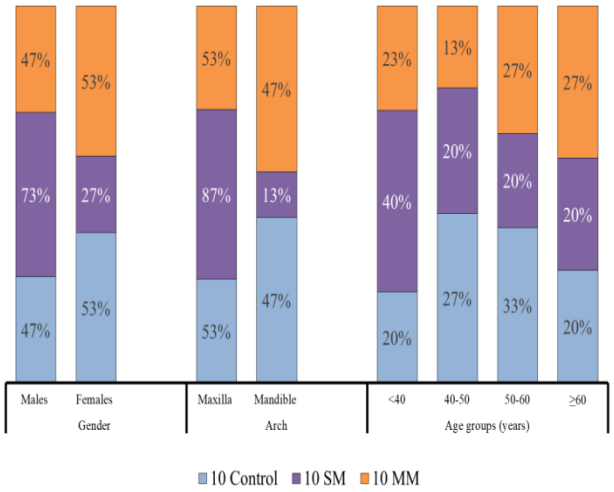

Fig. 3. Comparison of the sociodemographic characteristics among the studied groups

Table 1
Changes in keratinized tissue width (mm)
in the studied groups along the time intervals (mean ± SD)

Keratinized tissue thickness	Control	SM	MM	P-value*
Post-Op	5.28 ± 1.12	6.06 ± 1.32	5.31 ± 1.17	0.148
6 Weeks	6.05 ± 1.11	6.76 ± 1.95	5.94 ± 1.35	0.278
12 Weeks	5.70 ± 1.45	6.33 ± 1.33	6.02 ± 1.39	0.471
P-value†	0.245	0.466	0.280	–

Note: *one-way ANOVA test; †general linear model (repeated measures).

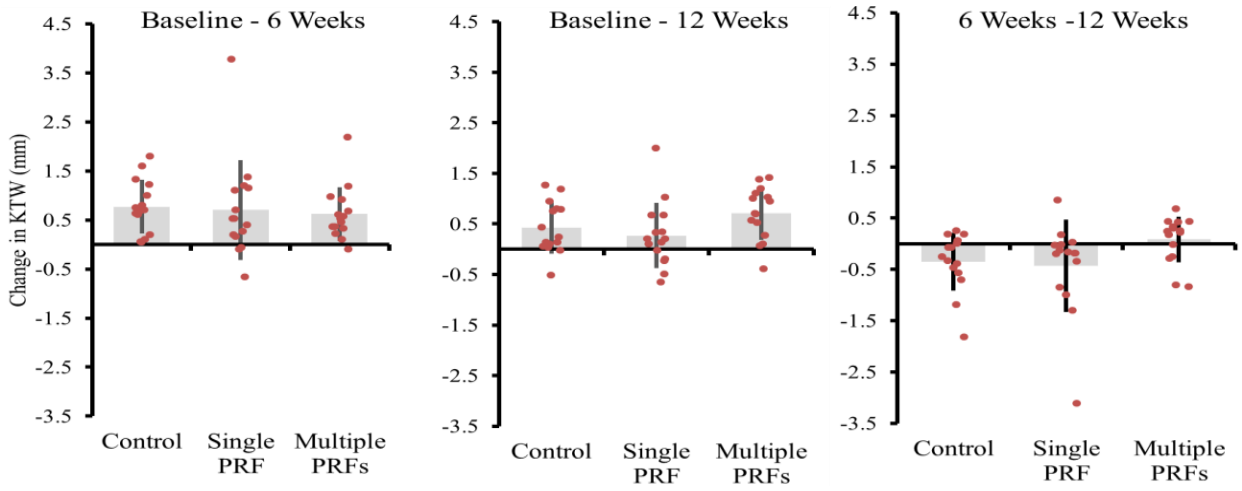

Fig. 4. Mean change in keratinized tissue width (KTW) with individual case distribution across study groups at different time intervals

Table 2 demonstrates the comparison of the tissue thickness at the crest, buccal, and lingual sites among the studied groups, revealing that the tissue thickness at the crest and buccal surface in the 1 PRF and 3 PRF groups were significantly higher than that in the control at 12 weeks which in turn were significantly higher than those at 0 week. At the same time, the tissue thickness on the lingual surface showed no significant statistical differences. Figure 5 demonstrates the changes in the soft tissue thickness at the three sites 12 weeks after the intervention.

Table 2
Changes in keratinized tissue thickness (mm): crest, buccal, and lingual among the studied groups along the time intervals (mean ± SD)

Tissue thickness	Control	SM	MM	P-value*
Crest (0 week)	2.06 ± 0.47^{aA}	1.79 ± 0.54^{aA}	1.83 ± 0.63^{aA}	0.368
Crest (12 weeks)	1.93 ± 0.44^{aA}	2.27 ± 0.64^{bB}	2.44 ± 0.39^{bB}	0.028
P-value†	0.469	0.007	0.005	–
Buccal (0 week)	1.49 ± 0.50^{aA}	1.34 ± 0.39^{aA}	1.41 ± 0.34^{aA}	0.606
Buccal (12 weeks)	1.66 ± 0.50^{aA}	1.65 ± 0.41^{aB}	1.96 ± 0.03^{bB}	0.049
P-value†	0.346	0.042	<0.001	–
Lingual (0 week)	1.82 ± 0.58^{aA}	1.81 ± 0.65^{aA}	1.48 ± 0.52^{aA}	0.198
Lingual (12 weeks)	1.84 ± 0.50^{aA}	2.04 ± 0.59^{aA}	1.75 ± 0.39^{aA}	0.282
P-value†	0.905	0.322	0.111	–

Notes: * – one-way ANOVA test; ^{a}, ^{b} – different lowercase superscript letters within a row indicate significant differences between groups (Tukey post-hoc test); † – paired t-test; ^{A}, ^{B} – different uppercase superscript letters within a column indicate significant differences over time.

Discussion

Achieving ideal parameters of the peri-implant soft tissue during the initial phases of healing could improve the long-term prognosis for implants (Jung et al., 2022). Despite various surgical and grafting options to increase keratinized tissues, clinicians are transitioning to using autologous biomaterials, such as platelet-rich fibrin (PRF), due to their lower invasive qualities and greater healing potential (Miron, Zucchelli, et al., 2017). Although PRF is associated with favorable results in soft tissue reconstruction, most studies evaluate the outcomes in a simplistic manner, that is, with PRF or without PRF, without considering if there is a dose-dependent relationship regarding the number of PRF layers and tissue outcomes (Thoma et al., 2018). Knowing if different amounts of PRF could be directly related to the KTW and KTT would provide a better biological guiding principle for clinicians to enhance the condition of peri-implant tissues.

This study sought to examine the impact of different numbers of layers of PRF membranes on soft tissue phenotype around the implant, as these are essential to the durability and esthetics of the dental implant (Schwarz & Ramanauskaite, 2022). Platelet-rich fibrin is an autologous matrix of fibrin that contains a high amount of platelets and leukocytes and is capable of stimulating the formation of new blood vessels, modifying inflammation, and healing the soft and hard tissues (Strauss et al., 2020). A clinical study design was utilized with three groups: one control group that did not receive PRF, a group that received one PRF membrane, and a group that received three stacked PRF membranes. The key parameters assessed included keratinized tissue width (KTW) and thickness (KTT) across at the baseline, 6-week, and 12-week intervals. The results revealed that while PRF had minimal effect on the keratinized tissue width, its application, particularly with three layers, significantly enhanced the thickness of the buccal and crestal tissues. This study is among the first to directly compare different PRF membrane quantities in a clinical implant context, highlighting the dose-responsive nature of PRF-mediated regeneration.

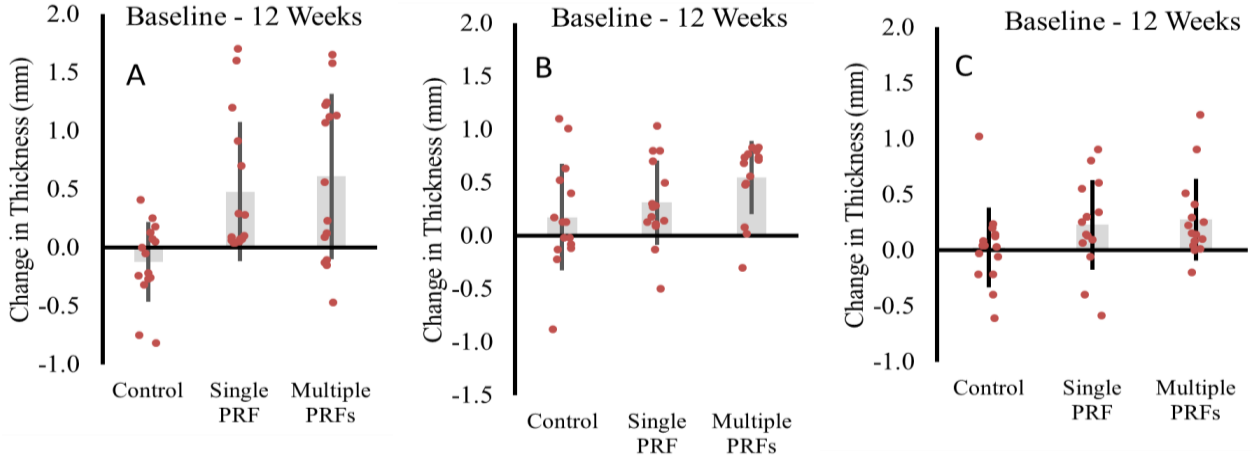

Fig. 5. Mean change in the tissue thickness with individual case distribution across study groups at different time intervals: *A* – crestal; *B* – buccal; *C* – lingual

In the current study, the use of PRF either as a single membrane or in three layers did not cause any significant increase in the keratinized tissue width (KTW) at the postoperative 6-week or 12-week time points. This suggests that the use of PRF alone might not increase keratinized mucosal dimensions to a significant degree in the short term. Although the PRF groups did demonstrate some minor numerical increases over time, these changes remained insignificant when compared with the control group and did not reach clinical relevance. The within-group analysis revealed that all groups had a stable KTW over time without a definitive upward or downward trend, indicating limited influence of PRF-mediated modulation on the natural healing for this parameter.

These results support the observations of Rodas et al. (2020), who in the systematic review and meta-analysis noted that PRF's performance was comparable to subepithelial connective tissue grafts for almost every periodontal parameter, while keratinized mucosa width was the exception. Likewise, Moeintaghavi et al. (2023) in a randomized controlled trial, reported no significant difference in KTW at the PRF-treated sites and control sites at baseline, one month, and three months after implant placement.

In comparison, the findings from the study by Cheruvu et al. (2023) presented positive results regarding the gain in keratinized tissue as the PRF membrane improved the healing of the peri-implant tissues and increased the KTW around the unsubmerged implants. Moreover, Al-Diasty et al. (2022) did report some increase in the width of keratinized mucosa around dental implants with the use of PRF membranes. The same study revealed, however, that free gingival grafts had a significantly greater potential to augment and enhance the keratinized mucosa around dental implants.

Possible reasons for the lack of notable results in our investigation include the anatomical constraints of the sites treated, short duration of the study's follow-up period, and the lack of a surgical flap advancement, which involves repositioning the flap to its original position.. This could explain why our results differ from Cheruvu et al. (2023), who used unsubmerged implants. Also, the impact of PRF on the tissues may be more significant regarding the thickness rather than the width, which is a critical distinction highlighted in the literature on soft tissue phenotypes. While the use of PRF in our study showed significant advantages regarding the dimensions of soft tissues, its effect on the increase of the KTW seems to be minimal when applied alone.

The findings in this study showed that at 12 weeks, the 1 PRF and 3 PRF groups had significantly greater increases in the thickenss of the crestal and buccal soft tissues compared with the control group. On the other hand, no group or time point showed significant differences in the thickness of the soft tissue on the lingual side. The increase in the crestal and buccal thickness indicates that PRF might have the potential to improve soft tissue augmentation in areas that are primarily subjected to surgical and prosthetic tension (Miron et al., 2017). Comparing the outcomes within the groups, both PRF-treated groups, in comparison with the control, had greater increases in crestal and buccal thickness by the 12-week mark. By contrast, this parameter in the control group did not significantly change over the same period.

The findings coincide with the systematic review demonstrating a significant soft tissue gain when PRF was used along with mucogingival surgery (Ardila et al., 2023). Similarly, Da Silva Lima et al. (2022) noted a marked enhancement in the buccal mucosa thickening following the use of PRF membrane during implant placement due to the PRF's presumed angiogenic and scaffolding features. The rationale in biology is noted with the increasing development of factors such as VEGF, EGF, and PDGF contained in PRF matrix, which enhance the fibroblast replication and collagen formation, as well as the formation of new blood vessels. All of these processes facilitate increase in soft tissue volume (Bayer et al., 2021).

Nevertheless, other contrasting evidence exists. One study reported no clinically measurable changes in the thickness of the palatal mucosa after the application of L-PRF (Alvarez-Medina et al., 2023). This might indicate anatomical and blood supply differences, as the lingual side typically undergoes less surgical manipulation, possibly explaining the lack of significant change in that area in our study as well (Rawaa & Mikdad, 2020). Another explanation could be the continuous pressure on the lingual side from the tongue during normal physiological actions such as swallowing and eating, which act like messaging to the newly vulnerable lingual area after surgery (Elkashty et al., 2023).

Possible increases in buccal and crestal thickness with the use of PRF in implants may result in better cosmetic results, improved mucosal sealing around implants, and a lesser chance of peri-implant mucosal recess in the future (Elkashty et al., 2023). Nevertheless, patient-specific factors such as BMI and body composition have been shown to influence dental implant outcomes, including healing. For example, obese patients tend to have poorer healing outcomes, which may be attributed to systemic inflammation and altered immune responses (Al-Radha, 2023).

These results support the use of PRF as a soft tissue modulator, particularly where buccal soft tissue support is critical, while also highlighting its limitations in augmenting lingual tissues. The dose-dependent effects of PRF warrant further study, especially when combined with bioactive surface modifications (e.g., nano-HA/TiO_2 coatings), which have demonstrated similar layer-dependent enhancements in bone to implant contact (Nasir & Rahman, 2016). Future research should explore hybrid approaches leveraging both biological and engineered interfaces.

Conclusion

The study revealed that while the application of platelet-rich fibrin resulted in only a minimal change to the width of the keratinized tissue, it produced a significant increase in the tissue thickness at both the buccal and crestal regions. This enhancement was especially pronounced after a three-layer application of the PRF.

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The authors declare no conflict of interests.

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