Innovative i-PRF semisurgical method for gingival augmentation and root coverage in thin periodontal phenotypes: A preliminary study

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Research Article

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Abstract

Objectives

The aim of this study was to evaluate the effect of injectable platelet-rich fibrin (i-PRF) on gingival thickness (GT) and gingival recession (GR) in individuals with thin periodontal phenotypes using a semisurgical approach.

Materials and methods

In this prospective study, i-PRF was applied via a semisurgical method to augment 53 tooth regions with thin periodontal phenotypes. i-PRF injection was applied to the relevant areas in 4 sessions at an interval of 10 days. GT, GR, keratinized tissue width and periodontal parameters were also recorded before treatment and at 6 months after the last injection.

Results

A statistically significant difference was observed in GT and GR values at the end of the study compared to baseline. Accordingly, an increase in GT was achieved in 92.5% of the areas treated with i-PRF, and the desired GT (0.8 mm) was achieved in 44.9% of these areas. In addition, significant reductions in the amount of recession were observed in 83.3% of the 12 GR areas (p = 0.005). Moreover, complete coverage was achieved in 60% of these regions.

Conclusion

With the new i-PRF semisurgical method, which we introduced in this first preliminary study, we showed that GT can be increased in tooth regions with thin gingiva and that areas of GR can be covered. Further comprehensive studies are needed to fully understand the role of i-PRF in enhancing angiogenesis and the histoconductive properties of this fully autogenous blood concentrate.

Clinical relevance

Classical periodontal plastic surgery applications cannot give predictable results in areas with thin periodontal phenotypes, especially in the case of bone dehiscence and fenestration. In some cases, undesirable progressive GR in these tooth regions also draws attention. With this new i-PRF semisurgical method, successful and predictable treatments can be applied in tooth areas with thin gingiva by increasing angiogenesis and taking advantage of the histoconductive properties of i-PRF.

Introduction

The term “periodontal phenotype” refers to the phenotypical characteristics of bone and the overlying gingival phenotype, which includes the keratinized tissue width (KTW) and gingival thickness (GT). The
periodontal phenotype may be modified by environmental factors and clinical interventions such as protruding restorations, orthodontics, or autogenous gingival grafting procedures [1].

A thin periodontal phenotype may contribute to gingival recession (GR) [2, 3]. In addition, many conditions, such as poor oral hygiene [4], periodontal disease [5], smoking [6], inadequate restorations [7] and improper tooth positions [8], are associated with the progression of GR. In a systematic review, researchers found that proclined teeth showed a higher incidence and severity of GR [9]. In a case-control study, it was found that the risk of GR in orthodontically treated teeth was found higher than untreated teeth with a significant odds ratio of 4.48 [10].

The periodontal phenotype also affects the mucogingival surgical technique to be chosen for the treatment of GR [11]. The initial GT has been found to be the most important factor affecting the likelihood of achieving complete root coverage, and a thicker flap is associated with a more predictable prognosis in root closure procedures. In coronally advanced flap operations for Miller Class I or II recession defects, it has been reported that a flap thickness of > 0.8 mm results in 100% root surface closure, whereas a thin flap (< 0.8 mm) results in partial root closure [12]. Thick gingival tissues are more resistant to mucosal regression or mechanical irritation. Therefore, there is a need to transform a thin phenotype into a thick phenotype to maintain healthy periodontal tissue [13].

In recent years, blood concentrates have been proposed to further accelerate tissue regeneration in dentistry and medicine. Platelet-rich fibrin (PRF) and its various forms have become very important in soft tissue augmentation [14–16]. Rodas et al. [17] previously reported that PRF exhibits excellent properties that enable it to be used as an important aid in the wound healing process, and therefore, its use should be considered an alternative to CTG in the treatment of GR. PRF is a second-generation platelet concentrate that does not use anticoagulants during its preparation [18–20].

Rapid neovascularization is vital for tissue repair and regeneration, as angiogenesis enables the migration of cells adjacent to the matrix, which allows repopulation in the injured area and the initiation of tissue repair [21]. However, cytokine adhesion and support for new vessels are required for angiogenesis [22]. Studies have shown that the three-dimensional structure of PRF allows the adhesion of new vessels and stimulates the activity of regulatory cytokines trapped in the fibrin network. For these reasons, PRF can be considered an ideal biomaterial for tissue engineering, i.e., a network that acts as a scaffold for living cells to support tissue repopulation and molecules that stimulate repair [23, 24].

In our first study [25], the application of only i-PRF or i-PRF + microneedling (MN) was found to be effective in surgical periodontal treatment in individuals with a thin periodontal phenotype. Both methods have been shown to be effective in improving GT independently of each other. Although gingival phenotype thickening was partially achieved, the i-PRF injection needed to be administered apical to the mucogingival composition because the lamina propria firmly adhered to the underlying bone and root in the areas of thin gingiva.
Therefore, the aim of this study was to increase the GT in regions with a thin gingival phenotype using repetitive i-PRF injections in addition to the VISTA technique. Second, we investigated the effect of this method on the treatment of GR in regions with a thin phenotype.

**Materials and methods**

This study, a prospective clinical study with a 6-month follow-up, was conducted in accordance with the Declaration of Helsinki and was approved for human subjects by the Clinical Research Ethics Committee of ... (name was deleted for blind review) University (approval no: 2023/01–08). All individuals participating in the study were informed about the purpose and method of the study and signed an informed consent form.

**Patient Selection**

A total of 53 tooth regions with a thin gingival phenotype in individuals who applied to ... (name was deleted for blind review) University Faculty of Dentistry Department of Periodontology for various reasons were included in this study. The inclusion criteria were as follows: 1) ≥ 18 years of age; 2) absence of systemic disease or pregnancy/breastfeeding; 3) nonsmoker; 4) full-mouth plaque index (PI) and bleeding on probing score ≤ 15%; 5) GT of mandibular teeth < 0.8 mm [12]; 6) absence of active orthodontic treatment, malocclusion, crowding, and filling; and 7) absence of a blood-borne condition.

All volunteers underwent Phase I periodontal therapy to eliminate any potential presence of gingival inflammation and were instructed to maintain oral hygiene using the nontraumatic tooth brushing technique.

**Determination of Periodontal Phenotype**

During measurement of the periodontal probing depth (PD), patients with high periodontal probe visibility in the buccal gingival sulcus were identified. The GT was measured in these patients using an endodontic spreader, and regions with GT < 0.8 mm were diagnosed as having a “thin phenotype” [12]. During the detection of regions with a thin gingival phenotype, areas of GR were also recorded. The risk of progressive GR of the regions was evaluated from the CBCT images of the patients.

**Clinical Measurements**

Clinical periodontal parameters included PI [26], GI [27], probing depth (PD), and GR recorded at six sites (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, distolingual) for each tooth. Measurements were made using a 10 mm periodontal probe (PWD6 Williams periodontal probe, Hu Friedy, Chicago, IL, USA). Digital callipers with a resolution of 0.1 mm were used to measure the GT and KTW (Fig. 1).
**Keratinized tissue width (KTW)**

The mucogingival junction was determined using Schiller's iodine solution. The distance from the free gingival margin to the mucogingival junction was determined using a periodontal probe.

**Gingival thickness (GT)**

To measure the GT, a No. 15 endodontic spreader was placed in the middle of a 3-mm-diameter silicone disc. The spreader was advanced from the vestibular midpoint of the gingiva perpendicular to the apical 1.5 mm of the gingival margin until a hard surface could be felt. Since the gingival margin coincided with the coronal edge of the silicone disc at that time, the GT measurement was made 1.5 mm apical to the gingival margin. The depth of penetration between the silicone disc and the spreader tip was measured [28].

**Preparation of i-PRF**

Venous blood samples, taken once from each patient with a 20 ml syringe, were transferred to two i-PRF tubes without anticoagulant and centrifuged with an Intra-Spin® L-PRF Centrifuge (Intra-Lock International, Boca-Raton, FL, USA, Germany) for 3 minutes at 2500 rpm (400 g) at room temperature. The obtained i-PRF samples were drawn into 2.5 cc dental injectors, and i-PRF injection was performed in the relevant area with 27 gauge dental injector needles.

**Treatment Protocol**

Treatment Protocol

After completing the Phase I periodontal treatment, local anaesthesia was applied to the planned area of i-PRF injection. With the help of a scalpel, a minimal incision was made in the apical region of the relevant region, and the periosteum was horizontally elevated by entering through the incision made with a microsurgical instrument. Thirty minutes after this procedure, the blood sample taken from the patient was subjected to the procedure described above, and i-RPF was obtained and injected into the relevant area using a dental injector (Fig. 2).

When it was necessary to control the bleeding caused by the needle tip after the procedure, a saline-soaked sponge was placed between the lip and the gingiva [29]. A total of 4 i-PRF injections were administered to the individuals over an interval of 10 days [25, 30] (Fig. 1). Illustration of the procedure was presented in Fig.x.

**Statistical Analysis**
The data were analysed using IBM® SPSS® Statistics for Windows (Version 20.0. Armonk, NY: IBM Corp.). Descriptive statistics were determined, and the distributions of the variables were calculated using the Kolmogorov–Smirnov test. The Wilcoxon test was used to compare data that were not normally distributed between baseline and the 6-month follow-up. The significance level was set at \( p < 0.05 \).

**Results**

The study included 53 mandibular tooth regions with a thin gingival phenotype in 6 patients (5 females, 1 male) aged 22–36 years (mean: 25.5 years). GR was observed in 12 of 53 defects. During the postoperative period, uneventful healing was observed without any complications, such as severe pain, bleeding, or excessive sensitivity, in the operated areas. The patients stated that they were extremely satisfied with the results and the treatment method (Fig. 2G, H, I).

The primary outcome parameters of this study were the GT and GR, while clinical periodontal parameters and the KTW were secondary outcome parameters.

A statistically significant increase in GT values was observed at 6 months after the last injection compared to baseline (from 0.39 ± 0.11 to 0.71 ± 0.21) \(( p = 0.000 \) ). Accordingly, an increase in GT was achieved in 92.5% of the regions, while the desired GT \(( \geq 8 \text{ mm} \) ) was achieved in 44.9% of these regions. In addition, a reduction was observed at 83.3% of the 12 detected GR sites (from 2.25 ± 0.97 to 0.63 ± 1.03) \(( p = 0.005 \) ). Moreover, complete coverage was achieved in 60% of these regions. Similarly, statistically significant improvements were found in the PI and GI \(( p = 0.000 \) and \( p = 0.000 \), respectively), while changes in the PD and KTW were not significant \(( p > 0.05 \) ) (Table 1) (Fig. 3). Severe dehiscence type defects in the treated areas were observed CBCT images (Fig. 4).

<table>
<thead>
<tr>
<th></th>
<th>Baseline mean ± SD (median)</th>
<th>6th month follow-up mean ± SD (median)</th>
<th>( p )-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingival thickness (mm)</td>
<td>0.39 ± 0.11 (0.40)</td>
<td>0.71 ± 0.21 (0.70)</td>
<td>0.000</td>
</tr>
<tr>
<td>Gingival recession (mm)</td>
<td>2.25 ± 0.97 (2.25)</td>
<td>0.63 ± 1.03 (0.25)</td>
<td>0.005</td>
</tr>
<tr>
<td>Keratinized tissue width (mm)</td>
<td>2.26 ± 0.89 (2.00)</td>
<td>2.22 ± 0.87 (2.00)</td>
<td>0.864</td>
</tr>
<tr>
<td>Probing depth (mm)</td>
<td>1.50 ± 0.36 (1.70)</td>
<td>1.57 ± 0.28 (1.70)</td>
<td>0.215</td>
</tr>
<tr>
<td>Plaque index</td>
<td>0.72 ± 0.46 (1.00)</td>
<td>0.26 ± 0.45 (0)</td>
<td>0.000</td>
</tr>
<tr>
<td>Gingival index</td>
<td>0.38 ± 0.49 (0)</td>
<td>0.04 ± 0.19 (0)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* Wilcoxon signed-rank test, Statistically significant difference: \( p < 0.05 \)

**Discussion**
According to the findings of this study, a statistically significant increase was observed in GT (92.5% of regions), while the amount of GR decreased significantly (83.3%) in areas with recession defects. Moreover, the desired GT (0.8 mm) was achieved in 44.9% of the regions where the increase was observed. Similarly, complete coverage was achieved in 60% of the GR regions with reductions. Therefore, the application of i-PRF with the VISTA technique can be successful in areas with a thin phenotype and/or a high risk of GR. No complications were observed in the treated areas. In addition, there were no regions in which the GT decreased or the amount of GR increased.

In our previous study [25], we applied the i-PRF and i-PRF + MN methods, and six months later, we achieved GT increases of 44.19% in the i-PRF group and 65.00% in the i-PRF + MN group. We believe that the reason for this limited increase in GT is because the i-PRF could not be injected between either the lamina propria and periosteum or the periosteum and alveolar bone because the attached gingiva was tightly attached to the underlying alveolar bone and did not allow histoconductive effects. Therefore, the i-PRF injection had to be made apical to the mucogingival junction, and we think that the achieved increase in GT was due to the long-term angiogenic effect rather than the histoconductive effect of i-PRF in these methods. In the method applied in the current study, unlike in the previous methods, the most important step was to create a gap with the VISTA technique that would allow the formation of a sufficient blood clot on the side of the gingiva facing the bone. Then, we ensured that the i-PRF reached that area as a result of repetitive injections. Thus, we achieved an increase in the GT not only through long-term exposure to angiogenic growth factors but also through the histoconductive effect of i-PRF and blood clots. In addition, we applied i-PRF nonsurgically to this space that we created with the help of microsurgery in the first session, thus ensuring the continuation of the histoconductive effect and the development of the semisurgical method. We think that the method we have developed is more acceptable because it is a semisurgical method and thus involves less trauma than surgery.

As for many other periodontal conditions, the aetiology of GR is multifactorial and complex, and the exact mechanism is not yet fully understood [31]. Therefore, the selection of the most appropriate soft tissue grafting procedure for root closure requires careful consideration of available techniques. The clinical aim is to create a functional, satisfactory, and aesthetically acceptable predictable result for the patient while causing minimal trauma to the periodontal tissues [32]. The techniques used for root closure are based on the use of absorbable and nonabsorbable membranes according to the principles of tissue regeneration guided by translation (pediculated flap procedures) and grafting (free gingival or connective tissue graft procedures). However, various modifications to conventional techniques have been developed to achieve optimal root closure and better aesthetic integration [33].

Systematic reviews [34, 35] have shown that CAF application is a safe and predictable approach for root closure, and the addition of a connective tissue graft increases the likelihood of achieving complete root closure in areas of Miller Class I and II GR but may compromise the aesthetic outcome. The aesthetic appearance of the bilaminar treated area may contrast with that of adjacent soft tissues for several reasons, including the chromatic difference between the uncovered epithelialized portion of the graft and the adjacent soft tissues [32, 36, 37], dichotomy associated with inadvertent CTG exposure due to flap
dehiscence [38–40], and the difference in thickness between the graft area and adjacent soft tissues [41]. In the current study, the abovementioned causes were unlikely to be found, patient satisfaction was good in terms of aesthetics, and there was no difference in terms of colour change. Similarly, Anuroopa et al. [42] stated that the use of PRF plays a vital role in early wound healing and the development and maturation of a normal vasculature and eliminates the possibility of any immune reaction.

The blood supply to the connective tissue graft is an important element of this technique (CAF + CTG) [43]. The connective tissue graft should extend at least 3 mm beyond the retraction defect margins to allow adequate overlap with the recipient connective tissue bed. This allows the graft to benefit from a double blood supply from both the surrounding connective tissue and the flap [44]. However, since postoperative pain and discomfort are related to the apicocoronal size and depth of the palatal donor site [28], an increase in the size of the connective tissue graft may increase patient morbidity. Indeed, Zucchelli et al. [41] stated that a less painful and more comfortable postoperative course can be expected with the use of shorter and thinner grafts. In addition, free autogenous soft tissue grafting requires a second surgical site with a risk of possible complications such as infection, pain, swelling and necrosis, which cannot be completely eliminated even with meticulous treatment planning and good surgical skills [45]. Moreover, Studer et al. [46] showed that palatal tissue in the first maxillary molar region can be as thin as 1.8 mm. In this case, the underlying anatomical structures and lack of access are the main challenges to obtaining a CTG. There is no need to worry about the limitations and postoperative complications mentioned above during treatment with i-PRF.

Connective tissue grafts can be applied with several different surgical techniques to close areas of GR [45, 47]. The CAF is one of the most widely used flap designs [48]. It involves vertical incisions that may cause fibrotic scars by disrupting the vascularization of the gingival margin in the early stages of wound healing. In addition, this flap design is indicated when treating patients with shallow recession defects less than 4 mm and a thick periodontal phenotype. The presence of insufficient keratinized tissue apical to the recession defect, the presence of a gingival cleft extending to the alveolar mucosa, and the presence of a high frenum pull, a prominent root, or a very shallow vestibule are contraindications for this application [49].

As another method, making a supra- or subperiosteal tunnel through the gingival sulcus causes problems such as difficulty achieving proper graft or membrane placement and increased risk of rupture or perforation of flaps, resulting in inappropriate healing of marginal gingival tissues [50]. To eliminate these negative results and minimize trauma to the surrounding tissues, it is recommended to use a minimally invasive method for flap preparation that can protect the underlying graft tissue and support rapid vascularization [51]. The concept of minimally invasive surgery encompasses all aspects of surgical techniques involving minimal wounds, minimal flap reflection, and gentle manipulation/handling of soft and hard tissues [52, 53]. Various tunnelling techniques have been described to maintain recipient site aesthetics and prevent relapse of recession [54]. A newer approach known as vestibular incision subperiosteal tunnel access (VISTA) has been developed to avoid some of the potential complications that occur with other intrasulcular tunnelling techniques [54, 55]. VISTA not only provides an adequate
blood supply but is minimally invasive, requiring only a small opening at the root closure site that prevents complete damage to the periosteum; this further assists in passive repositioning of the flap over the coronally exposed root [56]. Therefore, the VISTA technique, which is a minimally invasive method, was used in this study. In addition, since successful root closure also depends on the width of the keratinized gingiva [56], the VISTA technique was applied with i-PRF due to the presence of a thin phenotype.

Gupta et al. [57] reported that the combined application of a PRF membrane with the VISTA technique allows clinicians to successfully treat multiple recession defects. Patra et al. [58] also evaluated the effect of i-PRF with a collagen membrane compared to a collagen membrane alone in the VISTA method to cover areas if GR. The authors demonstrated a significant reduction in the amount of retraction in the test and control groups at 6 months post-surgery, as well as significant gains in the mean keratinized tissue thickness. In a recent study, the effect of i-PRF on GR and the KTW was investigated, and there was a 29% increase in GR at the 6th month, but no significant difference was observed in the KTW [59]. The results of the present study confirm these reports in the literature.

Although improvements in GT and GR were achieved over a period of 6 months in this study, other aspects, such as the sample size and level of patient comfort, should also be considered to confirm the effectiveness of the method. In addition, long-term studies are needed to evaluate the effect of the method on creeping attachment.

**Conclusion**

With the i-PRF semisurgical method, which we have newly developed and introduced with this first preliminary study, we prevent progressive GR by predictably thickening the gingiva in areas of thin gingiva. In addition, as we have shown in our study, some areas of GR can be covered. More comprehensive, well-designed studies with longer follow-up periods are needed to fully understand and develop this i-PRF semisurgical method.

**Declarations**

**Acknowledgements** We would like to thank Joseph Choukroun to shed light on our i-PRF researchs.

**Ethical approval** This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Clinical Research Ethics Committee of ... *(name was deleted for blind review)* (2023/01-08)

**Informed consent** Informed consent was obtained from all individual participants included in the study.

**Conflict of interest** The authors declare no competing interests.

**Author Contribution Statement**
The authors confirm their contribution to the paper as follows: All authors contributed to the study conception and design; patient selection was performed by Raif ALAN, Esra ERCAN, Mustafa TUNALI; clinical measurements were performed by Raif ALAN; material preparation, data collection and analysis were performed by Raif ALAN, Esra ERCAN, Yigit FIRATLI; manuscript preparation was performed by Raif ALAN, Esra Ercan, Yigit FIRATLI, Erhan FIRATLI. All authors reviewed the results and approved the final version of the manuscript.

References


Figures
Figure 1

The flow chart of study
Figure 2

Figure 3

Illustration of the i-PRF semisurgical method. A, B VISTA technique. C Waiting 30 min for blood clot stabilization. D i-PRF preparation. E i-PRF injections. F The area after i-PRF injections
Figure 4

Bar plots show parameters at baseline and 6<sup>th</sup> month follow-up. The asterisks (* and **) indicates a significant differences between baseline and 6th follow-up ($p<0.001$ and $p<0.05$, respectively)