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Research article

Efficacy of Concentrated Platelets-Rich Fibrin versus Injectable Platelets Rich Fibrin on Gingival Thickness and Keratinized Tissue Width in Subjects with Thin Gingival Phenotype: Split-Mouth Randomized Clinical Trial

Sajjad Ahmed Shakir[1]*, Suzan Ali Salman[1]

[1] *Department of Periodontics, College of Dentistry, University of Baghdad, Baghdad, Iraq*

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Abstract

Background: Platelet-rich fibrin (PRF) has regenerative characteristics and is used as an autologous source of growth factors for tissue regeneration and wound healing. ***Objective***: Evaluating the efficacy of C-PRF for increasing gingival thickness (GT) and keratinized tissue width (KTW) in subjects with a thin gingival phenotype in comparison with I-PRF injection. ***Methods***: Ten healthy participants with a thin gingival phenotype (GT ≤ 1.0 mm) were enrolled in this study. The upper and Lower arches of the participant's mouth were split into two sides, and each side was randomly injected with C-PRF, while the contralateral side was injected with I-PRF. GT and KTW were assessed before the treatment, 1 month later, and 3 months after the last injection session. ***Results***: Inter-group comparison between I-PRF and C-PRF groups revealed a statistically significant difference at the 3-month follow-up visit, with a mean difference between C-PRF and I-PRF of ±1.373 mm and an effect size of 0.200 at p= 0.048. Intra-group comparison was significant for both groups in both arches except for the I-PRF group in the upper arch for KTW, which was non-significant at p= 0.266. ***Conclusion***: In individuals with thin gingival phenotypes, C-PRF injections may influence an increase in both GT and KTW. The results suggest that the application of C-PRF may be beneficial as a non-surgical method for increasing GT and KTW. (ClinicalTrials.gov NCT05615155).

Keywords: Gingiva, Gingival thickness, Phenotype, Platelet-rich fibrin, Keratinized tissue width, Wound healing.

فعالية الفيبرين المركز الغني بالصفائح الدموية مقابل الصفائح الدموية القابلة للحقن على سمك اللثة وعرض انسجة الكيراتين في الأشخاص ذوي النمط الظاهري الرقيق للثة: تجربة سريرية عشوائية منقسمة الفم

الخلاصة

الخلفية: يتميز الفيبرين الغني بالصفائح الدموية (PRF) بخصائص متجددة ويستخدم كمصدر ذاتي لعوامل النمو لتجديد الأنسجة والتئام الجروح. **الهدف**: تقييم فعالية C-PRFلزيادة سمك اللثة (GT) وعرض الأنسجة الكيراتينية (KTW) في الأشخاص ذوي النمط الظاهري اللثوي الرقيق مقارنة بحقن I-PRF. **الطرائق**: تم تسجيل 10 مشاركين أصحاء مع النمط الظاهري اللثوي الرقيق (GT ≤ 1.0mmفي هذه الدراسة ، وتم تقسيم الأقواس العلوية والسفلية لفم المشارك إلى جانبين وتم حقن كل جانب بشكل عشوائي باستخدام C-PRF وتم حقن الجانب المقابل باستخدام I-PRF. تم تقييم GT و KTW قبل العلاج ، 1 شهر و 3 أشهر بعد جلسة الحقن الأخيرة. **النتائج**: كشفت المقارنة بين مجموعة I-PRF و C-PRF عن اختلاف ذو دلالة إحصائية بعد المتابعة لمدة 3 أشهر مع اختلاف متوسط C-PRF / I-PRF يبلغ ±1.373 مم وحجم التأثير 0.200 عند $p = 0.048$. كانت المقارنة داخل المجموعة ذات دلالة إحصائية لكلا المجموعتين في كلا القوسين باستثناء مجموعة I-PRFفي القوس العلوي ل KTW ، والتي كانت غير معنوية عند $p= 0.266$. **الأستنتاج**: في الأفراد الذين يعانون من الأنماط الظاهرية اللثوية الرقيقة، قد تؤثر حقن C-PRF على زيادة في كل من GT و KWT. تشير النتائج إلى أن تطبيق C-PRF قد يكون مفيدا لطريقة غير جراحية لزيادة GT و KTW .

* *Corresponding author*: Sajjad A. Shakir, Department of Periodontics, College of Dentistry, University of Baghdad, Baghdad, Iraq; Email: sajjad.shakir1959@gmail.com

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INTRODUCTION

The gingival phenotype encompasses not only clinical differences in gingival thickness and keratinized tissue breadth but also other characteristics such as bone morphotypes, tooth form, and gingiva and periodontium morphology. Teeth with thin gingival morphologies are more likely to develop gingival recessions, and teeth with thin gingival phenotypes may respond to surgery more gently, with less predictability of recovery when treating gingival recessions. Following tooth extractions, significant ridge resorption might be predicted. Different gingival phenotypes respond differently to inflammatory, restorative, traumatic, and parafunctional actions. These stressful events induce unique types of periodontal problems that respond differently to different treatments [1]. Platelet concentrates have been employed in dentistry for over thirty years as a regenerative method that can release supra-physiological quantities of growth factors. Platelet-rich fibrin (PRF) is recognized as a game-changing breakthrough in regenerative dentistry. It possesses regenerative properties and is employed as an autologous growth factor source for tissue regeneration and wound healing [2]. PRF has been used as a clot, in conjunction with a bone graft, or as a membrane to improve and promote tissue regeneration in the context of dental implants [3]. Platelet-rich fibrin injections into the socket can improve bone density [4]. When it comes to fixing intra-bony periodontal problems, PRF and decalcified freeze-dried bone allograft (DFDBA) work better together than DFDBA alone [5]. Results with a higher implant stability quotient (ISQ) show that PRF administration increased implant stability during the early stages of healing, and just applying this material seemed to speed up osseointegration [6]. Spin centrifugation was used to create injectable platelet-rich fibrin (I-PRF). In contrast to platelet-rich plasma (PRP), which is made by mixing thrombin and calcium, I-PRF increases the number of leucocytes and makes the body make too many growth factors [7]. According to Abdul Kareem and Al Hussaini, local administration of PRF can reduce the incidence of alveolar osteitis, but not significantly [8]. Several approaches to improving I-PRF platelet and leukocyte yields have been tried. For example, increasing the centrifugation time from 3 to 4 to 8 minutes has been recommended to further aggregate platelets in the top layer of the I-PRF [9], and when standard I-PRF protocols were followed, total platelet and leukocyte yields were increased by 35% and 30%, respectively, compared to baseline levels [9]. The concentrated PRF (C-PRF) was chosen as the working name for the PRF obtained through this method of harvesting [10]. Miron and colleagues demonstrated in 2019 that C-PRF can concentrate platelets more than 15 times above baseline, whereas the I-PRF procedure only concentrates platelets by roughly 2-3 fold. To the best of their knowledge, this is the highest platelet and leukocyte concentration observed in any PRF preparation [10]. An earlier study found that I-PRF with microneedling was helpful and effective for increasing gingival thickness in thin gingival biotypes. Using I-PRF before periodontal reconstructive surgery or orthodontics may improve their effectiveness [11]. The current study evaluated the effects of C-PRF injections on gingival thickness and keratinized tissue breadth in participants with a thin gingival phenotype who received I-PRF injections.

METHODS

Study design

This is a prospective split-mouth randomized clinical trial that will take place at the University of Baghdad's Department of Periodontology, College of Dentistry, from April 11, 2022 to January 1, 2023. The trial was carried out with the permission of the local ethics committee (Ref. 521, 10/4/2022) and in compliance with the Helsinki Declaration on Human Research. This is a self-funded study that has been registered at http://clinicaltrials.gov (NCT05615155). Subjects having a thin gingival phenotype on their maxillary and mandibular anterior teeth were injected with I-PRF in one site and C-PRF in the contralateral location for the study. All individuals were given thorough information about the study's objectives, and an informed permission form was signed, indicating the subject's willingness to participate in this clinical investigation. A total of 40 injection sites were studied, with clinically healthy and intact periodontium; BOP ≤10%, PPD ≤3.0 mm, and intact periodontal tissue (no probing attachment loss) [12]. Nonsmokers with non-adjacent anterior sites with a thin gingival phenotype (GT ≤1.0 mm) [13], no history of periodontal surgical therapy, and no gingival recession. Using the coin toss technique, the selected sites were randomly involved and assigned to experimental I-PRF and C-PRF sites.

Subject preparation (Baseline visit)

At the outset, we acquire a comprehensive medical, dental, and family history from each participant to determine the presence of any systemic disease or medication use that could jeopardize the study's outcomes. The plaque index (PI), bleeding on probing (BOP), gingival thickness (GT), and Keratinized Tissue Width (KTW) were also measured. Each subject had an impression taken for gingival thickness, and a stent (vacuum-formed retainer) was manufactured for each participant. The targeted sites were indicated on the stent, and then holes were drilled in each stent to ensure that the measurement was consistent across all injection visits. The drill holes were 1.5mm below the gingival border. The holes were marked on the participant's mouth with a periodontal probe, and the noted position was measured using the transgingival procedure using an endodontic spreader size 20 and a rubber stopper. A digital vernier caliper [14] was used to measure the distance (Figure 1). The KTW was evaluated using the functional

method (FM), the mucogingival junction was evaluated, and the length from the mucogingival junction to the tip of the marginal gingiva was measured with a vernier, one tip at the mucogingival junction and the other at the tip of the marginal gingiva [15].

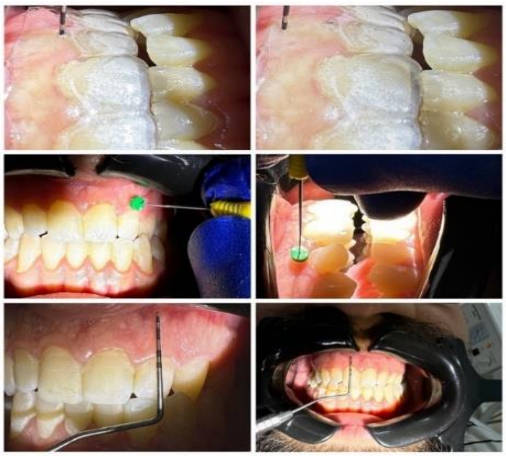

Figure 1: Gingival thickness and keratinized tissue width measurement using prefabricated stent.

I-PRF and C-PRF preparation and injection

Ten ml of venous blood was obtained in a plain plastic tube for I-PRF preparation, and the blood tube was centrifuged at 800 rpm for 3 minutes [11]. 10 ml of venous blood was taken in a 10 ml plain plastic tube for C-PRF preparation, then centrifuged at 2800 rpm for 8 minutes [9] (Figure 2).

Figure 2: PRF preparation and collection.

C-PRF and I-PRF were collected in plastic tubes and inserted in an insulin disposable syringe (1 mm/cc) with a 31-gauge needle, and injection sites were checked using a stent. C-PRF and I-PRF were injected into the alveolar mucosa near the mucogingival junction. The protocols described above were performed at the same places one and two weeks after the initial injection. After 1 month and 3 months following the last injection session, gingival phenotypic (GT and KTW) records were recorded and compared to baseline data.

Sample size justification

One of the clinical parameters (GT) was used as the primary outcome of the study with I-PRF injections. The mean gingival thickness was 0.55±0.504 mm at baseline, and the mean gingival thickness after I-PRF injections was 1.03±0.423 mm. It was found that the total sample size is 18 sites for I-PRF, and we also determined 18 sites for C-PRF injection at a 95% confidence level and 80% power when analyzed, which is rounded to 40 sites to avoid dropping out of the sample. Since the study is split-mouth, the subject's mouth was split into two sites, one for I-PRF injections and one for C-PRF injections, which means 40 sites for I-PRF and C-PRF. The following allocation ratio of 1:1 (20 sites for I-PRF injection and 20 sites for C-PRF injection) was followed: The sample size calculation was performed using G*Power (version 3.1.9.7).

Statistical analysis

Analysis of data was performed using one-way ANOVA and Bonferroni *post hoc* test. Significant difference was considered at $p<0.05$.

RESULTS

The mean gingival thickness was 0.5±0.504 mm at the baseline, and the mean gingival thickness after the I-PRF injections was 1.03±0.423 mm. It was found that the total sample size was 40 sites, 20 for I-PRF injections and 20 for C-PRF, at a 95% confidence level and 80% power when analyzed. Since the study is split-mouth, the subject's mouth was split into two sites, one for I-PRF injections and one for C-PRF. The following allocation ratio of 1:1 was followed: The sample size calculation was performed using G*Power (version 3.1.9.7). The results for the intragroup comparisons for GT were all statistically significant for both the I-PRF and C-PRF groups. The results for the intragroup comparisons for KTW were significant except for the I-PRF group in the maxillary arch, as shown in Table 1. According to outcome analysis of GT and KTW for I-PRF and C-PRF groups in the upper and lower arches, between baseline (1 month) and baseline (3 months), the results were statistically significant for all groups in both arches for GT and KTW except for GT in the upper arch in the I-PRF group between 1 month and 3 months, KTW in the Upper arch in the C-PRF group between 1 month and 3 months, and in the lower arch in the C-PRF group between 1 month and 3 months, as shown in Table 2.

Table 1: intragroup comparisons for gingival thickness and keratinized tissue width in the upper and lower arches for C-PRF and I-PRF groups

Tissue	Material	Visits	Range (mm)	Mean±SD (mm)	F	*p*-value	ES
GT Upper Arch	C-PRF	Baseline	0.70-0.94	0.82±0.09	57.27	0.00	0.871
		1 M	1.18-1.90	1.49±0.20			
		3 M	1.02-1.55	1.19±0.15			
	I-PRF	Baseline	0.71-0.98	0.87±0.08	30.15	0.00	0.780
		1 M	0.95-1.60	1.35±0.23			
		3 M	0.85-1.68	1.17±0.23			
GT Lower Arch	C-PRF	Baseline	0.44-0.73	0.584±0.10	49.012	0.00	0.852
		1 M	0.88-1.37	1.081±0.19			
		3 M	0.69-1.14	0.877±0.14			
	I-PRF	Baseline	0.50-0.81	0.68±0.10	38.756	0.00	0.820
		1 M	0.96-1.33	1.13±0.14			
		3 M	0.80-1.22	0.94±0.14			
KTW Upper Arch	C-PRF	Baseline	5.30-14.50	9.48±2.65	15.401	0.00	0.644
		1 M	7.90-14.80	11.32±2.45			
		3 M	7.50-14.70	11.16±2.58			
	I-PRF	Baseline	5.60-12.80	9.26±2.32	1.435	0.266	0.054
		1 M	6.90-12.80	9.82±1.92			
		3 M	6.50-12.80	9.76±2.01			
KTW Lower Arch	C-PRF	Baseline	2.80-7.20	5.13±1.74	12.469	0.00	0.595
		1 M	5.50-8.10	6.82±0.96			
		3 M	5.50-9.60	7.21±1.31			
	I-PRF	Baseline	2.30-7.20	4.81±1.68	5.756	0.012	0.404
		1 M	4.00-8.04	5.84±1.49			
		3 M	4.00-8.00	5.84±1.57			

GT: Gingival Thickness; KTW: Keratinized Tissue Width; C-PRF: Concentrated Platelets Rich Fibrin; I-PRF: injectable Platelets Rich Fibrin; Min: minimum; Max: Maximum; ES: Effect Size; $p<0.05$.

Table 2: Results of ANOVA and Bonferroni *post hoc* test for the GT and KTW in upper and lower arches for I-PRF and C-PRF groups.

Tissue	Material	(I) time	(J) time	Mean Difference (I-J)	*p*-value
GT Upper Arch	C-PRF	Baseline	1 M	-0.674	0.000
			3 M	-0.377	0.000
		1 M	3 M	0.297	0.001
	I-PRF	Baseline	1 M	-0.481	0.000
			3 M	-0.304	0.000
		1 M	3 M	0.177	0.057
GT Lower Arch	C-PRF	Baseline	1 M	-0.497	0.000
			3 M	-0.293	0.000
		1 M	3 M	0.204	0.000
	I-PRF	Baseline	1 M	-0.442	0.000
			3 M	-0.260	0.000
		1 M	3 M	0.182	0.000
KTW Upper Arch	C-PRF	Baseline	1 M	-1.840	0.00
			3 M	-1.680	0.00
		1 M	3 M	0.160	0.792
KTW Lower Arch	C-PRF	Baseline	1 M	-1.690	0.00
			3 M	-2.080	0.002
		1 M	3 M	-0.390	0.50
	I-PRF	Baseline	1 M	-1.034	0.019
			3 M	-1.027	0.19
		1 M	3 M	0.007	1.00

GT: Gingival Thickness; KTW: Keratinized Tissue Width; C-PRF: Concentrated Platelets Rich Fibrin; I-PRF: injectable Platelets Rich Fibrin; $p<0.05$.

Table 3: Inter-group comparison for gingival thickness and keratinized tissue width in the upper and lower arches between both C-PRF and I-PRF

Tissue	Time	Mean Difference (C-PRF\ I-PRF)	F	*P*-value	ES
GT Upper Arch	Baseline	-0.052	1.82	0.19	0.092
	1 M	0.141	2.17	0.16	0.107
	3 M	0.021	0.06	0.81	0.003
GT Lower Arch	Baseline	-0.099	3.56	0.05	0.057
	1 M	-0.044	0.34	0.57	0.018
	3 M	-0.066	1.16	0.29	0.060
KTW Upper Arch	Baseline	0.22	0.04	0.85	0.002
	1 M	1.50	2.33	0.14	0.115
	3 M	1.40	1.83	0.19	0.092
KTW Lower Arch	Baseline	0.32	0.18	0.68	0.010
	1 M	0.976	3.04	0.09	0.085
	3 M	1.37	4.51	0.04	0.200

GT: Gingival Thickness; KTW: Keratinized Tissue Width; C-PRF: Concentrated Platelets Rich Fibrin; i-PRF: injectable Platelets Rich Fibrin; MD: Mean Difference; ES: Effect Size; $p<0.05$.

DISCUSSION

In this split-mouth study, when the C-PRF and i-PRF were compared to see how they affected the gingival phenotype, the main results were that the C-PRF had better clinical results in some cases, but the results were not statistically significant. Only the KTW on the Mandibular arch revealed a significant difference when compared to the I-PRF at the 3-month follow-up, with a mean difference of 1.373 mm. This could be related to the C-PRF's higher concentrations of platelets, growth factors, and leukocytes as compared to the I-PRF. The use of C-PRF injections resulted in an improvement and a good result for improving gingival thickness and keratinized tissue width at 1 month and 3-month follow-up, respectively. In terms of GT, the results revealed a statistically significant rise in the mean GT values for all individuals at 1 month and 3 months' follow-up when compared to the baseline data. When compared to the baseline data, the results for the KTW showed an increase in the mean of the KTW for all subjects at 1 month and 3 months' follow-up; the largest width acquired was at 1 month following the last injection session. The biological features of the PRF itself may explain the favorable effect of C-PRF injection on the gingival phenotype. PRF's composition analysis reveals that it comprises platelets, leukocytes, immunological cytokines, and circulating stem cells, all of which are plentiful in fibrin clots. Although platelets and leukocytes are the principal cells engaged in the biologic activity of PRF, the fibrin matrix plays an important role in the therapeutic benefits of this platelet concentrate [16]. Some of the most important PRF growth factors are transforming growth factor (TGF), platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF1), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) [17]. In terms of the impacts of GT, the results revealed a statistically significant rise in the mean GT for all participants at 1 month and 3 months' follow-up as compared to the baseline data. The findings for the KTW revealed a variety of outcomes. Even though the application of I-PRF increased the mean KTW in the maxillary arch, the results were not statistically significant. The use of I-PRF injection, on the other hand, resulted in a statistically significant increase in mean KTW for the mandibular arch. These findings are consistent with earlier research. Ozsagir et al. used a 30-gauge needle on one side and a 24-gauge needle on the other to inject I-PRF into individuals with a thin gingival biotype in their study. Gingival thickness increased in both groups, and keratinized tissue breadth increased statistically significantly in the 30-gauge needle group [11]. Fotani *et al*. found that after 1 and 3 months of I-PRF injections, thin gingival biotype participants had a statistically significant increase in gingival thickness and keratinized tissue width. After one and three months [18]. The transient effect of both I-PRF and C-PRF may explain the decrease in gingival thickness after the 1-month follow-up visit when assessed at the 3-month follow-up visit. After injection, both I-PRF and C-PRF produce a fibrin clot that resorbs over time and releases growth factors that aid in tissue regeneration. Another study discovered that PRF can provide key development factors for at least one week and up to 28 days [19]. It could also be explained by the concept of tissue remodeling and maturation, as the remodeling phase of wound healing comes after the proliferative phase. Remodeling begins 21 days after damage, and the rate of collagen production reduces until it matches the rate of collagen breakdown. During the tissue remodeling phase, an unstructured array of tiny collagen fibers is gradually replaced by bigger fibers organized in an orientation paralleling stresses [20].

Study Limitations

To the best of the author's knowledge, this study contains the following limitations: 1) Because we couldn't find a study on the effect of C-PRF on gingival phenotype, we based the sample size on previous studies on the effect of I-PRF on gingival phenotype; 2) a relatively short follow-up period; and 3) some of the participants refused blood sampling, so they were excluded.

Conclusion

When compared to I-PRF, the application of C-PRF to improve gingival thickness and keratinized tissue width yielded more promising results. The intra-group comparison for the C-PRF yielded statistically significant findings for both GT and KTW. In the mandibular arch, a significant difference in KTW was seen for the C-PRF

group when compared to the I-PRF group, indicating that the C-PRF may have a favorable effect in this period. However, current findings should be regarded with caution until further research, including with a larger sample, is conducted.

Conflicts of interest

There are no conflicts of interest.

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Data sharing statement

All data are available upon reasonable request to the corresponding author.

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