Comparative analysis of fibroblast and blood vessel density in interdental papilla following injection of concentrated platelet-rich fibrin by fixed-angle and horizontal centrifugation: experimental study

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ABSTRACT

Introduction: Food intake is recognized as one of the key contributing factors to periodontal tissue disease. An open gingival embrasure (OGE) can result from various factors, including gingival biotype, poorly executed restorations, interproximal spacing, tooth loss, root angulation, the position of interproximal contacts, orthodontic treatment, and aging. Among minimally invasive approaches, the injection of concentrated platelet-rich fibrin (C-PRF) has shown potential for promoting periodontal tissue regeneration. Accordingly, this study aimed to analyze differences in fibroblast and blood vessel counts in the interdental papilla of Sprague–Dawley rats with experimentally induced OGE. **Methods:** This study employed a true experimental in vivo design involving 27 Sprague-Dawley rats, which were randomly allocated into three treatment groups. Each group received an injection of one of three test materials into the interdental papilla region: fixed-angle C-PRF, horizontally centrifuged C-PRF, or phosphate-buffered saline (PBS) as the control. Histological evaluations were then performed at three observation time points (days 1, 3, and 5), and the data were analyzed using two-way analysis of variance (ANOVA), followed by the least significant difference (LSD) post hoc test. **Results:** The horizontally centrifuged C-PRF group showed the greatest increase in fibroblast proliferation and blood vessel formation compared with the fixed-angle C-PRF and PBS groups. On day 5, the mean fibroblast counts were 239.06 ± 0.41, 200.60 ± 0.40, and 174.93 ± 0.30 in the horizontally centrifuged C-PRF, fixed-angle C-PRF, and PBS groups, respectively. The corresponding mean blood vessel counts were 43.33 ± 1.53, 37.67 ± 1.15, and 28.67 ± 0.58. Two-way ANOVA revealed significant differences among treatment groups and observation periods, as well as a significant interaction between treatment and observation period ($p < 0.05$). **Conclusion:** In a Sprague-Dawley rat model of experimentally induced OGE, the group receiving horizontally centrifuged C-PRF injections showed higher fibroblast and blood vessel counts than the fixed-angle C-PRF and PBS groups at all observation time points. These findings suggest that C-PRF prepared using the horizontal centrifugation method may have greater potential to promote periodontal tissue regeneration by enhancing fibroblast proliferation and angiogenesis within this experimental model.

Keywords

C-PRF, fibroblasts, blood vessels, open gingival embrasure, periodontal tissue regeneration

Perbedaan jumlah fibroblas dan pembuluh darah papilla interdental pasca injeksi concentrated platelet-rich fibrin hasil sentrifugasi fixed-angle dan horizontal: eksperimental laboratoris

ABSTRAK

***Pendahuluan**: Open Gingival Embrasure (OGE) menyebabkan masalah, akumulasi plak, retensi makanan, dan dipercaya menjadi awal mula penyakit jaringan periodontal. Beberapa faktor yang menyebabkan OGE: biotype gingiva, restorasi yang kurang baik, spacing interproksimal pada gigi, kehilangan gigi, angulasi akar, posisi kontak interproksimal, perawatan ortodontik, dan faktor usia Metode minimal invasif yang digunakan untuk memperbaiki kondisi OGE yaitu dengan injeksi Concentrated Platelet-Rich Fibrin (C-PRF) yang mempunyai potensi regenerasi jaringan periodontal. Tujuan penelitian ini untuk menganalisis perbedaan jumlah fibroblas dan pembuluh darah pada papila interdental tikus Sprague-Dawley yang dilakukan pemodelan OGE. **Metode**: Penelitian true experimental study in vivo pada 27 tikus Sprague-Dawley yang dibagi secara acak menjadi 3 kelompok. Masing-masing kelompok diberikan tiga kelompok bahan uji injeksi (C-PRF fixed-angle, C-PRF horizontal, dan Phosphate Buffered Saline (PBS)) pada bagian papila interdental, kemudian diamati pada tiga waktu pengamatan (hari ke-1, ke-3, dan ke-5). Analisis data mengunakan ANAVA dua jalur, kemudian dilanjutkan dengan uji Post Hoc Least Significance Difference (LSD). **Hasil:** Kelompok C-PRF horizontal menunjukkan peningkatan jumlah fibroblas dan pembuluh darah tertinggi dibandingkan kelompok C-PRF fixed-angle dan PBS. Jumlah fibroblas pada hari ke-5 berturut-turut adalah C-PRF horizontal (239,06 ± 0,41), C-PRF fixed-angle (200,60 ± 0,40), dan PBS (174,93 ± 0,30), sedangkan jumlah pembuluh darah masing-masing sebesar C-PRF horizontal (43,33 ± 1,53), C-PRF-fixed-angle (37,67 ± 1,15), dan PBS (28,67 ± 0,58). Analisis ANAVA dua jalur menunjukkan adanya perbedaan signifikan pada kelompok perlakuan, waktu pengamatan, dan interaksi keduanya ($p < 0,05$). Injeksi C-PRF horizontal pada papila interdental lebih unggul, dibandingkan C-PRF fixed-angle dan kelompok PBS (kontrol). **Simpulan:** Penelitian pada model OGE tikus Sprague-Dawley menunjukkan bahwa kelompok yang mendapatkan injeksi C-PRF horizontal memiliki jumlah fibroblas dan pembuluh darah yang lebih tinggi dibandingkan kelompok C-PRF fixed-angle maupun PBS pada seluruh waktu pengamatan. Hasil tersebut menunjukkan bahwa C-PRF yang diperoleh melalui metode sentrifugasi horizontal memiliki potensi yang lebih baik dalam mendukung proses regenerasi jaringan melalui peningkatan proliferasi fibroblas dan angiogenesis.*

Kata kunci

C-PRF, fibroblas, pembuluh darah, open gingival embrasure, regenerasi jaringan periodontal

INTRODUCTION

The loss of the interdental papilla, known as an open gingival embrasure (OGE), presents aesthetic, phonetic, and periodontal health challenges. Because its absence creates a "black triangle" between the teeth, it not only detracts from appearance but may also promote plaque accumulation, food retention, and inflammation of the periodontal tissues. OGE can arise from various factors, including a thin gingival biotype, suboptimal restorations, interproximal spacing, tooth loss, abnormal root angulation, improper positioning of interproximal contacts, orthodontic treatment, and aging.[1-3] Together, these factors make this condition complex and a continuing challenge in periodontology because of the limited vascularization of the interdental area.[4]

Various invasive and non-invasive approaches have been employed to address OGE. Invasive procedures, such as connective tissue graft (CTG) and free gingival graft (FGG), are often combined with platelet-rich fibrin (PRF) to enhance tissue regeneration. In contrast, non-invasive approaches involve injecting PRF or hyaluronic acid (HA) into the interdental papilla area for soft tissue augmentation.[5] Although HA can produce favorable aesthetic outcomes, its effects are temporary, necessitating repeated injections because it persists for only approximately six months.[6] By contrast, PRF has regenerative potential because it contains growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), and epidermal growth factor (EGF), which contribute to accelerated wound healing.[7-10]

PRF obtained using a fixed-angle centrifuge is known as leukocyte- and platelet-rich fibrin (L-PRF) and is processed at 2,700 rpm for 12 minutes.[7] However, this method has a limitation related to uneven cell distribution, as cells tend to accumulate against the distal wall of the tube because of the fixed centrifugation angle.[8-9] To overcome this limitation, Miron et al. introduced the horizontal centrifugation method, which enables more uniform density-based cell separation and increases platelet and leukocyte yields by up to fourfold.[8] The PRF produced by this method is known as concentrated platelet-rich fibrin (C-PRF). Derived from the buffy coat layer, C-PRF is considered to have a higher biological concentration and, therefore, greater potential for tissue regeneration.[9-13]

Tissue regeneration comprises three phases: inflammation, proliferation, and remodeling. Platelets play a crucial role in the initial inflammatory phase by releasing growth factors and cytokines that recruit immune cells, such as neutrophils and macrophages, to initiate tissue regeneration. The proliferation phase is characterized by fibroblast activity and angiogenesis, whereas the final phase involves collagen reorganization.[14,15] Although studies have demonstrated the effectiveness of HA and PRF injections in increasing interdental papilla volume, these approaches remain limited. Moreover, current methods do not fully address poor vascularization in the interdental area, and comparative research on PRF types produced using different centrifugation methods remains limited.

Consequently, regeneration of the interdental papilla requires more effective and efficient approaches. This study aimed to determine differences in fibroblast and blood vessel counts within the interdental papilla following injections of C-PRF prepared using fixed-angle and horizontal centrifugation methods in a Sprague-Dawley rat model of OGE. Accordingly, the hypothesis was that C-PRF obtained via horizontal centrifugation would result in greater increases in fibroblast and blood vessel counts than C-PRF obtained via fixed-angle centrifugation.

METHODS

This study was an *in vivo* true experimental study conducted at the Integrated

Testing and Research Laboratory (Unit IV), Universitas Gadjah Mada, Yogyakarta. Histological specimen preparation, staining, and microscopic observations were performed at the Anatomical Pathology Laboratory, Faculty of Medicine, Public Health, and Nursing (FK-KMK), Universitas Gadjah Mada, Yogyakarta. The research subjects were male Sprague-Dawley rats aged 2-3 months and divided into three treatment groups corresponding to observation periods of days 1, 3, and 5. To determine the sample size, Federer's formula (1967) was used: $(n - 1)(t - 1) \geq 15$, where n represented the number of samples per group and t represented the number of treatment groups.

Based on this formula, the minimum required sample size was three rats per group. Accordingly, 27 Sprague-Dawley rats were randomly selected to maximize data quality. Simple random sampling was performed by assigning identification numbers to the rats and using a lottery method. This procedure ensured that each rat had an equal chance of being assigned to any research group, thereby minimizing bias in sample allocation.

Male Sprague–Dawley rats weighing 250-300 g were housed in a breeding room maintained at 25°C and 64-80% relative humidity, with a 12-hour light/dark cycle. Throughout maintenance and treatment, the animals were provided with a standard pellet diet and tap water *ad libitum*. Prior to treatment, the animals were anesthetized via intramuscular injection of ketamine hydrochloride (90 mg/kg body weight) and xylazine (10 mg/kg body weight) to minimize discomfort. The autologous platelet-rich fibrin material used in this study was derived from human blood obtained from donors who met the following inclusion criteria: healthy, non-smokers, a normal platelet count (150,000-450,000 cells/µL of blood), and no regular use of medications affecting platelet function. The exclusion criterion was refusal to sign the informed consent form. Horizontal concentrated platelet-rich fibrin was prepared at 700 × *g* for 8 minutes, whereas fixed-angle concentrated platelet-rich fibrin was prepared at 2,700 × *g* for 12 minutes.

The OGE modeling procedure followed the method described by Sari et al. and involved inducing OGE in rat incisors using a U-shaped orthodontic wire (0.016-inch diameter and 20 mm in length) looped around the mandibular incisor surface, parallel to the tip of the interdental papilla. The wire was bonded using a bonding agent and resin composite and then activated to generate lateral pressure. OGE assessment was performed one week after induction. On day 7 after induction, body weight, OGE width, and SPD of the experimental animals were measured using a sliding caliper with a precision of 0.01 mm.

OGE samples were obtained from successfully established OGE models, which were confirmed through clinical observation of defect formation in the interdental papilla following the induction procedure. From these defects, tissue samples from the defective interdental papilla were subsequently collected for histological evaluation, specifically for measuring fibroblast and blood vessel counts as parameters of tissue regeneration.

After OGE was confirmed, each treatment group (n = 3) received a 20 µL injection of one of the test materials: horizontally centrifuged C-PRF, fixed-angle C-PRF, or PBS. The animals were observed on days 1, 3, and 5, and on each day after OGE induction, the experimental animals were sacrificed, and tissue samples were collected. Histological specimen preparation began with tissue fixation in 10% neutral-buffered formalin for 24 hours, followed by decalcification in a 10% EDTA solution for 30 days.[17] After the decalcification solution was removed, the tissue was dehydrated using graded alcohol concentrations (70%, 80%, 95%, and 100%) for 1.5 hours, followed by immersion in xylene and preparation of paraffin blocks.

The number of fibroblasts and blood vessels was counted in H&E-stained histological sections of periodontal papilla tissue obtained from the experimental animals. For this purpose, a light microscope equipped with an Optilab® camera at 400× magnification was used to observe five fields of view. Cell counting was performed by a single observer who was not part of the research team, using a blinded method. Fibroblasts appeared spindle-shaped, with eosinophilic nuclei and basophilic cytoplasm that tapered toward the ends.

Blood vessels were identified by the presence of a tunica intima lined with simple squamous endothelium, a lumen, and reddish-colored vasa vasorum.[18,19]

The research data were tested for normality using the Shapiro-Wilk test, while Levene's test was used to assess homogeneity of variance. Given that the data were normally distributed and homogeneous, two-way analysis of variance (ANOVA) was performed. To determine differences in mean values between groups, a post hoc test using the least significant difference (LSD) multiple-comparison method was applied. All statistical analyses were conducted at a 95% confidence level, with statistical significance defined as $p<0.05$.

RESULTS

This study received ethical approval. Then, platelet-rich fibrin (PRF) was prepared from human blood using fixed-angle and horizontal centrifugation methods. Finally, the resulting PRF was separated according to the layers formed.[14]

Table 1. Mean and standard deviation of fibroblast counts

Test material group	n	Mean and standard deviation		
		Day 1	Day 3	Day 5
C-PRF *fixed-angle*	9	120.93 ± 0.61	156.33 ± 0.50	200.60 ± 0.40
PBS	9	108.40 ± 0.72	135.60 ± 0.91	174.93 ± 0.30
C-PRF horizontal	9	137.00 ± 0.52	185.13 ± 0.61	239.06 ± 0.41

Table 1 shows that an increase in fibroblast counts occurred on days 3 and 5. Among the three observation time points, the horizontally centrifuged Concentrated Platelet-Rich Fibrin (C-PRF) group exhibited the greatest increase in fibroblast counts compared with the other groups. In contrast, the PBS group showed the smallest increase in fibroblast counts.

Table 2. Mean values and standard deviations of the differences in fibroblast counts

Test material group	n	Mean and standard deviation		
		Difference between Day 1 and Day 3	Difference between Day 3 and Day 5	Difference between Day 1 and Day 5
C-PRF *fixed-angle*	9	35.40 ± 0.40	44.26 ± 0.42	79.67 ± 0.61
PBS	9	27.20 ± 0.81	39.33 ± 0.61	66.53 ± 0.64
C-PRF horizontal	9	48.13 ± 0.76	54.27 ± 0.76	102.06 ± 0.90

Table 2 indicates that fibroblast counts increased across the observed time intervals. As in Table 1, the PBS group showed the smallest increase compared with the fixed-angle C-PRF and horizontally centrifuged C-PRF groups.

Table 3. Shapiro-Wilk normality test results based on observation time

Test Material	*Shapiro-Wilk*		Description
	Mean ± Standard Deviation	p-value	
C-PRF *fixed-angle*	36.80 ± 9.18	0.518	Normal
PBS	45.96 ± 6.61	0.507	Normal
C-PRF horizontal	82.76 ± 15.57	0.507	Normal

Table 3 shows p-values of 0.518 ($p>0.05$) for days 1 and 3, 0.507 ($p>0.05$) for days 3 and 5, and 0.507 ($p>0.05$) for days 1 and 5. Because these p-values were greater than 0.05, the data were normally distributed.

Table 4. Shapiro- Wilk normality test results based on test materials

Test Material	*Shapiro-Wilk*		Description
	Mean ± Standard Deviation	p-value	
C-PRF *fixed-angle*	53.00 ± 20.39	0.398	Normal
PBS	43.35 ± 17.45	0.508	Normal
C-PRF horizontal	68.16 ± 25.58	0.088	Normal

Table 4 shows normal distributions for all three injectable test material groups. The fixed-angle C-PRF group had a p-value of 0.390, the PBS group had a p-value of 0.508, and the horizontally centrifuged C-PRF group had a p-value of 0.088. Because all p-values were greater than 0.05, all three groups exhibited normally distributed data.

Table 5. Levene's test results for fibroblast homogeneity

p-value	Description
0,893	*p*>0,05

Based on Table 5, Levene's test yielded a p-value of 0.893 (p>0.05), indicating that the data across all treatment groups were homogeneous. With normality and homogeneity established, a two-way analysis of variance (ANOVA) was subsequently performed to determine whether the injection material groups influenced fibroblast counts across the different observation time points.

Table 6. Two-way ANOVA results for fibroblast counts

Variable	*p-value*
Treatment group	0.000*
Observation time	0.000*
Treatment group × Observation time	0.000*

Note: (*) = statistically significant difference (p<0.05)

The two-way ANOVA results in Table 6 indicate significant differences among the injection material groups, across the observation time points, and in their interaction (p=0.00; p<0.05). Accordingly, a post hoc least significant difference (LSD) test was performed to identify specific differences between the treatment groups and observation time points with respect to changes in fibroblast counts.

Table 7. Post hoc LSD test results for differences in fibroblast counts between groups at each observation time point

Group	CF (1-3)	CF (1-5)	CF (3-5)	PBS (1-3)	PBS (1-5)	PBS (3-5)	CH (1-3)	CH (1-5)	CH (3-5)
CF (1-3)		.000*	.000*	.000*	.000*	.000*	.000*	.000*	.000*
CF (1-5)			.000*	.000*	.000*	.000*	.000*	.000*	.000*
CF (3-5)				.000*	.000*	.000*	.000*	.000*	.000*
PBS (1-3)					.000*	.000*	.000*	.000*	.000*
PBS (1-5)						.000*	.000*	.000*	.000*
PBS (3-5)							.000*	.000*	.000*
CH (1-3)								.000*	.000*
CH (1-5)									.000*
CH (3-5)									

Note: (*): Significant; CF (1-3): C-PRF fixed-angle group, difference between day 1 and day 3; CF (1-5): C-PRF fixed-angle group, difference between day 1 and day 5; CF (1-3): C-PRF fixed-angle group, difference between day 3 and day 5; PBS (1-3): C-PRF fixed-angle group, difference between day 1 and day 3; PBS (1-5): C-PRF fixed-angle group, difference between day 1 and day 5; PBS (1-3): C-PRF fixed-angle group, difference between day 3 and day 5; CH (1-3): C-PRF horizontal group, difference between day 1 and day 3; CH (1-5): C-PRF horizontal group, difference between day 1 and day 5; CH (1-3): C-PRF horizontal group, difference between day 3 and day 5

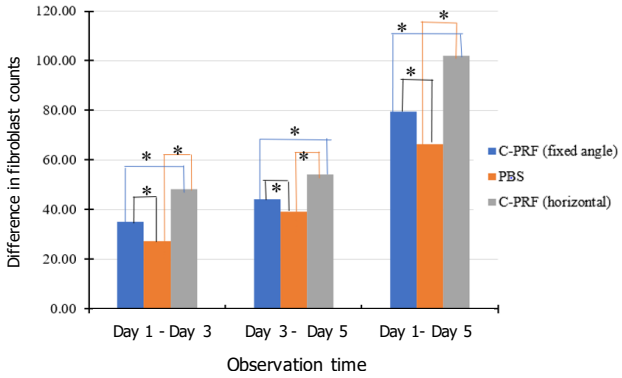


Figure 1. Bar chart showing the mean ± standard deviation of the differences in fibroblast counts across observation time points for the three injection material groups

The post hoc LSD results shown in Figure 1 indicate differences among the three injection material groups. These results yielded p-values <0.05, indicating statistically significant differences among the injection material groups. They also demonstrated significant differences among all groups (fixed-angle C-PRF, horizontally centrifuged C-PRF, and PBS) at the observation time points, as well as a significant interaction between the two factors.

Table 7 shows an increase in the differences in fibroblast counts across all groups during the observation period. Compared with the fixed-angle C-PRF and PBS groups, the horizontally centrifuged C-PRF group exhibited the highest mean difference in fibroblast counts. Overall, the horizontally centrifuged C-PRF group showed the greatest increase in the mean difference in fibroblast counts across all observation intervals.

Table 8. Mean values and standard deviations of blood vessel counts

Injection material	n	Mean and standard deviation		
		Day 1	Day 3	Day 5
C-PRF *fixed-angle*	9	30.67 ± 1.53	34.00 ± 1.00	37.67 ± 1.15
PBS	9	26.00 ± 1.00	26.67 ± 1.53	28.67 ± 0.58
C-PRF horizontal	9	33.67 ± 0.58	38.33 ± 0.58	43.33 ± 1.53

Table 8 shows that an increase in blood vessel counts occurred on days 3 and 5. Across the three observation time points, the horizontally centrifuged Concentrated Platelet-Rich Fibrin (C-PRF) group exhibited the greatest increase in blood vessel counts compared with the other groups. In contrast, the PBS group showed the smallest increase.

Table 9. Mean values and standard deviations of the differences in blood vessel counts

Test material group	n	Mean and standard deviation		
		Difference between Day 1 and Day 3	Difference between Day 3 and Day 5	Difference between Day 1 and Day 5
C-PRF *fixed-angle*	9	3.33 ± 0.58	3.67 ± 0.58	7.33 ± 1.00
PBS	9	0.67 ± 0.58	2.00 ± 1.00	2.67 ± 0.58
C-PRF horizontal	9	4.67 ± 0.58	5.00 ± 1.00	9.67 ± 1.15

Table 9 indicates an increase in blood vessel counts across the observation time points. As in the previous results, the PBS group showed the smallest increase compared with the fixed-angle C-PRF and horizontally centrifuged C-PRF groups. In contrast, the horizontally centrifuged C-PRF group showed the greatest increase.

Table 10. Shapiro-Wilk normality test results for blood vessel counts in the injection test groups

Test Material	*Shapiro-Wilk*			
	Mean ± Standard Deviation	***df***	***p-value***	**Description**
C-PRF *fixed-angle*	4.67 ± 1.87	9	0.740	Normal
PBS	1.78 ± 1.09	9	0.172	Normal
C-PRF horizontal	6.44 ± 2.55	9	0.504	Normal

Table 11. Shapiro-Wilk normality test results for mean blood vessel counts across the three observation time points

Observation Time	*Shapiro-Wilk*			
	Mean ± Standard Deviation	***df***	***p-value***	**Description**
Day 1–Day 3	2.89 ± 1.83	9	0.117	Normal
Day 3–Day 5	3.56 ± 1.51	9	0.327	Normal
Day 1–Day 5	6.44 ± 3.17	9	0.127	Normal

Based on the Shapiro-Wilk normality test results in Tables 10 and 11, the mean differences in blood vessel counts across the observation time points yielded p-values greater than 0.05, indicating a normal distribution. These results should be considered when interpreting blood vessel changes in relation to safety-related outcomes.

Table 12. Levene's test results for blood vessel homogeneity

p-value	**Description**
0.820	$p>0.05$

The homogeneity test results in Table 12 showed that the data across all treatment groups were homogeneous ($p>0.05$). Based on the normality and homogeneity tests, the blood vessel count data across all groups were normally distributed and homogeneous; therefore, they were subsequently analyzed using a two-way analysis of variance (ANOVA).

Table 13. Two-way ANOVA results for blood vessel counts

Variable	***p-value***
Group	0.000*
Observation Time	0.000*
Group × Observation time	0.007*

Note: (*): statistically significant difference ($p<0.05$)

The two-way ANOVA showed significant differences in blood vessel counts among the injection material groups and across the observation time points. as well as a significant interaction between the injection material groups and observation time points ($p<0.05$). Accordingly, a post hoc least significant difference (LSD) test was performed to identify specific differences between groups.

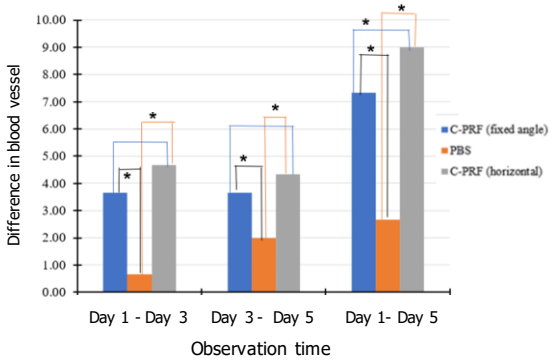


Figure 2. Bar chart showing the mean ± standard deviation of blood vessel counts differences across observation time points for the three injection material groups

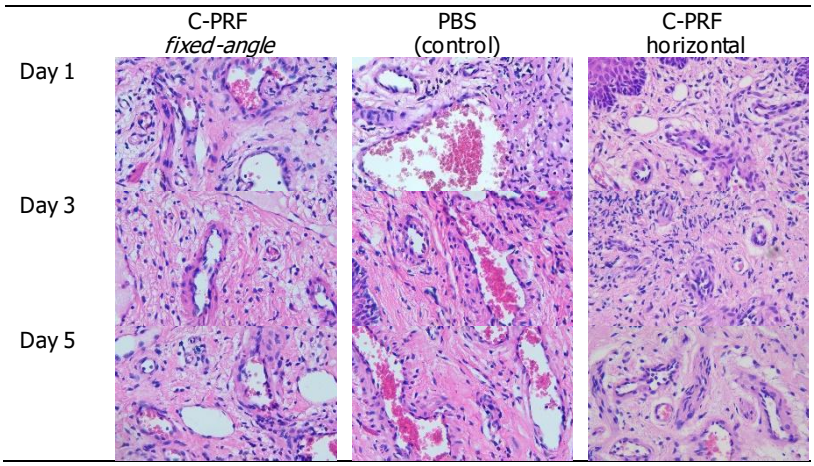


Figure 3. Histological specimens from the study on days 1, 3, and 5

DISCUSSION

The researchers utilized the OGE model as described by Sari et al.[16]. Although resilience was not assessed in this study, further research on this aspect is required. The results indicated that the horizontal C-PRF injection material group demonstrated a greater ability to stimulate fibroblast proliferation and blood vessel formation than both the fixed-angle C-PRF and PBS groups. This advantage could be attributed to the growth factors present in C-PRF, including PDGF, VEGF, TGF-β, IGF, and EGF. In particular, PDGF and TGF-β played crucial roles in stimulating fibroblast proliferation and promoting wound healing, while VEGF contributed to endothelial cell proliferation, differentiation, and migration following tissue injury. Furthermore, PDGF, in conjunction with angiopoietin-1, supported blood vessel maturation.[20-23]

These findings were consistent with reports by Kargarpour et al., which highlighted TGF-β as a key growth factor in oral fibroblast gene activation. They also aligned with research by Wang et al., which demonstrated that PDGF-BB could induce proliferation, migration, and collagen synthesis of oral mucosal fibroblasts through the PDGFR-β/PI3K/AKT pathway. Likewise, Pérez-Gutiérrez and Ferrara emphasized VEGF in regulating angiogenesis, including endothelial cell proliferation and migration, thereby supporting new blood vessel formation.[21,23,24]

This study shared similarities with the work of Song et al. and Pavlovic et al. in using PRF as a growth factor-rich biological material with tissue regeneration potential. However, it differed in its focus on comparing two C-PRF centrifugation techniques, horizontal versus fixed-angle, an area that had received limited attention in the previous literature.[21,22]

Fujioka-Kobayashi et al. stated that C-PRF was capable of inducing a higher release of growth factors than traditional injectable platelet-rich fibrin. Consistent with this, horizontal C-PRF induced cell proliferation by day 5.[25]. The PDGF and FGF growth factors present in fibroblasts, combined with the PDGF and TGF-β growth factors from C-PRF, enhanced fibroblast proliferation in the interdental papilla.

In addition to growth factors, the choice of centrifugation technique played a role in enhancing fibroblast and blood vessel proliferation. This view was supported by Lourenço et al., who found that horizontal centrifugation was superior in releasing higher levels of PDGF and FGF2 and reducing IL-1β compared with the fixed-angle technique.[26] Horizontal centrifugation also resulted in a more even distribution of cells than fixed-angle centrifugation.[8] This finding was consistent with Cho et al., who stated that horizontal centrifugation played a crucial role in periodontal regeneration.[27] Concentrated platelet-rich fibrin released VEGF, PDGF, and TGF-β1 growth factors, with levels peaking at day 10, thereby triggering fibroblast proliferation and type I collagen production.[10]

The results of this study indicated that the control group using PBS as the test material exhibited the lowest numbers of fibroblasts and blood vessels. This may be explained by PBS's nature: it contained no active substances and was non-reactive, making it incapable of directly stimulating cellular proliferation or inducing an inflammatory response. These findings were consistent with Dankai et al., who evaluated PBS as a liquid-based alternative medium for detecting HPV DNA. In that study, PBS was described as an isotonic solution that mimicked physiological body conditions and therefore elicited neither an inflammatory response nor significant cellular changes.[28]

The similarity between the two studies lay in the inert nature of PBS and its lack of significant biological activity. However, their research contexts differed. Dankai et al. emphasized PBS as a diagnostic medium for maintaining HPV DNA stability. In contrast, the present study assessed PBS effects on fibroblast proliferation and blood vessel formation. Thus, despite their different research objectives, both studies indicated that PBS did not provide biological stimulation to cells but functioned as a buffer solution that maintained physiological conditions.[28]

The analysis in Table 1 revealed significant differences among all test groups (horizontal C-PRF, fixed-angle C-PRF, and PBS) at each observation time point, including significant interactions between variables. These findings showed that both fixed-angle and horizontal C-PRF stimulated fibroblast proliferation, increasing cell numbers over time.

This conclusion was consistent with reports by Miron et al., which indicated that PRF exerted a stimulatory effect on fibroblast and osteoblast proliferation. The primary difference lay in the separation method employed; Miron et al. utilized a 1-mL separation technique, whereas this study adopted a direct separation approach based on the layers formed.[12,14] This methodological modification provided a simpler and more practical procedure that could be more easily implemented in clinical practice while maintaining biological effectiveness. In this context, increased fibroblast numbers played a crucial role in papilla regeneration and wound healing. Fibroblasts were involved in extracellular matrix synthesis and facilitated collagen formation, which was essential for interdental papilla regeneration.

Figure 1 showed that at the 1-day and 3-day observation points, the fixed-angle and horizontal C-PRF groups exhibited significantly greater increases in blood vessel numbers than the PBS group. This finding demonstrated the potential of C-PRF, using both fixed-angle and horizontal centrifugation methods, to stimulate early blood vessel formation (angiogenesis) compared with PBS. Similar results were observed on days 3 and 5, with the C-PRF groups showing greater increases in blood vessel numbers than the PBS group. The greatest increases in blood vessel numbers were observed in the fixed-angle and horizontal C-PRF groups at the 1-day and 5-day observation points.

The results of this study indicated that C-PRF prepared using either the fixed-angle or horizontal method had greater potential to enhance angiogenesis than PBS. This was evidenced by the greater increases in blood vessel numbers across the observation periods, with the highest values observed on day 5 post-injection. Notably, horizontal C-PRF resulted in significantly greater blood vessel formation than the other groups. This finding was consistent with reports by Yu et al., which indicated that the use of horizontal PRF bone blocks supported early tissue responses and the healing process by stimulating angiogenesis. Together, these studies shared a common finding regarding the ability of horizontal PRF to facilitate new blood vessel formation and support tissue regeneration.[29]

Conversely, the results were also consistent with the characteristics of PBS reported by Dankai et al., particularly its isotonic and inert nature, which did not trigger inflammatory responses or stimulate cell proliferation. Accordingly, this characteristic explained why the PBS control group exhibited the lowest fibroblast and blood vessel counts, suggesting limited biologic activity in this comparison.

When compared with the study by Sari et al., similarities were observed in the use of an open gingival embrasure model to evaluate interdental papilla regeneration. However,

a fundamental difference lay in the test materials used: Sari et al. compared L-PRF with hyaluronic acid (HA) injections, whereas the present study focused on C-PRF prepared using two different centrifugation techniques. In this regard, the focus on C-PRF offered a new perspective on the potential advantages of growth factor-rich materials, which could provide sustained biological activity, compared with HA, which primarily functions as a temporary filler. Therefore, this study provided further evidence that C-PRF, particularly when prepared using the horizontal method, showed greater regenerative potential than PBS and demonstrated greater blood vessel formation than the other treatment groups. Taken together, these findings indicated that both C-PRF preparation methods supported angiogenesis over the observation period and produced greater increases in blood vessel numbers than the PBS group.

These results indicated that C-PRF, prepared using either the fixed-angle or horizontal method, had greater potential to enhance angiogenesis than PBS, as evidenced by greater increases in blood vessel numbers across the observation periods. This increase was supported by reports indicating that optimal cell proliferation occurred on day 5 post-injection.[28] In addition, horizontal platelet-rich fibrin resulted in significantly greater new blood vessel formation.[29]

Fujioka-Kobayashi et al. reported that concentrated platelet-rich fibrin released growth factors, specifically VEGF, PDGF, and TGF-β1, with peak levels occurring on day 10.[9] According to Miron et al., the horizontal C-PRF fraction (0.3 mL above the buffy coat) could increase leukocyte concentration by more than 7-fold, platelet concentration by 16-fold, and monocyte concentration by more than 13-fold. These blood vessels played a crucial role in delivering nutrients and oxygen to tissues, thereby supporting periodontal tissue regeneration, particularly in the interdental papilla.[12,30] Accordingly, this study found that horizontal C-PRF stimulated new blood vessel formation, which could contribute to interdental papilla regeneration.

This study did not use hyaluronic acid (HA) as a positive control because the focus was on the growth factor content of C-PRF rather than a direct comparison with HA. This decision was based on the reported limitations of HA, which primarily functions as a tissue filler and is readily reabsorbed by the body, resulting in a temporary effect. This approach was consistent with Kim et al., who reported that HA injection into the interdental papilla primarily functioned as a tissue filler without providing sustained biological stimulation.[6]

Furthermore, research by Alhabashneh et al. demonstrated that the effectiveness of HA injections lasted for up to three months and declined between the third and sixth months after injection. This finding aligned with the present study's acknowledgment of the limitations of HA as an injectable material, particularly regarding the relatively short duration of its effects. A fundamental difference, however, was that this study did not include HA as a positive control. Instead, this study focused on C-PRF, which contained growth factors with potential regenerative effects.[31] Currently, no studies have compared the effects of horizontal C-PRF injections with HA on fibroblast and blood vessel counts in OGE; therefore, further research is warranted. To the best of the researchers' knowledge, no studies have investigated the effects of horizontal C-PRF injections on fibroblast and blood vessel counts in OGE over an extended observation period, highlighting the need for further research. A limitation of this study was the rapid physiological growth of rat teeth, which caused the orthodontic wire to become easily dislodged following activation.

CONCLUSION

Research using a Sprague-Dawley rat OGE model demonstrated that the group receiving horizontal C-PRF injections exhibited higher fibroblast and blood vessel counts than the fixed-angle C-PRF and PBS groups at all observation time points. Therefore, these findings indicate that C-PRF obtained using the horizontal centrifugation method has greater potential to support tissue regeneration by enhancing fibroblast proliferation and

angiogenesis. Consequently, these findings could serve as a foundation for future research and contribute to the development of regenerative therapies for OGE.

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REFERENCES

1. Ali MM, Nuhed FMSB, Alsheikhoon IAM, Alhuthali KKS, Almalki OAH. Effect of hyaluronic acid application on gingival black triangles: A systematic review. Sci Temper. 2023;14(3):975-84. https://doi.org/10.58414/SCIENTIFICTEMPER.2023.14.3.64
2. Zhang Y, Gao J, Wang X, Wang J, Zhang X, Fang S, et al. Biomechanical factors in the open gingival embrasure region during the intrusion of mandibular incisors: A new model through finite element analysis. Front Bioeng Biotechnol. 2023;11:1149472. https://doi.org/10.3389/fbioe.2023.1149472
3. Athar S, Jayadev S. Black gingival triangle in orthodontics: Its etiology, management and contemporary literature review. Saint's Int Dent J. 2020;4(1):17-22. https://doi.org/10.4103/sidj.sidj_17_20
4. Zuhr O, Rebele SF, Cheung SL, Hürzeler MB. Surgery without papilla incision: Tunneling flap procedures in plastic periodontal and implant surgery. Periodontol 2000. 2018;77(1):123-49. https://doi.org/10.1111/prd.12214
5. Hayei NAA, Yusof HM. Interdental papilla regeneration: The updates on several techniques. Eur J Gen Dent. 2023;12(3):17-22. https://doi.org/10.1055/s-0043-1773799
6. Kim SB, Cho J, Jue SS, Park JH, Kim JY. Effect of hyaluronic acid filler injection on the interdental papilla in a mouse model of open gingival embrasure. Int J Environ Res Public Health. 2020;17(14):4956. https://doi.org/10.3390/ijerph17144956
7. Choukroun J, Miron RJ. Platelet-Rich Fibrin in Regenerative Dentistry: A Second-Generation Platelet Concentrate. 1st ed. New Jersey: John Wiley & Sons; 2017. p. 4-5. https://doi.org/10.1002/9781119406792.ch1
8. Miron RJ, Chai J, Zhang P, Li Y, Wang Y, Mourão CFAB, et al. A novel method for harvesting concentrated platelet-rich fibrin (C-PRF) with a 10-fold increase in platelet and leukocyte yields. Clin Oral Investig. 2020;24(8):2819-28. https://doi.org/10.1007/s00784-019-03147-w
9. Fujioka-Kobayashi M, Kono M, Katagiri H, Schaller B, Zhang Y, Sculean A, et al. Histological comparison of platelet-rich fibrin clots prepared by fixed-angle versus horizontal centrifugation. Platelets. 2021;32(3):413-19. https://doi.org/10.1080/09537104.2020.1754382
10. Fujioka-Kobayashi M, Katagiri H, Kono M, Schaller B, Zhang Y, Sculean A, et al. Improved growth factor delivery and cellular activity using concentrated platelet-rich fibrin (C-PRF) compared with traditional injectable (i-PRF) protocols. Clin Oral Investig. 2020;24(12):4373-83. https://doi.org/10.1007/s00784-020-03303-7
11. Coucke B, Dilissen E, Cremer J, et al. Leukocyte- and platelet-rich fibrin for enhanced tissue repair: An in vitro study characterizing cellular composition, growth factor kinetics and transcriptomic insights. Mol Biol Rep. 2024;51(954):1-2. https://doi.org/10.1007/s11033-024-09890-y
12. Miron RJ, Cha J, Zhang P, Fujioka-Kobayashi M, Sculean A, Zhang Y. Evaluation of 24 protocols for the production of platelet-rich fibrin. BMC Oral Health. 2020;20(1):310. https://doi.org/10.1186/s12903-020-01299-w
13. Miron RJ, Gruber R, Farshidfar N, Sculean A, Zhang Y. Ten years of injectable platelet-rich fibrin. Periodontol 2000. 2024;94(1):92-113. https://doi.org/10.1111/prd.12538
14. Chaar EL. Periodontic and Implant Dentistry. New Jersey: John Wiley & Sons; 2023. p. 14-15.
15. Toma AI, Fuller JM, Willett NJ, Goudy SL. Oral wound healing models and emerging regenerative therapies. Transl Res. 2021;236:17-34. https://doi.org/10.1016/j.trsl.2021.06.003
16. Sari R, Sukorini U, Susilowati H, Suryono S. Comparative study on interdental papillae regeneration: Leukocyte platelet-rich fibrin by-product versus hyaluronic acid injections in modified open gingival embrasure model. Eur J Dent. 2025. https://doi.org/10.1055/s-0045-1802948
17. Savi FM, Brierly GI, Baldwin J, Theodoropoulos C, Woodruff MA. Comparison of different decalcification methods using rat mandibles as a model. J Histochem Cytochem. 2017;65(12):705-22. https://doi.org/10.1369/0022155417733708

18. Gartner LP, Hiatt JL. Textbook of Histology. 6th ed. Philadelphia: Elsevier; 2026 . p. 323.
19. Mescher AL. Junqueira's Basic Histology: Text and Atlas. 16th ed. New York: McGraw-Hill Education; 2023. p. 96-99.
20. Kargarpour Z, Nasirzade J, Panahipour L, Miron RJ, Gruber R. Liquid platelet-rich fibrin and heat-coagulated albumin gel: Bioassays for TGF-β activity. Materials (Basel). 2020;13(16):3466. https://doi.org/10.3390/ma13163466
21. Song P, He D, Ren S, Fan L, Sun J. Platelet-rich fibrin in dentistry. J Appl Biomater Funct Mater. 2024;22:22808000241299588. https://doi.org/10.1177/22808000241299588
22. Pavlovic V, Ciric M, Jovanovic V, Trandafilovic M, Stojanovic P. Platelet-rich fibrin: Basics of biological actions and protocol modifications. Open Med (Wars). 2021;16(1):446-54. https://doi.org/10.1515/med-2021-0259
23. Wang J, You J, Gong D, Xu Y, Yang B, Jiang C. PDGF-BB induces conversion, proliferation, migration, and collagen synthesis of oral mucosal fibroblasts through PDGFR-β/PI3K/AKT signaling pathway. Cancer Biomark. 2021;30(4):407-15. https://doi.org/10.3233/CBM-201681
24. Pérez-Gutiérrez L, Ferrara N. Biology and therapeutic targeting of vascular endothelial growth factor A. Nat Rev Mol Cell Biol. 2023;24(11):816-34. https://doi.org/10.1038/s41580-023-00631-w
25. Dashore S, Chouhan K, Nanda S, Sharma A. Platelet-rich fibrin, preparation and use in dermatology. Indian J Dermatol Online. 2021;12(Suppl 1):S55-S65. https://doi.org/10.4103/idoj.idoj_282_21
26. Lourenço ES, Alves GG, de Lima Barbosa R, Spiegel CN, de Mello-Machado RC, Al-Maawi S, et al. Effects of rotor angle and time after centrifugation on the biological in vitro properties of platelet rich fibrin membranes. J Biomed Mater Res B Appl Biomater. 2021;109(1):60-68. https://doi.org/10.1002/jbm.b.34680
27. Cho YD, Kim KH, Lee YM, Ku Y, Seol YJ. Periodontal wound healing and tissue regeneration: A narrative review. Pharmaceuticals (Basel). 2021;14(5):456. https://doi.org/10.3390/ph14050456
28. Dankai W, Khunamornpong S, Siriaunkgul S, Soongkhaw A, Aithin P, Lekawanvijit S. An evaluation of phosphate buffer saline as an alternative liquid-based medium for HPV DNA detection. Asian Pac J Cancer Prev. 2021;22(11):3441-45. https://doi.org/10.31557/APJCP.2021.22.11.3441
29. Yu S, Bd YT, Bd YW, Bd MF, BMed SL, BMed GT, et al. Early tissue and healing responses after maxillary sinus augmentation using horizontal platelet rich fibrin bone blocks. BMC Oral Health. 2023;23(1):589. https://doi.org/10.1186/s12903-023-03228-z
30. Ngeow WC, Tan CC, Goh YC, Deliberador TM, Cheah CW. A narrative review on means to promote oxygenation and angiogenesis in oral wound healing. Bioengineering (Basel). 2022;9(11):636. https://doi.org/10.3390/bioengineering9110636
31. Alhabashneh R, Alomari S, Khaleel B, Qinawi H, Alzaubi M. Interdental papilla reconstruction using injectable hyaluronic acid : A 6-month prospective longitudinal clinical study. J Esthet Restor Dent. 2022;33(3):531-37. https://doi.org/10.1111/jerd.12680