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ORIGINAL STUDY

Effects of Platelet-rich Plasma and Platelet-rich Fibrin Usage in Regenerative Endodontic Treatments: An Analysis of Root Length and Dentin Thickness

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Abstract

Background: **Regenerative endodontic treatment aims to relieve symptoms and maintain root development and regeneration of pulp tissue. This study aimed to retrospectively examine and compare the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in the regenerative endodontic treatment (RET) of immature necrotic teeth in terms of clinical and radiographic treatment results.**

Materials and methods: **This study included patients who underwent Regenerative Endodontic Treatment (RET) at Selcuk University Faculty of Dentistry between 2014 and 2019, totalling 38 cases. After apical bleeding is induced into the canal, PRP was utilized in 16 cases, while PRF was employed in 22 cases during the treatments. The changes in root length and dentin thickness were calculated by measuring the radiographs with the help of Image J software. The success rates of the treatments were evaluated in the range of 0−3 points based on the scoring index of Bezgin et al. Changes in root length and dentin thickness, apical closure, and success score were compared statistically in PRP and PRF treatment methods.**

Results: **There was no statistically significant difference between the group who had treatment with PRP and those who had treatment with PRF in terms of apical closure, root length increase, and success scoring, but there was a significant difference in terms of increased dentin thickness.**

Conclusions: **The use of PRP and PRF in RET showed similar results in terms of treatment success. Both treatment groups showed an increase in dentin thickness and root length.**

Keywords: Regenerative endodontics, Platetelet-rich fibrin, Platelet-rich plasma

1. Introduction

Endodontic treatments for immature teeth present challenges compared to traditional endodontic treatments in pediatric dentistry [1,2]. The lack of root apex closure creates difficulties in determining the working length, irrigation, and root obturation, while thin dentin walls make the teeth fragile [3].

Endodontic treatments continue to develop both scientifically and clinically with the contributions of advancements in regenerative medicine [4]. Regenerative endodontic treatment (RET) has come to this day due to two important published clinical case series that have established its relevance [5,6]. Many studies have shown that RET in immature teeth relieves symptoms and promotes root development and the vitality of teeth [7−9]. Clinical and radiographically successful treatment results suggest RET as the primary treatment option for immature teeth [10].

Tissue scaffold is one of the crucial components of regenerative endodontics [11,12]. The blood clot has been used as a scaffold in research [6,13], but apical bleeding may not always be achievable [8]. In such cases, alternative tissue scaffolds such as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF),

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which are abundant in growth factors, can be used [7,9]. The primary success criterion is that the teeth remain asymptomatic after the RET [14]. When evaluating treatment results, various factors, such as the resolution of symptoms, ongoing apical root development, and positive response to vitality tests, are considered [15,16]. This study aims to retrospectively compare PRP and PRF as scaffolds in the RET of immature necrotic teeth, considering clinical and radiographic assessments. The null hypothesis of this study is that there is no significant difference between the PRP and PRF treatment methods.

2. Materials and methods

This research was conducted with the approval of the Selcuk University Faculty of Dentistry Non-Invasive Clinical Research Ethics Committee (2020/51). Cases with clinical treatment and radiographic follow-up records were included in the research after obtaining informed consent for data use. This study included 38 cases of 34 children (14 boys and 20 girls) who had previously undergone RET in the Department of Paediatric Dentistry of Selcuk University between 2014 and 2019. Only immature teeth were included in the study. The selected cases consisted of teeth in which either PRP or PRF was used. Patients treated exclusively with blood clot scaffold, did not attend follow-up appointments, had incomplete data, and expressed unwillingness to participate in the study were excluded. A flow chart showing a summary of the methodology is presented in Fig. 1.

2.1. Treatment protocol

Cold test (Endo-Frost; Coltene-Roeko, Langenau, Germany) and electric pulp test (Digitest, Parkell

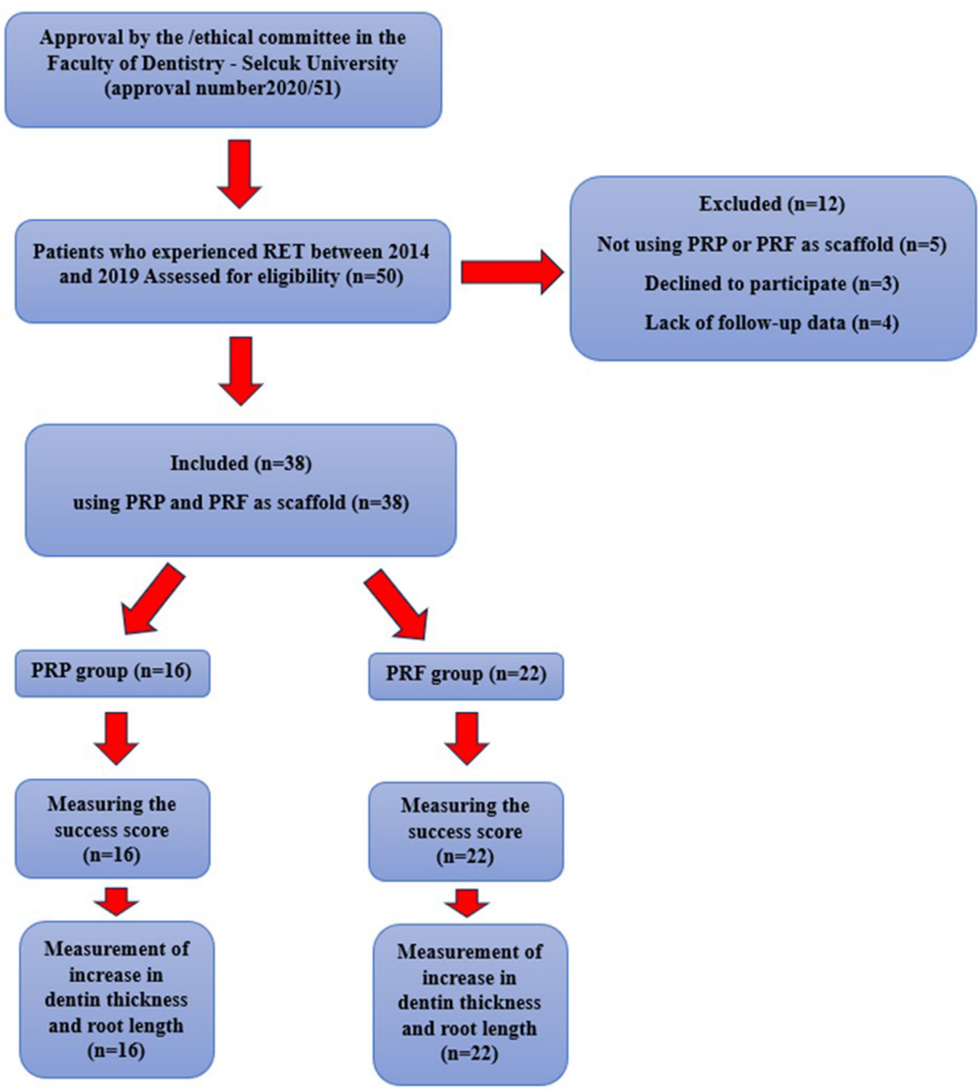

Fig. 1. Flowchart showing a summary of the methodology.

Inc, Brentwood, NY) were performed on patients with immature teeth who presented to our clinic with trauma or caries, and the diagnosis of necrosis was made following a negative response. RET was planned, and written informed consent was obtained from the patient's parents.

According to the clinical records, a standard protocol was applied to the patients treated according to the current treatment procedures of the American Association of Endodontists [15]. All treatments were completed in at least two sessions. In the first session, after local anesthesia (4% articaine with vasoconstrictor: Ultracaine DS Forte; Aventis, Istanbul, Turkey) was applied, the access cavity was opened under rubber dam isolation. After measuring the root canal length, the working length was 1 mm less than the apical. Each root canal was rinsed with 20 mL of 1.5% sodium hypochlorite, followed by 20 mL of saline solution for 5 min. After the root canals were dried with paper points, triple antibiotic paste (TAP) (a mixture of 250 mg ciprofloxacin, 400 mg metronidazole, and 50 mg minocycline in a 1:1:1 ratio) was used in the root canals for disinfection. Then, a temporary restoration was made, and a second appointment was planned in 3 weeks.

In the second appointment, after anesthesia (3% mepivacaine without vasoconstrictor: Citanest; AstraZeneca, London, UK), access to the root canals was achieved under rubber dam isolation. The root canals were irrigated with saline (20 mL/canal, 5 minutes) solution followed by 17% EDTA (20 mL/canal, 5 minutes) until complete elimination of TAP from within the root canals. Apical bleeding was induced by over-instrumentation 2 mm beyond the root apex with a K file. PRP or PRF was placed in the root canals as a tissue scaffold. The scaffold was sealed with MTA (ProRoot MTA; Dentsply Tulsa Dental, Tulsa, OK), and then the restorations were completed using glass ionomer cement and composite resin.

In the group with PRP (n = 16), 20 mL of blood taken from the patient was placed in sterile tubes containing citrate solution. Citrated blood was centrifuged at 1250 rpm for 15 min to obtain PRP without erythrocytes and leukocytes. The obtained PRP was placed in the root canal cavity 3 mm below the cementoenamel junction (CEJ) using sterile 27-G injectors [17].

In the group with PRF (n = 22), 20 mL of blood was taken into a sterile tube without anticoagulant and immediately centrifuged at 3000 rpm for 10 min. The PRF, which was removed from the test tube with the help of a pair of sterile tweezers, was placed in the root canal cavity 3 mm below the CEJ using small pieces of sterile pluggers [18].

Patients completing the treatment were called for follow-up appointments, and the clinical and radiological treatment results were recorded.

2.2. Data records and success score

The clinical follow-up results of the treatments were collected from the registered archives. Radiographic images were collected through the picture archiving and communication system in the Hospital Information Management System. In the records kept in the treatment follow-ups, the Presence of percussion tenderness, pain, and intraoral fistula, mobility score of teeth according to Miller classification [19], and responses of teeth to electric pulp test and cold test were evaluated. Periapical healing, periapical resorption, increase in root length and dentin thickness, and apical root closure were evaluated with radiographs.

A method developed by Bezgin et al. [7] was used to score the success of the treatments.

- Score 0 (unsuccessful): Clinically, the continuation of symptoms in the tooth and/or the presence of radiographic pathology findings.
- Score 1 (satisfactory): There are no clinical symptoms. There is evidence of bone healing. There is no increase in root length/width or no apical closure.
- Score 2 (good): There are no clinical symptoms. There is evidence of bone healing. There is a closure at the root apex with/without an increase in root length/width.
- Score 3 (excellent): Score 2 + It responds positively to cold and electric pulp tests.

2.3. Radiographic evaluation and measurements

Diagnostic radiographs could not be obtained with the paralleling technique, as it was usually referred to the clinic because of trauma. However, in the follow-up radiographs of the patients, film holder systems were used to keep the films parallel to the long axes of the teeth. Image analysis and calculation of all films included in the study were evaluated blindly by two dentists. Periapical healing, the presence of resorption, and apical closure were visually assessed. Root length and increase in dentin thickness were measured using ImageJ (version 1.52; National Institutes of Health, Bethesda, MD) software.

In this study, the increase in dentin thickness in the horizontal plane was calculated as a percentage based on the measurements made by Silujjai and

Linsuwanont [20]. When calculating the increase in root length in the vertical plane, vertical proportional measurements were made similar to those used by He et al. [21].

Appropriate films from the treatment sessions and final control films were selected for radiographic measurements. Since the paralleling technique was not used in the initial films, the one with the most precise image was selected among the first or second session (MTA control films). It was compared with the final control images taken with the paralleling technique. Since all images were taken with size two intraoral photographic film, image dimensions were calibrated on ImageJ software as 31 mm in the horizontal plane and 41 mm in the vertical plane [22].

2.4. Measurement of increase in dentin thickness and root length

Two fixed points that remained unchanged in both images were selected to calculate the magnification rates between the first and last films. These two fixed points were the mesial and distal CEJ in the horizontal plane. In the vertical plane, the mesial or distal CEJ and a fixed point on the coronal crown of the teeth were selected. The distance between these two fixed points was scaled using ImageJ software on calibrated images by combining them with a straight line. Horizontal (Fig. 2 a and b) and vertical (Fig. 3 a and b) magnification ratios between both images were obtained by proportioning the measurements in the films with each other.

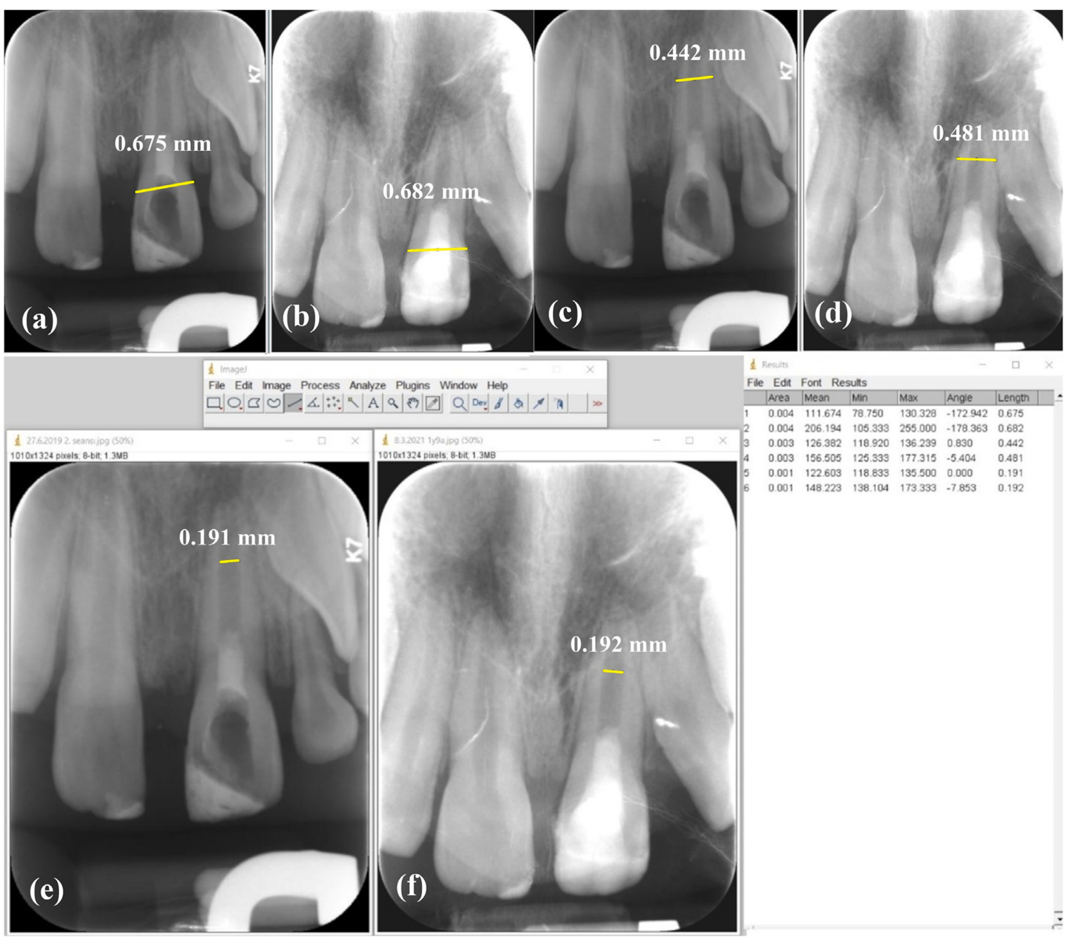

Fig. 2. (a,c,e) The first image is the radiograph of the second treatment session (MTA control). (b,d,f) The final image is the final follow-up radiograph of the treatment. Two fixed points in the horizontal plane (mesial and distal CEJ) were selected between the images to measure the dentin thickness in images with different magnification ratios. (a and b) The fixed points in the first and last images were proportioned for the horizontal magnification ratio, and the magnification ratio was found to be 0.675/0.682 = 0.989. Measurements were made from two-thirds of the root length to measure the dentin thickness. Dentin thickness was measured by subtracting the pulp space from the dentin thickness. (c and e) Dentin thickness in the first image = 0.442−0.191 = 0.252 mm. (d and f) Dentin thickness in the final image = 0.481−0.192 = 0.289 mm. When the final image was converted to the same magnification as the first image, the dentin thickness in the final image was found to be 0.289 × 0.989 = 0.285821 mm. The change in dentin thickness between both images was 0.285821−0.251 = 0.034821 mm. When calculated as a percentage according to the first image, (0.034821/0.251)×100 = 13.8729 was found. As a result, the dentin thickness increased by 13.87% compared to the first image.

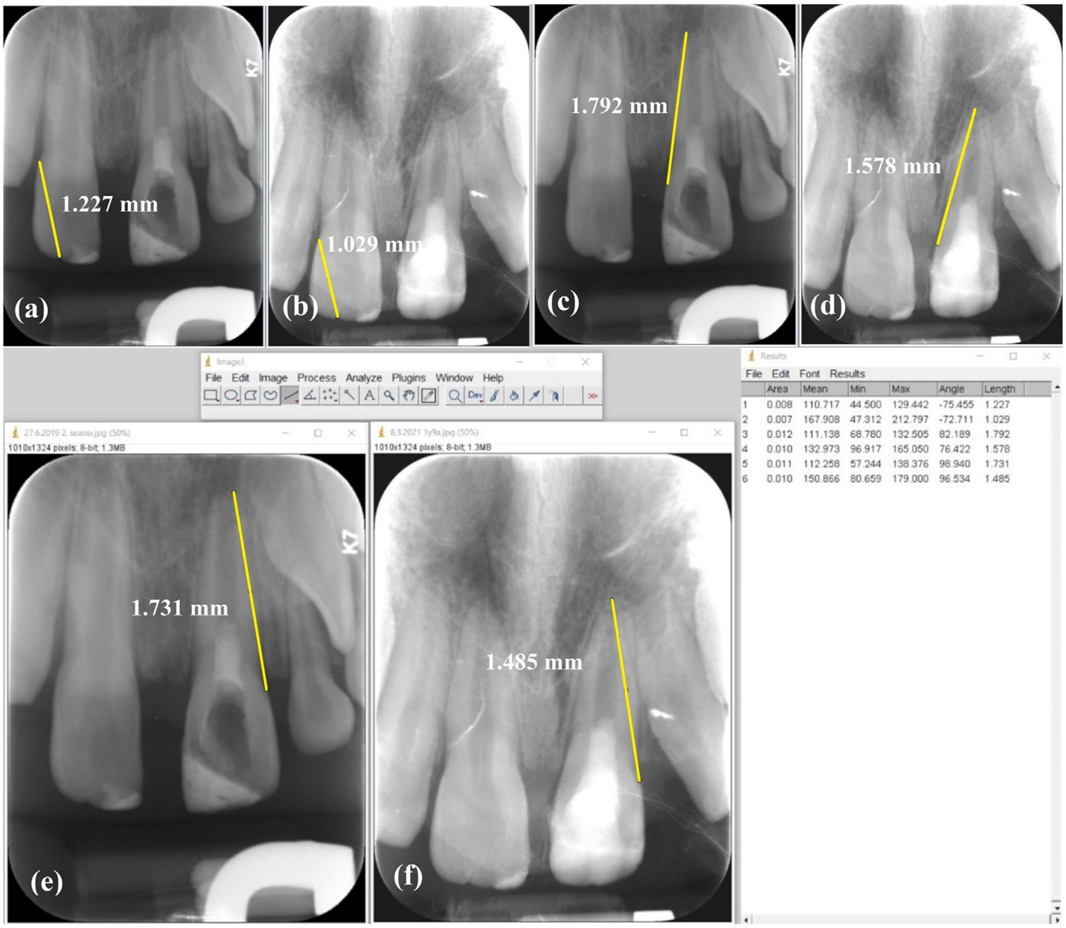

Fig. 3. (a,c,e) The first image is the radiograph of the second treatment session (MTA control). (b,d,f) The final image is the final follow-up radiograph of the treatment. Two fixed points in the vertical plane were selected between the images (such as CEJ and a fixed point on the coronal apex) to measure root length on images with different magnification ratios. (a and b) The fixed points in the first and last images were proportioned for the vertical magnification ratio, and the magnification ratio was 1.227/1.029 = 1.192. A straight line was drawn from the mesial and distal CEJ to the root apex to measure the root length. The mean root length was found by averaging this measurement. (c and e) Root length in the first image (1.792 + 1.731)/2 = 1.7615 mm. (d and f) Root length in the last image = (1.578 + 1.485)/2 = 1.5315 mm. When the final image was converted to the same magnification as the first image, the root length in the final image was found to be 1.5315 × 1.192 = 1.825548 mm. The change in root length between both images was found to be 1.825548−1.7615 = 0.064048 mm. When calculated as a percentage, it was found to be (0.064048/1.7615)× 100 = 3.6359. As a result, the root length increased by 3.63% compared to the first image.

When calculating the increase in dentin thickness, measurements were made from two-thirds of the root length (Fig. 2) [22]. When calculating the increase in root length, straight lines were drawn from the mesial and distal CEJ of the teeth to the root apex and the average of both measurements was taken (Fig. 3) [20]. Root length and dentin thickness increase measurements were calculated blindly by two different dentists at different times, and both measurements were averaged.

2.5. Statistical analysis

Using the calculated type I error of 0.05 and a power of 0.8, a minimum total sample of 36 subjects is required to detect a significant difference between the study groups. The sample size was calculated using the G*Power software version 3.1.9.2. The data obtained was loaded into the SPSS software (IBM SPSS, version 23; IBM, NY, USA). A P value of <0.05 was considered statistically significant. Following the Kolmogorov−Smirnov test results, it was determined that our variable of dentin thickness increase and root length increase did not follow a normal distribution. Thus, non-parametric tests were employed. The agreement among researchers measuring dentin thickness and root length was analyzed using the Wilcoxon test. The Mann−Whitney U test, one of the non-parametric tests, was utilized to statistically compare dentin thickness and root length between the PRP and PRF treatment groups. The variables about success scores and apical closure between the

PRP and PRF treatment groups were subjected to statistical analysis using the Chi-square test.

3. Results

Except for Case 12, no symptoms such as pain, sinus tract, or percussion sensitivity were observed in the PRP group. Case 12, the symptomatic patient, exhibited hypersensitivity to percussion and experienced spontaneous pain during the final follow-up. Furthermore, the mobility score of the symptomatic tooth was evaluated as Miller 1. In case 12, although symptomatic after 77 months, radiographic evaluation revealed increased dentin thickening and root length. In the PRF treatment group, only two cases (Case 3 and Case 5) demonstrated positive responses to cold and electric pulp tests.

Apical closure was seen in 50% ($n = 19$) of the treatment groups. Apical closure was 62.5% ($n = 10$) in the PRP group and 40.9% ($n = 9$) in the PRF group. The Chi-square test indicated no significant difference between the PRP and PRF treatment groups regarding apical closure ($P = 0.189$). Table 1 presents the demographic data, treatment follow-up information, apical closure, and success scores.

The agreement between dentin thickness and root length measurements conducted by both researchers was examined using the Wilcoxon test, and no statistically significant differences were found between the measures (P value for dentin thickness = 0.114, and P value for root length = 0.544). Therefore, the measurements were statistically consistent (Table 2). The average percentage increase in dentin thickness was 31.98% in the PRP group and 14.05% in the PRF group. Similarly, the average percentage increase in root length was 13.24% in the PRP group and 11.61% in the PRF group. The dentin thickness and root length variables between the PRP and PRF treatment groups were evaluated using the Mann–Whitney U test. Statistically, the PRP treatment group was found to be successful in increasing dentin thickness ($P = 0.025$). However, the increase in root length between the PRP and PRF treatment groups was statistically insignificant ($P = 0.701$) (Table 3).

The success scores of 1 and 2 were the most frequently observed. A case exhibiting symptoms of treatment failure received a score of 0. Two cases demonstrated a positive response in both vitality tests, resulting in a score of 3. The Chi-square test revealed that there was no significant difference between the PRP and PRF treatment groups in terms of success scoring ($P = 0.171$).

4. Discussion

A comprehensive evaluation of RET is essential for the predictability of treatment success [10]. A lot of research has been conducted to evaluate the

Table 1. Clinical follow-up and success scores.

	Platelet Rich-Plasma							Platelet Rich-Fibrin						
Case	A/S	E	TN	T	AC	V	SS	A/S	E	TN	T	AC	V	SS
1	14/M	T	22	59	+	−	2	19/M	T	11	24	-	−	1
2	13/F	T	21	48	+	−	2	9/M	T	11	22	-	−	1
3	18/M	T	11	48	-	−	1	9/M	T	21	13	+	+	3
4	13/F	D	35	46	-	−	1	14/M	T	21	21	-	−	1
5	15/F	T	11	30	-	−	1	10/F	T	22	19	+	+	3
6	14/F	D	35	30	-	−	1	12/M	T	11	39	-	−	1
7	13/M	T	11	51	-	−	1	12/M	T	21	39	-	−	1
8	14/M	T	21	31	+	−	2	9/F	T	11	20	-	−	1
9	11/M	T	21	36	+	−	2	9/F	T	21	15	-	−	1
10	11/F	T	21	55	+	−	2	16/F	D	34	31	+	−	2
11	14/M	T	21	55	+	−	2	11/F	T	21	46	+	−	2
12	14/F	T	21	77	+	−	0	10/M	T	21	21	+	−	2
13	12/F	D	45	49	+	−	2	13/F	D	45	33	+	−	2
14	13/F	T	11	57	-	−	1	11/F	T	11	32	-	−	1
15	14/M	T	21	58	+	−	2	11/F	T	21	32	-	−	1
16	12/F	T	21	55	+	−	2	14/M	T	21	32	+	−	2
17								10/M	T	21	13	+	−	2
18								14/F	T	21	12	-	−	1
19								14/F	T	21	33	-	−	1
20								17/F	D	34	30	-	−	1
21								11/F	T	11	24	-	−	1
22								10/F	T	21	25	+	−	2

A/S, age/sex (M, male; F, female); AC, apical closure; E, etiology (T, trauma; D; decay); SS, success score; V, vitality; T, time (month); TN, tooth no.

Table 2. Reliability analysis of measurements among researchers.

		Mean (%)	S.D.	z	P value
Increase in Root length	**Measurement 1**	11.09	1.75	−0.606	0.544
	Measurement 2	12.40	2.09		
Increase in dentin Wall thickness	**Measurement 1**	19.68	3.37	−1.581	0.114
	Measurement 2	22.63	4.45		

Wilcoxon test $p < 0.05$.

Table 3. Increase in root length and dentin thickness data.

	Scaffold	N	Mean (%)	S.D.	z	P value
Increase in Root length	**PRP**	16	13.24	2.78	−0.384	0.701
	PRF	22	11.61	2.19		
Increase in dentin Wall thickness	**PRP**	16	31.98	7.49	−2.247	0.025
	PRF	22	14.05	2.81		

Mann–Whitney U test $p < 0.05$.

success of this treatment radiographically and clinically [7,9,22–26]. Much research on regenerative endodontics shows excellent results regarding tooth survival. A scaffold that can support regeneration in root canals is essential for the success of regenerative endodontics. The blood clot formed in the root canals after apical bleeding is recommended as a scaffold by the American Association of Endodontists [15]. However, even if anesthesia without adrenaline is used, apical bleeding is not always easily achieved, and blood clots may not be formed [17]. In some research, blood transfer was performed from the distal root canal to the mesial root canals for mesial root canals where there was insufficient bleeding in molar teeth [27]. However, this is not possible in single canal teeth; in these cases, waiting until healthy tissue is formed, from which bleeding can be obtained in the periapical area, is recommended [8]. Uses alternative scaffolds rich in growth factors such as PRP and PRF if the blood clot cannot be formed [9,26]. In research, these scaffolds were compared, and no clinical or radiological superiority of PRP, PRF, and blood clots was observed [7,9,26]. Jadhav et al. [28] found PRP to be more successful than a blood clot, but since PRP was used together with a blood clot in this research, the success is not attributed to the use of PRP alone. Narang et al. [25] found PRF more successful than blood clots and PRP. Our study used PRP or PRF scaffolds combined with apical bleeding. The difference was insignificant between the groups in terms of treatment success.

Apical closure, increase in root length and increase in dentin thickness are frequently examined when evaluating root growth in regenerative endodontics [29,30]. Apical closure or apical narrowing is a physiological process that can be observed even in apexification treatments [31]. Apical closure can be seen even in unsuccessful RET [32]. Therefore, apical closure alone should not be considered as root development without increase in root length and increase in dentin thickness [14]. In our study, apical closure was seen in half of the cases. In some cases, the short follow-up period may have caused a decrease in the number of apical closures recorded.

Dentin thickness and root length increase, one of the most important results of RET, has been evaluated by various methods [9,23,24]. Some researchers used Image J computer software to assess root growth quantitatively [9,22–24]. Some researchers have also evaluated root development by proportioning changes in root length and dentin thickness in the teeth [20,21]. In research, the radiographic success of the treatment was evaluated by measuring radiographic findings such as increase in root length, increase in dentin thickness, decrease in apical diameter (apical diameter), radiographic root area, and periapical bone density [7,9,22–24]. Achieving radiographic standardization is difficult, especially for young and school-age children. The continuous bone and tooth development makes obtaining a parallel film to the first image difficult after years. Angulation errors made during radiography from young patients also cause image distortions [14]. Some researchers used ImageJ software's TurboReg (Biomedical Imaging Group, Swiss Federal Institute of Technology, Lausanne, VD, Switzerland) plug-in to reduce the error in radiographic film distortions [13,22]. With the TurboReg plug-in, dimensional changes caused by the angle difference between the preoperative and postoperative films were reduced by using three different fixed points in the films. However, it may not always be possible to find three different points that have remained unchanged on radiographs after years, especially in the anterior region. In the research by Silujjai and Linsuwanont [20], since three different fixed points for turboReg analysis could not be found, the magnification rate was calculated using two different fixed points (mesial and distal CEJ). Dentin thickness increase and root length increase were measured by this magnification rate. However, it can be misleading to calculate the size change in two planes using the magnification ratio in one plane. Due to angulation errors, image distortions may occur at different rates in the vertical and horizontal planes. In our study, dentin thickness increase measurement is made in the horizontal plane, and therefore, the magnification rate obtained with ImageJ software in the horizontal plane was used for dentin thickness measurements. Similarly, since the root length increase was measured in the vertical plane, the magnification rate obtained in the vertical plane was used.

Three basic methods were used with the help of ImageJ software to evaluate root growth quantitatively. Bose et al. [22] calculated the root length by drawing a straight line from the CEJ to the radiographic root apex and the root thickness by drawing a horizontal line from the apical parts of two-thirds of the roots. Differently, Alobaid et al. [3] used the mean of mesial and distal length while evaluating the root length. While assessing the root thickness, they used the mean value measured from three different root levels. Flake et al. [23] measured the total radiographic root area instead of separately evaluating the root length and width increase. However, this method cannot determine the amount of root length increase or dentin thickness increase. In addition, calculation difficulties may occur in molar teeth' root and canal areas. In the study conducted by Sutam et al. [33], it has been shown that all three methods complement each other. Our study calculated the mean root length increase from the mesial and distal CEJ. Dentin thickness was calculated from the two-thirds apical level. It was sometimes impossible to calculate radiographic root area or measure root thickness from three different levels because dentin barrier and calcifications were seen. Most studies comparing the increase in root length and dentin thickness between blood clots, PRP, and PRF treatment methods did not show a statistically significant difference [7,9,26]. Narang et al. [25] found the PRF group more successful than the blood clot and PRP group. In our study, while root length increase was statistically insignificant between PRP and PRF groups, PRP was statistically significant in dentin thickness increase. This may be because the follow-up period of the PRP group is much more extended than that of the PRF group. In addition, another reason for this difference may be that PRP as a scaffold is easier to spread on the dentin walls and root apex than gel-like PRF, thanks to its liquid consistency.

Although there are three goals in RET, such as symptom relief, root development, and vitality of the teeth, the most basic treatment success criterion is to keep the teeth asymptomatic [15]. Shivashankar et al. [26] evaluated the treatment success in two different ways: strict and loose criteria of success regarding the periapical health of the teeth. Bezgin et al. [7] scored the treatment success between 0 and 3, and Ulusoy et al. [9] is between 0 and 2 regarding the treatment goals achieved. In both studies, the difference between the treatment groups was statistically insignificant. In our study, treatment success was evaluated between 0 and 3 points, and the success score was statistically insignificant between the treatment groups.

The vitality of the teeth is one of the desired goals. However, the absence of a positive response to the tests does not necessarily indicate that there is no vitality. Thick restorative materials placed on root canals may adversely affect the vitality tests [17,34]. The formation of vital tissue at the apical part of the root and the formation of hard tissue with an accumulation of osteodentin in the canal walls may also adversely affect the response to vitality tests [35]. There was no positive response to vitality in our study, except for two cases. Thick restorations in teeth with excessive substance loss and MTA thickness may cause this. Finally, in some cases, calcifications and dentin barriers may have made obtaining results challenging. Despite all this, the production of hard tissue and the continuation of root development in the teeth can be a sign that the teeth are vital [26].

The use of PRP and PRF in RET showed similar results regarding tooth survival. Both treatment groups showed similar results in root length increase. However, the PRP group showed a significantly higher increase in dentin thickness. RET is a valuable treatment in terms of the future vision of endodontics. Despite numerous studies being published in this field today, longer-term case studies and research are needed to achieve all the treatment objectives. Conducting more research and innovative studies in this field will be guiding.

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Presentation at a meeting

Organisation: 4th Health Sciences and Innovation Congress.

Place: Baku, Azerbaijan.
Date: July 5-6 2021.

Conflicts of interest

The authors deny any conflicts of interest related to this study.

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