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ORIGINAL RESEARCH

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Clinical and radiographic evaluation of regenerative endodontic procedures (REPs) with or without concentrated growth factor (CGF) as scaffolds for non-vital immature mandibular premolars

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Abstract

This study aimed to perform clinical and radiographic investigations of the effect of regenerative endodontic procedures (REPs) with and without concentrated growth factor (CGF). Fifty-six non-vital and immature teeth from 56 patients were randomly categorized into two groups. Following chemical and mechanical preparation, REPs with and without CGF as a scaffold was induced in the blood clot (BLC) group and the CGF group. All patients were clinically and radiographically evaluated at 6-month and 12-month intervals to monitor their progress and treatment outcomes. When considering the total number of patients, the follow-up rate was 96.4% (54 out of 56 patients) over a 12-month period. Favorable clinical and radiographic outcomes were observed in 92.6% of patients (25 out of 27) in both the CGF and BLC groups; there were no significant differences between the two groups in these respects ($p > 0.05$). Notable differences were, however, observed in radiographic measurements relating to the development of root length and radiographic root area when compared between the CGF and BLC groups at both the 6-month and 12-month follow-up intervals ($p < 0.05$). REPs have been proven to represent a conservative and effective approach for promoting maturogenesis in non-vital and immature teeth. Furthermore, the incorporation of CGF as scaffolds holds promising potential for enhancing the desired biological outcomes of this regenerative technique. These findings highlight the clinical significance and potential benefits of CGF supplementation in REPs, further supporting its application in the field of endodontics.

Keywords

Maturogenesis; Concentrated growth factor; Immature teeth; Revascularization

1. Introduction

Pulp regeneration is an area of growing research interest, particularly in the field of regenerative endodontic procedures (REPs) [1, 2]. The concept of REPs was originally reported over half a century ago by Ostby [3], who demonstrated that a blood clot induced for bleeding in root canals undergoes gradual transformation from a granulation tissue into a fibrous connective tissue. The primary goals of REPs are to alleviate symptoms, promote bone healing, and achieve increased root wall thickness and/or root length, as well as increased levels of vitality [4–6]. REPs have proven to be a successful treatment for the treatment of necrotic immature teeth and have demonstrated consistently high survival and success rates [7–9].

REPs rely on three essential components: activators, stem cells and scaffolds [6]. Dental pulp regeneration can be achieved by either cell homing or transplantation [10]. The transplantation of cells involves the exogenous transplantation of stem cells into scaffolds loaded with signaling molecules within the root canal to facilitate the regeneration of the pulp system [11]. On the other hand, cell homing relies on the recruitment of endogenous stem cells into a specific anatomical compartment *via* specific biological signaling molecules [12]. Cell homing is particularly suitable in clinical applications where the *in vitro* isolation of stem cells is not required [13]. With regards to scaffolds, the initial step involves obtaining a blood clot through over-instrumentation; the blood clot subsequently serves as a delivery system for the blood column, growth factors, and stem cells within the pulp system [14]. However, achieving periapical bleeding into the pulp space is not always feasible [2]. Furthermore, erythrocytes within the blood clot can undergo necrosis and thus exert impact on key properties of the scaffolds [15]. To overcome these limitations, the use of concentrated growth factor (CGF), obtained from the patient's own blood, which contains a high concentration of platelets, can enhance the

blood column and improve the key properties of the scaffold.

CGF is an advanced second-generation platelet concentrate [16] and is obtained by the application of a centrifugal device that follows a specific centrifugation protocol [17]. This protocol harnesses the physical acceleration and deceleration necessary to activate alpha granules within the platelets, thus resulting in an autologous blood concentrate that is rich in higher concentrations of growth factors and cluster of differentiation 34+ (CD34+) cells [16]. CGF has demonstrated excellent regenerative capabilities in the promotion of bone tissue, soft tissue and skin regeneration and has gradually been applied in various fields, including dentistry, plastic surgery, wound repair and neural tissue regeneration [18]. CGF exhibits a denser fibrin matrix containing high concentrations of many key growth factors. Although some *in vitro* studies have investigated the effects of CGF on pulp cells [19], only one study has performed *in vivo* research to substantiate the effects of CGF in apexogenesis [20].

On the backdrop of these previous studies, we hypothesized that the introduction of CGF into a disinfected root canal during REPs would lead to improved outcomes. The specific aim of this study was to evaluate the efficacy of REPs performed with or without CGF as scaffolds by evaluating the range of key parameters by routine radiographic assessment, including an increase of root length (RL), closure of the apical foramen, and an increase of radiographic root area (RRA).

2. Materials and methods

This study was conducted as a randomized, controlled, single-blinded, and parallel clinical trial (RCT) using comparative analyses. Researchers carried out recruitment, treatment, and follow-up for the participants at Shaoxing Stomatological Hospital. A total of 56 teeth were treated between January 2022 and June 2022.

2.1 Sample size calculation

Based on a previous study [21], the effect size of periapical healing in REPs was determined to be 0.52. Using this effect size, a type I error of 0.05, and a power of 0.8, a minimum sample size of 48 subjects (24 subjects per group) was required to detect a significant difference between the study groups. Considering a potential dropout rate of 15%, the sample size was increased to 56 subjects (28 subjects per group). The sample size calculation was performed using G*Power software version 3.1.9.2 (Franz Faul, Berlin, Germany).

2.2 Randomization

A random number sequence ranging from 1 to 56 was generated by a co-investigator (MS) using computer software (http://www.random.org/). The sequence was then divided into two equal columns, labeled as Column 1 and Column 2. One column was designated for the CGF group, while the other column was assigned to the BLC group. The randomization table was held securely by the Co-investigator (MS). To allocate participants to their respective groups, four-folded numbered papers were placed inside opaque envelopes. At the beginning of the research study, each patient selected an envelope, and their allocation to either the BLC group or the CGF group was determined based on the random sequence generated earlier.

2.3 Inclusion criteria

Our inclusion criteria were as follows: healthy patients (Category: American Society of Anesthesiologists class 1) of both genders, aged between 7 and 16 years; mandibular premolars with an immature root apex (apical opening >1 mm); mandibular premolars not requiring a post or core for the final restoration; traumatically or cariously exposed mandibular premolars, and non-vital permanent anterior teeth with or without apical periodontitis.

2.4 Exclusion criteria

The exclusion criteria were as follows: patients with systemic diseases or currently undergoing systemic corticosteroid therapy; patients who reported bruxism or clenching; patients who had taken analgesics or other medications within the 12 hours prior to the procedure that could potentially alter pain perception; patients with a history of allergic reactions to any of the medications or materials used in the research; teeth with a vital pulp or complete root formation; teeth exhibiting internal or external root resorption, and non-cooperative patients.

2.5 Treatment

The patients in both groups received treatment from a single operator (Zhang) following the same protocol, with the exception of CGF application as a scaffold. The procedure began with access opening under a rubber dam using a round diamond. Minimal mechanical instrumentation was performed using an ISO #60 H-file (Kerr Corporation, Orange, USA), accompanied by irrigation with 20 mL of 2.5% sodium hypochlorite (NaOCl). The working length was determined radiographically by inserting a large file into the canal. Once the canal had been dried with paper points, an inter-appointment medication of triple antibiotic paste was applied using a sterile number 40 reamer (Kerr Corporation, Orange, USA). This standardized protocol, involving chemo-mechanical preparation and disinfection, employed consistently for all cases. In situations where canals exhibited weeping, an additional inter-appointment dressing was administered as required until the tooth became symptom-free and the canal was dry.

REPs in the BLC group were conducted using the following steps. Initially, a local anesthetic solution without adrenaline (X19990003, Septodont, Paris, France) was administered by infiltration around the apex of the incisor. Then, we prepared a fresh, sterile 23-G needle with a rubber stopper set 2 mm beyond the established working length. Using sharp, fine strokes, the file was gently pushed beyond the boundaries of the canal and into the periapical tissue. Once clear bleeding was observed at the cervical portion of the root canal, we inserted a cotton pellet approximately 3–4 mm in the canal which was held in place for a period of 5–7 minutes, thus allowing for the formation of a blood clot.

REPs with CGF as a scaffold were conducted as follows. Initially, 10 mL of venous blood was collected and transferred to sterile Vacuette tubes without the addition of anticoagulant

solutions. These tubes were then centrifuged using a one-step centrifugation protocol in a Medifuge machine. The centrifugation process involved several steps, including 30 seconds of acceleration, 2 minutes at 2700 rpm, 4 minutes at 2400 rpm, 4 minutes at 2700 rpm, 3 minutes at 3000 rpm, and 36 seconds of deceleration; then, the protocol was terminated. Upon completion of centrifugation, four distinct layers were obtained: the top layer consisted of serum, the second layer consisted of the fibrin buffy coat, the third layer contained the liquid phase with growth factors, and the fourth layer contained red corpuscles. Next, we carefully removed the CGF layer at the interface between the CGF and red corpuscle layers using sterile scissors. A small proportion of the red corpuscles was intentionally retained with the CGF, as these corpuscles are known to contain some vital growth factors. Following the previously described revascularization procedure and prior to clot formation, the CGF was sectioned into pieces and packed into the root canal, ensuring that it extended approximately 3–4 mm apical to the cementoenamel junction (CEJ).

After completing the procedure, the access opening was sealed using resin-modified glass ionomer cement (RMGIC, Shofu dental, Kyoto, Japan). To establish a baseline, an intraoral radiograph was acquired by a paralleling device (XCP-ORA® Instrument Kit, Dentsply Rinn, Elgin, IL, USA). Subsequently, all patients were scheduled for follow-up appointments at 1 week, 6 months and 12 months. During these visits, follow-up radiographs were obtained using the same device to ensure consistent alignment and positioning of the films and x-ray beam. This approach aimed to facilitate comparison of the radiographs with minimal distortion and magnification, thus enhancing the accuracy of evaluation.

2.6 Assessment of treatment success

2.6.1 Primary goal

Successful cases were defined as those demonstrating an absence or reduction of radiolucency, along with an asymptomatic clinical presentation (no sinus tract, swelling, and the absence of spontaneous, palpation or percussion pain). Pre- and postoperative pain levels were determined by applying the visual analog scale. Postoperative pain level was recorded at 1 week.

Postoperative radiographic outcomes were categorized into four types: (1) the absence of a periapical lesion; (2) reduction of the periapical lesion, (3) enlargement of the periapical lesion, and (4) uncertain (cases that could not be defined as either of the first three situations).

Conversely, cases exhibiting radiographic enlargement of the periapical lesion or a symptomatic tooth were classified as unsuccessful. This criterion allowed for clear differentiation between successful and unsuccessful outcomes.

2.6.2 Secondary goal

Radiographic data obtained from follow-up imaging were analyzed to evaluate changes in RL, apical foramen width (AFW) and RRA. A specialized radiologist, who was blinded to the grouping, performed the imaging measurements and evaluations. Image J software (version 2, the National Institutes of Health, Bethesda, MD, USA) was utilized for image calibration processing using the Turboreg plugin, following the method described by Bose [22]. Measurements of RL, AFW and RRA were conducted in accordance with the method described previously by Elsheshtawy [23] and Jun [24]. These standardized measurement techniques ensured the accurate and consistent assessment of radiographic parameters (Fig. 1).

2.7 Statistical analyses

Quantitative data are presented as mean ± standard deviation (SD). The *t*-test was used to identify statistically significant differences between quantitative data. Qualitative data are described in terms of frequencies and percentages. The Chi-squared (χ^2) test was used to identify significant differences among the qualitative data. The significance level was set at $p \leq 0.05$. Statistical analysis was performed using IBM SPSS software (version 28, IBM Corporation, NY, USA). A p-value < 0.05 was considered statistically significant, while a p-value ≥ 0.05 was considered not statistically significant.

3. Results

Of the 378 patients initially assessed, 56 patients met the inclusion criteria and were enrolled and included in the study, as depicted in Fig. 2.

3.1 Demographic data

Table 1 displays the baseline demographic data of the 56 patients. The demographic data, age, gender, periapical radiolucency presence, preoperative pain level and swelling or sinus tract distribution of the study subjects are displayed in Table 1. There were no statistically significant differences between the two groups with regards to these parameters (p = 1.00, 0.17, 1.00, 0.63 and 0.79 respectively).

3.2 Primary goal

At the end of the follow-up period, the clinical success rates were 92.59% for the BLC group and 92.59% for the CGF group; these were not statistically significant (p = 1.00), as shown in Table 2. There was no significant difference between the BLC group and the CGF group in terms of postoperative radiographic outcomes (p = 0.57) and the postoperative presence of pain (p = 1.00).

3.3 Radiological examinations

Line plots were used to illustrate the changes over time for RL, AFW and RRA in both groups (Fig. 3). The mean initial preoperative RL was 10.65 ± 1.83 mm in the BLC Group and 10.05 ± 2.00 mm in the CGF group; these were not significantly different (p = 0.257), as shown in Table 3. The mean increase rate in RL during the 6- and 12-month follow-up was 5.9 ± 6.2% and 10.9 ± 7.7% in the BLC group, and 8.3 ± 4.9% and 19.6 ± 12.7% in the CGF group, respectively. A statistically significant difference was observed between the BLC group and CGF group at the 12-month follow-up point (p = 0.005), but not at the 6-month follow-up (p = 0.122).

As shown in Table 3, the mean initial preoperative AFW was 2.47 ± 0.75 mm in the BLC Group and 2.57 ± 0.80 mm

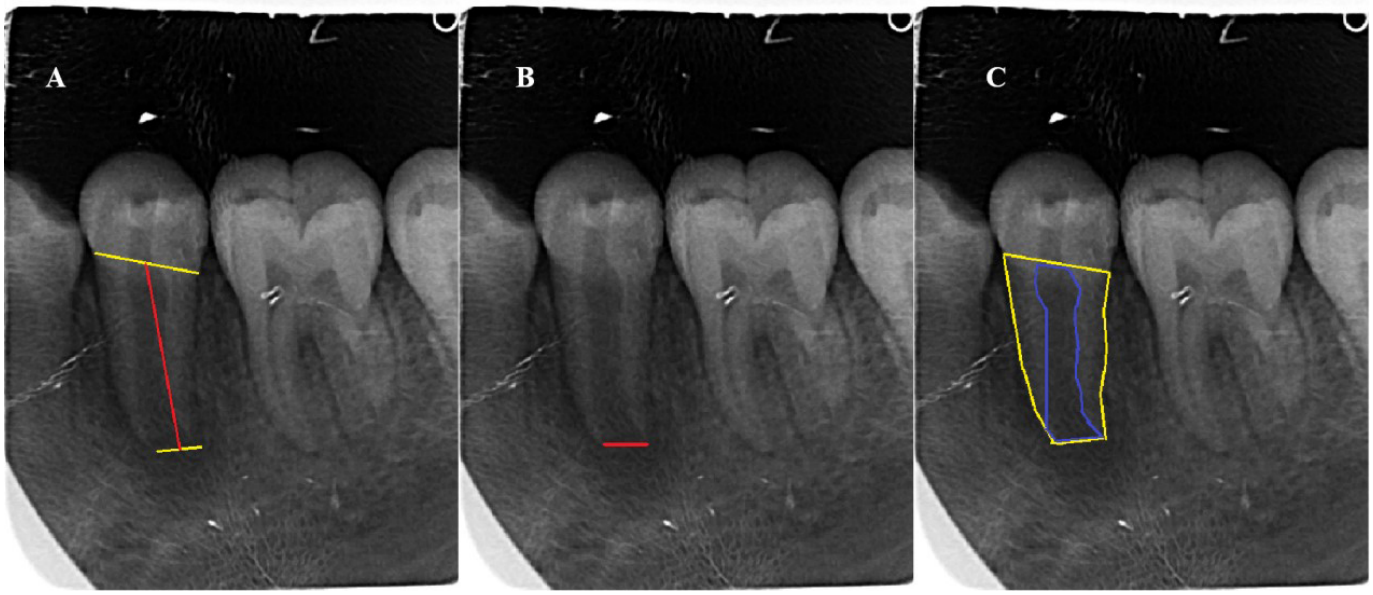

FIGURE 1. Schematic diagram of root measurements on periapical X-rays. (A) root length (RL)—the linear distance (red line) from the cementoenamel junction (CEJ) to the midpoint on the straight line from the apical foramen; (B) apical foremen width (AFW)—the linear distance (red line) between the apical foramen and the point on the straight line from the apical foramen; (C) RRA (radiographic root area)—over all root area (area marked by yellow line) minus pulp space (area marked by blue line). The area measured using Image J after outlining the contour of the root with multiple points.

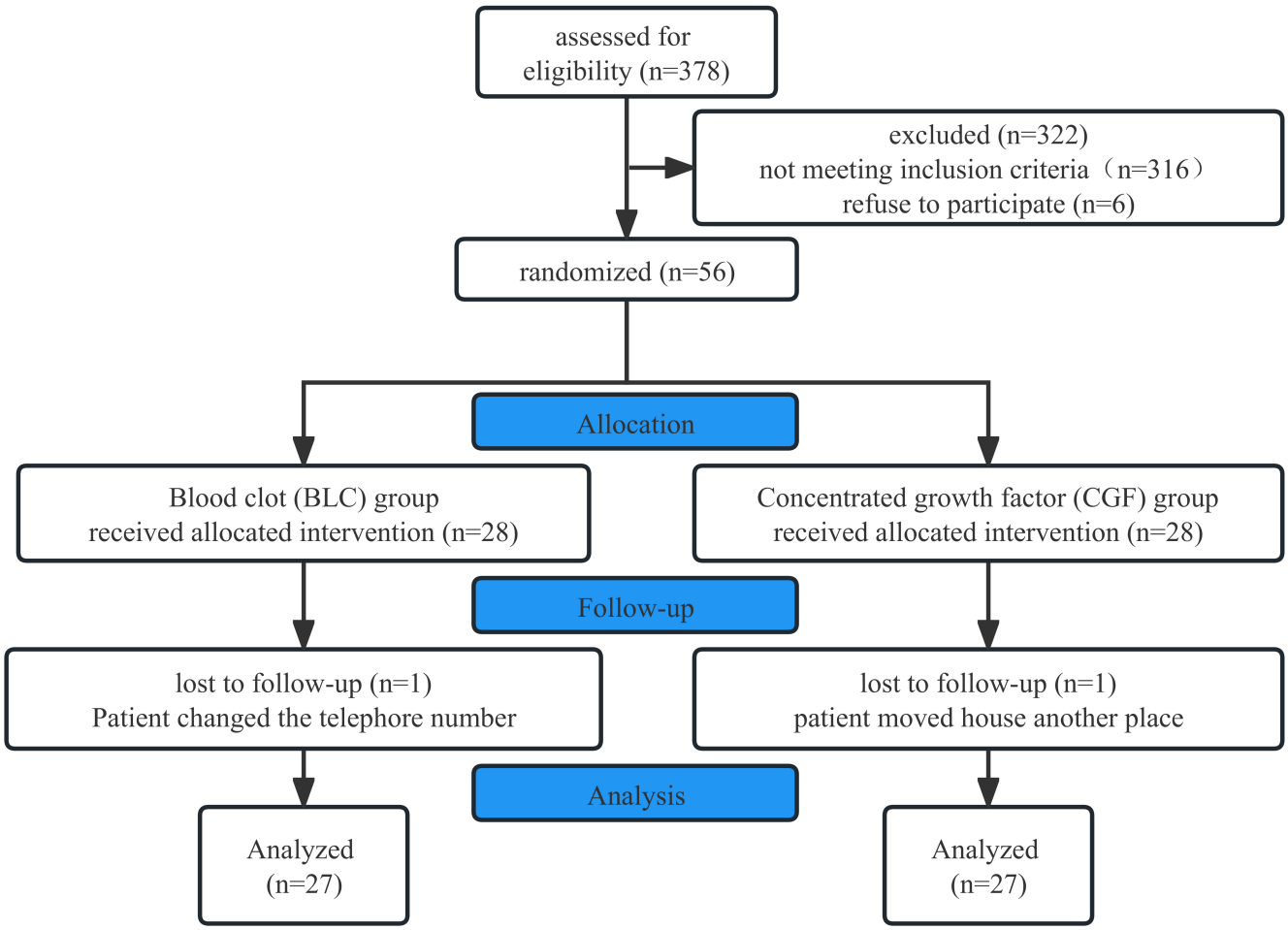

FIGURE 2. A flow diagram of the study.

TABLE 1. Demographic data, presence of periapical radiolucency, swelling, sinus tract, preoperative pain level, and radiographical data according to the groups.

Group	BLC	CGF	*p*-value
N	27	27	
Age, Mean ± SD	10.67 ± 1.21	10.67 ± 1.44	1.000
Sex, n (%)			
Male	10 (37.04%)	15 (55.56%)	0.172
Female	17 (62.96%)	12 (44.44%)	
Periapical radiolucency presence, n (%)			
Yes	23 (85.19%)	23 (85.19%)	1.000
No	4 (14.81%)	4 (14.81%)	
Swelling or sinus tract, n (%)			
Yes	15 (55.56%)	14 (51.85%)	0.785
No	12 (44.44%)	13 (48.15%)	
Preoperative pain, Mean ± SD	4.96 ± 2.16	4.63 ± 2.80	0.626
Preoperative RL, Mean ± SD	10.65 ± 1.83	10.05 ± 2.00	0.257
Preoperative AFM, Mean ± SD	2.47 ± 0.75	2.57 ± 0.80	0.626
Preoperative RRA, Mean ± SD	33.01 ± 5.68	31.36 ± 5.77	0.294

SD, standard deviation; BLC, blood clot; CGF, concentrated growth factor; RL, root length; AFM, apical foramen width; RRA, radiographic root area.

TABLE 2. The percentage of clinical success according to clinical and radiographic outcomes in BLC and CGF group.

Group	BLC	CGF	*p*-value
N	27	27	
Primary goal, n (%)			
Successful cases	25 (92.593%)	25 (92.593%)	1.000
Unsuccessful cases	2 (7.407%)	2 (7.407%)	
Postoperative radiographic outcome, n (%)			
Absence of the periapical lesion	11 (40.741%)	16 (59.259%)	
Reduction of the periapical lesion	14 (51.852%)	9 (33.333%)	0.570
Enlargement of the periapical lesion	1 (3.704%)	1 (3.704%)	
Uncertain	1 (3.704%)	1 (3.704%)	

BLC, blood clot; CGF, concentrated growth factor.

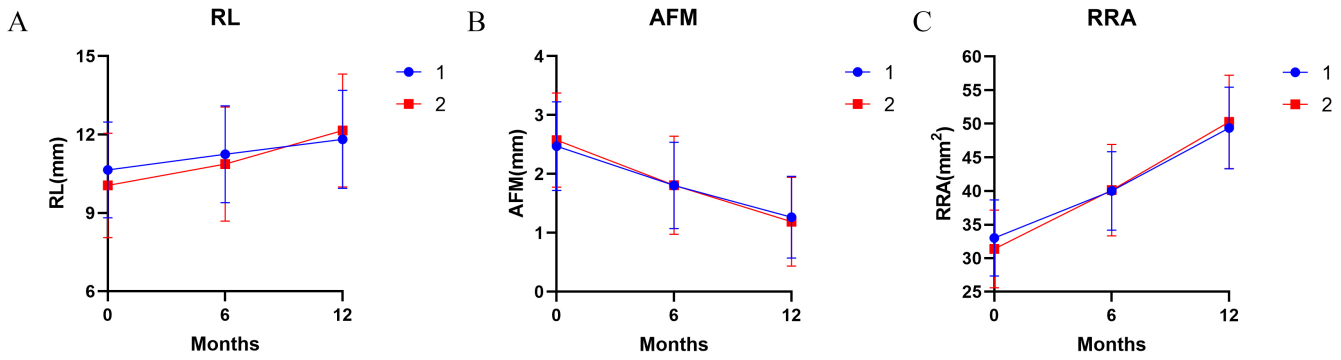

FIGURE 3. Change over time of the three dental outcomes (root length (RL)). (A) Apical foremen width (AFW). (B) Radiographic root area (RRA). (C) At baseline, 6 and 12 months.

TABLE 3. The percentage changes over time of the three dental outcomes measured using periapical radiographic method in both groups.

Group	BLC	CGF	*p*-value
N	27	27	
Mean increase in RL percent at 6 months (%) (Mean ± SD)	5.9 ± 6.2	8.3 ± 4.9	0.122
Mean increase in RL percent at 12 months (%) (Mean ± SD)	10.9 ± 7.7	19.6 ± 12.7	0.005*
Mean reduction in AFM percent at 6 months (%) (Mean ± SD)	27.5 ± 22.2	30.9 ± 20.3	0.567
Mean reduction in AFM percent at 12 months (%) (Mean ± SD)	47.8 ± 21.6	54.4 ± 21.5	0.282
Mean increase in RRA percent at 6 months (%) (Mean ± SD)	21.9 ± 10.3	28.4 ± 13.3	0.039*
Mean increase in RRA percent at 12 months (%) (Mean ± SD)	50.4 ± 18.0	59.4 ± 22.6	0.112

*SD, standard deviation; BLC, blood clot; CGF, concentrated growth factor; RL, root length; AFM, apical foramen width; RRA, radiographic root area; *p-value < 0.05.*

in the CGF group; these were not significantly different (p = 0.626). The mean reduction rate in AFW during the 6- and 12-month follow-up was 27.5 ± 22.2% and 47.8 ± 21.6% in the BLC group, and 30.9 ± 20.3% and 54.4 ± 21.5% in the CGF group, respectively. No statistically significant difference was observed between the BLC group and CGF group at either time point (p = 0.475 and 0.282, respectively).

As shown in Table 3, the mean initial preoperative RRA was 21.9 ± 7.5 mm^2 in the BLC Group and 31.36 ± 5.77 mm^2 in the CGF group; there was no significant difference between the two groups (p = 0.294). The mean increase rate in RRA during the 6- and 12-month follow-up was 21.9 ± 12.5% and 50.4 ± 18.0% in the BLC group, and the mean reduction rate in RRA during the 6-month and 12-month follow-up was 28.4 ± 17.1% and 59.4 ± 22.6% in the CGF group, respectively. A statistically significant difference was observed between the BLC group and CGF group at the 6-month follow-up point (p = 0.039), but not at the 12-month follow-up (p = 0.112).

4. Discussion

Initially proposed for dental pulp revascularization in immature permanent teeth, REPs have shown promising results for inducing root development, developing dentinal wall thickness, and continued apical closure [25]. More recently, there has been growing interest in exploring the potential application of REPs [25]. By regenerating dental pulp, it is possible to establish an innate immune system within the pulp system which can help to reduce the incidence of reinfections [26]. The primary objective of REPs is to promote induced root development and achieve apical closure. This process is facilitated by the presence of mesenchymal stem cells (MSCs) derived from the apical papilla, which differentiate into odontoblasts when provided with a suitable matrix. In the current study, CGF was utilized as a scaffold for REP.

The suitability of BLC as a scaffold system for pulp revascularization has raised concerns in previous research, as it is believed to primarily increase healing rather than control pulp regeneration [27]. Consequently, there is an urgent need to develop a more effective scaffolding system that can effectively promote the formation of pulp-like tissue and induce the differentiation of odontoblastic cells [28].

Concentrated growth factor (CGF) is an advanced type of platelet concentrate that belongs to the second generation [29]. Unlike platelet-rich fibrin or platelet-rich plasma, CGF is produced by alterations in centrifugation speed ranging from 2400 to 2700 rpm. This modified centrifugation process leads to the formation of a denser matrix with a higher concentration of growth factors compared to platelet-rich fibrin or platelet-rich plasma [20]. CGF has a unique fiber structure that is tightly packed, making it relatively stiffer than platelet-rich fibrin or platelet-rich plasma [16]. One of the notable features of CGF is its sustained release of growth factors for approximately 14 days, with the peak concentration occurring on the 5th day [30]. This sustained release profile is particularly beneficial as it supports long-term cell proliferation, matrix regeneration, and angiogenesis; this ability relies on the abundant presence of Transforming growth factor beta 1 (TGF-β1) and Vascular Endothelial Growth Factor (VEGF), which play crucial roles in these processes [31]. Immunohistochemical analysis demonstrated that CD34+ cells are present in CGF; these cells are vital for maintaining vascular supply and promoting angiogenesis [32]. The quantification of CGF contents is relatively simple because the centrifugation conditions for CGF preparation are constant. Consequently, when CGF is applied in a clinical situation, the outcomes could be more predictable when compared to other platelet concentrates.

In the CGF group, a faster increase in RL and RRA was observed than in the BLC group at both 6 and 12 months. This could be attributed to the fact that CGF possesses a denser fibrin matrix with a higher concentration of growth factors when compared to the BLC group [20]. The presence of the enriched matrix in CGF may contribute to enhanced tissue regeneration and accelerated root development, thus resulting in more favorable radiographical outcomes. In addition, the release of inflammatory cytokines, such as Interleukin-1α (IL-1α) and TNF-β, may be associated with the inhibition of stem cells and could potentially have adverse effects on the biological function of the Hertwig epithelial root sheath (HERS) [33, 34]. It is possible that these effects may be reversible after the resolution of infection. Further research is now needed to evaluate the influence of periapical lesions on the survival and function of HERS. In addition, it is important to investigate the effects of key infection factors, such as the type of infection, the characteristics of infection, the size of the

periapical lesion, and the duration of infection, on the survival of HERS. Understanding these factors will provide valuable insights into the overall health and function of the HERS in relation to infection, thus contributing to better treatment approaches in the future.

The present research has certain limitations. Firstly, the follow-up period was relatively short, thus limiting our ability to assess long-term differences in clinical outcomes. Future studies with extended follow-up periods would provide valuable insights into the long-term prognosis of the treatments under comparison. Secondly, the current research was carried out in a single center; this may impact the generalizability of our findings. Further research, involving multiple centers, would enhance the reliability and applicability of our results. In addition, in this study, we focused primarily on clinical and radiographic outcomes; further investigations are now needed to investigate the specific mechanisms underlying the observed effects. Future studies incorporating mechanistic research would provide a deeper understanding of the biological processes involved. Despite these limitations, this study contributes valuable insights into the comparison of treatment outcomes and paves the way for future research addressing these gaps.

5. Conclusions

REPs are effective and conservative methods for improving maturogenesis in non-vital and immature teeth. The application of CGF as scaffolds can potentially increase the biological outcome of REPs.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analyzed during the current study are not publicly available due individual privacy can be compromised but are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

MS and YZ—designed the research study, wrote the manuscript. YZ—performed the research, analyzed the data. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This was a randomized controlled single blinded parallel clinical trial (RCT) performed using comparative tests. The study protocol received approval from the Institutional Review Board/Ethical Committee at Shaoxing Stomatological Hospital, Shaoxing, China (ID: 2022-33-02). All patients or their parents/legal guardians provided informed consent after receiving a thorough explanation of the treatment procedures, potential side effects and available treatment alternatives.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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