Received: 1 April 2022 | Revised: 25 September 2022 | Accepted: 12 November 2022

DOI: 10.1111/ipd.13039

INTERNATIONAL JOURNAL OF PAEDIATRIC DENTISTRY WILEY

ORIGINAL ARTICLE

Evaluation of the characteristics of root canal calcification after regenerative endodontic procedures: A retrospective cohort study over 3 years

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Abstract

Aim: This study aimed to evaluate the characteristics of root canal calcification after regenerative endodontic procedures (REPs) during long-term follow-up.

Design: Data of children who underwent REPs and were followed up for >3 years in the Department of Pediatric Dentistry, Peking University School and Hospital of Stomatology, Beijing, China, from January 2013 to January 2019, were collected. All the patients were treated by the protocol of REPs based on the American Association of Endodontists (AAE) protocol. A total of 91 teeth of 54 boys and 37 girls (average age 10.4 ± 1.9 years) with follow-up duration >3 years were included. The follow-up duration ranged from 36 to 92 months (average, 53.2 ± 13.4 months). The prevalence, contributing factors, and long-term prognoses of root canal calcification after REPs are discussed. Independent *t*-test and χ^2 test were used for statistical analysis.

Results: The incidence of root canal calcification was 78% (71/91). The use of calcium hydroxide paste was significantly correlated with the occurrence of root canal calcification ($p < .05$). Some teeth showed aggravation of calcification with time; however, not all teeth showed calcification after longer follow-up duration.

Conclusions: Teeth treated with REPs had a relatively high probability of root canal calcification detection during the long-term follow-up. The occurrence of calcification is related to the use of calcium hydroxide paste but does not affect the long-term prognosis of teeth.

KEYWORDS

immature permanent teeth, regenerative endodontic procedures, root canal calcification

1 | INTRODUCTION

Regenerative endodontic procedures (REPs) were designed to treat immature permanent teeth with pulp necrosis or apical lesions. The current American Association of Endodontists (AAE) Glossary of Endodontic Terms defines regenerative endodontics as "biologically-based procedures designed to physiologically replace damaged tooth structures, including dentin and root structures, as well as cells of the pulp-dentin complex."

In recent years, root canal calcification has been reported as a common phenomenon after REPs, and the incidence of root canal calcification after REPs ranges from 20% to 91%.[1–7] There is concern that the occurrence of calcification may compromise future endodontic therapy, if indicated.[8,9] During REPs, the intracanal medications

used for root canal disinfection include calcium hydroxide and antibiotic paste (triple antibiotic paste/double antibiotic paste). In 2017, Song et al.[2] analyzed 29 teeth with an average follow-up duration of 24.9 months after REPs and found that the incidence of root canal calcification in teeth with induced bleeding (69.6%) was higher than that in teeth without induced bleeding (33.4%). In addition, the incidence of calcification in teeth treated with calcium hydroxide (76.9%) was higher than that in teeth treated with antibiotic paste (46.2%). Due to the limitation of the sample size, the authors, however, failed to draw any statistical conclusions.

At present, there is a lack of systematic and convincing data related to the characteristics of root canal calcification after REPs. This retrospective study aimed to evaluate and summarize the characteristics of root canal calcification after REPs by reviewing cases with follow-up duration >3 years to gain a more comprehensive understanding of the same. The points explored in this study are as follows: first, the prevalence of root canal calcification in teeth followed up for >3 years after REPs; second, the long-term prognosis of calcified teeth after REPs; third, whether root canal calcifications will occur in all teeth undergoing REPs with the extension of follow-up duration; fourth, contributing factors related to root canal calcification; and fifth, the effect of root canal calcification on the formation of the root and whether conventional root canal therapy is needed after root formation.

Why this paper is important to paediatric dentists

- This retrospective study evaluated the characteristics of root canal calcification after REPs by reviewing cases with follow-up duration >3 years.
- Teeth treated with REPs had a relatively high probability of root canal calcification during the long-term follow-up. The occurrence of calcification is related to the use of calcium hydroxide paste. The extent of calcification may depend, in part, on the length of the follow-up duration. Not all teeth that undergo REPs, however, will result in calcification with the extension of follow-up duration.
- Root canal therapy is not conventionally needed in teeth that achieve root formation after REPs.

2 | MATERIAL AND METHODS

2.1 | Ethics

The study protocol was approved by the Ethics Committee of Peking University School and Hospital of Stomatology (ref. PKUSSIRB-202054062).

2.2 | Clinical protocol

Clinical treatment and follow-up details were obtained from medical and radiographic records. All of the REP protocols in this study were performed according to the "AAE Clinical Considerations for a Regenerative Procedure" advised by the AAE. REPs were explained to all patients and their caregivers, and parental consent was obtained before treatment.

At the first appointment, chemical disinfection was performed under dental dam isolation. The root canal was copiously and gently irrigated with NaOCl (1.5%–3%) and saline, and then medicated with calcium hydroxide paste or low-concentration triple or double antibiotic paste.

At the second appointment (1–4 weeks after the first visit), if clinical signs or symptoms persisted, the procedures performed in the first appointment were repeated.

If the clinical signs and symptoms disappeared, copious, gentle irrigation was provided under dental dam isolation with 17% ethylenediamine tetraacetic acid (EDTA) with or without saline. The canal was dried with paper points. Bleeding in the canal system was induced by rotating a precurved K-file 2 mm past the apical foramen. Intracanal bleeding (sufficient or insufficient bleeding) was recorded by measuring the level of intracanal bleeding in the evoked-bleeding step: Bleeding reaching the middle of the root was recorded as sufficient intracanal bleeding; else, it was recorded as insufficient bleeding. Different scaffolds were applied, including blood clots, platelet-rich fibrin (PRF), and collagen membranes.[10] ProRoot MTA (Dentsply Tulsa Dental), iRoot BP (Innovative Bioceramix), or Fuji IX GIC (Fuji Corporation) was used as a capping material. A layer of Filtek Z250 composite resin (3M ESPE; 3–4 mm) was placed over the capping material for the final restoration, and follow-ups were arranged.

2.3 | Case inclusion

Children who underwent REPs in the Department of Pediatric Dentistry, Peking University School and Hospital of Stomatology, Beijing, China, between January 2013 and January 2019 were enrolled. The deadline for data collection was January 2022. The inclusion criteria were as follows: (1) single-rooted teeth followed up for >3 years after REPs, (2) the disappearance of clinical symptoms and

healing of apical lesions, and (3) complete medical and radiographic records. The exclusion criteria were as follows: (1) teeth that suffered trauma or received external treatment during follow-up, (2) those that underwent <3 years of follow-up, (3) those that failed to achieve the primary goal, and (4) incomplete medical or radiographic records. There was no restriction on age or sex ratios.

Ninety-seven teeth with a follow-up duration of 3 years after REPs were initially collected. During follow-up, six teeth suffered crown fractures, all of which involved the anterior teeth; thus, these were excluded. Finally, 91 teeth of 54 boys and 37 girls (average age 10.4 ± 1.9 years) with follow-up duration >3 years were included.

2.4 | Data collection

The collected data included demographic information and pre- and postoperative medical and radiographic records, which included tooth position, clinical symptoms, contributory etiologies, diagnoses, apical lesions, stage of root development,[11] treatment information, root formation conditions, and follow-up duration.

Treatment information included: (1) the type of intracanal medication: calcium hydroxide paste, antibiotic paste, or combined application; (2) intracanal bleeding conditions, divided by the level of intracanal bleeding in the evoked-bleeding step; and (3) type of scaffold: blood clot, PRF, or collagen membrane.[10]

2.5 | Evaluation of treatment outcomes

Treatment outcomes were evaluated using medical and radiographic records. All included cases first achieved the primary goal of clinical symptom resolution and periapical lesion healing. The identification of periapical lesions was based on comparing the pre- and postoperative periapical films. If necessary, the relevant medical records, including clinical symptoms and signs, and cold and thermal test reactions, should also be reviewed to differentiate between apical lesions and physiological radiolucent areas.

The classification of calcification types was determined by the pre- and postoperative periapical films.[2] Root canal calcification in this study was divided into three categories: no calcification, partial calcification, and complete calcification (root canal obliteration), with the following criteria: (1) "no calcification" meant clear root canal images with no calcified bodies; (2) "partial calcification" included the presence of high-density calcified bodies in the root canal, but the root canal image was still visible; and (3) "complete root canal calcification" (obliteration) meant severe calcification with complete disappearance of root canal images.

Partially calcified teeth were divided into three categories according to the location of the calcified bodies: (1) calcification in the upper half of the root, (2) calcification in the lower half of the root, and (3) diffuse calcifications.

The radiographic assessment of the presence and pattern of intracanal calcification was performed in a blinded manner by two independent reviewers, both were our researchers. When an evaluation was not unanimous, a consensus was reached through discussion.

2.6 | Statistical analyses

The data collected included demographic information, follow-up duration, tooth type, contributory etiologies, diagnosis, apical lesions, root development stage,[11] intracanal medications, intracanal bleeding conditions, type of scaffolds, root formation conditions, and root canal calcifications. Differences in continuous variables between the groups were analyzed using independent sample *t*-tests. Differences in categorical variables were evaluated using the chi-squared test. The percentage difference was estimated based on the 95% confidence interval, and $p < .05$ was considered a significant difference.

3 | RESULTS

The follow-up duration ranged from 36 to 92 months, with an average follow-up duration of 53.2 ± 13.4 months. There was no significant difference in the follow-up duration between calcified (53.4 ± 13.4 months) and noncalcified teeth (52.5 ± 13.5 months). Root canal calcification occurred in 78% of the cases (71/91), including partial and complete root canal calcifications (Figures 1–4). Partial calcification occurred in 63% (57/91) of teeth, of which the incidence of calcification in the lower half of the root was the highest (30/57). Complete calcification occurred in 15% (14/91) of the included teeth (Table 1). The average follow-up duration of calcified teeth was 53.4 ± 13.4 months, with the longest duration of 92 months. The occurrence of root canal calcification does not affect the long-term prognosis of teeth. The main cause of long-term failure is crown fractures, which are more likely to occur in anterior teeth with a history of dental trauma.

The factors contributing to root canal calcification in REPs were analyzed. The type of intracanal medication used significantly correlated with postoperative root canal calcification. The root canal calcification rate of teeth treated with calcium hydroxide was 86% (50/58), which was significantly higher than that of teeth treated with

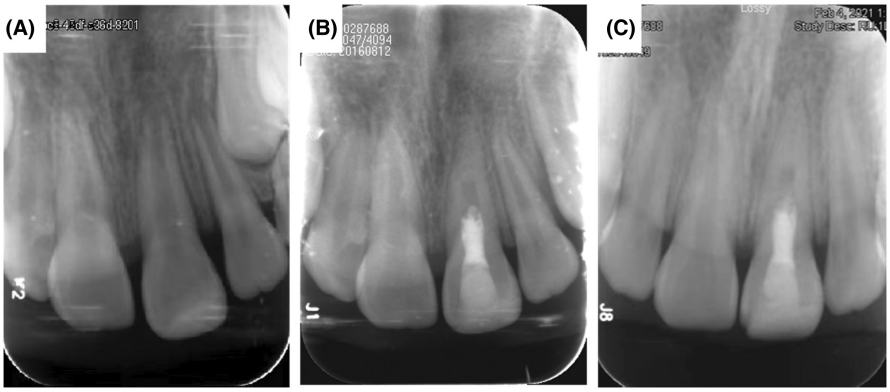

FIGURE 1 A 9-year-old male patient diagnosed with symptomatic apical periodontitis. Periapical films of tooth 21 over a 68-month follow-up duration. (A) A preoperative periapical film showing an incompletely developed root with a noticeable periapical lesion and an open apical foramen. (B) A 14-month follow-up periapical film showing complete resolution of the apical lesion: the root achieved complete development, and calcification occurred in the lower half of the root. (C) A 68-month follow-up periapical film: diffuse calcification occurred in the lower half of the root, and the root canal images show that the upper half of the root was still visible.

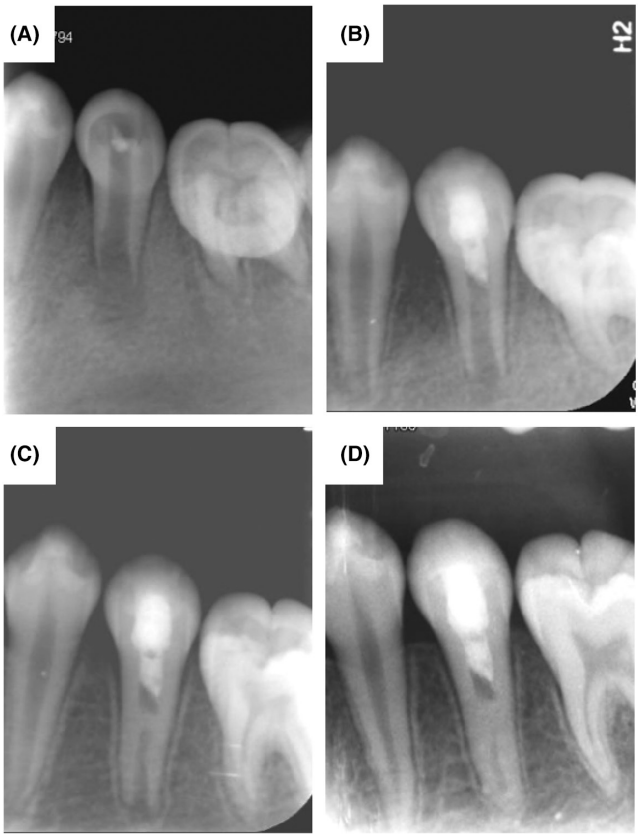

FIGURE 2 A 10-year-old female patient diagnosed with an acute apical abscess. Periapical films of tooth 35 over a 51-month follow-up duration. (A) A preoperative periapical film showing an incompletely developed root with an extensive periapical lesion and an open apical foramen. (B) A 4-month follow-up periapical film showing complete resolution of the apical lesion and healing of the periapical bone, in which the root continued to develop. (C) A 13-month follow-up periapical film: a calcific barrier formed in the canal between the coronal sealing material and the root apex. (D) A 51-month follow-up periapical film: the root achieved complete development, and the extent of calcification increased, but the root canal image was still visible.

antibiotic paste (61%) or combined application of calcium hydroxide and antibiotic paste (67%; $p < .05$). There was no significant correlation between root canal calcification and tooth type, etiology, diagnosis, apical lesions, initial root development stage,[11] intracanal bleeding conditions, or scaffolds. In addition, there was no significant difference in root formation conditions between teeth with or without root canal calcification (Table 2).

4 | DISCUSSION

In 2017, Song et al.[2] analyzed 29 teeth after REPs with an average follow-up duration of 24.9 months and found that the incidence of root canal calcification was 62.1%. They also found that the incidence and extent of calcification may depend, in part, on the length of the follow-up duration.[2] In the present study, the higher incidence (78%) of calcification after REPs may be related to the longer follow-up duration. Moreover, the phenomenon of root canal calcification aggravation with time was also observed in the present study (Figures 5–7). This suggests that there may be a correlation between the severity of root canal calcification and follow-up duration. On the contrary, the average follow-up duration of teeth without calcification in the present study was 52.5 ± 13.5 months, with the longest duration of 78 months, which indicates that not all teeth that undergo REPs end up with calcification on the extension of follow-up duration.

A previous study reported that post-traumatic teeth are more likely to have intracanal calcification. It, however, was impossible to draw a conclusion regarding this due to the limited sample size.[2] Thirty-seven anterior teeth and 54 posterior teeth were included in the present study. The etiology of the anterior teeth was commonly trauma, and that of posterior teeth was dens evaginatus. It was thought

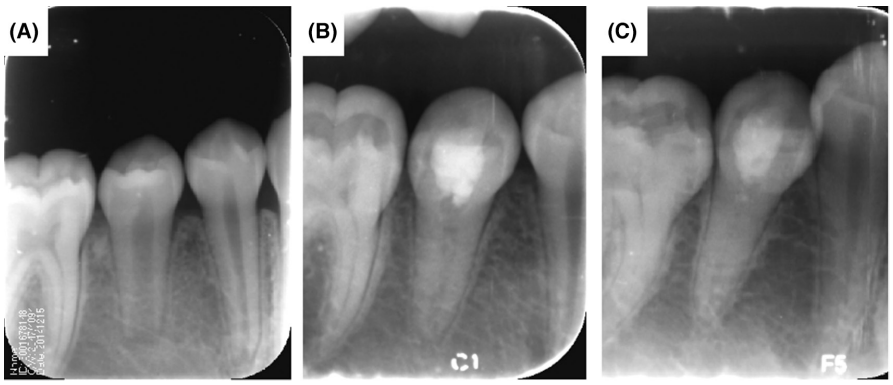

FIGURE 3 A 14-year-old male patient diagnosed with pulp necrosis. Periapical films of tooth 45 over a 68-month follow-up duration. (A) A postoperative periapical film showing an incompletely developed root with an open apical foramen. (B) A 16-month follow-up periapical film: the root achieved complete development, and diffuse calcification occurred in the root canal. (C) A 68-month follow-up periapical film: the extent of calcification increased, and root canal images were still visible but significantly narrower.

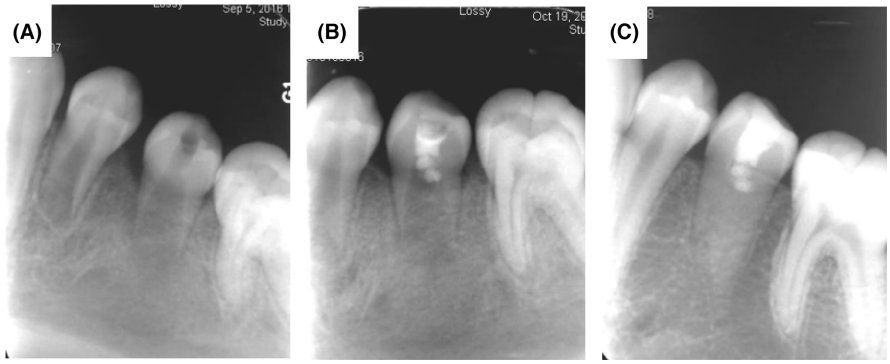

FIGURE 4 A 9-year-old female patient diagnosed with an acute apical abscess. Periapical films of tooth 35 over a 51-month follow-up duration. (A) A preoperative periapical film showing an incompletely developed root with an extensive periapical lesion and an open apical foramen. (B) A postoperative periapical film: the size of the apical radiolucent area decreased. (C) A 51-month follow-up periapical film showing complete resolution of the apical lesion: the change in the root dimension was not noticeable, and severe root canal calcification (obliteration) occurred.

TABLE 1 Distribution of root canal calcification in all teeth

Types, *n* (%)	
No calcification	20 (22)
Partial calcification	57 (63)
Lower half of the root	30
Upper half of the root	8
Diffuse calcification	19
Complete calcification	14 (15)

that vascular injury of the root tip of the traumatic teeth might lead to a higher incidence of calcification, but our results showed that the different etiologic categories did not lead to a different incidence of root canal calcification.

The outcome of radiographic root development was less predictable when immature permanent teeth with periradicular pathosis were treated with REPs.[12] It has been reported that residual bacteria in the root canal may lead to the osteogenic differentiation of stem cells, resulting in the formation of hard tissue and root canal calcification.[13–17] Theoretically, the residual bacteria in the root canal of teeth with apical lesions at the initial visit may be higher than those without apical lesions at the initial visit after chemical disinfection. The present study compared the incidence of root canal calcification in teeth with and without apical lesions at the initial visit, and no significant difference was found between the two groups. The occurrence of root canal calcification was not significantly related to apical lesions at the initial visit. Residual bacterial factors may not be the decisive factor for root canal calcification in REPs; however, further basic research is needed to confirm this.

It was reported that root canal calcification occurred more frequently in cases treated with calcium hydroxide paste than in those medicated with antibiotic pastes.[2,18] In the present study, the incidence of calcification of teeth medicated with calcium hydroxide paste was 86% (50/58),

TABLE 2 Analysis of related factors of root canal calcification

Variable	Calcified	Noncalcified	*p*-Value
Age (years)			
Range	8–15	6–15	.933[a]
Mean ± SD	10.4 ± 1.8	10.4 ± 2.5	
Sex, *n* (%)			
Male	42 (59)	12 (60)	.946[b]
Female	29 (41)	8 (40)	
Follow-up periods (months)			
Mean ± SD	53.4 ± 13.4	52.5 ± 13.5	.803[a]
Range	36–92	36–78	
Tooth type, *n* (%)			
Anterior	27 (38)	10 (50)	.336[b]
Premolar	44 (62)	10 (50)	
Etiologies, *n* (%)			
Trauma	25 (35)	10 (50)	.456[b]
Dens evaginatus	43 (61)	9 (45)	
Caries	3(4)	1(5)	
Diagnosis, *n* (%)			
AAP	9 (13)	1 (5)	.268[b]
SAP	20 (28)	10 (50)	
AAA	21 (30)	5 (25)	
CAA	20 (28)	3 (15)	
PN	1 (1)	1 (5)	
Apical lesions, *n* (%)			
With	66 (93)	16 (80)	.086[b]
Without	5(7)	4 (20)	
Stage of root development[c], *n* (%)			
7	16 (23)	2 (10)	.458[b]
8	42 (59)	14 (70)	
9	13 (18)	4 (20)	
Intracanal medication, *n* (%)			
Calcium hydroxide	50 (70)	8 (40)	.041[b]
Antibiotic paste (TAP/DAP)	11 (16)	7 (35)	
Combined application	10 (14)	5 (25)	
Intracanal bleeding conditions, *n* (%)			
Insufficient	12 (17)	7 (35)	.079[b]
Sufficient	59 (83)	13 (65)	
Scaffold, *n* (%)			
Blood clot	49 (70)	16 (80)	.456[b]
Collagen membrane	11 (15)	3 (15)	
PRF	11 (15)	1 (5)	
Root formation, *n* (%)			
Yes	49 (69)	12 (60)	.449[b]
No	22 (31)	8 (40)	

Abbreviations: AAA, acute apical abscess; AAP, asymptomatic apical periodontitis; CAA, chronic apical abscess; DAP, double antibiotic paste; PN, pulp necrosis; PRF, platelet-rich fibrin; SAP, symptomatic apical periodontitis; SD, standard deviation; TAP, triple antibiotic paste.

[a]Independent *t*-test.

[b]Chi-squared test.

[c]Nolla stage of root development.[11]

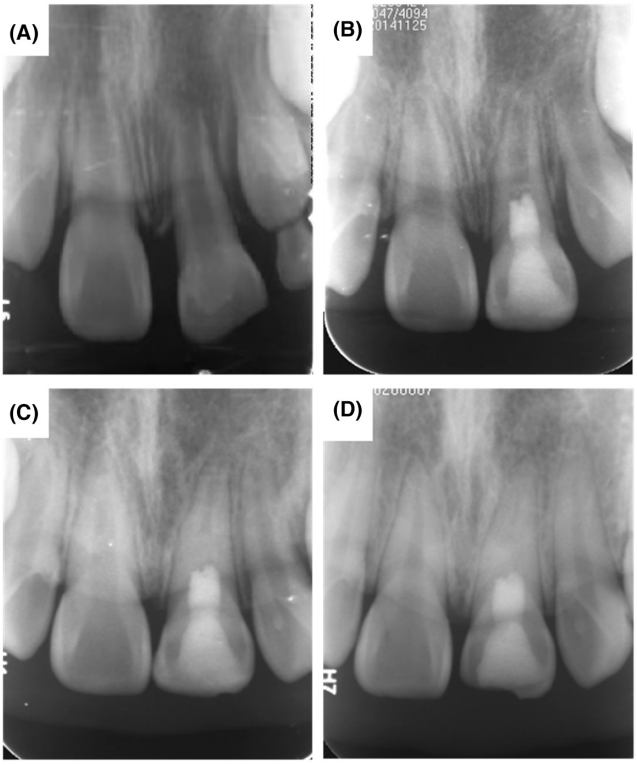

FIGURE 5 A 9-year-old male patient diagnosed with symptomatic apical periodontitis. Periapical films of tooth 21 over a 73-month follow-up duration. (A) A preoperative periapical film showing an incompletely developed root with an extensive apical radiolucent area. (B) A 4-month follow-up periapical film showing complete resolution of the apical lesion: a calcific barrier formed in the canal between the coronal sealing material and the root apex, and the root continued to develop. (C) A 19-month follow-up periapical film: the root achieved complete development, and the extent of calcification increased. (D) A 73-month follow-up periapical film: severe root canal calcification (obliteration) occurred.

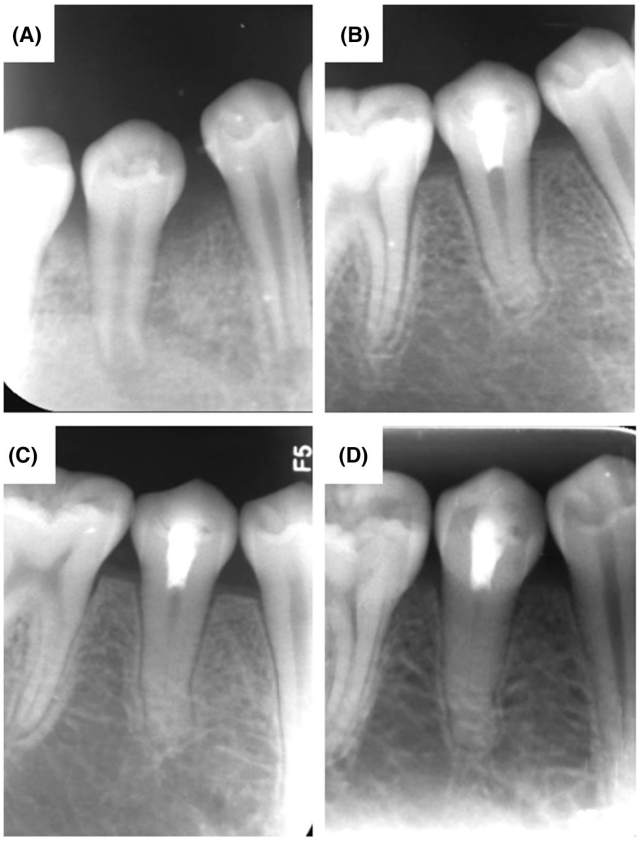

FIGURE 6 A 13-year-old male patient diagnosed with symptomatic apical periodontitis. Periapical films of tooth 45 over a 47-month follow-up duration. (A) A preoperative periapical film showing an incompletely developed root with a noticeable apical radiolucent area. (B) A 13-month follow-up periapical film showing complete resolution of the apical lesion: the root achieved complete development, and calcification occurred in the lower half of the root. (C) A 28-month follow-up periapical film: the extent of calcification increased. (D) A 47-month follow-up periapical film: severe root canal calcification (obliteration) occurred.

which was significantly higher than that of teeth treated with antibiotic paste (11/18, 61%) or teeth using combined application methods (10/15, 67%). In vitro results showed that calcium hydroxide promotes the migration and osteogenic differentiation of dental pulp stem cells,[19] and calcium ions induce mineralization of dental pulp stem cells,[20] which may be related to the higher proportion of calcification in teeth treated with calcium hydroxide. Since this was a retrospective study, further prospective research is needed to verify this conclusion.

A previous study reported that mesenchymal stem cells from different tissue sources retain their innate differentiation potential, which reflects the tissue of origin.[15,21] Bleeding from the periapex carries periodontal ligament stem cells and bone marrow stem cells from the alveolar bone, and induced bleeding in REPs recruits cells with cementogenic and osteogenic differentiation capabilities into the root canal space, resulting in ectopic bone formation and cementogenesis inside the lumen of the root canals. It has been reported that induced bleeding may contribute to root canal calcification after REPs.[2] The procedures they carried out, however, were not REPs; in the field of REPs, there are no relevant data on whether different bleeding conditions will lead to different incidences of root canal calcification. The present study analyzed the relationship between different bleeding conditions (sufficient and insufficient bleeding) and the incidence of calcification and found no significant correlation between these. Induced bleeding from the periapex may introduce stem cells into the root canal, but it may not be the decisive factor for root canal calcification following REPs.

Some researchers compared the prognoses of teeth using different scaffolds in REPs; they found that the imaging area in the root canal of teeth using blood clots as

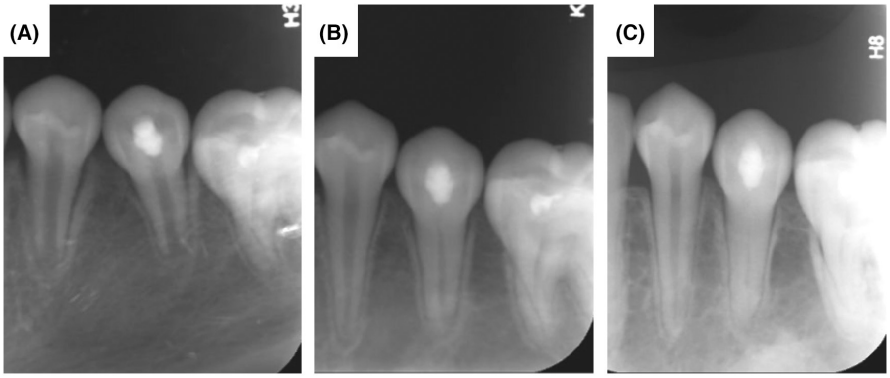

FIGURE 7 An 11-year-old female patient diagnosed with symptomatic apical periodontitis. Periapical films of tooth 35 over a 90-month follow-up duration. (A) A postoperative periapical film showing an incompletely developed root with an open apical foramen. (B) A 20-month follow-up periapical film: the root achieved complete development. (C) A 90-month follow-up periapical film: diffuse calcification occurred, and root canal images were still visible but significantly narrower.

scaffolds decreased more significantly after REPs and proposed that the occurrence of root canal calcification may be related to the blood clot scaffold.[22] At present, the most commonly used scaffold in REPs is blood clots formed after bleeding induced from the periapex. The disadvantage of using a blood clot as a scaffold is that bleeding conditions are uncontrollable. Many teeth could not induce enough blood into the root canal space and failed to form a blood clot with enough strength to guide the formation of new tissues. In addition to blood clot scaffolds, two types of scaffolds were used in the present study: PRF and collagen membrane scaffolds (Bio-Gide). PRF is known as the second generation of platelet concentrates.[23] Its unique three-dimensional structure provides a scaffold for tissue regeneration and slowly releases various growth factors during degradation to promote tissue repair and regeneration.[24] In 2017, Jiang et al.[10] evaluated the clinical efficacy of a collagen membrane (Bio-Gide) as a scaffold material and found that, compared with traditional blood clots, the thickness of the root canal wall in the middle third of the root increased significantly in teeth using collagen membranes. There, however, was no significant difference in root length and apical foramen width. The present study compared the incidence of root canal calcification in teeth using blood clots, PRF, and collagen membranes as scaffolds and found that different scaffolds did not directly lead to different incidences of root canal calcification. It, however, should be noted that in teeth using PRF and collagen membranes as scaffolds, the induced bleeding step was also carried out before PRF and collagen membrane application. Therefore, the effect of blood clots on the occurrence of root canal calcification cannot be completely excluded. This point needs to be further analyzed through prospective research.

In the present study, 67% (61/91) of the included teeth achieved complete root formation. As normal root formation is detectable after a mean of 16 months of follow-up after REPs,[8] the absence of root formation in the other 30 cases was not due to an inadequate follow-up duration. No correlation was observed between root formation and root canal calcification. The occurrence of root canal calcification did not affect continued root formation after REPs. Moreover, the calcification of some teeth occurred after root formation (Figures 6 and 7).

Teeth treated with REPs had a relatively high probability of root canal calcification during the long-term follow-up. The occurrence of calcification is related to the use of calcium hydroxide paste but does not affect the long-term prognosis of teeth. Therefore, although the occurrence of root canal calcification may compromise endodontic therapy if indicated in the future, root canal therapy is not conventionally needed in teeth that achieve root formation after REPs.

AUTHOR CONTRIBUTIONS

Xijun Jiang: study design, treatment outcomes evaluation, data collection and article writing. Yunfei Dai: study design and data collection. He Liu: study design, treatment outcomes evaluation and guidance in the process of article writing.

CONFLICTS OF INTEREST

The authors deny any conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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How to cite this article: Jiang X, Dai Y, Liu H. Evaluation of the characteristics of root canal calcification after regenerative endodontic procedures: A retrospective cohort study over 3 years. *Int J Paediatr Dent*. 2023;33:305-313. doi:10.1111/ipd.13039