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Continued root development of immature permanent teeth after regenerative endodontics with or without a collagen membrane: A randomized, controlled clinical trial

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Background: Recent studies evaluated the efficacy of a collagen membrane (Bio-Gide) to promote root development in regenerative endodontics (REPs). However, the influence of etiology and tooth type on the results was not assessed.

Aims: To evaluate the quantitative and qualitative effect of a collagen membrane (Bio-Gide), with and without the effect of etiology and tooth type, in promoting root development in immature teeth after REPs.

Design: Eighty nonvital immature teeth were divided randomly into two groups (40 teeth in each group). REPs were performed with (experimental group) and without (control group) a collagen membrane. All teeth were subjected to regular clinical and radiographical follow-up.

Results: Seventy-six teeth were included in the final analyses. The average follow-up periods were 33.1 ± 21.8 months for the control group and 28.1± 18.6 months for the experimental group. Quantitative analysis of root development in the experimental group showed a greater increase in dentin wall thickness in the middle one-third of the root compared to the control group, with or without the effect of etiology and tooth type. Six types of root development were observed. There was no significant difference in the type of tooth development between the two groups.

Conclusion: The use of a collagen membrane could promote an increase in dentin wall thickness in the middle one-third of the root, and it had no significant effect in terms of the type of subsequent root development.

KEYWORDS
collagen membrane, continued root development, regenerative endodontics

1 | INTRODUCTION

Regenerative endodontics (REPs) is an effective treatment method for immature, non-vital permanent teeth and was introduced in 2001.[1] Since then, many clinical studies and systematic reviews have confirmed its utility.[2-6] The key steps in REPs include chemical disinfection of the canals, introduction of a scaffold in the root canal space, and creation of a coronal seal that restricts bacterial entry.[2-6] The most commonly used scaffold is a blood clot created by laceration of periapical tissues after disinfection of the canal and resolution of symptoms.[7,8] In clinical practice, however, we cannot always induce sufficient intracanal bleeding to serve as a scaffold.[3,9,10] Therefore, researchers are investigating alternative scaffolds for promoting root development.

In 2017, Jiang et al[11] conducted a clinical study to evaluate the efficacy of a collagen membrane (Bio-Gide; Geistlich Pharma AG) as a scaffold to promote root development in REPs. They found that the use of a collagen membrane led to increased dentin wall thickness in the middle one-third of the root.[11] Owing to the limitations of sample size and follow-up time, it was not possible to exclude the influence of etiology (whether or not is it caused by trauma) and tooth type on the results at that time. Further, the influence of etiology or tooth type on the results should be excluded as REP outcomes of traumatized anterior teeth are less predictable and less favorable than those of non-traumatized premolar teeth with dens evaginatus–induced pulp necrosis and infection. The aim of this study was to evaluate the quantitative and qualitative usefulness of the Bio-Gide collagen membrane in promoting root development in teeth that have undergone REPs by using a larger sample size and prolonged follow-up time compared to previous study.[11] The null hypothesis was that the continued root development of immature teeth after REPs would be consistent with or without a collagen membrane.

Why this paper is important to paediatric dentists

- This randomized, controlled clinical study evaluated the efficacy of the Bio-Gide collagen for use as a scaffold in promoting dentin formation in the middle one-third of the root in REPs and provided evidence for its clinical application.
- The use of a collagen membrane had no significant effect in terms of the type of continued root development.

2 | MATERIALS AND METHODS

2.1 | Ethics

This randomized, controlled clinical study (Ref no. ChiCTR 2000034180) was performed in the Department of Pediatric Dentistry, Peking University School and Hospital of Stomatology, Beijing, China; the study protocol was approved by the Ethics Committee of Peking University School and Hospital of Stomatology (ref no. PKUSSIRB-201523072).

2.2 | Sample size

Sample size was determined by statistical power analysis according to a previous study.[12] The increases in root thickness in the control and experimental groups after 6 months were 5.8% ± 1.2% and 4.5% ± 1.6%, respectively.[12] To achieve 90% power and a 5% significance level using the *t* test for testing 2 independent means, a sample size of 46 teeth was needed, with an anticipated loss to follow-up of 10%.

2.3 | Patient recruitment

Patients were recruited from January 2014 to October 2020. The inclusion criteria were single-rooted permanent tooth with necrotic pulp and an immature apex; pulp space not needed for post/core, final restoration; compliant patient/parent; and patients not allergic to medicaments and antibiotics necessary to complete procedure (ASA 1 or 2).

Regenerative endodontics were explained to all patients and their caregivers. We had comprehensive discussions on the risks, complications, and alternative treatment options, and parental consent was obtained. A total of 80 teeth were included in the study.

2.4 | Randomization

The envelope method was used for randomization. A total of 80 group information notes (40 for the experimental group and 40 for the control group) were packed randomly into 80 opaque envelopes after generating a random sequence; each envelope was numbered sequentially and allocated to each patient according to the time the participant joined the study.

2.5 | Intervention

A total of 80 teeth were distributed randomly into the experimental and control groups. Clinical examinations, pulp sensitivity, and radiographic examinations were performed. Medical history, clinical symptoms, and preoperative examination of the tooth were collected prior to the REP.

Regenerative endodontics were performed with (experimental group) and without (control group) a collagen membrane. All REPs were performed in accordance with the 'AAE Clinical Considerations for a Regenerative Procedures' established by the American Association of Endodontists.[7]

At the first appointment, we performed chemical disinfection of the root canal under rubber dam isolation.[11] Teeth were reviewed after 1-2 weeks to assess the response to initial treatment. If clinical signs or symptoms persisted, the procedures performed in the first appointment were repeated.

For the second appointment, anesthesia was induced with lidocaine (2%) without a vasoconstrictor. We removed the temporary seal under rubber dam isolation. Copious, gentle irrigation was provided with 20 mL of 17% EDTA. The canal was dried with paper points. At the evoked-bleeding step, bleeding in the canal system was induced by mechanical irritation of periapical tissue and rotational movement of a sterilized 40# K-file. The level of intracanal bleeding was recorded. The Bio-Gide collagen membrane was placed at the middle one-third of the root over the blood clot, and ProRoot MTA (Dentsply Tulsa Dental) was used as a capping material. A layer of Filtek Z250 composite resin (3M ESPE; Irvine, CA; 3-4 mm) was placed over the capping material for the final restoration.

the tooth with a resolution of the periapical lesion and the absence of any clinical signs or symptoms.

Radiographic examinations were performed by taking pre- and postoperative parallel periapical radiographs, which were transferred to ImageJ software (version 1.41; National Institutes of Health) and corrected and calibrated using the TurboReg plug-in (Biomedical Imaging Group, Swiss Federal Institute of Technology).[11] Changes in root dimensions were evaluated by measuring changes in the root length, dentin wall thickness (measured at one-third and two-thirds of the preoperative root length), and apical foramen width (Figure 1). The values were calculated as follows:

$$\text{Percentage increase in root length} = (\text{postoperative length} - \text{preoperative length}) \div \text{preoperative length} \times 100\%$$

$$\text{Percentage increase in dentin wall thickness} = (\text{postoperative thickness} - \text{preoperative thickness}) \div \text{preoperative thickness} \times 100\%$$

$$\text{Percentage increase in apical foramen width} = (\text{postoperative width} - \text{preoperative width}) \div \text{preoperative width} \times 100\%$$

For the control group, all steps were the same as those for the experimental group, except that Bio-Gide was not used before adding the MTA.

All patients were evaluated at 3-month intervals for at least 6 months. Treatment outcomes were assessed clinically and radiographically at each follow-up visit.

2.6 | Evaluation of treatment outcomes

Treatment outcomes were assessed in a blinded manner by two independent reviewers, both paediatric dentists.[11] Clinical success of the treatment was defined as survival of

Each measurement was made in duplicate, and all results were averaged. Root development was quantitatively analyzed by comparing the changes in the root dimensions (root length, dentin wall thickness, and apical foramen width) between the two groups.

In 2012, Chen et al[13] summarized five types of responses to pulp regeneration therapy in young permanent teeth. Root development in the present study was qualitatively evaluated by categorizing the types of continued root development in the two groups according to Chen's classification.[13] Other clinical findings such as crown discoloration, restoration failure, and root canal calcification were also evaluated.[11]

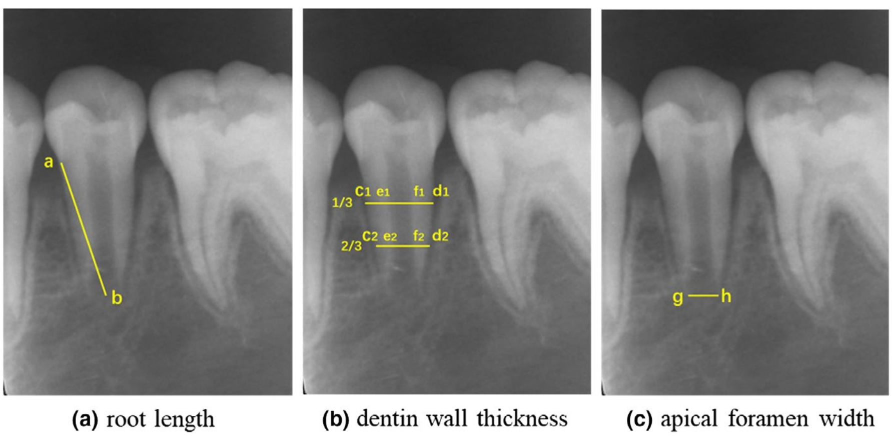

FIGURE 1 The cementoenamel junction (A) and the midpoint of the radiographic apex (B). The measurements of root length were made along the straight line from a to b. The straight line from c to d indicates the root width at the level of one-third or two-thirds of the length of the preoperative root canal. The straight line from e to f is the pulp space width at the same level. Measurements of dentin wall thickness were made by subtracting ef from cd. The straight line from g (distal apical end of the root) to h (mesial apical end of the root) indicates the apical foramen width (C)

2.7 | Data collection and statistical analyses

Data collected included demographic information, tooth type, contributory etiologies, stage of root development, diagnoses, follow-up periods, treatment outcomes, crown discoloration, root canal calcification, and electric pulp test.[11] Differences in continuous variables between the groups were analyzed using the *t* tests. Differences in categorical variables were evaluated using the chi-square test. The percentage difference was estimated based on the 95% confidence interval, and $P < .05$ was considered a significant difference. Statistical analyses were performed using SPSS software (version 20; IBM).

3 | RESULTS

3.1 | Demographic and clinical characteristics

The CONSORT flow diagram of this study (Figure 2) shows that 83 patients were initially recruited. Three patients were excluded, of these, two declined to participate, and one patient with severe intrusive luxation injury in the control group was excluded during follow-up because this type of trauma has been associated with poor outcomes after REPs and could affect the reliability of our results. Reasons for exclusion are shown in Figure 2.

Ultimately, 76 teeth were included in the analysis (Table 1 and Figure 2): 38 teeth each in the control group and the experimental group. The average follow-up periods were 33.1 ± 21.8 months for the control group and 28.1 ± 18.6 months for the experimental group; the longest follow-up period was 76 and 68 months in the control and experimental groups, respectively. The demographic and clinical characteristics are listed in Table 1. No significant differences in demographic or clinical characteristics were noted between the groups at baseline (all $p > .05$).

3.2 | Quantitative analysis of continued root development

There were no significant differences in the mean pretreatment root dimension parameters between the two groups (*t* test, $p > .05$) (Table 2).

3.3 | Quantitative analysis of continued root development in all teeth

At the time of analysis, all teeth were asymptomatic with complete resolution of signs and symptoms. The teeth in the experimental group showed significantly (*t* test, $p < .05$) greater increase in dentin wall thickness in the middle one-third of the root (23.4% ± 17.2%) than those in the control group did (12.1% ± 13.6%), whereas the remaining three root development parameters were comparable between the groups (Table 3).

3.4 | Quantitative analysis of continued root development in premolars

There were 25 premolars in the control group and 30 premolars in the experimental group. The premolars in the experimental group showed significantly (*t* test, $p < .05$) greater increase in dentin wall thickness in the middle one-third of the root (25.1% ± 18.1%) than the premolars in the control group did (14.2% ± 14.9%), whereas the remaining three root development parameters were comparable between the groups (Table 4).

3.5 | Qualitative analysis of continued root development

Four types of continued root development based on Chen's classification (types 1-4) were observed in the present study: type I, increased thickening of the canal walls with a significant increase in root length, with the root apex becoming closed; type II, increased thickening of the canal walls without a significant increase in root length, with the root apex becoming blunt and closed; type III, continued root development with the apical foramen remaining open; and type IV, severe calcification (obliteration) of the canal space. One type of continued root development in Chen's classification was not observed (type 5). In addition, two new types of continued root development were observed in the present study: type V, no continued root development; and type VI, an uncommon type of segmental root development (Figures 3-8).

There was no significant difference in the types of tooth development between the two groups, with or without the effect of etiology and tooth type (Tables 5 and 6).

3.6 | Other clinical and radiographic findings

No restoration failure was observed in either group. A total of 28 teeth (74%) in the control group and 28 (74%) in the experimental group showed crown discoloration. In addition, 20 teeth (53%) in the control group and 18 (47%) in the experimental group showed pulp canal calcification. Seven teeth (18%) in the control group and 12 (32%) in the experimental group showed positive electric pulp test (EPT) reactions.

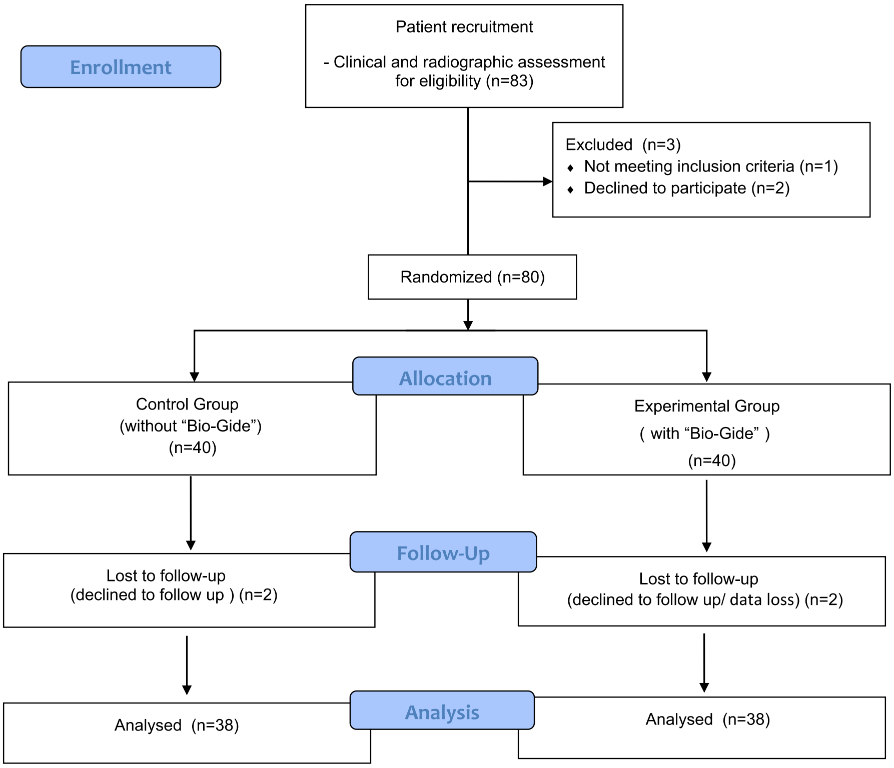

FIGURE 2 A CONSORT flow diagram showing the patient recruitment and follow-up process (http://www.consort-statement.org/)

Three teeth in the control group showed mineral trioxide aggregate (MTA) collapse, whereas there was no collapse in teeth in the experimental group. None of the three teeth with MTA collapse showed the entry of sufficient blood into the pulp space. There were no statistically significant differences between the two groups in any of these parameters (Table 7).

4 | DISCUSSION

The present study included almost twice as many teeth as that in previous study and prolonged follow-up duration.[11] The teeth with collagen membranes showed a greater increase in dentin wall thickness in the middle one-third of the root than those without the membrane did; that is, the use of a collagen membrane improved the deposition of new mineralized tissue in the middle one-third of the root wall. Meanwhile, the success rate and other indicators (root length, dentin wall thickness in the apical one-third of the root, apical foramen width, discoloration, calcification, and EPT) were comparable between the groups. The current studies have shown that the clinical success rate of young permanent teeth after REPs varies from 76.47% to 100%.[14,15] Poor control of root canal microbial infection is the cause of failure in most cases. In addition, coronal microleakage is also a possible source of infection.[14,16] In the present study, the clinical success rate was 100%. All cases in both groups were asymptomatic, with complete resolution of signs and symptoms, and the use of a collagen membrane did not affect the clinical outcomes. In the process of placing the collagen membrane, an aseptic operative technique should be strictly followed to prevent root canal infection.

This study included 13 anterior teeth and 25 premolars in the control group and 8 anterior teeth and 30 premolars in the experimental group. The etiological factor common to all anterior teeth and premolars was trauma and dens evaginatus, respectively. In order to draw an accurate and reliable conclusion, the influence of etiology and tooth type on the results should be excluded, as after REPs, traumatized teeth are associated with less predictable and less favorable outcomes than the non-traumatized premolar teeth with dens evaginatus–induced pulp necrosis and infection do. In this study, we excluded all the anterior teeth and analyzed the continued root development with or without the collagen membrane in only the premolars. After excluding the influence of etiology and tooth type on the results, the premolars in the experimental group showed a greater increase in dentin wall thickness in the middle one-third of the root than the premolars in the control group did. The use of a collagen membrane promotes the deposition of hard tissue in the middle of the root canal, and this reduces the risk of root fracture as the root is strengthened by the deposition of new mineralized tissue in the root canal. In the present study, the collagen membrane was placed in the middle one-third of the root to avoid damaging the physiological structure of the root apical region and to serve as a scaffold. It has been reported that Bio-Gide can promote adherence, migration, proliferation, and differentiation of ectomesenchymal cells. It can also stabilize blood clots, maintain growth factor levels, and promote tissue regeneration.[17] These characteristics may be the

TABLE 1 Demographic and clinical characteristics

Variable	Control group	Experimental group	*p*-value
Age (y)			
Range	8-15	7-15	.411[b]
Mean ± SD	10.6 ± 1.7	11.0 ± 1.9	
Sex, n (%)			
Male	20 (57)	18 (50)	.546[a]
Female	15 (43)	18 (50)	
Tooth type, n (%)			
Anterior	13 (34)	8 (21)	.200[a]
Premolar	25 (66)	30 (79)	
Etiologies, n (%)			
Trauma	13 (34)	8 (21)	.200[a]
Broken central cusp	25 (66)	30 (79)	
Stage of root development[c]			
7	5 (13)	5(13)	.693[a]
8	29(76)	31(82)	
9	4(11)	2(5)	
Diagnosis, n (%)			
Asymptomatic apical periodontitis	30 (79)	27 (71)	.376[a]
Symptomatic apical periodontitis	7 (18)	7 (18)	
Pulp necrosis	1 (3)	4 (11)	
Follow-up period (mo)			
Mean ± SD	33.1 ± 21.8	28.1 ± 18.6	.278[b]
Range	6-76	6-68	

Abbreviation: SD, standard deviation.

[a]Chi-square test.

[b]Independent *t* test.

[c]Nolla stage of root development.

TABLE 2 Mean pre-treatment root dimension parameters (mm) in both groups

Variable	Control group mean ± SD	Experimental group mean ± SD	*p*-value[a]
Root length	12.4 ± 2.3	11.7 ± 2.7	.228
Dentin wall thickness in the middle one-third of the root	1.5 ± 0.2	1.4 ± 0.3	.174
Dentin wall thickness in the apical one-third of the root	1.1 ± 0.3	1.1 ± 0.3	.720
Apical foramen width	2.3 ± 0.7	2.2 ± 0.9	.517

Abbreviation: SD, standard deviation.

[a]Independent *t* test.

TABLE 3 Quantitative analysis of continued root development in all teeth (mm)

Variable	Control group (n = 38) mean ± SD	Experimental group (n = 38) mean ± SD	*p*-value[a]
Root length	14.2 ± 10.4%	14.8 ± 10.9%	.799
Dentin wall thickness in the middle one-third of the root	12.1 ± 13.6%	23.4 ± 17.2%	.002
Dentin wall thickness in the apical one-third of the root	25.0 ± 24.8%	25.1 ± 24.4%	.979
Apical foramen width	–61.7 ± 40.1%	–73.3 ± 34.5%	.180

Abbreviation: SD, standard deviation.

[a]Independent *t* test.

TABLE 4 Quantitative analysis of continued root development in premolars (mm)

Variable	Premolars in control group (n = 25) mean ± SD	Premolars in experimental group (n = 30) mean ± SD	*p*-value[a]
Root length	15.2 ± 11.4%	16.2 ± 10.4%	.737
Dentin wall thickness in the middle one-third of the root	14.2 ± 14.9%	25.1 ± 18.1%	.020
Dentin wall thickness in the apical one-third of the root	25.3 ± 25.7%	25.7 ± 22.4%	.943
Apical foramen width	–67.3 ± 38.3%	–71.4 ± 34.4%	.680

Abbreviation: SD, standard deviation.

[a]Independent *t* test.

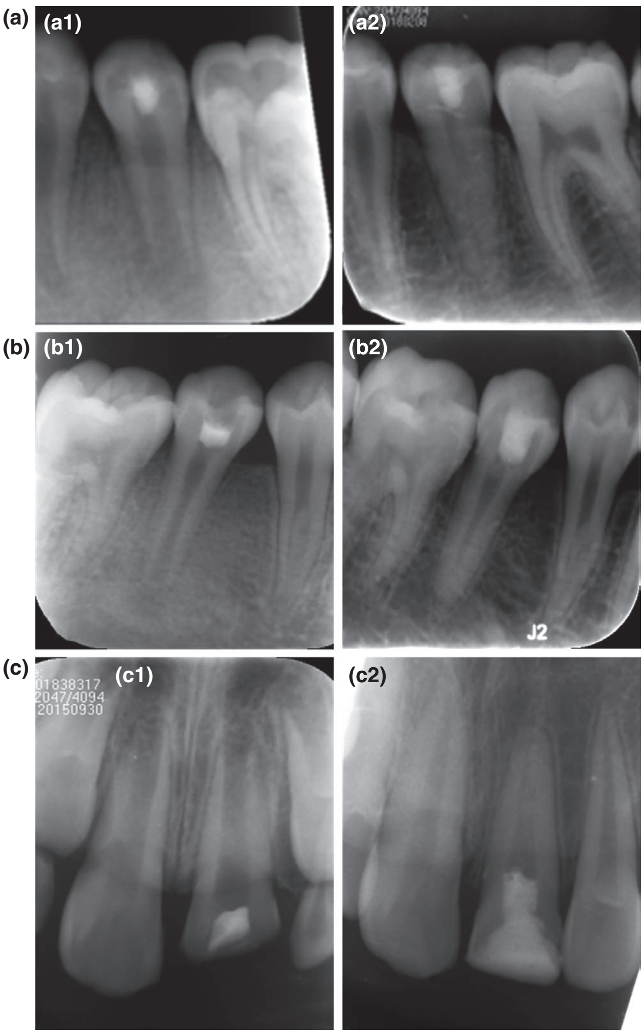

FIGURE 3 Type I: Increased thickening of the canal walls with a significant increase in root length, with the root apex becoming closed. A, Tooth 35. A1, Postoperative radiograph. A2, Thirty-six-month follow-up radiograph. B, Tooth 45. B1, Postoperative radiograph. B2, Twenty-one-month follow-up radiograph. C, Tooth 21. C1, Postoperative radiograph. C2, Fifty-two-month follow-up radiograph

reasons why it promotes dentin formation in the middle one-third of the root.

Regarding the types of continued root development, Chen et al[13] summarized five types of responses to pulp regeneration therapy in 20 young permanent teeth during 6-26 months of follow-up. In the present study, 76 teeth were categorized into six types of continued root development based on the changes in root dimensions. The teeth that achieved apical foramen closure with increased root length and wall thickness were classified as type I—the root morphology of this type of tooth is closest to the physiological state. The teeth that achieved apical foramen closure and thickened root canal wall but no significant increase in root length were classified as type II; here, the root development was completed, but the final root length was smaller than that in healthy teeth. In this study, this type of development occurred only in the anterior teeth. The teeth that achieved increased root length and/or thickness of the root canal wall with persistent opening of the apical foramen were classified as type III. The teeth with severe calcification (obliteration) of the canal space were classified as type IV; these teeth were placed into a separate category because once complete obliteration occurred, the root canal disappeared, and the thickness of the root canal wall and size of the apical foramen could not be measured; therefore, in the quantitative analysis of continued root development, we removed all the teeth under this category in both groups. Types I–IV were consistent with previous Chen's classification.[13] The teeth with no significant changes in root dimensions were classified as type V. The teeth with an uncommon type of segmental root development were classified as type VI. The changes in the root dimensions in this type of teeth were calculated by measuring the main root. There were no significant differences in the types of root development between the two groups with or without the effect of etiology and tooth type. The teeth with complete root canal obliteration should be classified as type IV regardless of the changes in the root length, root canal wall, and apical foramen. With time, all teeth may achieve type IV development, but type IV teeth do not change to other types.

Nolla divided the development process of permanent teeth into 10 stages using X-ray films.[18] When the apical foramen was closed, the tooth was considered to have reached stage 10 and completed its development. In a prospective study by Saoud et al[19], who observed patients for 12 months after REPs, 55% (11/20) of the teeth achieved apical foramen

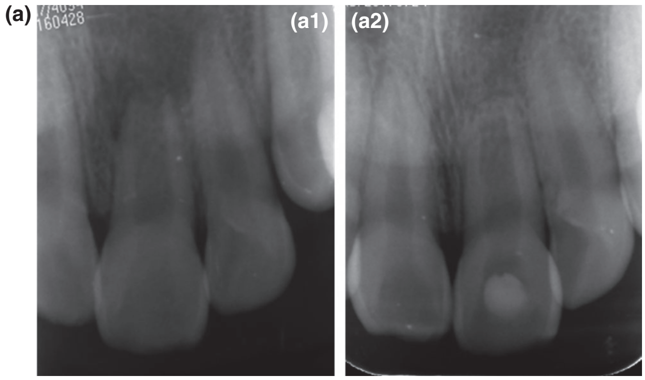

FIGURE 4 Type II: Increased thickening of the canal walls without a significant increase in root length, with the root apex becoming blunt and closed. A, Tooth 21. A1, Preoperative radiograph. A2, Twenty-one-month follow-up radiograph

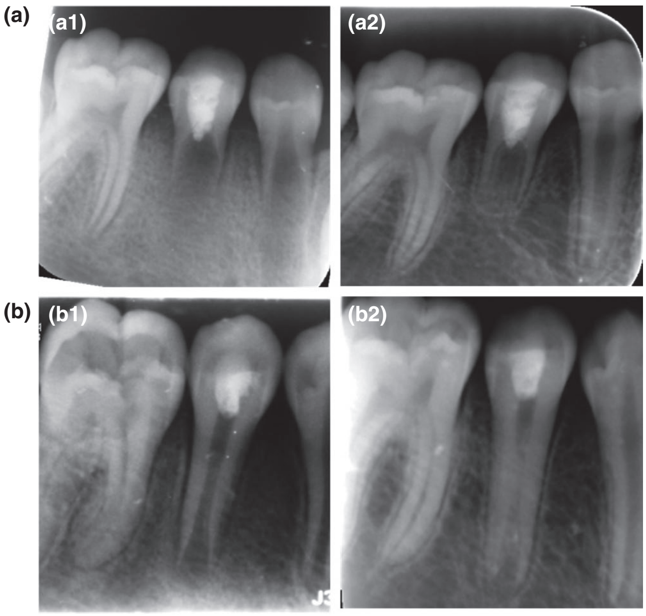

FIGURE 5 Type III: Continued root development with the apical foramen remaining open. A, Tooth 45. A1, Postoperative radiograph. A2, Thirty-one-month follow-up radiograph. B, Tooth 45. B1, Postoperative radiograph. B2, Twenty-four-month follow-up radiograph

closure as early as 6 months after the procedure (15). In the present study, 36 teeth (16 in the control group and 20 in the experimental group) achieved apical foramen closure, accounting for 47% of the total teeth. Apical foramen closure was first observed at 6 months postoperatively, similar to the findings of Saoud et al. Besides, most teeth (96%) achieved apical foramen closure within 24 months.

Calcification is a common occurrence in teeth that have undergone REPs. Chen[13] observed 20 teeth for 6-26 months and found a 35% incidence of root canal calcification. In the present study, 20 teeth in the control group and 18 in

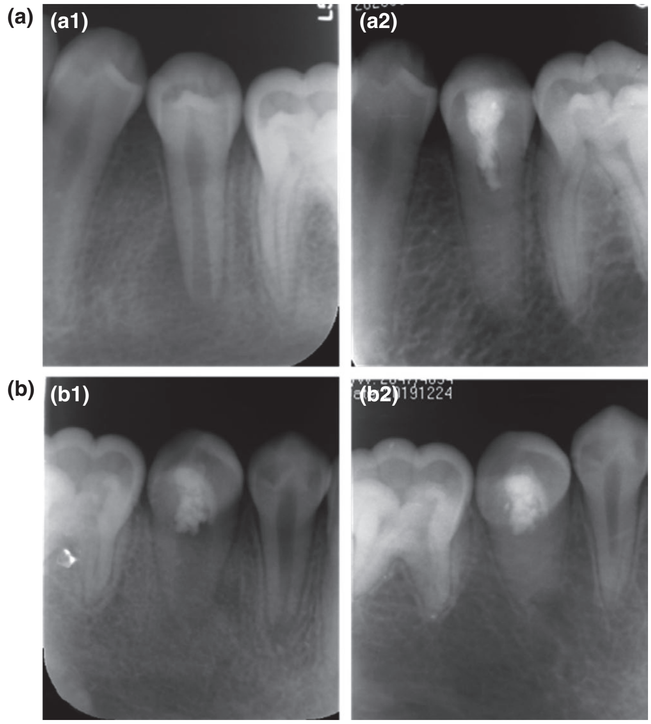

FIGURE 6 Type IV: Severe calcification (obliteration) of the canal space. A, Tooth 35. A1, Preoperative radiograph. A2, Seventy-three-month follow-up radiograph. B, Tooth 45. B1, Postoperative radiograph. B2, Fifty-three-month follow-up radiograph

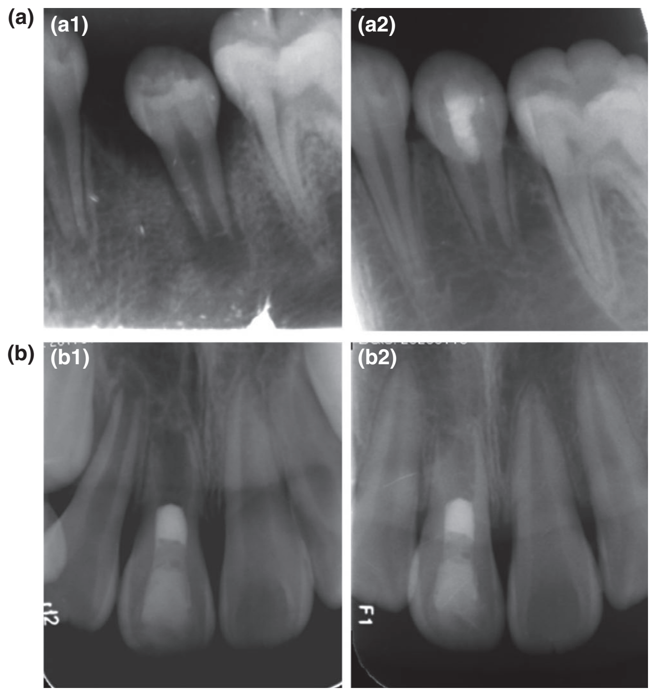

FIGURE 7 Type V: No continued root development. A, Tooth 35. A1, Preoperative radiograph. A2, Fifty-seven-month follow-up radiograph. B, Tooth 11. B1, Postoperative radiograph. B2, Fifty-eight-month follow-up radiograph

the experimental group showed root canal calcification. The total incidence of root canal calcification in this study

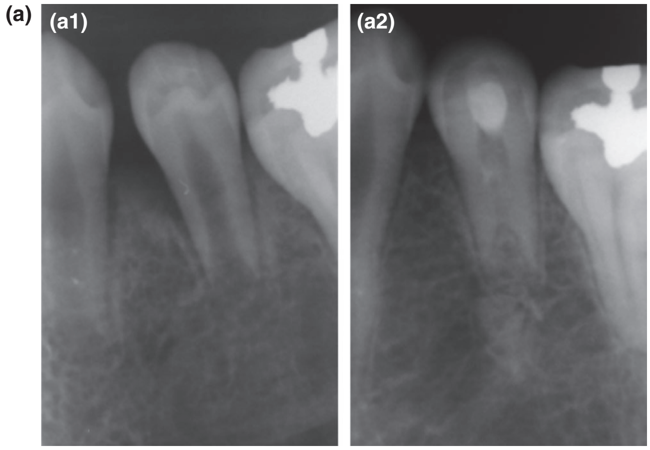

FIGURE 8 Type VI: An uncommon type of segmental root development. A, Tooth 35. A1, Preoperative radiograph. A2, Thirty-two-month follow-up radiograph

TABLE 5 Types of continued root development in all teeth

Types	Control group (n = 38)	Experimental group (n = 38)
I	15	19
II	1	1
III	14	14
IV	4	1
V	3	2
VI	1	1

TABLE 6 Types of continued root development in premolars

Types	Control group (n = 25)	Experimental group (n = 30)
I	10	16
II	0	0
III	8	11
IV	4	1
V	3	1
VI	1	1

TABLE 7 Comparisons between other clinical and radiographic findings

Variable	Control group (n = 38)	Experimental group (n = 38)	*p*-value[a]
Discoloration, n (%)	28 (74)	28 (74)	1.000
Calcification, n (%)	20 (53)5	18 (47)	.646
EPT, n (%)	7 (18)	12 (32)	.185
MTA collapse, n (%)	3 (8)	0 (0)	.077

Abbreviations: EPT, electric pulp test; MTA, mineral trioxide aggregate.
[a]Chi-square test.

was 50%, which may be a consequence of long observation time in this study. Five premolars developed complete root canal obliteration. The follow-up time was 6, 22, 53, 58, and 73 months, respectively. Thus, complete root canal obliteration may occur as soon as 6 months after an REP. Biological analysis of the causes of root canal calcification has shown that blood from the apical foramen carries periodontal stem cells and alveolar bone-derived bone marrow stem cells, which result in the formation of osseous and cementum tissue within the root canal.[20-26] In addition, a recent study showed that the presence of residual biofilms and antigens is related to root canal calcification, stem cells from the apical papilla showed robust expression of markers of osteoblast-like cells in the presence of residual biofilms and antigens.[27] The long-term prognosis and optimal treatment of such teeth have not been determined yet. Of all the calcified teeth in the present study, six were followed up for more than 5 years and 15 for more than 3 years; none of the calcified teeth in this study showed periapical lesions in the follow-up period.

The use of the Bio-Gide collagen membrane could promote an increase in dentin wall thickness in the middle one-third of the root. Six types of continued root development after REPs were observed. The use of a collagen membrane had no significant effect in terms of the type of continued root development.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

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

AUTHOR CONTRIBUTION

Xijun Jiang designed the study, performed the clinical operation, analyzed and evaluated the treatment outcomes, collected the data, and wrote the article. **He Liu** designed the study, helped guiding in the process of study, and wrote the article. **Chufang Peng** helped guiding in the process of study and wrote the article.

ETHICAL APPROVAL

This randomized, controlled clinical study was performed in the Department of Pediatric Dentistry, Peking University School and Hospital of Stomatology, Beijing, China; the study protocol was approved by the Ethics Committee of the Peking University School and Hospital of Stomatology (ref no. PKUSSIRB-201523072).

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REFERENCES

1. Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. *Dent Traumatol*. 2001;17:185-187.
2. Jung IY, Lee SJ, Hargreaves KM. Biologically based treatment of immature permanent teeth with pulpal necrosis: a case series. *J Endod*. 2008;34:876-887.
3. Petrino JA, Boda KK, Shambarger S, Bowles WR, McClanahan SB. Challenges in regenerative endodontics: a case series. *J Endod*. 2010;36:536-541.
4. Chueh LH, Huang GT. Immature teeth with periradicular periodontitis or abscess undergoing apexogenesis: a paradigm shift. *J Endod*. 2006;32:1205-1213.
5. Kim DS, Park HJ, Yeom JH, et al. Long-term follow-ups of revascularized immature necrotic teeth: three case reports. *Int J Oral Sci*. 2012;4:109-113.
6. Miltiadous ME, Floratos SG. Regenerative endodontic treatment as a retreatment option for a tooth with open apex - a case report. *Braz Dent J*. 2015;26:552-556.
7. Law AS. Considerations for regeneration procedures. *J Endod*. 2013;39(3):S44-S56.
8. Kontakiotis EG, Filippatos CG, Tzanetakis GN, Agrafioti A. Regenerative endodontic therapy: a data analysis of clinical protocols. *J Endod*. 2015;41:146-154.
9. Nosrat A, Seifi A, Asgary S. Regenerative endodontic treatment (revascularization) for necrotic immature permanent molars: a review and report of two cases with a new biomaterial. *J Endod*. 2011;37:562-567.
10. Cehreli ZC, Isbitiren B, Sara S, Erbas G. Regenerative endodontic treatment (revascularization) of immature necrotic molars medicated with calcium hydroxide: a case series. *J Endod*. 2011;37:1327-1330.
11. Jiang X, Liu H, Peng C. Clinical and radiographic assessment of the efficacy of a collagen membrane in regenerative endodontics: a randomized, controlled clinical trial. *J Endod*. 2017;43:1465-1471.
12. Nagy MM, Tawfik HE, Hashem AA, Abu-Seida AM. Regenerative potential of immature permanent teeth with necrotic pulps after different regenerative protocols. *J Endod*. 2014;40:192-198.
13. Chen MY, Chen KL, Chen CA, Tayebaty F, Rosenberg PA, Lin LM. Responses of immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/abscess to revascularization procedures. *Int Endod J*. 2012;45:294-305.
14. Silujjai J, Linsuwanont P. Treatment outcomes of apexification or revascularization in nonvital immature permanent teeth: a retrospective study. *J Endod*. 2017;43(2):238-245.
15. Chan EKM, Desmeules M, Cielecki M, Dabbagh B, Ferraz dos Santos B. Longitudinal cohort study of regenerative endodontic treatment for immature necrotic permanent teeth. *J Endod*. 2017;43(3):395-400.
16. Bukhari S, Kohli MR, Setzer F, Karabucak B. Outcome of revascularization procedure: a retrospective case series. *J Endod*. 2016;42(12):1752-1759.
17. Liu Q, Humpe A, Kletsas D, et al. Proliferation assessment of primary human mesenchymal stem cells on collagen membranes for guided bone regeneration. *Int J Oral Maxillofac Implants*. 2011;26:1004-1010.
18. Nolla CM. The development of the permanent teeth. *J Dent Child*. 1960;27:254-266.
19. Saoud TM, Zaazou A, Nabil A, Moussa S, Lin LM, Gibbs JL. Clinical and radiographic outcomes of traumatized immature permanent necrotic teeth after revascularization/revitalization therapy. *J Endod*. 2014;40(12):1946-1952.
20. Wang X, Thibodeau B, Trope M, Lin LM, Huang GTJ. Histologic characterization of regenerated tissues in canal space after the revitalization/revascularization procedure of immature dog teeth with apical periodontitis. *J Endod*. 2010;36:56-63.
21. Martin G, Ricucci D, Gibbs JL, Lin LM. Histological findings of revascularized/revitalized immature permanent molar with apical periodontitis using platelet-rich plasma. *J Endod*. 2013;39:138-144.
22. Yamauchi N, Nagaoka H, Yamauchi S, Teixeira FB, Miguez P, Yamauchi M. Immunohistological characterization of newly formed tissues after regenerative procedure in immature dog teeth. *J Endod*. 2011;37:1636-1641.
23. Diogenes A, Hargreaves KM. Microbial modulation of stem cells and future directions in regenerative endodontics. *J Endod*. 2017;43:S95-S101.
24. Thibodeau B, Teixeira F, Yamauchi M, Caplan DJ, Trope M. Pulp revascularization of immature dog teeth with apical periodontitis. *J Endod*. 2007;33:680-689.
25. Da SL, Nelson-Filho P, Da SR, et al. Revascularization and periapical repair after endodontic treatment using apical negative pressure irrigation versus conventional irrigation plus triantibiotic intracanal dressing in dogs' teeth with apical periodontitis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;10:779-787.
26. Song M, Cao Y, Shin SJ, et al. Revascularization-associated intracanal calcification: assessment of prevalence and contributing factors. *J Endod*. 2017;43:2025-2033.
27. Vishwanat L, Duong R, Takimoto K, et al. Effect of bacterial biofilm on the osteogenic differentiation of stem cells of apical papilla. *J Endod*. 2017;43:916-922.

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