Clinical and Radiographic Assessment of the Efficacy of a Collagen Membrane in Regenerative Endodontics: A Randomized, Controlled Clinical Trial

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Abstract

Introduction: Recent reviews confirm a general lack of randomized, controlled clinical studies on the efficacy of regenerative endodontics in immature teeth affected by pulp and periapical diseases. Moreover, we have no evidence of the curative efficacy of collagen membranes used as scaffolds in regenerative endodontics. Here, we evaluated whether a Bio-Gide collagen membrane (Geistlich Pharma AG, Wolhusen, Switzerland) has efficacy in promoting dentin formation in regenerative endodontics. **Methods:** Forty-three patients yielding a total of 46 nonvital immature teeth were divided randomly into 2 groups. Subsequent to chemomechanical preparation, regenerative endodontics with (the experimental group) and without (the control group) Bio-Gide were performed. All cases were followed up clinically and radiographically every 3 months for at least 6 months. Quantitative analyses using an imaging program yielded percentage changes in root dimensions based on a comparison between preoperative and recall radiographs. **Results:** The results of 40 patients (43 teeth) were included in the final analyses. All patients from both groups showed clinical success with complete resolution of signs and symptoms. Radiographically, the thickness of the dentin wall at the middle third of the root was higher for the experimental group than the control group. However, other indicators were comparable between both groups. **Conclusions:** The use of the Bio-Gide collagen membrane promoted the development of the dentin wall in the middle third of the root in patients undergoing regenerative endodontic procedures. The convenience of operation and the assured positioning of the sealing material make the Bio-Gide collagen membrane especially suitable for handling wide root canals. *(J Endod 2017;43:1465–1471)*

Key Words

Dental pulp necrosis, immature teeth, radiography, regenerative endodontics, revascularization

Significance

This randomized, controlled clinical study evaluated the efficacy of a Bio-Gide collagen in promoting dentin formation in regenerative endodontics and provided evidence to support the use of collagen matrix in regenerative endodontics.

Pulp necrosis in an immature tooth with an open apex can lead to devastating consequences for the patient and represents a distinct challenge for the endodontist. Before 2004, clinicians relied on a "traditional" apexification procedure or the use of apical barriers (1, 2). However, neither procedure allows for thickening of the root wall or continued development of the root. In 2004, a novel treatment procedure for immature, nonvital teeth called "regenerative endodontics" (revascularization) was introduced by Banchs and Trope (3). In contrast to traditional apexification or the use of apical barriers, this procedure allowed for increasing both the length of the root and dentin wall thickness. Many case reports have since shown favorable outcomes after the eradication of bacteria from the root canal system, the formation of a scaffold in the canal space, and the creation of a bacteria-tight coronal seal (4–8). Although following a similar concept of regenerative endodontics, their treatment protocols vary in terms of the use of irrigants (8–10), intracanal disinfectant medications (3, 4, 6, 10), canal scaffolds (7, 11), and sealing materials (4, 9, 10, 12, 13). The search for an optimal revascularization protocol is ongoing.

In terms of the scaffold, most reported cases induce a blood clot from the apical foramen to allow the growth of a new tissue into the canal. However, in clinical practice, we cannot always induce sufficient blood to serve as a scaffold, which also increases the likelihood of sealing material collapse. In 2008, a resorbable collagen membrane was introduced to prevent the mineral trioxide aggregate (MTA) from collapsing and to serve as a scaffold to allow new tissue growth into the pulp space in cases with insufficient blood (5). However, to date, no randomized, controlled clinical study has provided persuasive evidence of the efficacy of collagen membranes in inducing root maturation.

The Bio-Gide (Geistlich Pharma AG, Wolhusen, Switzerland) (Fig. 1) is an absorbable, pure collagen membrane used widely as a scaffold material in periodontal tissue regeneration (14, 15). It consists of types I and III collagen extracted from quarantined pigs and refined to remove antigens; it has no antigen-sensitizing effects. Its degradation products include carbon dioxide and water, and the degradation period is in the range

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2017.04.011

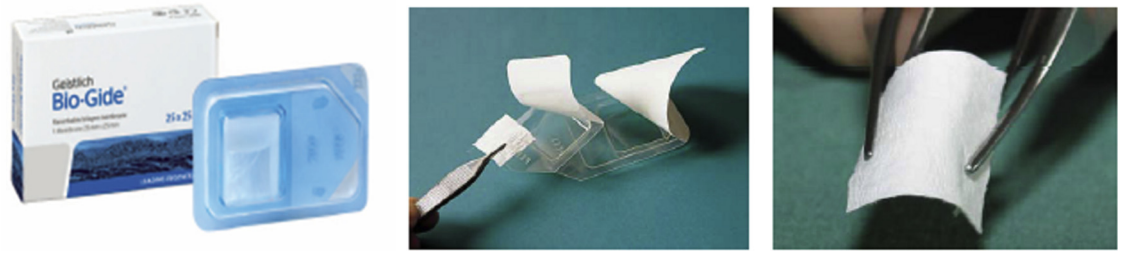


Figure 1. The Bio-Gide.

of 4 to 6 months to provide time for tissue reconstruction (16, 17). To date, no study of the curative efficacy of it used as a scaffold in regenerative endodontics has been reported. Experiments have shown that it can promote the adherence, migration, proliferation, and differentiation of ectomesenchymal cells (17, 18). It has many advantages, such as the ability to stabilize blood clots, maintain growth factor levels, and promote tissue regeneration (14–17). Therefore, its use in regenerative endodontics is desirable, especially in cases in which we cannot induce sufficient blood into the pulp space. We decided to use the Bio-Gide membrane as a scaffold material because it may promote the formation of the dentin in case we could not induce enough blood into the pulp space and may even help to promote the overall curative effect.

In this randomized, controlled clinical study, we investigated the efficacy of the Bio-Gide collagen membrane in promoting dentin formation in regenerative endodontics following the Consolidated Standards of Reporting Trials guidelines. The null hypothesis was that the use of the Bio-Gide collagen membrane would not promote dentin formation in regenerative endodontics.

Materials and Methods

Study Design and Sample Size

This 2-arm, parallel, randomized, controlled clinical study compared dentin formation in a regenerative endodontic procedure between an experimental group in which a Bio-Gide collagen membrane served as a scaffold and a control group without its use.

This 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 Peking University School and Hospital of Stomatology (ref no. PKUSSIRB-201523072).

The present study was designed based on the work of Nagy et al (19) to have 80% power in detecting a difference between the 2 groups. The increases of root thickness in the 2 groups after 6 months were 5.8% ± 1.2% and 4.5% ± 1.6%, respectively. A sample size of 46 teeth to detect differences at the 5% level of significance using the *t* test for testing 2 independent means was calculated with an anticipated loss to follow-up of 10% included.

Children who underwent dental treatment at the Department of Pediatric Dentistry, Peking University Hospital of Stomatology, from January 2014 to February 2016 were recruited. In all cases, the following protocol was followed. It was explained to all patients and their caregivers that regenerative endodontic procedures were relatively new and that, to date, there were no guidelines for treatment protocols in the literature. A comprehensive discussion of the risks, complications, and alternative treatment options was undertaken, and parental consent was obtained. The informed consent form also explained that data from the procedure would be used for research purposes and that patient anonymity would be protected.

A simple random sampling method was used to recruit patients. Forty-six group information notes (23 for controls and 23 for the experimental group) were packed randomly into 46 opaque envelopes after generating a random sequence, and each envelope was numbered sequentially and handed over to each patient according to the time the participant joined the study. Inclusion criteria were an open apex >2 mm in diameter, absence of systemic or immune disease, and absence of known allergies to the medications used in the procedure. Exclusion criteria were a closed apex, apical cysts, and existing disease that needed long-term anti-inflammatory treatment.

Interventions

All of the regenerative endodontic procedures were performed by experienced faculty members of the department following similar protocols according to the clinical considerations for a regenerative procedure advised by the American Association of Endodontists. Clinical examinations, pulp sensitivity, and radiographic examinations were performed. A medical history, clinical symptoms, and preoperative examination of the tooth were collected before the operation.

During the first treatment visit, the access cavity was prepared under rubber dam isolation using 4% articaine with epinephrine 1:100,000. Copious, gentle irrigation was provided twice: first with 1.25% sodium hypochlorite (20 mL, 5 minutes) using an irrigating needle positioned ~1 mm from the root end to minimize cytotoxicity to stem cells in the apical tissues and then again with saline (20 mL/canal, 5 minutes). Canals were dried with paper points. Calcium hydroxide paste was delivered into the canal system using a syringe, and the canal was temporarily sealed using a 3–4 mm glass ionomer.

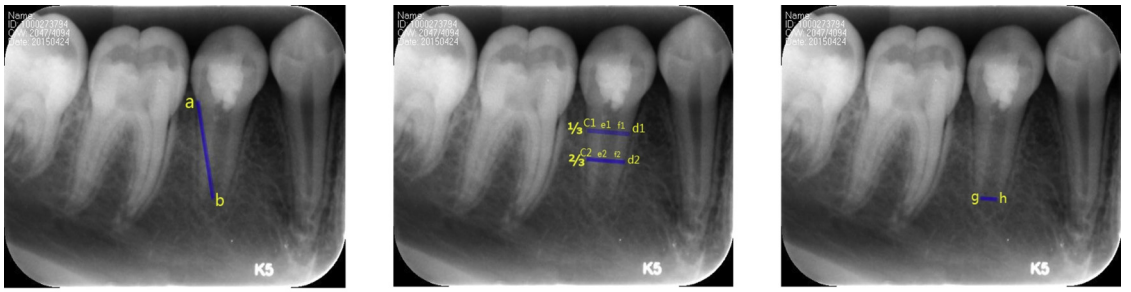
Teeth were reviewed 2 weeks later to assess the response to the initial treatment. If there was any sign or symptom of persistent infection, additional treatment was considered with intracanal medications.

For the second surgery, anesthesia was induced with 2% lidocaine without a vasoconstrictor. The tooth was isolated with a dental dam. Copious, gentle irrigation was provided with 20 mL 17% EDTA. The canal was dried with paper points. Bleeding in the canal system was infused by rotating a precurved K-file at 2 mm past the apical foramen. The Bio-Gide collagen membrane was placed at the middle third of the root, over the blood clot, and ProRoot MTA (Dentsply Tulsa Dental, Johnson City, TN) 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. For the control group, all steps were similar as for the experimental group, except that no Bio-Gide was 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.

Evaluation of Treatment Outcomes

Treatment outcomes were assessed in a blinded manner by 2 independent reviewers, both pediatric dentists. When an evaluation was not



(1) root length (2) dentin wall thickness (3) apical foramen width

Figure 2. 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.

unanimous, consensus was reached through discussion. Clinical success of the treatment was defined as survival of the tooth with resolution of the periapical lesion and the absence of any clinical sign or symptom.

Radiographic examinations were performed by taking pre- and postoperative parallel periapical films. All films were recorded using the same dental X-ray machine (eXpert DC; Gendex Dental Systems, Hatfield, PA) available at the Radiology Department of the Peking University Hospital of Stomatology. Preoperative and final postoperative periapical images in JPEG format were transferred to ImageJ software (version 1.41; National Institutes of Health, Bethesda, MD), mathematically corrected using the TurboReg plug-in (Biomedical Imaging Group, Swiss Federal Institute of Technology, Lausanne, Switzerland) according to the method described by Bose et al (20), and calibrated. Changes in root dimensions between the preoperative and final postoperative images were evaluated by measuring the changes in root length, dentin wall thickness (measured at one third and two thirds of the preoperative root length), and apical foramen width (Fig. 2).

Changes between the postoperative and preoperative values are reported as a percentage. The values were calculated as follows:

$$\text{Percentage increase in root length} = (\text{postoperative length} - \text{preoperative length} / \text{preoperative length}) \times 100\%$$
$$\text{Percentage increase in dentine wall thickness} = (\text{postop thickness} - \text{preop thickness} / \text{preop thickness}) \times 100\%$$
$$\text{Percentage change in apical foramen width} = (\text{postop width} - \text{preop width} / \text{preop width}) \times 100\%$$

Each measurement was made in duplicate, and all results were averaged. Other clinical findings such as crown discoloration, restoration failure, and root canal calcification were also evaluated from the postoperative radiographs.

Data Collection and Statistical Analyses

The data collected included demographic information, contributory etiologies and diagnoses, teeth types, follow-up periods, treatment outcomes, change in tooth color, root canal calcification, and an electric pulp test. Dental data were converted into a Microsoft Excel (Microsoft, Redmond, WA) spreadsheet. Radiographs obtained from patients were scanned and saved in JPEG format. Data from the Excel spreadsheet were imported into SPSS software (Version 20; IBM, Armonk, NY) for further analyses. Differences in continuous variables between groups were analyzed using *t* tests. Differences in categoric variables were evaluated using the Fisher exact test. Percentage changes in root length, dentin wall thickness, and apical foramen width were analyzed using the Mann-Whitney *U* test. *P* values <.05 were considered statistically significant. The mean values are reported.

Results

Demographic and Clinical Characteristics

All patients were evaluated in 3-month intervals for at least 6 months. The follow-up period ranged from 8 to 28 months for the control group and 7 to 26 months for the experimental group. One patient (1 tooth) in the control group and 2 patients (2 teeth) in the experimental group were lost to follow-up because their parents declined to return for it.

Ultimately, 40 patients with 43 teeth were included in the final analyses (Table 1 and Fig. 3): 20 patients (22 teeth) in the control group (average age, 9.82 ± 1.5 years; average follow-up time, 16.1 ± 8.8 months) and 20 patients (21 teeth) in the experimental group (average age, 10.3 ± 1.9 years; average follow-up time, 15.0 ± 5.8 months). No significant differences in study variables, such as average age, sex, distribution of

TABLE 1. Demographic and Clinical Characteristics

Variable	Control group	Experimental group
Age (y)	9.82 ± 1.5	10.3 ± 1.9
Sex, *n* (%)		
Male	8 (40)	9 (45)
Female	12 (60)	11 (55)
Tooth type, *n* (%)		
Anterior	9 (41)	5 (24)
Premolar	13 (59)	16 (76)
Cause, *n* (%)		
Trauma	9 (41)	5 (24)
Broken central cusp	13 (59)	16 (76)
Diagnosis, *n* (%)		
CAP	15 (68)	10 (48)
AAP	7 (32)	5 (24)
CP	0 (0)	6 (28)
Follow-up period (mo)	16.1 ± 8.8	15.0 ± 5.8

AAP, acute apical periodontitis; CAP, chronic apical periodontitis; CP, chronic pulpitis.

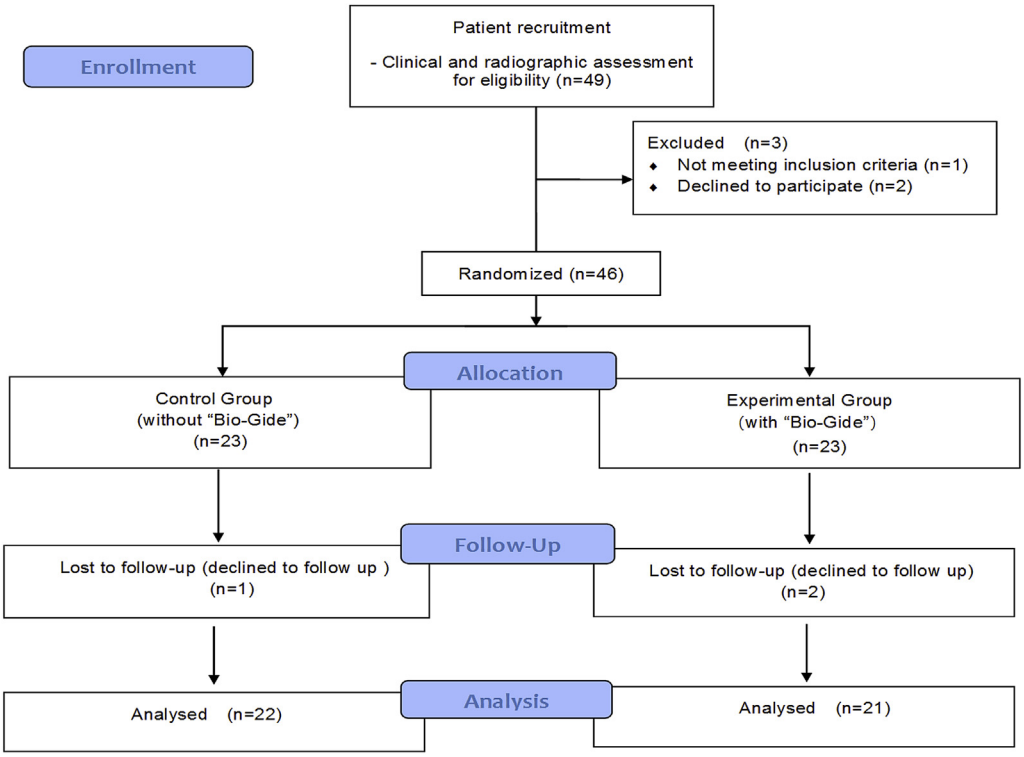


Figure 3. A flow diagram indicating patient recruitment and follow-up, according to the Consolidated Standards of Reporting Trials (http://www.consort-statement.org/).

teeth type, diagnosis, root development stage, or follow-up period, were identified between the groups (all $P > .05$).

Treatment Outcomes

At the end of treatment, all cases were asymptomatic with complete resolution of signs and symptoms (Table 2). Radiographically, all teeth were found to have longer roots. Five teeth in the control group and 8 in the experimental group achieved complete root development. Moreover, 91% (20/22) of cases in the control group and 86% (18/21) in the experimental group showed increased dentin wall thickness in the apical third of the root, 55% (12/22) of cases in the control group and all in the experimental group showed increased dentin wall thickness in the middle third of the root, and 91% (20/22) of cases in the control group and all in the experimental group showed narrowing of the apical foramen width.

TABLE 2. Treatment Outcomes

Variable	Control group (*n* = 22)	Experimental group (*n* = 21)
Increase in root length, *n* (%)	22 (100)	21 (100)
Increase in dentin wall thickness in the apical third of the root, *n* (%)	20 (91)	18 (86)
Increase in dentin wall thickness in the middle third of the root, *n* (%)	12 (55)	21 (100)
Narrowing of apical foramen width, *n* (%)	20 (91)	21 (100)
Discoloration, *n* (%)	14 (64)	15 (71)
Calcification, *n* (%)	12 (55)	10 (48)
EPT, *n* (%)	4 (18)	7 (33)

EPT, electric pulp test.

Postoperative crown discoloration was observed in 64% (14/22) of cases in the control group and 71% (15/21) in the experimental group. No restoration failure occurred in either group. Pulp canal calcification was observed in 55% (12/22) of cases in the control group and 48% (10/21) in the experimental group. An electric pulp test was achieved in 18% (4/22) of cases in the control group and 33% (7/21) in the experimental group. There were no statistically significant differences between the 2 groups in terms of any of the parameters studied (all $P > .05$).

Changes in Root Dimensions

Radiographically, cases in the experimental group showed a slightly greater change in root length (16.40% ± 13.6% in the experimental group and 15.4% ± 13.6% in the control group), a slightly greater change in dentin wall thickness in the apical third of the root (21.5% ± 22.5% in the experimental group and 21.2% ± 19.5% in the control group), and a slightly greater change in the apical foramen width (−65% ± 34% in the experimental group and −55% ± 34% in the control group) (Table 3 and Fig. 4). However, these differences were not statistically significant (Mann-Whitney *U* test, $P > .05$). The difference in dentin wall thickness in the middle third of the root between the 2 groups (23.8% ± 21% in the experimental group and 6.9% ± 14% in the control group) was statistically significant (Mann-Whitney *U* test, $P < .05$).

Other Radiographic Findings

During the follow-up period, no significant differences between anterior and posterior teeth in terms of continued root development were identified. In addition, the effect of apical lesions on continued root development was also nonsignificant.

TABLE 3. Changes in Root Dimensions

Variable	Control group, mean ± SD	Experimental group, mean ± SD
Root length (%)	15.4 ± 13.6	16.4 ± 13.6
Dentin wall thickness in the apical third of the root (%)	21.2 ± 19.5	21.5 ± 22.5
Dentin wall thickness in the middle third of the root (%)	6.9 ± 14	23.8 ± 21
Apical foramen width (%)	−55 ± 34	−65 ± 34

SD, standard deviation.

Discussion

The collagen matrix was introduced into pulp regenerative endodontics to avoid apical displacement of the MTA and promote tissue ingrowth in cases with insufficient blood for a clot (5, 8). In our study, no cases of collapse occurred in either group; dentin wall thickness at the middle third of the preoperative root canal showed a significant increase in the experimental group. However, there were no significant differences between the groups in the success rate or other clinical indicators such as pulp vitality, root canal calcification, or tooth discoloration. There were also no significant differences in parameters of continued root development such as root length, dentin wall thickness at the apical third of the root, or apical foramen width. This suggests that use of the collagen membrane did not directly improve the success rate or development at the apical third of the root.

In our study, 14 cases in the experimental group and 15 in the control group did not induce sufficient blood into the pulp space. In order to compare the effect of the collagen membrane in cases with or without sufficient blood, we performed a further analysis. Statistical analysis found that the result in cases with or without sufficient blood is consistent with the overall result (the increase in root canal wall thickness in the middle third of the root was significantly higher in the experimental group than the control group, whereas other parameters are comparable). Although the difference was not significant, the increase in root length was more obvious in cases with insufficient blood than the overall result in the experimental group compared with the control group. Further follow-up appointments and studies with a larger sample size are scheduled to confirm these results.

When Jung et al (5) first introduced the collagen matrix into regenerative endodontics to grow a new tissue into the pulp space in cases with insufficient blood for a clot, they microscopically observed that blood was oozing from the periradicular tissue and wetting the matrix. Collagen is a bioactive material; it can improve the rate of revascularization and helps in releasing the growth factors (13). We placed the Bio-Gide in the middle third of the root to avoid damaging the physiological structure of the root apical region and serve as a scaffold. The Bio-Gide in our study may have helped to promote blood filling into the root canal (5), stabilize the blood clot (16), and aid in releasing the growth factors (13) to promote the development of the root, especially in the middle third of the root where we placed it. However, we should note that a scaffold is not the only factor providing the final curative effect because several teeth in our study did not

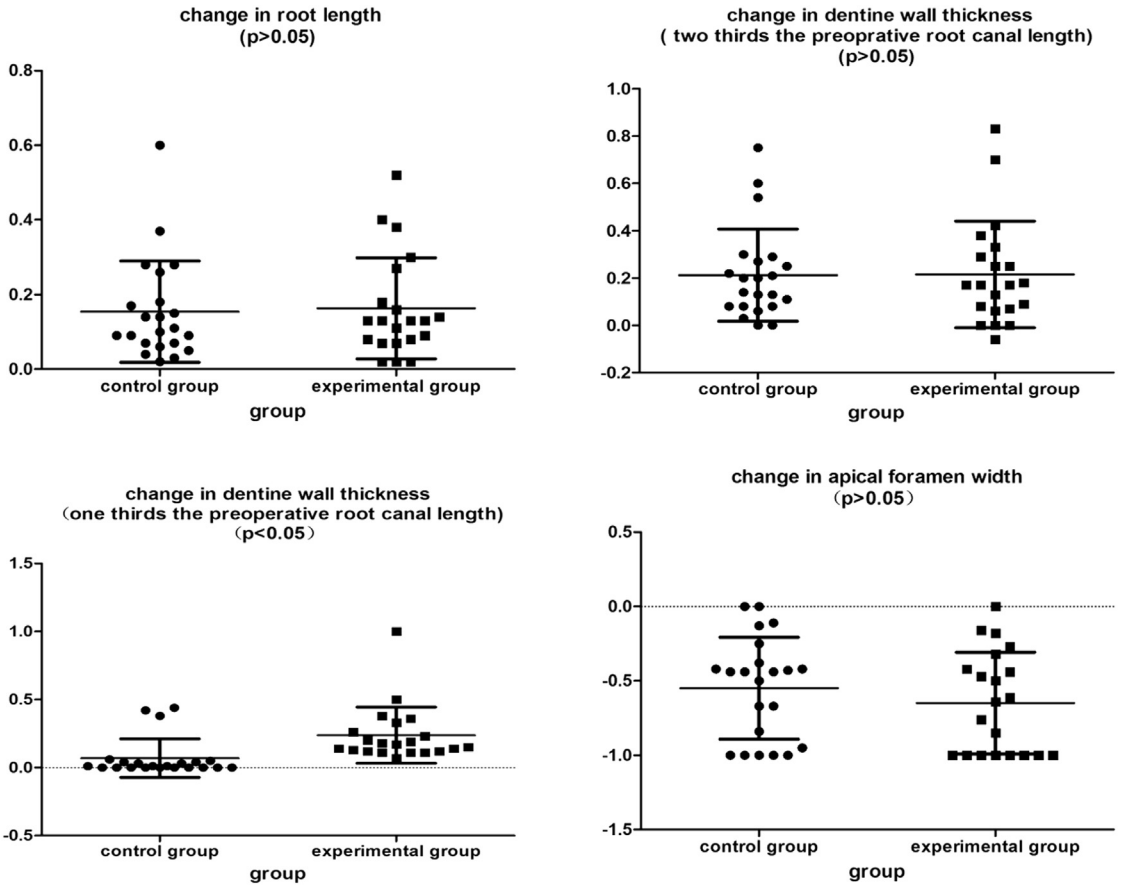


Figure 4. Changes in Root Dimensions.

achieve sufficient root formation although sufficient blood was induced in the pulp space and a collagen membrane was used as a scaffold.

Our study included 14 anterior and 29 posterior teeth; we found no differences between anterior and posterior teeth in terms of continued root development. This confirms the value of regenerative endodontics in anterior teeth affected by dental trauma involving pulp and periodontitis.

We observed 2 main patterns of continued root development in our study; some achieved complete development, with morphology similar to the control teeth (Figs. S1 and S2 are available online at www.jendodon.com). Others failed to show a significant increase in root length; rather, dentin wall thickness increased, and the root end became blunt (Fig. S3 is available online at www.jendodon.com), illustrating the uncertain effects of regenerative endodontics.

Our study included 25 cases with and 18 without apical lesions, with no differences in curative effects identified between the groups. This confirms the curative effects of regenerative endodontics in teeth with apical lesions. However, 1 tooth with a severe apical lesion showed continued root development after being separated from the main root (Fig. S4 is available online at www.jendodon.com). The viability of the Hertwig epithelial root sheath and apical papilla depends on the severity and duration of apical periodontitis/abscesses (21). In regenerative endodontics, these structures must survive to regulate root development. A long history and a wide range of apical lesions can result in the separation of the Hertwig epithelial root sheath and apical papilla, leading to abnormal root growth (22).

Ten cases in the experimental group and 12 in the control group showed root canal calcification. In animal studies, tissues that form in the canals of revascularized immature teeth with apical periodontitis are described as cementoid/osteoid tissues and periodontal ligament–like tissues (23–27). Martin et al (27) found that tissues that form in the canals of human teeth are mineralized tissues with some fibrous connective tissue. No pulplike tissue, characterized by the presence of odontoblastlike cells, has been observed lining the dentinlike mineralized tissue. To date, specific factors for calcification and their effects on the prognosis of those undergoing regenerative endodontics treatment remain unknown.

Regenerative endodontics is a conservative yet effective method for dealing with immature teeth affected by pulp and periapical diseases. The use of the Bio-Gide collagen membrane did not significantly improve the success rate or growth in the apical third of the root, but it promoted the development of the dentin wall at the middle third of the root, which has an advantage in avoiding cervical root fractures. It also increased our convenience in the operation and ensures the positioning of the sealing material, making it especially suitable for teeth with wide root canals. However, the curative effects of regenerative endodontics are still uncertain. We need to further explore factors that affect the clinical course of this procedure.

Conclusions

The null hypothesis that the use of the Bio-Gide collagen membrane would not promote dentin formation in regenerative endodontics was partly rejected. The use of the membrane promoted dentin formation in the middle third of the root. Because most of the teeth in our study were not fully developed during the short period of follow-up, further follow-up appointments are scheduled.

Acknowledgments

The authors thank the faculty of the Department of Pediatric Dentistry, Peking University School and Hospital of Stomatology for helping with subject recruitment.

Supported by a grant from Capital Health Development Research Special Fund (grant no. 2014-3-4104).

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

Supplementary Material

Supplementary material associated with this article can be found in the online version at www.jendodon.com (http://dx.doi.org/10.1016/j.joen.2017.04.011).

References

1. Ghose LJ, Baghdady VS, Hikmat YM. Apexification of immature apices of pulpless permanent anterior teeth with calcium hydroxide. J Endod 1987; 13:285–90.
2. Yates JA. Barrier formation time in non-vital teeth with open apices. Int Endod J 1988;21:313–9.
3. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? J Endod 2004;30:196–200.
4. Chueh LH, Huang GT. Immature teeth with periradicular periodontitis or abscess undergoing apexogenesis: a paradigm shift. J Endod 2006;32: 1205–13.
5. Jung IY, Lee SJ, Hargreaves KM. Biologically based treatment of immature permanent teeth with pulpal necrosis: a case series. J Endod 2008;34: 876–87.
6. 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;4:109–13.
7. Miltiadous ME, Floratos SG. Regenerative endodontic treatment as a retreatment option for a tooth with open apex - a case report. Braz Dent J 2015; 26:552–6.
8. Petrino JA, Boda KK, Shambarger S, et al. Challenges in regenerative endodontics: a case series. J Endod 2010;36:536–41.
9. Ding RY, Cheung GS, Chen J, et al. Pulp revascularization of immature teeth with apical periodontitis: a clinical study. J Endod 2009;35:745–9.
10. Shah N, Logani A, Bhaskar U, Aggarwal V. Efficacy of revascularization to induce apexification/apexogensis in infected, nonvital, immature teeth: a pilot clinical study. J Endod 2008;34:919–25. discussion 1157.
11. Zhujiang A, Kim SG. Regenerative endodontic treatment of an immature necrotic molar with arrested root development by using recombinant human platelet-derived growth factor: A case report. J Endod 2016;42:72–5.
12. 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;37:562–7.
13. Jadhav G, Shah N, Logani A. Revascularization with and without platelet-rich plasma in nonvital, immature, anterior teeth: a pilot clinical study. J Endod 2012;38: 1581–7.
14. Tonetti MS, Cortellini P, Lang NP, et al. Clinical outcomes following treatment of human intrabony defects with GTR/bone replacement material or access flap alone. A multicenter randomized controlled clinical trial. J Clin Periodontol 2004;31:770–6.
15. Sculean A, Schwarz F, Chiantella GC, et al. Five-year results of a prospective, randomized, controlled study evaluating treatment of intra-bony defects with a natural bone mineral and GTR. J Clin Periodontol 2007;34:72–7.
16. Behring J, Junker R, Walboomers XF, et al. Toward guided tissue and bone regeneration: morphology, attachment, proliferation, and migration of cells cultured on collagen barrier membranes. A systematic review. Odontology 2008;96:1–11.
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;26:1004–10.
18. Xu F, Yang DS. Effect of Bio-Gide collagen membranes on the proliferation and osteogenetic differentiation of rabbit bone marrow mesenchymal stem cells. Zhongguo Zuzhi Gongcheng Yanjiu 2014;18:4797–803.
19. 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;40:192–8.

20. Bose R, Nummikoski P, Hargreaves K. A retrospective evaluation of radiographic outcomes in immature teeth with necrotic root canal systems treated with regenerative endodontic procedures. J Endod 2009;35:1343–9.
21. Chen MY, Chen KL, Chen CA, et al. Responses of immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/abscess to revascularization procedures. Int Endod J 2012;45:294–305.
22. Jung IY, Kim ES, Lee CY, Lee SJ. Continued development of the root separated from the main root. J Endod 2011;37:711–4.
23. Thibodeau B, Teixeira F, Yamauchi M, et al. Pulp revascularization of immature dog teeth with apical periodontitis. J Endod 2007;33:680–9.
24. da Silva LA, Nelson-Filho P, da Silva RA, 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; 109:779–87.
25. Wang X, Thibodeau B, Trope M, et al. 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.
26. Yamauchi N, Nagaoka H, Yamauchi S, et al. Immunohistological characterization of newly formed tissues after regenerative procedure in immature dog teeth. J Endod 2011;37:1636–41.
27. 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;39:138–44.