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ORIGINAL ARTICLE

Histologic and radiological observations of a human immature premolar with pulp necrosis treated with regenerative endodontic procedure – A case report of a four-year follow-up

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Abstract *Background:* Reports are found on long-term radiographic follow-up of teeth treated with regenerative endodontic techniques, but scarce literature is observed regarding the tissue formed in the root canal. A four-year radiographic follow-up with histologic findings in an immature mandibular premolar with dens evaginatus that underwent a regenerative endodontic procedure (REP) is presented here. After four years following REP, the tooth was extracted for orthodontic purposes thereby presenting an opportunity to report the histologic findings.

Summary: The radiographic changes included a slight root lengthening and thickening and apex closure. Histologic evaluation revealed a fibrous connective tissue with an inflammatory infiltrate in the canal space. In the apical region, cementum-like irregular mineralization developed toward the center, giving the appearance of a closed apex. Root thickening, increase in length, and radiographic closure of the apex occurred due to cemental hyperplasia.

Conclusion: After 4 years following REP in an immature mandibular premolar with apical periodontitis, granulation tissue had formed in the root canal, indicating repair and not regeneration.

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1. Introduction

One of the most challenging domains in pediatric dentistry and endodontics is managing non-vital and immature permanent teeth. Endodontic treatment modalities such as calcium hydroxide ($CaOH_2$) / mineral trioxide aggregate (MTA) apexification contribute to the healing of apical periodontitis. This treatment is time-consuming, technique-sensitive, does not contribute to root length improvement or dentinal wall thickness, and leaves the treated immature permanent tooth vulnerable to fractures (Dabbagh et al., 2012; Andreasen et al., 2002; Cvek., 1992).

Regenerative Endodontic Procedure (REP) promotes continued root development while regenerating the pulp-dentin complex (Nosrat et al., 2011). Based on the treatment goals outlined for regenerative procedures (Amer. Assoc. endo., 2021), successful REP leads to continued root development, increased root wall thickness, periodontal healing, root apex closure, and return of tooth vitality. Clinical and radiological success with periodontal healing was reported in 79–100% of cases, whereas root development and thickening of dentinal walls were less frequently achieved (Nazzal, Duggal, 2017; Kontakiotis et al., 2014).

Clinicians report favorable clinical and radiographic outcomes with REP for immature permanent teeth. However, regeneration is a histological observation that cannot be ascertained either clinically or radiographically. Histological studies of some cases of REP showed connective tissue within the root canal walls, deposition of cementum, and ectopic hard tissue formation (Yamauchi et al., 2011). Long-term follow-up and tooth extraction are mandated to obtain definitive evidence of histologic changes post-REP. A four-year follow-up of clinical, radiographic, and histological findings is reported here. A necrotic immature mandibular first premolar with dens evaginatus underwent REP and had to be subsequently extracted for orthodontic therapy.

2. Case report

A 10–year–old female of Chinese lineage was brought for a routine check-up to the outpatient department of the Faculty of Dentistry, Manipal University College Malaysia. Her medical and family history was non-contributory. Soft tissue examination revealed a draining sinus on the buccal mucosa in relation to a non-carious mandibular left first premolar. Dens evaginatus was present on both the erupted mandibular first premolars. No history of pain or swelling was observed to be associated with the mandibular left first premolar. The pulp sensibility tests (i.e., heat, cold, and electric tests), percussion, and palpation tests were negative. A gutta–percha point used to trace the sinus tract led to the apex of the mandibular left first premolar. (Fig. 1A). A pre-operative cone beam computed tomography (CBCT) (Fig. 2A) of the left lower premolar revealed a development groove with dens evaginatus that might have led to the non–vitality of the mandibular left first premolar.

A clinical diagnosis of chronic periapical periodontitis was confirmed with pulpal necrosis of the mandibular left first premolar. Treatment options such as MTA apexification and REP were explained to the parents. The parents gave consent to proceed with REP for the child.

2.1. First appointment

Local anesthesia was administered using 3% Mepivacaine (Scandonest® 3% Plain, Septodont Inc, Novocol Pharmaceutical, Cambridge, Ontario, Canada). Rubber dam was applied during access opening of the mandibular left first premolar. No vital tissue was encountered, and the existing necrotic pulp was removed. The canal was flushed with 1.5% sodium hypochlorite (NaOCl) and normal saline and then dried with sterile paper points. ($CaOH_2$) (UltraCal™ XS, Ultradent, South Jordan, UT) was placed in the canal and the access cavity was closed with a sterile cotton pellet and Intermediate Restorative Material (IRM)® (Dentsply International, Milford, DE).

2.2. Second appointment

Based on American Association of Endodontists (AAE) guidelines, the patient was followed up after one week (AAE, 2021). The tooth was asymptomatic, the sinus tract had healed, the provisional restoration was intact and hence, it was decided to induce bleeding in the canal. Local infiltration was done using 3% Mepivacaine without vasoconstrictor (Scandonest® 3% Plain, Septodont Inc, Novocol Pharmaceutical, Cambridge, Ontario, Canada) and the access cavity was reopened under rubber dam isolation. The root canal was irrigated with sterile saline to remove the $CaOH_2$ paste and flushed with 2.5% NaOCl for 3 mins. No bleeding was encountered in the canal. After completing the instrumentation, the root canal was copiously irrigated with 17% Ethylenediaminetetraacetic acid (EDTA) for 5 mins (Wei et al., 2022). The canal was dried with sterile paper points.

A sterile #15 K file was then introduced into the canal 2 mm beyond the working length to induce bleeding from the periapical tissues. A sterile cotton pellet was placed in the pulp chamber to obtain hemostasis and to facilitate blood clot formation (Wei et al., 2022). Once the clot had formed, the pulp chamber was cleared of any remnants of blood that could cause future discoloration of the crown. The access was sealed with mineral trioxide aggregate (MTA) (ProRoot®MTA Dentsply), glass ionomer cement (GC Fuji II®), and composite (3 M™ Filtek™ Z350 Universal restorative).

2.3. Follow-up visits and radiographic evaluation

The patient was followed up after one week and periodic visits were scheduled. After 3 months, the periapical radiolucency had significantly reduced, and the tooth was asymptomatic. A discolored crown was observed within 6 months of the visit, probably due to bismuth oxide (BiO_2) in MTA. Follow-up sequential radiographs revealed marginal increase in root wall length and thickness. Observations of follow-up radiographs after 1, 2, 3, and 4 years revealed a normal periapical appearance and significant narrowing of the root apex. Over 4 years, the tooth was asymptomatic with normal periapical condition, and a visible increase in the thickness of the root walls and marginal narrowing of the root apex (Fig. 1A-I). However, pulp sensibility tests were negative.

It was decided to extract the mandibular first premolars, as part of the patient's orthodontic treatment plan. Before the extraction, pulp sensibility tests and CBCT were done (Fig. 2) for records. Local infiltration with 2% Mepivacaine

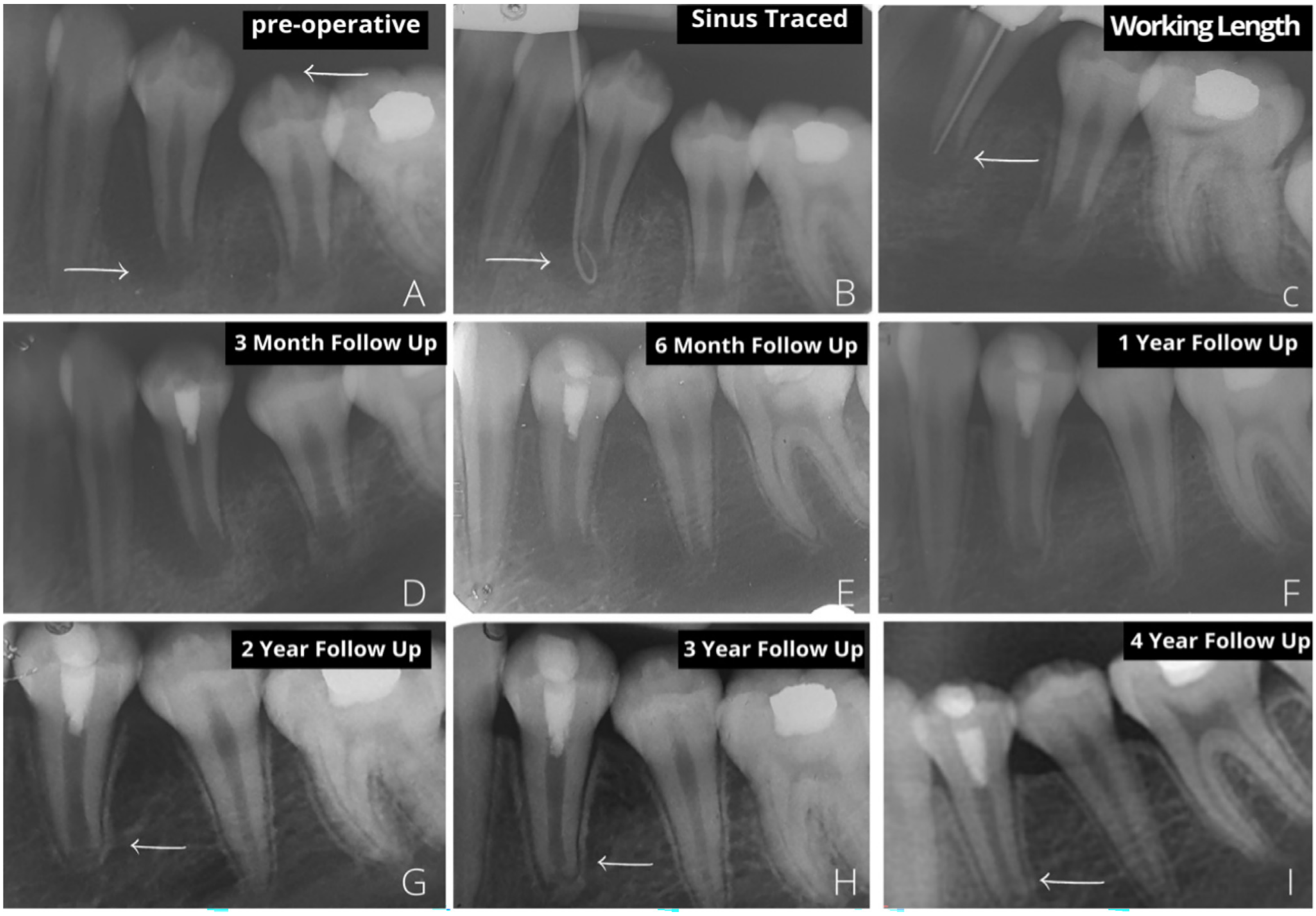

Fig. 1 Radiographic follow-up (A-B) Diagnostic pre-treatment intraoral periapical radiograph taken with gutta-percha cone inserted into the sinus tract. (C) K file was introduced into the canal through the apical foramen. (D-E) 3-month and 6-month follow-up radiographs showed gradual resolution in radiolucency (F-G) 1-year and 2-year follow-up radiographs showed complete resolution of radiolucency. The tooth was asymptomatic and periodontal healing was observed along with a slight thickening of root dentin. (H) A 3-year follow-up radiograph showed narrowing of the apex and significant thickening of root dentin. (I) Apex appears to have closed.

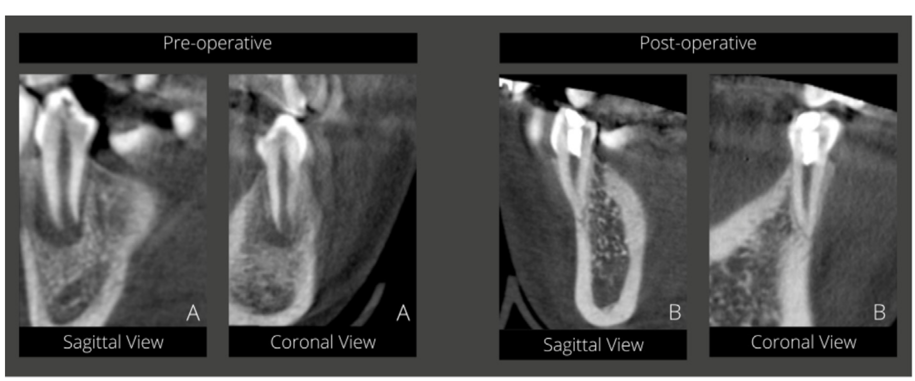

Fig. 2 CBCT Evaluation (A) Pre-Operative CBCT showing the presence of dens evaginatus, and periapical abscess in immature mandibular left first premolar. (B) Post-Operative CBCT taken at 4-year follow-up showed an increase in root wall thickness and closure of the apex.

and 1:20,000 Levonordefrin was used while extracting both mandibular first premolars. Informed consent was obtained for histologic examination of the extracted tooth from the patient's parents. Photographs of the extracted tooth were taken (Fig. 3A-C). The crown was separated from the root at the cementoenamel junction (CEJ) using a diamond bur with distilled water as a coolant so that the specimen is fixed

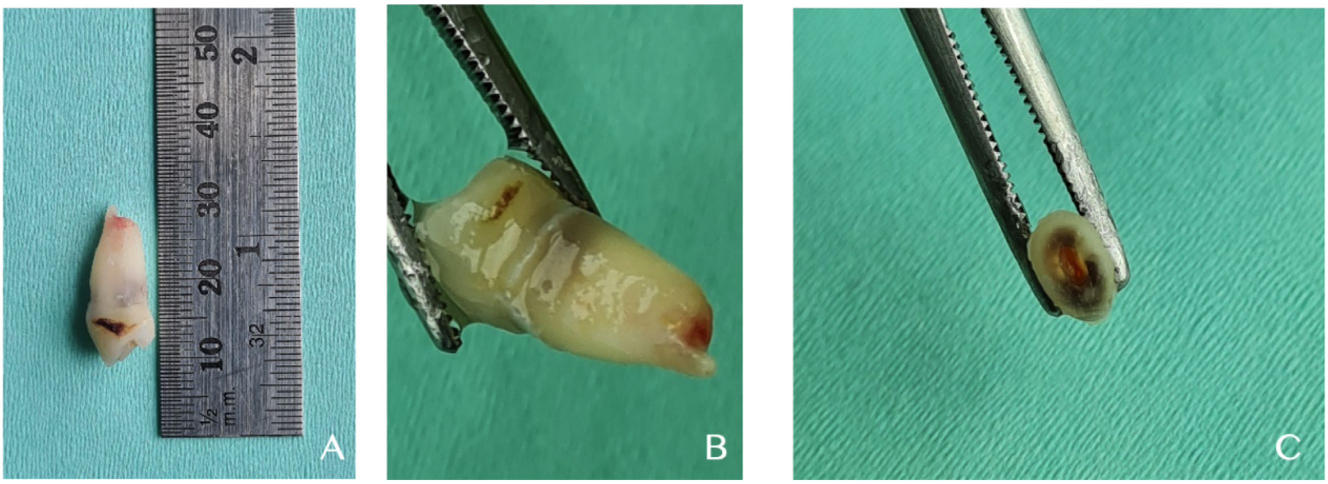

Fig. 3 **Extracted Tooth** (A-B): Extracted tooth #34 showing the longer buccal side of root apex (C): Crown portion removed showing vital tissue at the coronal third of the root.

properly. (Fig. 3C) The separated root was stored in 10% buffered formalin and sent for histopathological investigation.

2.4. Tissue processing

The single-rooted specimen (measuring 1.20 × 0.7 × 0.2 cm) was decalcified using a rapid decalcifying solution [hydrochloric acid (HCl) 5–9% (w/w)]. The decalcified specimen was bisected along the long axis of the tooth in the mesiodistal plane. The bisected specimen was then embedded on the cut surface and sectioned using a rotary microtome. Routine 4 µm sections were then stained with Hematoxylin and Eosin (H&E) stain.

2.5. Histologic evaluation

2.5.1. Coronal third

In the coronal third area, fibrous connective tissue was seen within the root canal interspersed with numerous spindle-shaped fibroblasts, intense, mixed inflammatory plasma cells, and macrophages. The tissue also appeared highly vascularized; as several blood vessels of various calibers, congested with red blood cells and a few lymphatic channels were observed. Occasional cholesterol clefts were also observed within the pulpal core of the coronal third area. Specifically, the presence of globular eosinophilic material consistent with foreign body material (MTA) was observed over the induced blood clot (Fig. 4).

2.5.2. Middle third

A thick layer of newly formed, homogenous cementum-like matrix containing cementocytes housed in lacunae was seen to extend along the lateral walls to the apical third area.The tissue was significantly less cellular and vascular compared to the coronal third.

2.5.3. Apical third

In the apical third, globules of basophilic, malformed cementum-like material were deposited within the tissue. Towards the root apex, the cementum layer was thicker and showed irregular mineralization and it formed towards the center of the canal, hence it appeared as a constricted root apex.

3. Discussion

Regenerative Endodontic Procedure (REP) is a favored treatment option for non-vital immature permanent teeth and the present case report focuses on radiographic and histological changes observed 4 years post-REP. Previous studies have reported histologic observations following REP with a follow-up of up to 2 years (Shimizu et al., 2012; Becerra et al., 2014; Martin et al., 2013; Torabinejad, Faras, 2012; Lei Lishan et al., 2015) and only 1 other histologic study is documented with 54 months follow-up after REP in immature teeth. (Austah et al., 2018).

In the present case, the patient initially presented with an asymptomatic draining sinus and continued to be asymptomatic for the duration of the follow-up, which lasted over 4 years. Sequential radiographs over four years showed a gradual postoperative resolution of the radiolucency and an attempt at radiographic root closure. In the radiograph taken at the 4-year follow-up, the root end appears to have closed, along with an increase in the length and the thickness of the root wall (Fig. 1). However, examination of the extracted mandibular left second premolar confirmed the radiographic findings of attempted root apex closure with increased length on the buccal side only (Fig. 3). This was further confirmed histologically with cementoid/ dentinoid deposits observed in the middle and apical third of the root along the inner walls of the radicular dentin and within the tissue in the root's apical part which was well-demarcated from primary dentin. No new dentin deposition was observed in the canal. At the apical third, the calcified tissue (cementoid/ dentinoid) appeared to be thickest and cellular cementum predominated with cemental hyperplasia leading to the constricted root appearance (Fig. 3-A-B) and an increase in root length in the radiographs. (Fig. 4-B-F), respectively. Similar findings have been reported in humans (Shimizu et al., 2012; Becerra et al., 2014; Martin

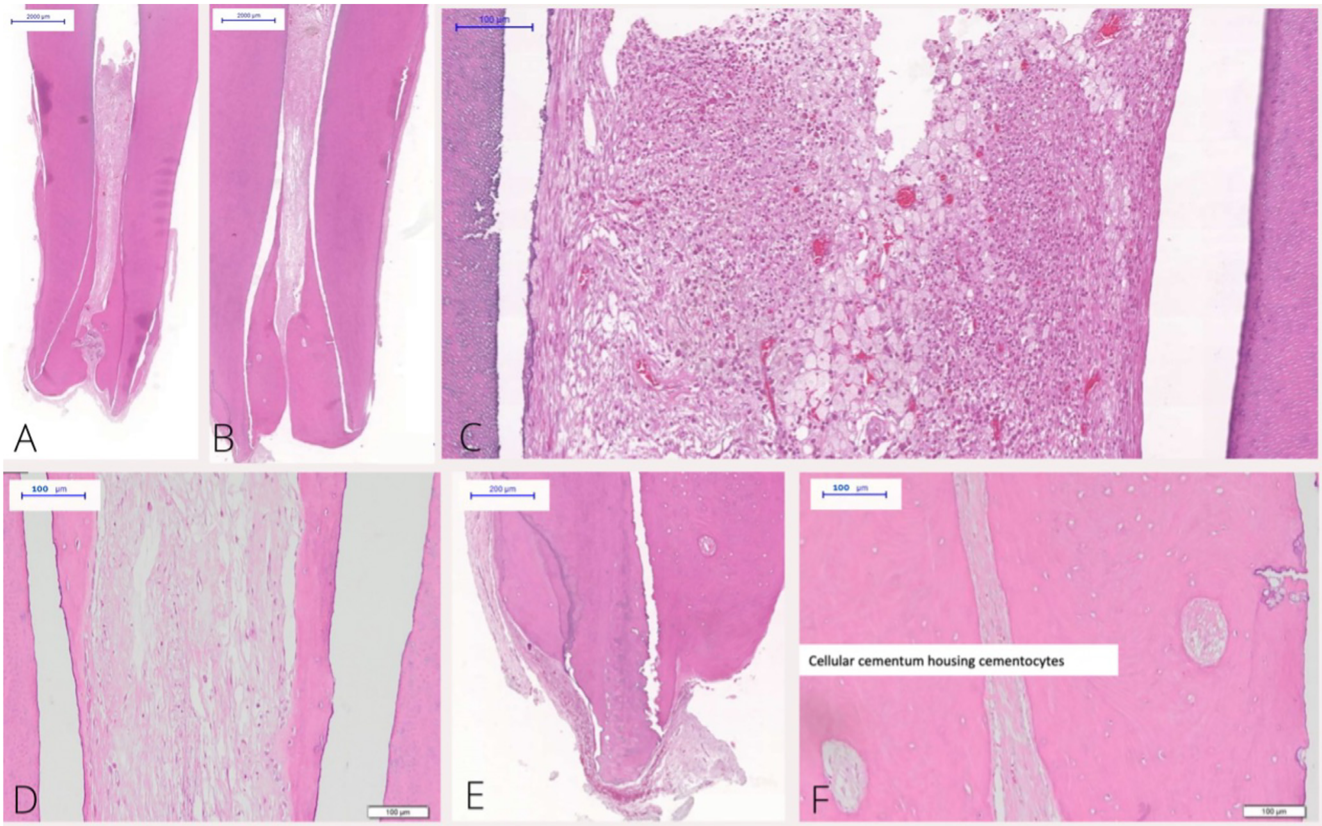

Fig. 4 Histological Examination of the specimen (A), (B) Root seen in different sections; the calcified tissue (cementoid/dentinoid) appears to be the thickest at the apical third, giving it a constricted root appearance like forming a calcific barrier at the apex. Clearly, demarcated newly formed mineralized material is seen in both the apical and the middle third. **(C)** Higher magnification of the coronal third of the root shows inflamed pulp tissue consisting of fibrous mesenchymal tissue, multiple vascular channels, and inflammatory cell infiltrate of lymphocytes as well as foamy histiocytes with no evidence of calcification. There is no evidence of regenerated tissue. **(D)** Flattened cells can be seen lining the surface of the newly formed mineralized material (cementoid/dentinoid). The spindle-shaped and flat cells appear to be cementoblasts lining the cementum. **(E)** The apical third of the root shows uneven cemental hyperplasia. **(F)** Within the apical third of the root, the cementum formed mainly consists of cellular cementum which is seen housing the cementocytes.

et al., 2013; Torabinejad, Faras, 2012; Lei Lishan et al., 2015; Peng Chufang et al., 2017; Schmalz et al., 2020) and animal studies (Yamauchi et al., 2011; Wang et al., 2010).

Histological study revealed a highly vascularized fibrous connective tissue that filled the canal space and exhibited inflammatory infiltrate in the coronal third of the root canal. This indicated granulation tissue within the coronal part of the tissue confirming tissue repair and not regeneration (Becerra P et al., 2014). Periapical infection and the use of strong irrigants that destroy the stem cells in the apical tissue or remaining vital pulp tissue can lead to the formation of repair tissue (Peng et al., 2017; Shimizu et al 2012). The stem cells stimulated by REP may develop into osteo/ cementogenic cells in the absence of residual pulp tissue as observed in our case (Zhu et al., 2013). Mesenchymal cells brought into the canal by induction of bleeding adheres to the dentinal walls and differentiates into pre-osteoblasts and pre-cementoblasts (Lovelace et al., 2011). This explains cementoid/ dentinoid tissue formation and the cemental hyperplasia in the apical region in our case.

Hertwig's epithelial root sheath (HERS) which determines the root shape is susceptible to trauma. The amount of vascularity and cellularity in the apical region may allow root formation even in the presence of infection and necrosis. On the other hand, damage to HERS may prevent periodontal ligament stem cells to differentiate into odontoblasts or cementoblasts, thus preventing continued root development (Nazzal, Duggal 2017; Rafter Mary 2005; Saoud et al., 2014). In our case, no HERS-like cells were observed within the periodontal ligament surrounding the apex possibly due to the damage caused by apical periodontitis present initially. Therefore, the REP led to repair, even after 4 years.

In the present case, radiographic findings revealed a marginal increase in the root wall thickness and root length. A recent *meta*-analysis of REP stated that standardized, radiographic interpretation of an increase in length and root wall thickness must be done with caution as these are dependent on the X-ray beam angulation and radiographic positioning (Ong et al., 2020). Although standard CBCT guides were applied for our radiographic evaluation, what appeared as root thickening and root elongation on periapical radiography was found to be irregular dentinoid/ cementoid formation on histologic examination. The histologic examination also revealed continued deposition of irregular new tissue along the root wall which would have eventually led to complete obliteration of the canal. After REP, clinically significant root development is unpredictable with an additional risk of hard tissue formation inside the root canal (Schmalz et al., 2020; Ong et al., 2020).

Our case with a 4-year follow-up demonstrated REP to form dentinoid/ cementoid-like tissue instead of pulp-like tissue. While this may give an appearance of increased root wall thickness, it does not give mechanical strength to the root walls as this is a weak disorganized tissue (Schmalz et al., 2020).

4. Clinical relevance

A four-year follow-up of REP led to dentinoid/ cementoid-like tissue formation. However, REP is currently accepted as a treatment modality for young permanent teeth with pulpal necrosis with a risk of hard tissue deposition within the canals to maintain the alveolar bone (Schmalz et al., 2020 Mittmann et al., 2020). However, it is an acceptable risk, and the treatment option is offered to parents as many practitioners may not have access to the advanced and predictable modalities shown with tissue engineering, stem cells, customized scaffolds, and growth factors. Further research should focus on immunohistochemistry while investigating the histological tissue obtained after the REP.

5. Declaration

Preferred Reporting Items for Case reports in Endodontics (PRICE) 2020 guidelines (Nagendrababu et al. 2020) have been followed in documenting this report.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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