Histologic Findings of a Human Immature Revascularized/Regenerated Tooth with Symptomatic Irreversible Pulpitis

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Abstract

Introduction: Pulp revascularization/regeneration in immature permanent teeth with necrotic pulp and/or apical periodontitis is an effective approach for inducing root maturation. Previous histologic studies showed cementoid/osteoid tissue and/or periodontal ligament–like tissue formed within the root canals. This case report describes the histologic findings of a human symptomatic irreversible pulpitis immature permanent tooth with most of the pulp removed after a revascularization/regeneration procedure. **Methods:** A human immature permanent mandibular premolar (tooth #29) was diagnosed as symptomatic irreversible pulpitis with symptomatic apical periodontitis at the emergency department. Most of the pulp was removed. The tooth was treated with revascularization/regeneration. **Results:** At the 12-month recall, the radiographic examination revealed thickening of the root canal wall, narrowing of the root apex, and lengthening of the root. The tooth was extracted at 12 months for orthodontic treatment. The specimens were processed for histologic examination. Histologically, the apical third of the root canal was filled with newly formed dentinlike and pulplike tissue. There was a layer of flattened odontoblastlike cells lining the dentinal wall. In the midportion of the root canal, the newly formed dentinlike tissue gradually changed to cementumlike tissue. In the upper third of the root canal, there was a presence of cementocytelike cells housed in the lacunae of cementumlike tissue around the loose connective tissue. **Conclusions:** In the present case, regeneration of the pulplike tissue and the periodontium existed after a revascularization/regeneration procedure in an immature permanent tooth clinically diagnosed as symptomatic irreversible pulpitis. *(J Endod 2017;43:905–909)*

Key Words

Immature permanent teeth, irreversible pulpitis, regeneration, revascularization

Significance

This case report presents histologic findings of an immature permanent tooth with irreversible pulpitis after pulp revascularization, which is valuable to help clinicians understand the results of pulp revascularization and optimize the revascularization procedure accordingly.

The management of immature permanent teeth with necrotic pulp is a challenging task for dental professionals. The young pulpless tooth with an open apex frequently has thin, fragile walls, which make it difficult to debride adequately and obtain the necessary apical seal using conventional endodontic treatment methods. Calcium hydroxide–based apexification and mineral trioxide aggregate apexification are usually used for these cases. However, neither treatment can solve the problem of the cessation of root development and the subsequent thin and fragile root canal wall (1, 2).

A novel treatment of revascularization/regeneration of immature nonvital teeth was introduced by Iwaya et al (3) and Banchs and Trope (4). They showed that a human necrotic immature permanent tooth after a revascularization/regeneration procedure could achieve increased thickening of the canal walls and continued root development. Since then, many successful cases have been reported (5–8). Radiographically, revascularized human immature permanent teeth had apical closure, root length increasing, and canal wall thickening and gave the impression that a normal, functional pulp had regenerated. However, animal histologic studies of revascularized teeth showed that the new tissue growing in the pulp space was cementoid/osteoid tissue and/or periodontal ligament–like tissue (9–14) regardless of the presence or absence of apical lesion, with or without the introduction of stem cells and scaffold. Almost all human studies of immature teeth with pulp necrosis and apical periodontitis after a revascularization/regeneration procedure showed that the tissues formed in the canal space were similar to the animal studies (15–18), except Torabinejad and Faras (19) in which pulplike tissue was observed in a revitalized tooth.

In an irreversible pulpitis tooth with normal periapical tissues, which was treated with revascularization/regeneration after a deep pulpotomy or a partial pulpectomy, the canal was filled with loose connective tissue similar to the pulp tissue (20). Based on the histologic findings in the case, regeneration of pulplike tissue is possible after a revascularization/regeneration procedure.

This case report describes the histologic observation of a human immature permanent tooth clinically diagnosed as symptomatic irreversible pulpitis after a revascularization/regeneration procedure.

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Materials and Methods

An 11-year-old girl was referred from the emergency department to the pediatric department at Peking University School and Hospital of Stomatology, Beijing, China, for the treatment of the lower right second premolar (tooth #29). The child had spontaneous and mastication-evoked pain in the lower right second premolar for 1 month. According to the case records, tooth #29 had a fracture of dens evaginatus. Pain could be induced by the thermal test. The tooth was diagnosed as symptomatic irreversible pulpitis with symptomatic apical periodontitis. The dentist at the emergency department removed most of the pulp and placed a cotton pellet in the pulp cavity of tooth #29 to relieve the pain. The patient went to the department of pediatric dentistry 2 days later. Tooth #29 was sensitive to percussion but negative to palpation. It showed no response to thermal or an electric pulp tester (Vitality Scanner; SybonEndo, Glendora, CA). Radiographic examination revealed an open apex of tooth #29 without noticeable periradicular radiolucency (Fig. 1*A*). A cotton pellet was found in the access cavity of tooth #29. Bleeding was noted when the root canal was irrigated with 5.25% sodium hypochlorite under rubber dam isolation without administration of a local anesthetic. The patient felt pain at the apical third of the root canals, which was 3–4 mm to the apex when exploring using a file, indicating vital pulp tissue in the apical portion of the root canal. It appeared that a partial pulpectomy might have been previously performed on tooth #29. Indications, procedures, advantages, and disadvantages of apexification and the revascularization/regeneration procedure were explained to the patient and her parents. They chose the revascularization/regeneration procedure, and informed consent was obtained.

Briefly, the root canal was gently irrigated with 20 mL 5.25% sodium hypochlorite solution with an irrigation syringe 2 mm above the residual pulp tissues and then dried with sterile paper points. A mixed antibiotics paste of ciprofloxacin, metronidazole, and minocycline based on the method Hoshino et al (21) previously described was placed in the root canal space, and the access cavity was sealed with Coltosol F (Coltene-Whaledent, Altstatten, Switzerland). A week later, the temporary filling was missing for 3 days. The root canal was disinfected using 20 mL 5.25% sodium hypochlorite irrigation and triple antibiotic paste intracanal medication 1 more time. At the third visit, the tooth showed mild sensitivity to percussion. Local infiltration with 2% lidocaine without vasoconstrictor was administered. After removing the remaining antibiotics paste under rubber dam isolation, the root canal was irrigated with 10 mL 5.25% sodium hypochlorite solution and 10 mL sterile saline and dried with sterile paper points. A size #30 K-file was then introduced into the root canal and pushed beyond the apex toward the periapical tissue to create bleeding into the canal. However, there was no bleeding noted in the root canal except on the file. After 10 minutes, no more bleeding was observed, and the accessed cavity was sealed with Fuji IX glass ionomer cement (Fuji Corporation, Osaka, Japan) followed by Filtek Z250 composite resin restoration (3M ESPE, Irvine, CA).

Clinical examination of the patient at the 3-month recall showed the tooth was asymptomatic. The tooth was not sensitive to percussion or palpation and showed no response to ice, heat, or an electric pulp tester. The radiographic examination revealed minimal lengthening of the root without thickening of the canal wall (Fig. 1*B*).

At the 12-month recall, the tooth was asymptomatic. The tooth was still not sensitive to percussion or palpation and showed no response to thermal or electric stimuli. The radiographic examination revealed thickening of the root canal wall, narrowing of the root apex, and lengthening of the root, which showed the continual development of the root (Fig. 1*C*).

Twelve months later, the tooth was planned to be extracted because of orthodontic treatment. After an inferior alveolar block using 2% lidocaine with 1:100,000 epinephrine, bleeding was observed from the pulp cavity when the filling was removed. There was no calcified bridge formed when explored with a file. After extraction, the tooth was immediately fixed in 4% formaldehyde for 48 hours and decalcified in 10% EDTA (pH = 7.5) for 6 months at 4°C. Then, the specimen was dehydrated in ethanol, embedded in paraffin, and serially sectioned (to 5-μm thickness) along the long axis of the tooth. The sections were stained with hematoxylin-eosin and examined under a light microscope.

Results

Histologically, the apical third of the root canal was filled with newly formed dentinlike and pulplike tissue (Fig. 2*A* and *B*). There was a clear line between the new dentinlike tissue and the previous dentin (Fig. 2*C*). There was a layer of flattened odontoblastlike cells lining the dentinal wall of the apical root canal. The newly formed dentinlike tissue contained dentinal tubules (Fig. 2*D*). The pulplike tissue with hypercellular and hypervascular connective tissue appeared to be an extension of periapical tissue. In the midportion of the root canal, there was amorphous tissue enveloped with fibers and phagocytes and inflammatory cells (Fig. 2*E* and *F*). The newly formed dentinlike tissue gradually changed to cementumlike tissue (Fig. 2*G*), which was not well connected with the dentinal wall and extended into the root canal space. In the upper third of the root canal, there was a presence of cementocytelike cells housed in the lacunae of cementumlike tissue. The loose connective tissue was around the cementumlike tissue (Fig. 2*H*). The increased root length and thickness were caused by apical deposition of newly formed dentinlike and cementumlike tissue.

Discussion

The present study showed that there were 4 types of tissues formed in the root canal after the pulp revascularization/regeneration procedure: dentinlike tissue, pulplike tissue, cementumlike tissue, and loose

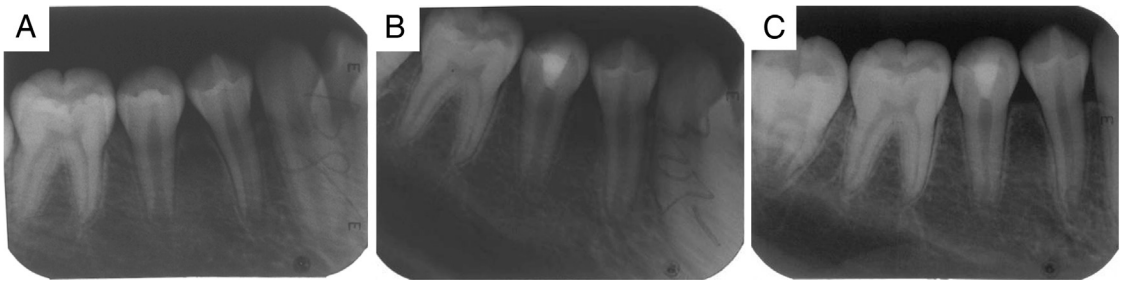

Figure 1. (*A*) The preoperative radiograph of tooth #29. (*B*) The postoperative radiograph at the 3-month recall. (*C*) The postoperative radiograph at the 12-month recall.

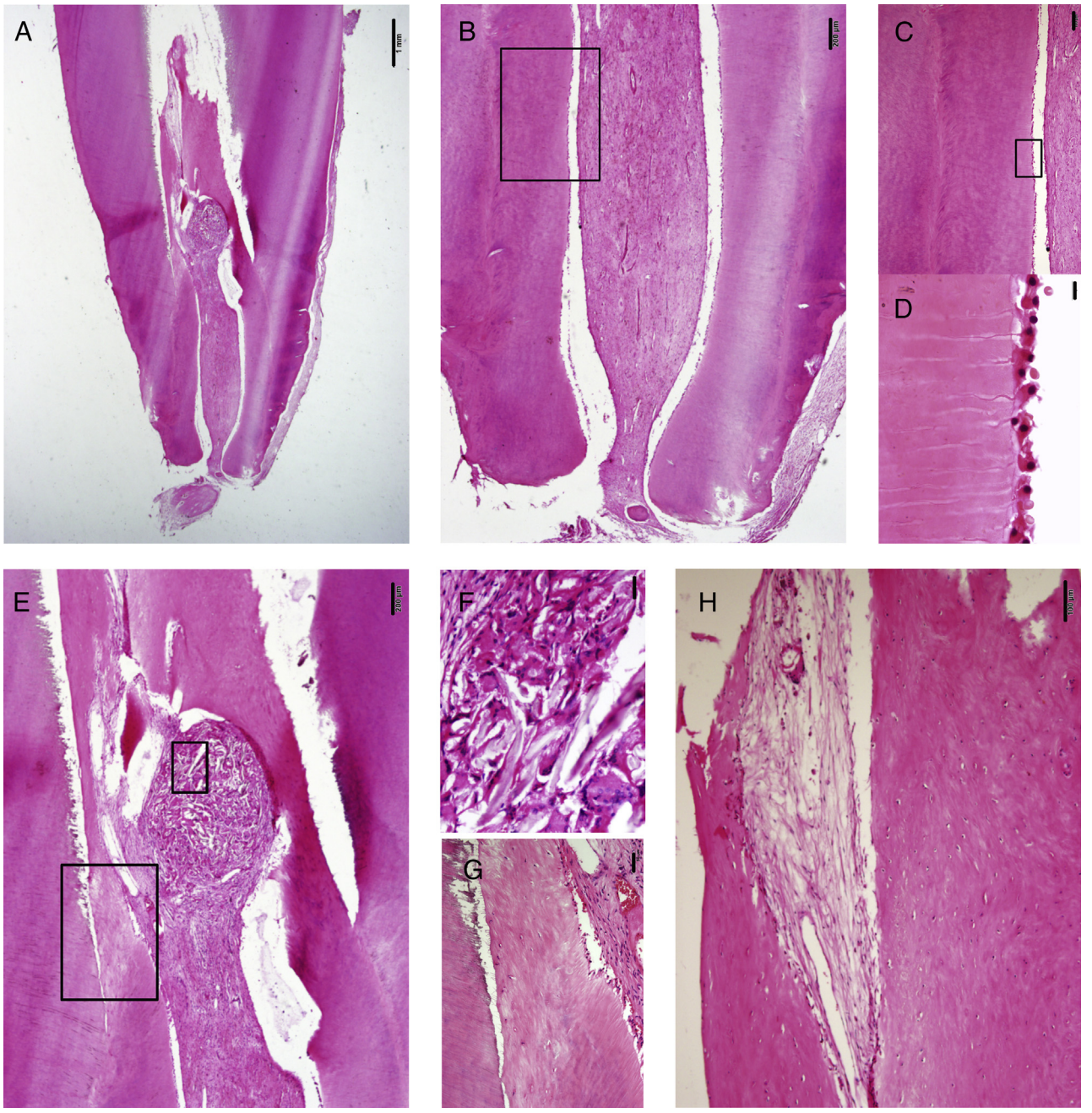

Figure 2. (*A*) Hematoxylin-eosin staining of extracted revascularized/regenerated tooth #29. Bar: 1 mm. (*B*) A detailed view of the apical third of the root in *A*. The canal was filled with newly formed dentinlike and pulplike tissue. Bar: 200 μm. (*C*) A high magnification of the rectangular area from *B*. There was a clear line composed by the reparative dentin between the new dentinlike tissue and the previous dentin. Bar: 100 μm. (*D*) A high magnification of the rectangular area from *C*. Flattened odontoblastlike cells lined along the newly formed dentinlike tissue. Bar: 10 μm. (*E*) A detailed view of the middle third of the root in *A*. Bar: 200 μm. (*F*) A high magnification of the smaller rectangular area from *E*. Amorphous tissues and phagocytes. Bar: 25 μm. (*G*) A high magnification of the larger rectangular area from *E*. The newly formed dentin gradually changed to the cementumlike tissue. Bar: 50 μm. (*H*) A detailed view of the upper third of the root in *A*. The mineralized tissue resembling cementum tissue and loose connective tissue were noted in the upper third of the root canal. Bar: 100 μm.

connective tissue. The newly formed dentinlike and pulplike tissue was found in the apical third of the canal. The cementumlike tissue and the loose connective tissue filled in the rest of the canal. These histologic results are different from animal studies and other human case reports on pulp revascularization/regeneration procedures. In previous animal and human histologic studies of the necrotic immature revascularized/regenerated teeth with apical periodontitis, the newly formed tissues were described as cementumlike tissue, bonelike tissue, and/or periodontal ligament–like connective tissue in root canal (10–12,14–18). Continuation of the cementumlike tissue from the outer root surface into the inner canal surface and bonelike tissue in the root canal or stuck to the root canal was observed. There was only 1 exception in which the tissue extirpated from the root canal was described as pulplike tissue in a human tooth treated with regenerative endodontics using platelet-rich plasma as a scaffold although with no evidence of odontoblasts lining the surface of the predentin (19). The

possible reason of no pulp tissue regeneration was the lack of dental pulp progenitor cells or stem cells derived from the remaining vital pulp and apical papilla, which are destroyed by severe endodontic infection and chemical agents. The adjacent mesenchymal stem cells from the periapical tissues may migrate into the pulp space in greater number and generate ectopic periodontal/periapical tissues. However, the human and animal studies showed that, in teeth with pulp extirpation in the absence of periapical inflammation, the new tissue formed in the root canal after the pulp revascularization/regeneration procedure was also not pulplike tissue (13, 22). However, in Shimizu et al's case report (20), the histologic findings of an immature permanent tooth with irreversible pulpitis without apical periodontitis after a revascularization/regeneration procedure showed more than half of the canal was filled with loose connective tissue similar to the pulp tissue. Regeneration of pulplike tissue seems possible after a revascularization/regeneration procedure with the survival of Hertwig's epithelial root sheath and the apical papilla.

In our case report, histologic analysis showed a clear boundary between newly formed dentinlike tissue and the primary dentin. The newly formed dentinlike tissue exhibited a similar structure as the primary dentin. Although on the boundary, there was reparative dentin with irregular tubular structures, which was not continuous with primary dentin. Along the inner surface of the new dentinlike tissue, there were flattened odontoblastlike cells. Whether these cells were newly differentiated progenitor cells from the remaining pulp or newly differentiated odontoblasts originated from the apical papilla after the revascularization/regeneration procedure was unclear. In the midportion of the root canal, mineralized tissue deposited on the surface of the canal wall gradually transitioned from dentinlike tissue to cementumlike tissue, which was not well connected with the primary dentin and extended into the root canal. These phenomena suggest that vital apical papilla have limited potential of pulp regeneration; periodontal regeneration exceeds pulp regeneration.

Compared with Shimizu et al's report, the amount of pulp regeneration in our patient was less. The possible explanations are as follows. First, the amount of remaining pulp tissues was different. In our case, most pulp was extirpated with some residual pulp tissues present in the apical third of the canal as proved by probing tenderness at the first appointment. In Shimizu's study, pulp was noted near the midroot. Second, at the second appointment, our temporary filling was missing, which may cause pulp recontamination and more tissue destruction. Third, the times of the irrigation using sodium hypochlorite and intracanal medication were longer in our study. It is reported that a high concentration of sodium hypochlorite and antibacterial dressing would affect the survival of stem cells in the apical papilla (23–25). Furthermore, dentin conditioning with 5.25% sodium hypochlorite considerably influenced the differentiation from dental pulp stem cells into odontoblastlike cells (26).

In our case report, there was cementumlike tissue formation on the mid and coronal canal dentinal walls, which may be because of 2 reasons. One possible reason is that the microenvironment around the residual pulp and the nonpulp root canal wall after irrigation with the high concentration of sodium hypochlorite was different. The former favors pulp regeneration, and the latter tends to induce periodontal regeneration. The stem cells from the remaining pulp or apical papilla may differentiate into osteo/cementogenic cells under a nonpulp microenvironment (11). Another possible reason is mesenchymal stem cells from the periapical tissues were brought into the canal space through hemorrhage induced at the periapex by the file used (27). These cells adhere to the dentin wall more rapidly than the stem cells migrating from the existing residual pulp and apical papilla and differentiate into preosteoblasts and precementoblasts.

Based on histologic observation of the present case, regeneration of the pulplike tissue and periodontal tissue both exist after a revascularization/regeneration procedure even if the residual pulp and the apical papilla survived in an immature permanent tooth clinically diagnosed as symptomatic irreversible pulpitis.

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

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