Histologic and Immunohistochemical Findings of a Human Immature Permanent Tooth with Apical Periodontitis after Regenerative Endodontic Treatment

Lishan Lei, MDS,[*†] *Yuemin Chen, BDS,*[*†] *Ronghui Zhou, BDS,*[*†] *Xiaojing Huang, DDS, PhD,*[*†] *and Zhiyu Cai, MDS*[*†‡]

Abstract

Specimens of human immature permanent teeth after regenerative endodontic treatment (RET) are sparse. This case report describes the histologic and immunohistochemical findings of tissue formed in the canal space of a human immature permanent tooth with apical periodontitis after RET. A patient presenting with immature human permanent tooth #29 with apical periodontitis underwent RET. At the 10-month follow-up visit, radiographic examination revealed complete resolution of the periapical lesion, marked narrowing of the apical foramen, increased thickness of the canal walls, and minimal lengthening of the root. Notably, the tooth regained pulp sensibility. Tooth #29 was extracted for orthodontic reasons and processed for histologic and immunohistochemical examination. The canal space was filled with newly formed cementumlike tissue, bonelike tissue, and fibrous connective tissue. The apical closure, thickness, and length increment of the root were caused by the deposition of cementumlike tissue without dentin. Furthermore, neurons and nerve fibers were observed in the canal space; this observation was confirmed by immunohistochemistry. Based on the findings in the present case, after RET, the newly formed tissues in the canal space of the human immature permanent tooth with apical periodontitis were primarily fibrous connective tissue, cementumlike tissue, and bonelike tissue. Nerve regeneration was identified. *(J Endod 2015;41:1172–1179)*

Key Words

Apical periodontitis, cementumlike tissue, human immature permanent tooth, nerve regeneration, regenerative endodontic treatment

The interest in regenerative endodontics has increased because of case reports with successful outcomes (1, 2). A number of case reports and case series regarding the regenerative endodontic treatment of human immature permanent teeth with pulp necrosis or apical periodontitis have been published. Radiographically, many of these cases have shown favorable results as evidenced by the resolution of apical radiolucency, root lengthening, apical closure, and hard tissue deposition on the canal walls (3, 4). In addition, the recovery of pulp sensibility has been observed in a few cases (1, 2, 5–8). The newly formed soft tissue might be regenerated by pulp tissue or periodontal ligament (PDL) tissue, whereas the hard tissue deposition could be caused by the ingrowth of dentin, cementum, or bone (9). Histologic studies in animal models (10–12) showed that the tissues growing in the canal space were cementumlike or bonelike hard tissue and PDL-like connective tissue. However, because only human histologic studies could directly answer the question of tissue identity after regenerative endodontic treatment in patients, samples obtained at rare opportunities are valuable for accumulating evidence of tissue identity. Recently, 5 case reports described histologic findings after successful regenerative endodontic treatment (RET) in humans (13–17). These reports showed that the tissue growing in the pulp space was different (Table 1). Moreover, where these tissues come from and whether nerve regeneration in the pulp space occurs after RET remain unknown. The aim of this study was to describe histologically and immunohistochemically a human immature permanent mandibular premolar that initially had apical periodontitis and then regained sensibility after RET. To our knowledge, this is the first histologic and immunohistochemical study of a human immature permanent tooth after RET that has positive responses to cold and pulp vitality tests similar to those of the adjacent tooth.

Case Report

A 10-year-old girl was referred by a general dentist to the Department of Endodontics and Operative Dentistry, School and Hospital of Stomatology, Fujian Medical University, Fuzhou, Fujian, China. The patient's chief complaint was the presence of pain during mastication for 2 weeks. The patient suffered from pain for 2 weeks before visiting her general dentist. The general dentist made an opening to access tooth #30 based on the diagnosis of irreversible pulpitis. However, because the pain persisted, the dentist referred this patient to our hospital. Clinical examination revealed extensive caries in tooth #30, and the access point was kept open with a cotton pellet. Tooth #30 was not sensitive to percussion and palpation and did not have mobility. Tooth #29 was

From the *School and Hospital of Stomatology, and †Key Laboratory of Stomatology, Fujian Medical University, Fujian Province University; and ‡Department of Stomatology, Fujian Medical University Union Hospital, Fuzhou, Fujian, China.

Address requests for reprints to Dr Xiaojing Huang, School and Hospital of Stomatology, Fujian Medical University, 246 Yangqiao Zhong Road, Fuzhou, Fujian 350002, China. E-mail address: hxiaoj@163.com

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TABLE 1. Current Published Histologic and Immunohistochemical Reports of Regenerative Endodontic Treatment in Humans

Authors	Diagnosis	Scaffold	Post-treatment vitality responses	Histologic findings	Immunohistochemical findings
Torabinejad et al, 2012 (17)	Pulp necrosis and symptomatic apical periodontitis	Platelet-rich plasma	Yes	Collagen fibers, cells, and blood vessels in pulplike connective tissue. No odontoblastlike cells could be observed in the canal (only soft tissue in the canal).	NA
Shimizu et al, 2012 (16)	Symptomatic irreversible pulpitis	Blood clot	No	Connective tissue, odontoblastlike cells and epithelial-like HERS. No nervelike fibers and hard tissue was formed in the canal.	Strol-1–positive cells were observed in the connective tissue near the apical foramen.
Martin et al, 2013 (14)	Pulp necrosis and symptomatic apical periodontitis	Platelet-rich plasma	NA	Cementoid/osteoid tissue and uninflamed fibrous connective tissue. No HERS or odontoblastlike cells could be observed in the canal.	NA
Shimizu et al, 2013 (15)	Pulpal necrosis and chronic apical abscess	Blood clot	NA	Cementum- or bonelike tissue and fibrous connective tissue. No pulplike tissue was present as characterized by the presence of polarized odontoblastlike cells.	Positive immunoreactivity for BSP was observed, whereas DSP and neurofilament immunoreactivity were negative.
Becerra et al, 2014 (13)	Pulpal necrosis and a chronic apical abscess	Blood clot	NA	Connective tissue similar to that in the periodontal ligament and cementumlike or bonelike hard tissue. No tubulelike structures of mineralized tissue or odontoblastlike cells could be observed in the canal.	NA

NA, information not available.

free of caries but had dens evaginatus with a fractured occlusal tubercle. Localized swelling and redness were present in the buccal mucosa facing its apex. This tooth was tender to percussion with class 2 mobility. Pulp sensibility tests with an ice stick and an electric pulp test (EPT) were performed on teeth #20, #28, #29, and #30. Positive responses were elicited in teeth #20 and #28 but not in teeth #29 and #30. Periodontal probing depths were within normal limits for all the teeth. Radiographic examination (Fig. 1*A*) revealed that tooth #29 had an incomplete apex and periapical radiolucency. After clinical and radiographic examination, the diagnoses were as follows: tooth #29 had pulpal necrosis with symptomatic apical periodontitis, and tooth #30 had pulpal necrosis. Root canal treatment was proposed for tooth #30. For tooth #29, treatment options and procedures, including conventional calcium hydroxide apexification, an artificial apical barrier technique, and RET were explained to the parents and the child. RET was finally chosen for the child, and informed consent was obtained.

First Treatment Visit

No anesthesia was administered initially to evaluate whether vital tissue was present in the root canal of tooth #29. When the access cavity was made under rubber dam isolation, a purulent hemorrhagic exudate was discharged from the pulp chamber. Observation with a Zeiss surgical microscope (Carl Zeiss Meditac Inc, Dublin, CA) confirmed the absence of vital tissue in the pulp chamber and canal. Without mechanical instrumentation, the pulp chamber and canal were gently irrigated with 20 mL 1% sodium hypochlorite. Then, the canal was dried with sterile paper points. Subsequently, a triple antibiotic paste that consisted of a powder of 100 mg each of ciprofloxacin, metronidazole, and cefaclor mixed with 1 mL sterile water was placed into the apical portion of the canal and filled to the level directly below the cementoenamel junction using a syringe under a microscope. The access cavity was temporized with 3 mm Cavit (3M ESPE, Seefeld, Germany) and 2 mm glass ionomer (Fuji IX; GC, Tokyo, Japan). Tooth #30 received root canal treatment; this tooth is not discussed further in this report.

Second Treatment Visit

The patient returned to the clinic 4 weeks later. The tooth was asymptomatic with intact temporary fillings. Tooth #29 was not tender to percussion or palpation. No mobility was noted. Local anesthesia was administered with 2% lidocaine without a vasoconstrictor. After isolation with a rubber dam, the temporary filling was removed, and no sign of inflammatory exudate was observed. Then, the antibiotic paste was gently flushed out of the canal with copious amounts of sterile normal saline. Next, the canal was irrigated with 10 mL 17% EDTA solution and dried with sterile paper points. Under a surgical microscope, a sterile #35 K-file was introduced into the canal through the apical foramen with a push and pull motion to provoke bleeding from the periapical tissue into the canal up to 3 mm below the cementoenamel junction. A sterile moist cotton pellet was applied with gentle pressure for 15 minutes to form a stable blood clot in the canal. The blood clot was covered by a layer of CollaPlug (Zimmer Dental, Carlsbad, CA) and then by a 3-mm thickness of a ProRoot mineral trioxide aggregate (MTA) mixture (Dentsply Tulsa Dental Specialties, Tulsa, OK). A moist