Histologic Study of a Human Immature Permanent Premolar with Chronic Apical Abscess after Revascularization/Revitalization

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Abstract

Introduction: Histologic studies of teeth from animal models of revascularization/revitalization are available; however, specimens from human studies are lacking. The nature of tissues formed in the canal of human revascularized/revitalized teeth was not well established.

Methods: An immature mandibular premolar with infected necrotic pulp and a chronic apical abscess was treated with revascularization/revitalization procedures. At both the 18-month and 2-year follow-up visits, radiographic examination showed complete resolution of the periapical lesion, narrowing of the root apex without root lengthening, and minimal thickening of the canal walls. The revascularized/revitalized tooth was removed because of orthodontic treatment and processed for histologic examination.

Results: The large canal space of revascularized/revitalized tooth was not empty and filled with fibrous connective tissue. The apical closure was caused by cementum deposition without dentin. Some cementum-like tissue was formed on the canal dentin walls. Inflammatory cells were observed in the coronal and middle third of revascularized/revitalized tissue.

Conclusions: In the present case, the tissue formed in the canal of a human revascularized/revitalized tooth was soft connective tissue similar to that in the periodontal ligament and cementum-like or bone-like hard tissue, which is comparable with the histology observed in the canals of teeth from animal models of revascularization/revitalization.

Key Words
Bone-like tissue, cementum-like tissue, chronic apical abscess, human immature permanent tooth, revascularization/revitalization

Many case reports and case series of revascularization/revitalization of human immature permanent teeth with apical periodontitis have been reported in the literature. However, a high level of evidence concerning the outcome of revascularization/revitalization is lacking. The resolution of apical periodontitis, thickening of the canal walls, and continued root development are often reported in published cases after revascularization/revitalization of immature permanent teeth with apical periodontitis. Five types of hard-tissue responses of human immature permanent teeth with apical periodontitis after revascularization/revitalization have been described (1). In some cases, no to minimal thickening of the canal walls and closure of the root apex without root lengthening were observed radiographically (1–4). Some authors speculated that this was because of the blood clot in the canal breaking down, leaving no scaffold for new tissue to grow into the empty canal space (4). Histologic studies of revascularized teeth in animals showed that the tissue growing in the pulp space was fibrous soft connective tissue and cementum-like or bone-like hard tissue (5–8). Most recently, 2 case reports described the histology after successful revascularization procedures in humans (9, 10). It was confirmed that the tissue formed in the canal space was cementum-like or bone-like tissue. No dentin-like tissue or odontoblast-like cells were observed.

The purpose of this case report was to describe histologically a human immature mandibular premolar after a revascularization/revitalization procedure that initially had infected necrotic pulp and a chronic apical abscess. Radiographically, the revascularized/revitalized tooth showed resolution of the periapical lesion, minimal thickening of the canal walls, and narrowing of the root apex without root lengthening.

Case Report

An 11-year-old girl was brought to the dental office because she noted a localized swelling on the mucosa in the area of the mandibular left premolars. The patient did not have any spontaneous or evoked pain or other symptoms. Clinical examination showed that the crown of tooth #20 was caries free but had a dens evaginatus with a fractured cusp. A sinus tract was present buccally in association with the mandibular second premolar. The tooth was not tender to percussion (vertical and lateral) or palpation. No mobility was noted. The tooth showed no response to sensitivity tests (ie, heat, cold, and electric pulp test). A periapical radiograph of tooth #20, taken after tracing the sinus tract with a gutta-percha point, showed that a large radiolucent lesion was present in the periapical area (Fig. 1A). The root apex was open, and the canal walls appeared to be thin (Fig. 1A).

The clinical diagnosis of tooth #20 was pulp necrosis and a chronic apical abscess with a sinus tract. Treatment options and procedures, including apexification and revascularization/revitalization, were explained to the patient and patient’s parents. They chose the revascularization/revitalization procedure of the tooth for the child, and informed consent was obtained.

First Session

No anesthesia was administered to see if vital tissue was present in the apical portion of the canal in the first session. The tooth was isolated with a rubber dam, and an adequate access cavity was prepared. No vital tissue was observed in the apical
third of the canal using a surgical microscope. The pulp chamber and the root canal were gently irrigated with 20 mL 5.25% sodium hypochlorite using a 27-G needle (Appli–Vac; Vista Dental Products, Racine, WI) without mechanical instrumentation. The canal was dried with sterile paper points and irrigated with 1.2 mL 2% chlorhexidine gluconate solution (CHX-Plus, Vista Dental Products). A mixture of ciprofloxacin (200 mg), metronidazole (500 mg), and minocycline (100 mg) paste was placed into the apical portion of the canal with a Lentulo spiral as intracanal medication. The access cavity was closed with a sterile cotton pellet and Intermediate Restorative Material (IRM) (Dentsply International, Milford, DE). A radiograph was taken at this time (Fig. 1B).

Second Session

The patient was scheduled for a second visit after 26 days. The tooth was asymptomatic during the entire postoperative period, and the temporary filling was intact. The sinus tract had disappeared. Local anesthesia was administered with 3% mepivacaine without a vasoconstrictor. After isolation with a rubber dam, the access cavity was reopened. The canal was irrigated first with sterile saline to remove the triple antibiotic paste and then with 5.25% sodium hypochlorite, which remained in the root canal approximately for 3 minutes. The canal was dried with sterile paper points. No bleeding was noted. A #40 K-file was introduced into the canal through the apical foramen with a push and pull motion (Fig. 1C) to provoke bleeding from the periapical tissue into the canal up to the pulp chamber under a surgical microscope. A sterile cotton pellet was applied with gentle pressure in the pulp chamber for 15 minutes to obtain hemostasis and a blood clot formation in the canal. A paste of white mineral trioxide aggregate (MTA) (MTA Blanco; Angelus Indústria de Produtos Odontológicos S/A, Londrina, Brazil) and sterile distilled water, prepared according to the manufacturer’s instructions, was placed over the blood clot and gently condensed with a no. 3/4 plugger (Dentsply International) to a depth of approximately 3 mm up to the cementum-enamel junction. A moist cotton pellet was placed over the MTA in the pulp chamber, and the access cavity was closed with IRM.

Figure 1. (A) A diagnostic radiograph taken with a gutta-percha cone inserted into the buccal sinus tract. (B) A radiograph taken at the end of the first session. (C) Second session: a K-file was introduced into the canal through the apical foramen. (D) A radiograph taken at the end of third section after restoration of the access cavity with composite. (E) The 1-month follow-up radiograph showing that the periapical radiolucency decreased. (F) A radiograph taken after 1 year. The periapical radiolucency healed completely. The tooth was asymptomatic. (G–M) Radiographs taken after 18 and 24 months, respectively. Normal periapical conditions and some thickening of the root canal walls. (I–M) Cone-beam computed tomographic examination showing that in some scans the root apex seems closed, whereas in others the root apex, in fact, is open. Note the calcification present in the canal lumen short of the foramen.
Third Session

The patient was asked to return 1 week later. The tooth was asymptomatic. IRM was removed and replaced with a bonded resin restoration (Spectrum TPH3; Dentsply Brasil, Petrópolis, Brazil) (Fig. 1D).

Follow-up Visits

One month postoperatively, the periapical radiolucency had reduced considerably (Fig. 1E), and the tooth was asymptomatic. A follow-up radiograph taken 1 year later showed that the periapical radiolucent lesion had healed completely with some indication of thickening of the root canal walls (Fig. 1F). The tooth was asymptomatic. Successive follow-up radiographs taken after 18 months and 2 years showed normal periapical condition and narrowing of the root apex (Fig. 1G–H). At this time, the patient needed orthodontic treatment, and the treating orthodontist, after a complete case study including a cone-beam computed tomographic scan, requested extraction of the mandibular left second and right first premolars.

The cone-beam computed tomographic images provided more detailed information concerning radicular and apical anatomy 2 years after the revascularization procedures. Although in some scans it seemed that the apical structure was formed, in others it was clear that the apex was immature, with a large foramen open buccally. The lingual portion of the root canal wall was thicker and longer than the buccal one (Fig. 1I–M). A calcified structure was present approximately at the center of the canal lumen, just before the wide foramen (Fig. 1I–M).

Permission for histologic examination of extracted teeth was obtained from the patient’s parents. Photographs and radiographs were taken of the extracted tooth (Fig. 2A–C). The specimens were

Figure 2. (A) A mesial view of the extracted tooth. Note the discoloration of the root in the coronal two thirds. (B) A radiograph of the extracted tooth taken with a mesiodistal projection. (C) A detailed view of the foraminal area. (D) A section of the apical third taken on the buccolingual plane, passing through the center of the canal and foramen (overview: hematoxylin-eosin, original magnification ×8). (E) A detailed view of the apical canal showing the abundance of nonmineralized fibrous tissue. Note the island of calcified tissue characterized by a mass of dystrophic calcification with numerous dentin spicules embedded with dentinal tubules cut in all directions (original magnification ×25, inset ×400). (F) A high-power view of the area indicated by the left arrow in E. Concentration of chronic inflammatory cells (mainly plasma cells) (original magnification ×400). (G) A high-power view of the area indicated by the right arrow in E. The accumulation of foam cells surrounded by collagen fibers (original magnification ×400).
immediately immersed in a 10% neutral buffered formalin solution. The mandibular right first premolar, which was caries free, was used as the control. To obtain proper fixation of the coronal pulp tissue with a fully formed apex, a hole was drilled on the occlusal surface with a #5 round diamond bur mounted on a high-speed handpiece under water spray until the pulp chamber was reached.

**Tissue Processing**

Demineralization was performed in an aqueous solution consisting of a mixture of 22.5% (vol/vol) formic acid and 10% (wt/vol) sodium citrate for 4 weeks. The endpoint was determined radiographically. At the end of the demineralization process, the crowns of both teeth were separated from the roots with a sharp razor blade at the cementum-enamel junction. The roots were then divided transversally into 3 portions.

The radicular segments were washed in running water for 48 hours, dehydrated in ascending grades of ethanol, infiltrated, and embedded separately in paraffin (melting point 56°C) according to standard procedures.

With the microtome set at 4–5 μm, longitudinal serial sections were cut on a buccolingual plane for the apical portion until the specimen was exhausted. Special precautions were undertaken to obtain sections passing through the main apical opening. For the middle and coronal portions, 200 cross-cut sections were taken in a coronal direction. The specimens were then removed from the paraffin blocks and re-embedded upside down to obtain cross-cut serial sections in an apical direction. Approximately an additional 200 cross-cut sections were taken for each of the 2 portions.

Every fifth slide was stained with hematoxylin-eosin for screening purposes and an assessment of tissues formed in the canals. Selected slides were stained with the Taylor modified Brown and Brenn technique for bacteria. Slides were examined under a light microscope.

**Results**

**Histologic Observations**

A fibrous connective tissue characterized mainly by fibroblasts and collagen fibers had filled the radiographically empty canal space up to the coronal MTA plug (Figs. 2 and 3). This tissue was largely nonmineralized, with the exception of an island of dystrophic calcification apically, close to the foramen (Fig. 2D and E). Dense collagen bundles were discerned within this tissue, interspersed with blood vessels (Fig. 2E).

Longitudinal sections of the apical third of the canal showed that the newly formed calcified tissue on the canal walls was both cellular (Fig. 2H–K) and acellular cementum-like tissue. In some areas, the...
cellular cementum-like tissue was clearly demarcated from the canal dentin by a darkly stained line (Fig. 2H and I). On the pulpal side, large cells resembling cementoblasts or fibroblasts lined the cementum, and the collagen bundles were inserted into the cementum-like tissue at a right angle (Fig. 2J and K). No tubule-like structures of mineralized tissue or odontoblast-like cells could be observed in the canal. Mild to moderate concentrations of chronic inflammatory cells (mainly plasma cells and foam cells) could be observed in the majority of the tissue, whereas a dense concentration of foam cells could be observed on 1 side (Fig. 3A). A thin layer of cellular cementum was present on the majority of the canal dentin walls and partly detached from the dentin because of shrinkage during histologic processing (Fig. 3B). No odontoblast-like cells (rather only fibroblasts) could be seen layering the newly formed cementum (Fig. 3B). Cementum-like tissue was completely absent on that portion of the canal wall where severe inflammation was present (Fig. 3A). For comparison, cross-cut sections taken at the middle third of the intact tooth #28 revealed the presence of a normal dentin-pulp complex, with clearly distinguishable tubular dentin/predentin, a normally aligned odontoblast layer, and a central pulp rich of neurovascular structures with no inflammatory cells (Fig. 3C and D).

Figure 3. (A) The cross-cut section from the middle third of the root passing approximately at the level of line 1 in Figure 2A and B. Fibrous tissue fills the canal lumen. This tissue appears surrounded by a layer of cementum for two thirds of the canal circumference. Severe inflammation is present in the left portion of the lumen characterized by foam cells (inset). The empty spaces are shrinkage artifacts (original magnification ×16; inset ×400). (B) The right portion of the canal lumen. The area of the canal wall indicated by the arrow is magnified in the circle. The dentin wall is layered by acellular cementum (original magnification ×50; inset ×400). (C and D) The control premolar tooth. The cross-cut section taken at the middle third. Normal features of the pulp-dentin complex, with predentin, dentin, odontoblast layer, and central pulp with an abundance of neurovascular structures (original magnification ×16 and ×50; inset ×400). (E) The cross-cut section taken from the coronal third at the level of line 2 in Figure 2A and B. Amorphous material is present in the canal, with some scattered polymorphonuclear leukocytes (Taylor modified Brown and Brenn stain, original magnification ×400). (F) The other area of the canal wall (original magnification ×100). (G) A high-power view of the area of the canal wall indicated by the arrow in F. Few bacterial cells can be recognized, colonizing the entrance of dentinal tubules (original magnification ×400).
Case Report/Clinical Techniques

Cross-cut sections taken through the coronal third, encompassing the apical portion of the coronal MTA filling, showed amorphous material colonized by numerous hyperemic vessels and scattered polymorphonuclear leukocytes (Fig. 3F). Sections from the most coronal area of the canal showed few bacteria on the root canal walls (Fig. 3F and G).

Discussion

In the present revascularized/revitalized case, the postoperative radiographic appearance of the canal space did not appear to reduce significantly relative to preoperative images. Importantly, the canal space was not empty but filled with fibrous connective tissue, with minimal mineralization other than an island of mineralized tissue near the apical foramen. This might indicate that the blood clot in the canal after induced periapical bleeding was not broken down. Wound healing processes, including blood clot formation, granulation tissue ingrowth, and regeneration with parenchymal cells or repair with different cells, did occur in the canal space. The fibrous connective tissue in the apical canal appears to connect to the periodontal ligament through the apical foramen. The dentin chips embedded in the island of mineralized tissue in the apical portion of the canal were likely caused by the push and pull motion of the file through the canal during the induction of periapical bleeding.

Some cellular and acellular cementum-like tissue was formed on the canal walls in the apical third of the canal. In the middle third of the canal, cementoblast-like cells were observed along the surface of the cellular cementum-like tissue and periodontal ligament-like fibers were inserted into the cementum-like tissue at a right angle. The junction between the intracanal cementum-like tissue and the canal dentin walls in some areas was heavily stained with hematoxylin. The cementum-like tissue was partially detached from the canal walls, which was not observed on the root surface between the junction of the dentin and cementum. Yamauchi et al. (8) also observed the detachment of dentin-associated hard tissue from the canal dentin walls after revascularization/revitalization procedures. Therefore, the strength of the junction between intracanal cementum-like tissue and the canal dentin walls in revascularized/revitalized teeth is unknown.

Cementum is produced by cementoblasts, which could be differentiated from 3 sources: (1) neural crest-derived ectomesenchyme of the dental follicle cells (11), (2) progenitor/stem cells in the periodontal ligament (12), or (3) Hertwig’s epithelial root sheath (HERS)—mesenchymal transformation (13, 14). In the present case, cementoblast-like cells were likely differentiated from progenitor/stem cells in the periodontal ligament and produced the intracanal cementum-like tissue. It is not known if most progenitor/stem cells in the periodontal ligament enter the canal immediately after induced periapical bleeding or after blood clot formation. The blood clot contains many growth factors, cytokines, provisional extracellular matrix, and fibronectin and fibrins, which are capable of attracting progenitor/stem cells. However, the cellular and molecular mechanisms that signal progenitor/stem cells in the canal to differentiate into cementoblast-like or osteoblast-like cells are not known (15).

In the present case, the narrowing of the root apex was caused by deposition of cementum without dentin, which was also shown in animal studies (5–8) and human studies (9, 10). HERS regulates tooth root development (11, 13, 14) and most likely survived the chronic apical abscess in the present case. HERS and its products, enamel matrix-like proteins, could direct progenitor/stem cells in the surrounding periodontal ligament or in the ectomesenchymal dental follicle if not destroyed to differentiate into cementoblasts and produce cementum. After maturation of the root apex, HERS becomes apoptotic and remains as remnants of epithelial cell rests of Malassez in the peri-odontal ligament. In calcium hydroxide apexification, the hard-tissue barrier formed in the apical area of the canal is similar to cementoid or osteoid tissue histologically (16).

MTA has been shown to have excellent physical properties and sealing ability (17). In the present case, inflammatory cells were observed mainly in the coronal area of revascularized/revitalized tissue, indicating that coronal leakage occurred after the completion of revascularization/revitalization. At the time of tooth extraction, the margins of occlusal composite appeared deteriorated; therefore, the sealing ability of MTA in resisting bacterial penetration is questioned based on this finding. It is possible that, if not extracted, the present case might fail as follow-up time continues because of coronal leakage.

Inflammation and wound healing have a seesaw-like action. Infection/inflammation causes tissue destruction and prevents wound healing (18, 19). Although stem/progenitor cells can be recruited to the wound site by soluble factors such as stromal cell-derived factor-1–CXCR4 and hepatocyte growth factor–cMET axes released by damaged tissue and cells (20–23), they are not able to differentiate into tissue-committed cells (24–26). Therefore, infection/inflammation must be under control for stem cells to function properly and wound healing to occur. This might be the reason why no cementum-like tissue was formed on the canal walls in the area of severe inflammation.

Conclusion

Based on the present case and previous case reports (9, 10), the tissues formed in the canal of revascularized/revitalized human teeth are similar to the tissues observed in the canals of teeth from animal models undergoing revascularization/revitalization. These tissues are fibrous connective tissue similar to that found in the periodontal ligament and cementum-like or bone-like tissue.

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

References