Histologic Characterization of Regenerated Tissues in Canal Space after the Revitalization/Revascularization Procedure of Immature Dog Teeth with Apical Periodontitis

Xiaojing Wang, DDS, *‡ Blayne Tbibodeau, DMD, MS, ‡ Martin Trope, DMD, ‡ Louis M. Lin, DMD, PhD, †§ and George T.-J. Huang, DDS, MSD, DSc* †

Abstract

Introduction: Recently, it has been shown that it is possible to treat an immature tooth with an infected pulp space and apical periodontitis in such a way as to heal and promote the ingrowth of new vital tissue into the pulp space. However, the type of new-grown tissue is unclear. Methods: Based on the samples of a previously reported study, we further investigated histologically the types of tissues that had grown into the canal space. Results: The canal dentinal walls were thickened by the apposition of newly generated cementum-like tissue termed herein “intracanal cementum (IC).” One case showed partial survival of pulp tissue juxtaposed with fibrous connective tissue that formed IC on canal dentin walls. The IC may also form a bridge at the apex, in the apical third or midthird of the canal. The root length in many cases was increased by the growth of cementum. The generation of apical cementum or IC may occur despite the presence of inflammatory infiltration at the apex or in the canal. These cementum or cementum-like tissues were similar to cellular cementum. Bone or bone-like tissue was observed in the canal space in many cases and is termed intracanal bone (IB). Connective tissue similar to periodontal ligament was also present in the canal space surrounding the IC and/or IB. Conclusions: Our findings explained in part why many clinical cases of immature teeth with apical periodontitis or abscess may gain root thickness and apical length after conservative treatment with the revitalization procedure. (J Endod 2010;36:56–63)

Key Words
Bone, cementum, dog, periodontal ligament, root canal space, tissue regeneration

A different clinical approach to treat cases indicated for apexification has been advocated (1–7). This approach involves the use of an antibiotic paste to disinfect the canal, and, after which, no artificial materials were used to fill the canal space. This allows the vital tissue to generate or regenerate in the canal space. Although an apexification procedure has been used by clinicians for decades (8, 9), this new approach that is more conducive to tissue regeneration has been considered to be a better option for dealing with immature teeth with nonvital pulp and even for cases with severe periapical infection (5, 6). A significant number of case reports and a pilot clinical study have been published (10–12). The advantage of this approach is twofold: (1) it takes only 2 to 3 visits within a few weeks span, and (2) it gains root thickness and potentially root length. Overall, this approach outweighs apexification, which takes multiple visits in a time span of up to several years. Although the recent modification of apexification using mineral trioxide aggregate (MTA) as the apical plug saves time (13–15), the outcome measure still falls short when considering the gain of root thickness and length. From this perspective, it has been recently proposed that apexification is no longer much needed in the near future if not at present (7).

One question that has been puzzling clinicians is what type of tissues is actually generated in the pulp space after antibiotic paste therapy. It was hypothesized that remaining vital pulp and/or apical papilla may exist in the canal space. These tissues may reconstitute pulp and dentin after the infection is eradicated. If the pulp and apical papilla are both destroyed, only periodontal tissues including bone, periodontal ligament (PDL), and cementum would grow into the canal space (7, 16–18). Although human samples are difficult to collect, animal models may shed some light to address this issue. In this study, we examined the type of tissues that are generated in the canal space of dog teeth that had been infected by periodontal plaque, developed periapical lesions, and were disinfected with an antibiotic paste.

Materials and Methods

The present study focused on the histologic interpretation of the tissues grown into the canal space after healing. The histologic materials were based on samples from a previous report (19). Experimental procedures on animals were performed in our previous studies. No new animal procedures were conducted in the present study. The following is a summary of the animal experiments performed previously.

From the *Division of Endodontics, Section of Oral and Diagnostic Sciences, Columbia University, College of Dental Medicine, New York, NY; †Department of Prosthodontics, School of Stomatology, Fourth Military Medical University, Xi’an, China; ‡College of Dentistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada; §Department of Endodontics, University of North Carolina-Chapel Hill, Chapel Hill, NC; and †Department of Endodontics, New York University College of Dentistry, New York, NY.

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Address requests for reprints to Dr George T.-J. Huang, Boston University School of Dental Medicine, Department of Endodontics, 100 E. Newton St. G-705, Boston, MA 02118. E-mail address: gtjhuang@bu.edu.

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Animals and Treatment of Teeth

Six purpose-bred mixed breed canine model dogs aged approximately 6 months were obtained from Covance Research Products in Virginia. Sixty double-rooted premolars were randomly divided into five treatment and control groups. All experimental teeth were mechanically exposed and pulp tissue was disrupted by an endodontic file. Supragingival plaque scaled from the dogs’ teeth was placed and sealed temporarily in the pulp chambers with intermediate restorative material (Dentsply Caulk, Milford, DE). Approximately 3 weeks later when apical periodontitis was evident, all previously infected teeth were re-entered and disinfected. Canals were filled with a mixture of equal parts of metronidazole, ciprofloxacin, and minocycline mixed with sterile saline (Professional Compounding Centers of America, Houston, TX). Twelve teeth (24 roots) per group were randomly assigned into four experimental groups. In group 1, teeth were closed permanently at that appointment with a double coronal seal consisting of white MTA (Dentsply Tulsa Dental, Johnson City, TN) and silver amalgam (Sybraloy; Kerr Corporation, Orange, CA). The teeth in groups 2 through 4 were closed temporarily with intermediate restorative material for 4 weeks. Subsequently, the teeth were re-entered and canals irrigated with 10 mL of 1.25% NaOCl and 10 mL of sterile saline per tooth. In the 24 roots

Figure 1. Normal maturation of root. (A) The demarcation of root dentin (D) and cementum is indicated by arrowheads. (B) Radiographs showing the increased thickness of root canal walls. Ba and Bb indicate the x-rays taken before the experiment and 19 weeks after respectively. *Studied root in A. Scale bar: 500 μm. (Sample from group 5.)

Figure 2. The apical extension of root structure after healing. Canal space filled with soft tissues and the apical extension resulting from the apposition of cementum and cementum-like tissue. D, dentin. Blue arrowheads indicate demarcation between dentin and new cementum/cementum-like. Black arrows indicate the continuation of the EC into IC. Yellow arrows and dashed lines indicate the apical length generated by the cementum. (A) A sample from group 4. (Aa): An x-ray before the experiment and (Ab) after the experiment and healing. *Studied root. Scale bars: 500 μm.
randomly assigned to group 2, a sterile #30 stainless steel endodontic hand file was inserted past the canal terminus into the periapical tissues to induce bleeding to fill the canal spaces as much as possible. A type I collagen solution (rat tail type I collagen; BD Biosciences, Bedford, MA; 2.33 mg/mL in 2× phosphate-buffered saline) was placed in the 24 roots randomly assigned to group 3. The 24 roots randomly assigned to group 4 had the collagen solution placed in the canals before the induction of bleeding from the periapical tissues into the canal space. All of these teeth were then closed with a double coronal seal of white MTA (Dentsply Tulsa Dental) and silver amalgam (Sybraloy). The 12 teeth randomly assigned to group 5 were negative controls. These teeth were never operated but were left untouched to develop naturally for comparison with the experimental teeth. The first radiographs of the teeth were taken right before the experimentation. All of the teeth were monitored radiographically after the treatment on a monthly basis for 3 months before the animals were sacrificed, and tissues were harvested for histologic examination. All animal procedures followed a protocol approved by the Institutional Animal Care and Use Committee at the University of North Carolina, Chapel Hill.

Histological Processing and Image Analysis

After the removal of all soft tissue and excess hard tissue from the specimens, the tooth samples were fixed in Formical (Decal Chemical Corporation, Congers, NY) for 6 days followed by decalcification in ImmuNoc (Decal Chemical Corporation, Tallman, NY) for 2 months. The specimens were then processed, embedded, longitudinally sectioned along the long axis of the teeth, and stained by hematoxylin and eosin. Each sample was analyzed under a light microscope for the presence or absence of vital tissues in the canal space.

Criteria for identification of dentin, cementum, bone, and PDL histologically are as follows: dentin: presence of dentinal tubules; cementum: absence of dentinal tubules and adherence onto dentin, presence of cementocyte-like cells; bone: presence of Haversian canals with uniformly distributed osteocyte-like cells; and PDL: presence of Sharpey’s fibers and fibers bridging cementum and bone.

Results

Previously, we reported 43.9% of experimental teeth having hard tissue and 29.3% having vital tissues in the canal spaces. The remaining teeth did not show any observable newly generated tissue in the space (19). To determine the types of tissues generated in the pulp space after disinfection of the root canal system of immature permanent dog’s teeth with induced apical periodontitis, histologic examination was conducted. Of those healed cases, only one case showed partial survival of pulp tissue. No regenerated pulp tissue was discernable or observed in the canal space in other samples in which the infection appears to destroy the entire pulp and apical papilla.

Thickened Root by Deposition of Cementum-like Tissue on the Canal Walls

In the control group, teeth were allowed to develop normally and the roots reached their maturity as shown in Figure 1. The root dentin acquired its functional thickness, which resulted in narrowing of the pulp space. In contrast, teeth in the experimental groups showed arrested dentin development. However, there was a gain of thickness of the root because of the ingrowth of cementum-like tissue from the root surface into the canal walls. Samples presented in Figures 2 through 5 are typical cases showing the ingrowth of a layer of IC.
cementum-like tissue that appears to be more irregular in thickness compared with cementum on the root surface (orthotopic cementum). This ectopic cementum-like tissue in the canal space is hereby termed “intracanal cementum” in contrast to the orthotopic cementum which is referred as “extracanal cementum (EC).” A continuity of the cementum transition from the outer root into the inner canal surface can be seen in most cases. In addition to the increased root thickness by the extent of cementum-like tissue into the canal wall, this tissue also extended apically causing the vertical increase of root length (Figs. 2 and 3). Some IC formed projections into the center of the canal (Fig. 5A) or became quite bulky near the apex (Fig. 5B). The radiographs did not reflect accurately the actual amount of new grown hard tissues in the canal or at the root apex because of the angulation and image resolution.

**Figure 4.** Thickened root resulting from the deposition of IC onto dentin. Intracanal bone-like tissues (IB) scattered in the root canal space along with intracanal PDL-like tissues (IPL). (A) A sample from group 2. A magnified view of the boxed region is shown in Aa. Black arrows indicate cementocyte-like cells in IC; blue arrowheads indicate cementoblast-like cells lining against the IC. (B) A sample from group 1 showing well-generated IPL extending from extracanal periodontal ligament (PL). (Ba) The boxed region is shown in the magnified view. The yellow arrowheads indicate Sharpey’s fibers. Yellow dashed lines, angles of ligament fibers. The black arrows indicate cementocyte-like cells in IC. Scale bars: (A, Aa, and B) 500 μm; (Ba) 200 μm.

**Figure 5.** The formation of IC with extension toward canal center. (A) Yellow arrowheads indicate external resorption with loss of EC. Green arrowheads indicate bridge formed by IC in the canal space (sample from group 4). (B) The IC extends toward canal center narrowing the canal opening (sample from group 4). Scale bars: 500 μm.
Many cementocyte-like cells are present in the IC, and in some samples cementoblast-like cells can be observed on the surface of the IC (Fig. 4Aa and Ba). Along with the ingrown IC on the canal walls, a layer of soft tissue resembling PDL is present adjacent to the IC in some samples. Sharpey’s fibers from the PDL are inserted into the IC at an angle similar to that into the EC (Fig. 4Ba). The density of these fibers appears to be higher in the EC than IC in most samples. As reported previously, teeth in groups 2 and 4 filled with a blood clot after disinfection tended to form hard tissue in the canal. In the present study, we confirmed that the hard tissue is cementum or cementum-like tissue on the canal walls.

**Vital Pulp Present in the Canal after Disinfection**

One case from group 2 revealed partially survived pulp tissue in the canal evidenced by the presence of odontoblasts lining against one side of the dentin wall (Fig. 6A and D). In contrast, the other side of the canal space does not have the odontoblast layer. Instead, the canal wall appears to be deposited with a layer of IC that spans from the apex to the coronal region where it extends toward the other side of the canal wall forming a bridge (Fig. 6A and C). The entire bridge appears to consist of cementum-like tissue. In the apical region, there is a junction where dentin and cementum merge (Fig. 6B).

**Generation of Cementum or Cementum-like Tissue in the Presence of Inflammation**

A considerable number of cases showed the presence of both healing with tissue regeneration and significant amounts of inflammatory infiltrates adjacent to the newly generating tissues. As indicated in Figure 7A, IC is present along the canal dentinal wall with the bulkiest amount near the apex and becomes thinner toward the coronal end. There are highly vascularized inflammatory tissues at the apex and in the canal adjacent to the newly generated IC. Cementoblast-like cells are seen on the surface of the cellular cementum. Immediately adjacent to the cementoblast layer in the canal, fewer inflammatory cells are present, whereas a more condensed extracellular matrix appears to have been produced. External root resorption occurred with the presence of inflammatory infiltrates adjacent to it. Another sample presented in Figure 7B, however, shows that no IC was noticeable with the presence of chronic inflammation. Generated IC may be resorbed away by the presence of inflammation (Fig. 7C).

**Generation of Bone-like Structure in Canal Space**

Bone-like tissue with trabecular formation is scattered in the canal space in many cases of the experimental groups. We term this structure ”intracanal bone-like tissue (IB)” (Figs. 3, 4, and 7). Many trabeculae of the bone-like tissue are woven bone (Fig. 4). Some IB was difficult to differentiate from cellular IC because no bone marrow space was present. Histologically, the IB appears to have more cells trapped inside the hard tissue than in IC (Fig. 4).

**Bridge Formation by Cementum- and/or Bone-like Tissue**

The formation of hard tissue bridges inside the root canals was observed in some samples of experimental groups 2 through 4. The sample presented in Figure 8A shows that a thin layer of hard tissue or IC formed beneath the MTA in the coronal area. A bridge similar to cellular IC can also be seen at the apical canal or right at the apex (Fig. 8B and C).

**Discussion**

Using a dog study model, disinfection of the root canals with an antibiotic paste led to healing/improvement of existing apical...
periodontal lesion in ~60% of the tested teeth (19). Based on our histologic examination in the present study, mainly three types of tissue were generated in the canal space: (1) IC along the dentinal walls causing the thickening of the root, (2) bone-like tissue, and (3) PDL-like tissue. The experimental period was only 3 months (from treatment to tissue collection); therefore, in most cases, the maturation of the generated tissues in the pulp space appeared incomplete. Loose connective tissue or the collagen gel was still present in the canal space. One may predict that given a longer time, more cementum-like, PDL-like, and bone-like tissues will become more organized and matured in the canal space. Pulp may also partially survive after the infection and recover as evidenced in one case in our studies.

The ingrowth of periodontal tissues in empty canal space has been long observed (20). Studies regarding tissue generation in the canal after replantation of avulsed immature teeth have shown that well-organized bone, PDL, and cementum can grow into the canal space (17, 20, 21). However, tissue generation in the canal space of immature teeth with apical periodontitis after infection and disinfection has not been well investigated. It was speculated that PDL, bone, and cementum would also grow into the canal space of infected immature teeth after conservative treatment (18). Our findings explained, at least in part, the reason why the root structure became thickened after treatment. It was not because of the apposition of new dentin unless the pulp survived but rather because of cementum-like tissue. Additionally, the gained root length was also the result of newly formed cementum or cementum-like tissue at the root apex. The formation of cemental bridges at various levels of the canal space was possibly the result of the osteoinductive activity of the MTA, which in many cases was pushed into the canal space during the sealing of the access opening. This phenomenon is similar to using MTA as an apical plug that induces the formation of a cemental bridge at the open apex of teeth undergoing apexification treatment (22). This should be avoided in the clinical setting to prevent apical bridge formation, which would impede the vital tissue ingrowth into the canal space. There are cases, however, in which apical IC tended to build significant thickness such that it may eventually close the apical opening if given time. One may reason that this activity is a type of defense mechanism to separate the contaminated root canal system from the more internal periapical tissues.

In most cases examined, the hard tissue deposited onto the canal dentinal walls is cementum-like tissue based on the histologic characteristics and the continuation of the tissue from the external root surface to the internal canal walls. Some samples show discontinuity of the IC from the EC (Fig. 5). Two types of cementogenesis can occur after cementum damage (23). One is when there is dentin resorption, which exposes dentin matrix. The cementoblast-like cells produce cementum matrix to intermingle with dentin matrix. The hydroxylapatite crystals later deposit in the mixture of dentin and cementum matrix. This type of cementum formation is similar to embryonic dentin-cementum complex formation. Therefore, histologic cleavage plane between the primary dentin and IC may occur. The other is when there is no resorptive process of dentin. The cementoblast-like cells produce mineralized cementum directly onto the dentin. This type of dentin-cementum complex formation depends on the interlocking of mineral crystals. Accordingly, artifactual splits, located between the layer of new

**Figure 7.** Intracanal healing with chronic inflammatory infiltrate. (A) IC (yellow arrowheads) deposition along with numerous lymphocyte infiltrate (magnified view in Aa). External resorption of the apex exists at the location of inflammation (blue arrowhead). The space between the IC and inflammatory tissue is an artifact (sample from group 4). (B) A sample with no IC deposition with heavy inflammatory infiltrate is noted. External resorption of the apex exists at the location of inflammation (blue arrowheads) (sample from group 3). (C) Resorption of IC. The yellow arrowhead (left) is pointing at the resorptive surface of the IC. The yellow arrowhead (right) indicates the intact IC (sample from group 2). Scale bars: 500 μm (A-C); 50 μm (Aa).
cementum and the dentin surface, may be observed in histologic sections (Fig. 4 and Fig. 8).

For definitive identification of odontoblasts from cementoblasts, the specific marker dentin sialophosphoprotein that is only expressed by odontoblasts in the canal needs to be examined to verify the nature of the hard tissue. Differentiating IC and IB may be difficult in many cases. The typical alveolar bone trabeculae in the periapical region contained small bone marrow spaces or Haversian canals with uniformly distributed osteocytes. This was not the feature of the IC. Samples were more frequently found to have IB dispersed in the canal space, with typical osteoblast-like cells lining against the trabecular bone-like surface revealed under higher magnification. Some IB tissue closely resembled the alveolar bone in the periapical area with respect to cell density.

Although direct deposition of bone-like tissue onto the canal dentin walls (internal ankylosis or replacement resorption) was not observed in the present study, many islands or trabeculae of bone-like tissue are present in the canal. Bone can form directly onto the root surface if PDL is severely damaged such as in luxated or avulsed teeth (24). Ingrowth of bone and PDL into the canal space after pulp revascularization resulting in internal ankylosis was observed in luxated or avulsed teeth (24). Therefore, there is a possibility that internal replacement resorption may occur. Currently, there is a lack of evidence or report of this phenomenon.

Those clinical cases reported to date showing the healing of immature teeth via revitalization have the characteristics of gaining root thickness and length resembling a normal maturation of the root. This leads to the speculation that some survived pulp tissue and likely the apical papilla must have been present after dissection. These vital tissues contributed to the maturation of root development (18, 25). In this dog study model, only one case showed the partial survival of pulp tissue. The recovered pulp tissue exhibited a normal odontoblast layer. However, the other half of the pulp space appears to be filled by fibrous connective tissue that generated a layer of IC on the dentin wall. This suggests that pulp and other regenerated nonpulp connective tissue can coexist in the canal space. In all the rest of the cases in this study, no typical pulp tissue or new dentin was found regenerated in the canal space. Three possible situations may have occurred: (1) the infection involving the insertion of supragingival plaque into the pulp chamber caused severe tissue damage, (2) the use of the highly concentrated antibiotic paste may have been toxic to the live tissues, and (3) vital tissue including apical papilla has difficulty surviving when the infection is heavy and spread into the periapical tissues.

An interesting finding on the coexistence of heavy inflammatory infiltration and the generation of hard tissue deserves discussion. The persistent presence of lymphocyte infiltration indicates the existence of unresolved irritating materials including microbes or foreign bodies. However, adjacent to the heavy infiltrates, new cementum-like tissue was able to be laid down by the cementoblast-like cells, suggesting that the presence of those inflammatory cells does not interfere or may even serve as a stimulus of the involved cells to make cementum-like tissue. This has been clinically observed as enlarged

Figure 8. Bridge formation. (A) Bridge formation in the coronal third of the canal (sample from group 3). Green arrowheads indicate bridge formation by IC and scattered IB. Yellow arrowheads indicate thin layers of IC on dentin walls. (B) Bridge formation by cementum at the apex (sample from group 2). (C) Bridge formation at the apical third of the canal by ingrowth of IC. The dark blue arrow (upper left) indicates voids of external resorption of the EC. The blue arrowheads indicate demarcation of original dentin. The blue arrows indicate cementocyte-like cells in IC. Note the periapical tissue is regenerated with loose soft tissue at the endpoint of the experiment. D, dentin. Sample from group 2. Scale bars: 500 μm.
cementum associated with chronic apical periodontitis, although no report to date specifically discusses this phenomenon. Inflammation may provide factors to guide the differentiation of stem/progenitor cells in the healing soft tissue into cementoblasts. Stressed odontoblasts caused by trauma or other irritations may accelerate their production of dentin, which is generally termed tertiary dentin. This type of dentin has less regular dentinal tubules. Some samples were found to have IC deposited onto different layers of dentin that shows differential characteristics of dentinal tubules in terms of their number and organization. It is possible that when the pulp tissue was disrupted by the endodontic files, irritated odontoblasts rapidly deposited new dentin (tertiary) before they underwent necrosis by the incoming pulp infection. After this, the generated IC was laid over the tertiary dentin (Fig. 3D). Overall, hard tissue deposition appears to respond to infection and inflammation and most likely serves as part of the defense mechanism.

In summary, the revitalization approach for managing immature permanent teeth with infected pulp and/or apical periodontitis allows ingrowth of vital tissue consisting of tissues resembling cementum, PDL and bone. These tissues are not pulp parenchymal tissue. They do not function like a pulp tissue. Therefore, revitalization is not tissue regeneration but wound repair. Pulp tissue may survive the infection, recover, and remain healthy. The clinical case reports showing severe narrowing of the canal space cannot be explained by the present study as the experimental period was short. Although the revitalization treatment may be more favorable than traditional apexification procedures in terms of having vital tissues including cementum-like tissue deposited on canal walls, the long-term outcome of these new tissues in the canal space needs further investigation. The biological functions of cementum are to provide anchorage of Sharpey’s fibers of PDL to keep the tooth in the socket, to maintain occlusal relationship, and to repair damaged cementum (25, 26). Cellular cementum is formed in response to cemental damage on the root surfaces caused by periodontal disease or external root resorption. Besides the fact that these periodontal tissues are just filling into an empty canal space, the biological significance of cellular cementum-like tissue formed on the canal walls is unclear. Specifically, further research should be directed toward whether the root thickened by cementum-like tissue provides needed physical strength and how to manage a tooth if it becomes reinfected by recurrent caries.

References