Complex Apical Intraradicular Infection and Extraradicular Mineralized Biofilms as the Cause of Wet Canals and Treatment Failure: Report of 2 Cases

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Abstract
This article describes 2 cases that showed persistent intracanal exudation (wet canal) even after several visits of antimicrobial endodontic treatment. Histologic and histobacteriologic investigation was conducted for determination of the cause. The 2 cases involved teeth with apical periodontitis lesions, which presented persistent exudation refractory to treatment after several visits. In case 1, it was not possible to achieve a dry canal, and surgery had to be performed. In case 2, attempts to dry the canal succeeded and the canal was filled, but follow-up examination showed an enlarged apical periodontitis lesion and extraction was performed. Biopsy specimens consisting of the root apex and apical periodontitis lesion for case 1 and the whole root for case 2 were subjected to histologic and histobacteriologic analyses. Both cases showed complex bacterial infection in the apical root, affecting both the intraradicular space and the outer root surface. Case 1 showed bacterial biofilms in ramifications, on untouched walls, and extending to the external root surface to form a thick and partially mineralized structure with high bacterial density. Different bacterial morphotypes were evidenced. Case 2 had a ledge on the apical canal wall created during instrumentation, which was filled with necrotic debris, filling material, and bacteria. The walls of the apical portion of the canal were covered by a bacterial biofilm, which was continuous with a thick extraradicular biofilm covering the cementum and dentin in resorptive defects. The extraradicular biofilm showed areas of mineralization and was dominated by filamentous bacteria. The 2 cases with wet canals and treatment failure were associated with complex persistent infection in the apical part of the root canal system extending to form thick and partially mineralized biofilm structures (calculus) on the outer apical root surface. (J Endod 2016;42:509–515)

Key Words
Extraradicular infection, post-treatment apical periodontitis, treatment outcome, wet canal

Apical periodontitis is a disease caused by bacterial infection of the root canal system. The infection is usually restricted to the intraradicular space, but occasionally it can spread to the extraradicular space (1). When formed, the extraradicular infection is usually an extension of the intraradicular infection. In some cases, an extraradicular biofilm can be formed on the outer root surface area surrounding the apical foramina (2–4).

Extraradicular biofilms have been associated with post-treatment apical periodontitis (4) and are usually associated with symptoms (1, 3). Some reports indicate that the extraradicular biofilm can become mineralized. Harn et al (5) reported a case of post-treatment apical periodontitis associated with a sinus tract in which a calculus-like deposit was observed on the root surface during surgery. Ricucci et al (2) described 2 cases in which calculus (mineralized biofilms) occurred on the outer apical root surface of teeth with post-treatment apical periodontitis. In their study on the prevalence of biofilms in treated and untreated teeth, Ricucci and Siqueira (1) also reported 6 cases of extraradicular biofilms (4 from untreated teeth and 2 from treated teeth). Two cases showed areas of mineralization with a calculus-like appearance. An untreated tooth with an extensive calculus-like deposit on the external apical root surface was shown in a review article on endodontic biofilms (6). None of these reported cases had deep periodontal pockets reaching the apex, and most of them were associated with sinus tracts.

The purpose of this article was to contribute to the knowledge of the causes of persistent apical periodontitis by describing 2 cases that showed continuous intracanal exudation refractory to orthograde root canal treatment. Histologic and histobacteriologic analysis of specimens obtained by apical surgery and extraction showed the occurrence of apical intraradicular infection associated with extraradicular mineralized biofilm structures as the cause of persistent symptoms and disease.

Case Reports

Case 1
A 29-year-old man attended a general dental office complaining of several episodes of swelling in the right anterior maxilla and the taste of purulent exudate. He reported that he was self-medicating with antibiotics and had no history of trauma. His medical history was noncontributory.
At the intraoral examination, a moderate swelling fluctuant to palpation was noted in the periapical area of tooth #7. Tooth #9 had an access cavity on the palatal side, and teeth #7 and #8 did not respond to pulp sensitivity tests. Apical palpation in the periapical area of tooth #7 provoked drainage of purulent exudate through a sinus tract present buccally. The general dentist initiated the root canal treatment of tooth #7 but was not able to control drainage from the root canal; he decided to temporize the access cavity and refer the case to the Advanced Course in Endodontics, Institute of Dental Studies and Services of Fortaleza, Brazil, for evaluation and further treatment.

A periapical radiograph taken by the endodontic specialist revealed a large round radiolucency surrounding the apex of tooth #7 with corticated margins also involving the apices of teeth #6 and #8 (Fig. L). Tooth #7 showed an access cavity sealed with a temporary

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**Figure 1.** (A) Preoperative radiograph. Extensive calcification is associated with the mesial apical profile. (B) After flap elevation and exposure of the root, a dark structure covering the apex appeared. (C) Resected apex. Buccal view. (D) Mesial view. (E) View from bottom. (F–K) Sections taken approximately 50 μm from each other from root tip in a coronal direction (Taylor-modified Brown and Brenn, original magnification × 25). (L) Detail of the area demarcated by the rectangle in H. The bacterial biofilm shows varying densities (original magnification ×100). (M) Magnification of the rectangular area in L (original magnification × 400). (Inset) High-power view showing the different bacterial morphotypes (original magnification ×1000) (continued).
material, whereas extensive composite coronal restorations were present in tooth #8. A radiopaque area could be clearly distinguished on the mesial apical profile of the lateral incisor, suggesting a calculus-like deposit (Fig. 1A). Periodontal probing did not reveal pockets exceeding 2 mm deep around the roots of all the maxillary anterior teeth. Thermal and electric pulp tests gave no response for teeth #7 and #8, whereas tooth #6 responded normally.

Orthograde root canal treatment was scheduled for the maxillary right incisors. Root canal treatment of the lateral incisor was initiated first. After anesthesia and rubber dam isolation, the canal was reaccessed and gently flushed with 20 mL 2.5% sodium hypochlorite (NaOCl) solution. The working length was established with an electronic apex locator (RomiApex A-15; Romidan Ltd, Kiryat Ono, Israel). The root canal was instrumented with the Reciproc R50 file (VDW, Munich, Germany) and copious irrigation with 2.5% NaOCl. During all procedures, apical patency was maintained with a #10 K-file. At completion of canal preparation, an intracanal exudate was observed, and the canal was filled with calcium hydroxide mixed with sterile saline and the access cavity temporized. The canal was reaccessed after 15 days, and after canal revision, exudate was still present in the canal. Calcium hydroxide medication was placed once again. After 45 more days, exudate was still present in the canal, and at this point a mixture of iodoform and calcium hydroxide was packed into the canal. During each visit, the root canal was instrumented and copiously irrigated with 2.5% NaOCl. Passive ultrasonic irrigation with the Irrisonic tip (Helse Dental Technology, Santa Rosa de Viterbo, SP, Brazil) was used in all appointments for 1 minute. At the next visit, after another 30 days, drainage of purulent exudate was still observed, making it impossible to obtain dry conditions for root canal obturation. In the meantime, treatment of tooth #8 was completed uneventfully in 2 visits. Periradicular surgery for the lateral incisor was scheduled at this point.

The region was anesthetized with 2% mepivacaine with epinephrine (1:100,000). A mucoperiosteal flap was raised following osteotomy with a round bur under copious irrigation with saline solution to expose the periapical pathologic tissue. The lesion seemed not to be firmly adhered to the root tip and was gently removed from the bone crypt with curettes. A black, apparently mineralized, amorphous structure was observed covering the buccal and mesial apical surfaces (Fig. 1B). Root end resection was accomplished with an ultrasonic tip (W1; GVDentus, São José dos Campos, SP, Brazil) at a 90° angle coronally to the calcified structure. A 4-mm-long fragment of the apical root was removed (Fig. 1C–E). The root tip and the fragments of soft pathologic tissue were immersed in fixative for histopathologic and histotaxiologic analyses. The root canal preparation was then recaptured with hand files (Dentsply Maillefer, Ballaigues, Switzerland) and copious irrigation with saline. Next, 17% EDTA was used for 3 minutes, and the root canal was dried with sterile paper points and filled with cold lateral condensation and sealer (EndoFill, Dentsply Maillefer). The excess apical gutta-percha point was removed and burnished and the flap sutured. A postoperative radiograph was taken. Amoxicillin 500 mg/8 h for 7 days, ibuprofen 600 mg/8 h for 3 days, and oral rinses with 0.12% chlorhexidine digluconate 3 times a day for 7 days were prescribed. The postoperative period was uneventful.

After 6 months, the patient reported no discomfort. Vertical and lateral percussion as well as buccal and palatal palpation of the periapical area gave normal responses. Periodontal probing was within normal limits. A periapical radiograph revealed that healing was in progress, with bone formation from the margin of the original radiolucency, in a centripetal direction.

Case 2

A 70-year-old man presented to the dental office of 1 of the authors (C.B.) because “a bridge in the right mandible was decemented.” The patient declared that the bridge had been prepared several years before and that recently he felt it was loose. He did not experience any pain.

At inspection, the abutment of tooth #29 appeared completely destroyed by caries, and an explorer could be inserted into the exposed pulp chamber without provoking any pain or bleeding. Percussion and palpation gave negative responses. No sinus tract could be seen buccally or lingually. Tooth #27 did not show any caries lesion and responded normally to thermal and electric pulp tests. A periapical radiograph showed a radiolucent area located on the mesial apical profile of tooth #29 (Fig. 2A). The diagnosis of pulp necrosis with asymptomatic apical periodontitis was made, and root canal treatment was scheduled for tooth #29.

After anesthesia and rubber dam isolation, the carious tissue was removed, and the access cavity was prepared. The working length was established with a #15 K-file and an electronic apex locator (DentaPort ZX; Morita, Tokyo, Japan). A radiograph was taken to confirm the working length, which showed the apical foramen ending at the mesial apical profile (Fig. 2B). Instrumentation was initiated with the ProTaper S1 instrument (Dentsply Maillefer), but it became evident that successive instruments (ProTaper S2 and F1) could not reach the established working length because of ledge formation. Irrigation was performed with 5% NaOCl (Niclor; Ogna, Muggiò, MB, Italy) followed by 10% EDTA (Tubuliclen, Ogna). A sterile cotton pellet was placed in the orifice, and the access was sealed with Cavit (3M ESPE, Seefeld, Germany).
At the next visit 1 week later, an attempt was made to bypass the ledge with GT hand files (Dentsply Tulsa Dental Specialties, Tulsa, OK); this resulted in root canal instruments taken beyond the limits of the canal as confirmed by radiography after attempts to flatten the ledge and maintain apical patency (Fig. 2C). It was not possible to obtain a dry canal after instrumentation because of continuous seepage of exudate from the periapical inflammatory tissue; therefore, a sterile cotton pellet was placed and the access temporized as described previously.

The canal was revised 2 more times at intervals of 1 week, but the situation remained unchanged, with fluid flooding the canal from the apex. Irrigation was repeated with the EndoVac system (Discus Dental, Culver City, CA).

At the fifth visit, the canal was finally dry and obturated using Thermafil (Dentsply Maillefer) and Pulp Canal Sealer EWT (Kerr Corp, Orange, CA) (Fig. 2D). The access cavity was temporized, and the patient was referred to the prosthodontist for placement of a new bridge (Fig. 2E).

The patient presented after 14 months for follow-up, declaring that there were no symptoms over the whole postoperative period. A radiograph showed that the radiolucency had enormously increased, destroying the alveolar bone along the entire mesial radicular profile (Fig. 2F). No sinus tracts could be observed, but a periodontal probe penetrated over 10 mm from the mesial side with purulent exudation and bleeding. Root canal deviation as a result of post preparation was also evident.

**Figure 2.** (A) Preoperative radiograph. (B) Working length radiograph. (C) Checking the working length after an attempt to pass an apical obstacle showed that the instruments were taken beyond the root canal limits. (D) Postobturation radiograph. (E) Radiograph taken after cementation of the new bridge. Note that the post space was prepared at the expenses of the distal wall, but perforation did not occur. (F) Fourteen-month follow-up. A large osteolytic lesion is present on the mesial profile of the root. (G) Radiograph of the extracted tooth. (H) Photograph of the mesial aspect of the root. A calculus-like structure can be appreciated in the apical third. (I) Close-up of the apical area (continued).
The patient was informed about the poor prognosis of the tooth and decided on extraction. A radiograph (Fig. 2G) and photographs (Fig. 2H and I) were taken of the extracted tooth. Grayish/brownish deposits, apparently calcified, were observed on the mesial apical surface located concentrically around the anticipated foramen. These deposits were absent in the middle and coronal third of the root (Fig. 2H and I).

The tooth was immersed in fixative for histobacteriologic examination.

**Tissue Processing**

The biopsies (consisting of the root tip and several soft tissue fragments for case 1 and the whole tooth for case 2) were kept in a 10% neutral buffered formalin solution for about 4 weeks. For case 2, after fixation the root was separated from the crown in the cervical region with a diamond disk under water spray, and the root was arbitrarily further subdivided into 3 portions (apical, middle, and coronal thirds), which were processed separately. With the exception of the soft tissues obtained for case 1, demineralization of the specimens 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 with the end point being determined radiographically. The specimens were washed in running tap water for 24 hours, after which the metal post segments were gently removed from the middle and coronal thirds of case 2. They were then dehydrated in ascending grades of ethanol, cleared in xylene, infiltrated, and embedded in paraffin (melting point of 56°C) according to standard procedures. With the microtome set at

![Figure 2. (Continued).](image)
4–5 μm, serial sections of the 2 apices were cut transversally from the root tip in a coronal direction until they were exhausted. More than 800 sections were cut for each apical sample. For case 2, an additional 200 sections were cut from the middle third and another 200 from the coronal third. To prevent detachment of the small apical sections during the several baths in acetone-containing solutions, all sections were collected on special polarized slides (Superfrost Plus; Gerhard Menzel GmbH, Braunschweig, Germany).

Slides were stained with the Taylor-modified Brown and Brenn technique for bacteria (7, 8) and hematoxylin-eosin. Sections were examined under the light microscope, and photomicrographs were taken.

Results

Minimal artifacts were observed in some sections from the 2 apices because of partial detachment of the calcified matter from the apical surface during dehydration of the specimens. Bacterial staining was deemed successful (Figs. 1 and 2). Histologic sections confirmed that the dark structure adhering to the apical surface of both apices was a mineralized biofilm (Fig. 1F–M and 2K–T).

Case 1

The initial sections of the series, cut tangentially to the very apical portion, displayed an eccentric foramen, ending on the mesial aspect (Fig. 1F–H). The apical profile was irregular because of previous resorption, and these areas were covered by a dense biofilm, the thickness of which exceeded 1 mm in some areas (Fig. 1G–I). Depressions could be observed, corresponding to the exits of major apical ramifications (arrows in Fig. 1G–H). Sections cut approximately 1 mm coronally to the geometric top showed 3 large apical ramifications, all containing necrotic tissue and bacterial biofilms (Fig. 1I, N, and O). Several minor or tiny ramifications could also be observed (Fig. 1J).

Sections cut more coronally (at about 2 mm from the top) revealed that only 1 large elongated canal was present at this level, with the palatal portion enlarged by instruments and a buccal extension apparently not affected by instrumentation (Fig. 1J–K).

High-power view of the exuberant extraradicular biofilm revealed a high bacterial density with only a few areas in which the extracellular matrix component was prevailing. In some areas, a “sandwich-like” structure with distinct layers could be observed (Fig. 1L and M). The apical calculus was composed of bacteria with different morphotypes (ie, cocci [more abundant], rods, and filaments [inset in Fig. 1]). Cocc were the dominant bacterial morphotype in the biofilms layering the walls of the large apical ramifications (Fig. 1N and O).

Histologic examination of the soft tissue fragments displayed an inflammatory tissue with an abundance of polymorphonuclear leukocytes and epithelial strands equally infiltrated by polymorphonuclear leukocytes. Because only fragments of the pathologic periapical tissue were obtained, the diagnosis of the type of lesion could not be made.

Case 2

Photographs of the cleared apical third of the root immersed in xylene and before paraffin infiltration revealed that a ledge had been created in the apical dental wall during instrumentation, which appeared partly filled (Fig. 2J). The first sections, cut through the ledge

Figure 2. (Continued). (Q) Detail of the apical canal indicated by the rectangle in M. The filling material at the foramen is covered by a bacterial biofilm extending uninterruptedly on the root surface (original magnification ×100). (R) High-power view of the area of the apical canal indicated by the arrow in Q. A biofilm fills an irregularity of the wall and is covered by filling material (original magnification ×400). (S) Section cut at the level of line 4 in J passing coronally to the foramen (original magnification ×25). (T) High-power view from the area of the apical profile indicated by the arrow in S. Filamentous bacteria prevail in the biofilm (original magnification ×400).
and not encompassing the foramen (located more coronally), revealed necrotic debris, filling material, and bacteria in this iatrogenically created space (Fig. 2K and N). Sections cut more coronally and incorporating the foramen showed that the walls of the apical portion of the canal were covered by a bacterial biofilm in some areas, and this was lined by filling materials toward the lumen (Fig. 2Q and R). A bacterial biofilm also lined the filling material at the foraminal opening. This was continuous with the biofilm covering the external apical root (Fig. 2M and Q). In some sections, this biofilm extended for approximately half of the root circumference and exhibiting varying thickness, covering the cementum or the dentin in resorptive defects denuded from the cementum (Fig. 2S). Filamentous bacteria were the prevailing morphotype in the apical calculus (Fig. 27). Biofilm films were observed on the apical external surface up to a distance of approximately 3 mm from the root tip and were absent in the middle and coronal thirds.

Discussion
The clinical condition referred to as “wet canals” is characterized by continuous seepage of inflammatory exudate into the canal persisting despite treatment. The cause is arguably persistent infection, and the 2 cases reported in this article significantly contribute to the understanding of the etiology of this clinical condition. Both teeth had persistent intracanal exudation, which was associated with apical intraradicular infection continuous with a thick extraradicular biofilm as determined by histobacteriology. In case 1, root canal filling had to be placed during the surgical procedures. In case 2, exudation was controlled after several visits, but the lesion increased in size as revealed at the first follow-up examination.

The extraradicular biofilms associated with the 2 cases were thick and presented areas of mineralization that gave them a calculus-like appearance similar to previous reports (1, 2). Different bacterial morphotypes were found in the biofilms, indicating the occurrence of a multispecies bacterial community. These extraradicular biofilms were contiguous to intraradicular biofilms, which were unaffected by treatment procedures. These observations give support to the assumption that extraradicular biofilms are usually an extension of the intraradicular infection (9). Whether or not these complex extraradicular bacterial structures exhibiting areas of mineralization become independent of the intraradicular infectious process remains to be determined. Given the apparently high degree of organization of these mature bacterial structures, it is reasonable to believe that they may not be significantly influenced by intracanal procedures that succeeded in eradicating the intraradicular infection. Consequently, these mature and mineralized extraradicular biofilms could become an independent infectious entity. It is important to point out that this assumption is not supported by the present findings because there was an intraradicular infectious component in both cases. However, a previous article showed 1 case of extraradicular biofilm in a tooth with no detectable intraradicular infection (3). Other studies should elaborate on this issue.

The extraradicular biofilm is a relatively rare occurrence (1) because bacteria leaving the apical foramen are directly combated and eliminated by the host defense mechanisms. When developed, it is likely to be a consequence of massive infection of the root canal system associated with prolonged exposure of the canal space to the oral environment (10). The fact that most cases of extraradicular biofilms are associated with sinus tracts may indicate that these cases are resulting from chronicization of a previous abscess, a condition known to be caused by egress of pathogenic bacteria from the canal to the periapical tissues (11). Moreover, the extraradicular occurrence of bacteria may also be precipitated by overinstrumentation (3); this may have occurred in case 2. This reinforces the need for controlled apical length of instrumentation in order to avoid postoperative complications.

Calculation is a bacterial biofilm that underwent mineralization. In supra- or subgingival calculus, separate foci of mineralization increase in size and coalesce to form solid mineralized masses (12). Crystals form by precipitation of mineral salts initially in the intercellular matrix of the biofilm, then on the bacterial cell surface, and eventually within the bacterial cells (13). Whether or not the same mechanism happens in extraradicular calculus cannot be inferred, but the presence of mineralization foci in these apical structures may suggest likewise. There are some possible ways by which mineralization can develop on the extraradicular biofilm. A possible source may be the inflammatory exudate and periradicular tissue fluids saturated with minerals from bone solubilization. In addition, long-standing sinus tracts may function as a route of communication between the periradicular area and the external environment, permitting the passage of minerals and salts from the oral fluids into the apical periodontitis lesion. Although it is possible that bacteria may play some role in mineralization of the biofilm, the most common opinion on periodontal calculus is that bacteria are only passively involved, being included in the mineralization process along with the biofilm intercellular matrix (14). Communication of the apical periodontitis lesion with a secondary periodontal defect may also have been a source of mineralization in case 2.

In conclusion, complex persistent infection in the apical part of the root canal system extending to form thick and partially mineralized biofilm structures on the outer apical root surface was the cause of wet canals and endodontic treatment failure in the cases reported. Several attempts to control infection and persistent exudation were performed, and the treatment was significantly arrested.

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