Persistent Extraradicular Infection in Root-filled Asymptomatic Human Tooth: Scanning Electron Microscopic Analysis and Microbial Investigation after Apical Microsurgery

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Abstract

Introduction: Procedural accidents have a negative effect on healing and might contribute to the persistence of infections in inaccessible apical areas, requiring surgical intervention. This report describes a case of persistent apical periodontitis of a lower left first molar associated with the sinus tract and a periapical lesion that required nonsurgical endodontic retreatment and apical surgery for resolution. **Methods:** The tooth had received endodontic treatment 3 years ago and had to be retreated using the crown-down technique with chemical auxiliary substance (2% chlorhexidine gel), foramen patency, and enlargement and was filled in a single appointment. The occlusal access cavity was immediately restored with composite resin. After 1 month, it could be observed that the sinus tract persisted and, radiographically, the lesion remained unaltered. Therefore, endodontic microsurgery was indicated. Apical microsurgery was performed under magnification with the use of a dental operating microscope including apicectomy, root end with ultrasound, and sealing with mineral trioxide aggregate. A microbiological sample was collected from the apical lesion. The resected distal root apex was observed by scanning electron microscopy. **Results:** The following species were detected: *Actinomyces naeslundii* and *Actinomyces meyeri*, *Propionibacterium propionicum*, *Clostridium botullinum*, *Parvimonas micra*, and *Bacteroides ureolyticus*; scanning electron microscopic analysis revealed bacterial biofilm surrounding the apical foramen and external radicular surface. Gutta-percha overfilling at the apex because of a zip caused during initial endodontic treatment could be observed. A 6-month follow-up showed apparent radiographic periapical healing, which progressed after 24 months. **Conclusion:** Gram-positive anaerobic bacteria and extraradicular biofilm seem to participate in the maintenance of persistent periapical pathology, and endodontic retreatment followed by periapical microsurgery proved to be a successful alternative in the resolution of persistent extraradicular infections. *(J Endod 2011;37:1696–1700)*

Key Words

Apical periodontitis, apical surgery, endodontic failure, endodontic outcome, nonsurgical retreatment, root canal infection

The failure of nonsurgical root canal treatment is commonly related to the presence of residual bacteria (persistent infection) or the reinfection of a previously disinfected root canal environment (secondary infection) (1). Unsuccessful outcomes can be attributed to persistent intraradicular infections found in previously uninstrumented canals, dentinal tubules, or the complex irregularities of the root canal system (2–4). The extraradicular causes of endodontic failures include periapical actinomycosis (5), a foreign-body reaction caused by extruded endodontic materials (6), the accumulation of endogenous cholesterol crystals in the apical tissues (7), and an unresolved cystic lesion (8). Formerly treated teeth with persistent periapical lesions might be preserved with nonsurgical retreatment, assuming the tooth is restorable and periodontally sound. Previous procedural accidents have a negative effect on healing (9). Besides, they might contribute to the establishment of infections at inaccessible apical areas, requiring a surgical intervention (10).

Periradicular surgery is indicated in cases of unsuccessful outcomes after primary root canal therapy followed by nonsurgical retreatment. The goal of periradicular surgery is the removal of diseased periapical tissues and the sealing of the apical root canal system to facilitate the regeneration of hard and soft tissues, including the formation of new attachment cells (11).

This clinical article reports a case of persistent apical periodontitis on the lower left first molar associated with a sinus tract, which was treated with nonsurgical endodontic retreatment and surgical procedures. This case report shows the limitations imposed by inadequate clinical procedures such as transportation and ledging of the main canals to achieve an adequate disinfection during first root canal treatment.

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Case Report

A 38-year-old female patient was referred to the Endodontic Department of the University of Campinas complaining about a persistent sinus tract on the buccal alveolar mucosa associated with the distal apex of the lower left first molar. The patient did not report the presence of spontaneous pain. Periapical pathology could be observed in the preoperative radiograph. The dental history indicated that the lower left first molar had previously received root canal treatment 3 years ago. The patient's medical history was noncontributory. There was no history of drug allergies. Radiographic examination showed an apparent radiolucency around the distal apex and widening of the periodontal ligament at the apex of the mesial root (Fig. 1*A*). The quality of the root canal filling was inadequate because, radiographically, the limit of the filling was below the standard required with a possible deviation in the apical third of the mesial and distal canals. Clinical examination showed a negative response to percussion and palpation tests. Periodontal examination revealed probing depths of 3 mm or less without mobility. The tooth had been restored with amalgam. Based on the history, clinical tests, and radiographs, a diagnosis of a root-filled tooth with persistent periapical periodontitis was established.

The patient was informed that conventional root canal retreatment and follow-up was the initial approach to reach infection control and apical healing. However, the persistence of the sinus tract and clinical symptoms would lead to apical endodontic surgery as a complementary approach to the resolution of the pathology. The patient consented to the treatment plan.

Nonsurgical Endodontic Retreatment

After local anesthesia (2% lidocaine with 1:100.000 epinephrine), a rubber dam was placed, and the tooth and surrounding field were disinfected with 30% hydrogen peroxide followed by a 2.5% sodium hypochlorite solution for 30 seconds each. The coronal restoration and root canal filling materials were removed. Close inspection under high magnification with the dental operating microscope (D F Vasconcellos S/A, São Paulo, Brazil) showed an untreated distolingual canal. The root canal filling material was removed with Gates-Glidden drills in the coronal two thirds and K-files in the apical third. A further root canal retreatment was performed using the crown-down technique with 2% chlorhexidine gel as an auxiliary chemical substance followed by irrigation with a sterile physiological solution, patency, and foramen enlargement for appropriate cleaning of this area (ie, mesiobuccal, mesiolingual, and distolingual). Apical patency could not be accomplished in the distobuccal canal because of ledging. The working length was established with an apex locator (Novapex; Forum Technologies, Rishon le-Zion, Israel). The first file that bound at the working length was #25 K-file (mesiobuccal), #25 K-file (mesiolingual), #30 K-file (distobuccal), and #30 K-file (distolingual). Three additional manual K-files (Dentsply Maillefer, Ballaigues, Switzerland) were used after the first instrument that fitted the working length. Therefore, the final file used for apical preparation at the working length was #40 K-file (mesiobuccal), #40 K-file (mesiolingual), #45 K-file (distobuccal), and #45 K-file (distolingual). A solution of 17% EDTA was used for 3 minutes to remove the smear layer. The canals were dried with sterile paper points and then filled with gutta-percha and Endomethasone sealer (Septodont, Saint-Maur-des-Fossés, France) using a warm vertical combined with a lateral condensation technique. After endodontic retreatment, the tooth was restored with composite resin (Filtek Z250; 3M ESPE, St Paul, MN), which was clinically adequate. Figure 1*B* shows a postoperative radiograph after nonsurgical retreatment. After 1 month, it could be observed that the sinus tract persisted and the patient reported the persistence of discomfort upon vertical percussion. The endodontic microsurgery was indicated.

Periradicular Microsurgery and Sampling Procedures

Immediately before surgery, the gingival and mucosa were washed with 0.2% chlorhexidine gluconate for disinfection followed by a local rinse with 5% Tween 80 and 0.07% soy lecithin (to reduce the carryover effect of chlorhexidine). After local anesthesia with 2% lidocaine with 1:100,000 epinephrine for the left mandibular nerve block and buccal infiltration, a mucoperiosteal flap was made. Periapical pathology was noted at the apex of the distal root with cortical bone fenestration. The periapical tissues were removed with a sterile curette, and a microbial sample was obtained by rubbing sterile paper points against the root apex, which was held in place for 60 seconds inside the surgical cavity. The paper points were pooled in a sterile tube containing 1 mL of VMGA III (12) transport medium for microbial cultivation. The sterility of the operative field was checked by collecting a periosteal tissue sample from an area adjacent to the surgical site using curettes and paper points to test for bacterial contamination.

The granulation tissue was excised, and osteotomy was performed. Three millimeters of the distal root apex was resected orthogonally to their longitudinal axis (Fig. 1*C*) using a tungsten-carbide straight fissure drill (Maillefer Zekrya; Dentsply-Maillefer Instruments, Ballaigues, Switzerland) under constant 0.9% sodium chloride solution irrigation with the aid of a surgical operating microscope and micromirror. The root tip was then removed with sterile tweezers, rinsed in sterile saline, and placed in 0.2% trypsin solution for 24 hours for later scanning electron microscopic (SEM) analysis.

The resected root surfaces were examined at high magnification ($\times 12$). The root-end cavity was prepared with microsurgical ultrasonic tips (Pro Ultra Surgical Tips; Dentsply, Tulsa, OK) and subsequently filled with mineral trioxide aggregate (MTA) (ProRoot; Dentsply). Flap closure was obtained with 5-0 nylon sutures. Postoperative radiographs were taken. The patient received postoperative instructions. Additional antibiotics and analgesics were provided to the patient (amoxicillin 500 mg, 3 times a day for 5 days, and ibuprofen 600 mg for pain, 2 times a day as needed). The patient returned 1 week later for suture removal and reported slight postoperative pain. Healing of the surgery was uneventful. The patient was examined clinically and radiographically at the 6- and 24-month recall visits. The tooth was asymptomatic. Periapical healing around the apical root area could be observed in the follow-up radiographies (Fig. 1*D*).

Microbiological Identification

The isolation and identification of the microorganisms were performed by the use of culture techniques for phenotypic characterization as described previously (13). In summary, inside an anaerobic chamber, the samples were vortexed for 60 seconds and diluted in fastidious anaerobe broth (Lab M, Bury, UK) by 10-fold serial dilution to 10^{-4}. A volume of 50 μL of each dilution was spread onto 5% defibrinated sheep blood (Fastidious Anaerobe Agar [FAA], Lab M) containing 5 mg/mL of hemin (final concentration of 5 μg/mL) and 1 mg/mL) of vitamin K1 (final concentration of 1 μg/mL). Selective culture media were also used as follows: 5% sheep blood FAA + NAL (0.001% w/v nalidixic acid) + vancomycin (0.5 mg/L) to select gram-negative anaerobic bacteria, 5% sheep blood FAA + kanamycin + vancomycin to select "black-pigmented bacteria," 5% sheep blood FAA + neomycin (0.0075% w/v neomycin) for clostridia and other anaerobes, and 5% sheep blood FAA + nalidixic acid (0.001% w/v nalidixic acid) for gram-positive anaerobes and *Actinomyces* involved. The plates were incubated at 37°C in an anaerobic atmosphere for up to