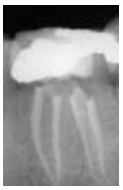

CASE REPORT

Resolution of persistent periapical infection by endodontic surgery

F. B. A. Ferreira, A. L. Ferreira, B. P. F. A. Gomes & F. J. Souza-Filho
Endodontic Unit, Piracicaba Dental School, State University of Campinas-UNICAMP, Piracicaba, SP, Brazil

Abstract

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Aim To examine the surfaces of a root tip removed during surgical endodontic treatment for the presence of microorganisms.

Summary The present clinical case illustrates an endodontic retreatment of a maxillary premolar tooth with a fistula and periapical reaction. The case was under treatment for 1 year, during which an intracanal medicament was replaced several times. As the lesion did not decrease and exudate was persistent through the fistula and root canal, root end resection with root end filling was performed. Microbiological samples were collected from the fistula, where *Propionibacterium acnes*, a species associated with endodontic failures, was detected by appropriate anaerobic technique. The resected root apex was observed by scanning electron microscopy (SEM), which revealed cocci and fungal forms surrounding one of the foramina. After 12 months, the periapical lesion had reduced.

Key learning points

- Persistent extraradicular infections are not affected by the action of antimicrobial agents such as irrigants and medicaments used during root canal treatment.
- Apical surgery is a suitable alternative for definitive removal of an established refractory infection, promoting repair of difficult cases.

Keywords: endodontics, extraradicular infection, microorganisms, periapical plaque.

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Introduction

When microorganims enter the pulpal space, they contaminate the main root canal, proliferating through the dentinal tubules and ramifications of the root canal system until they reach the periapical area. At this site, the resorption lacunae formed in the apical cement by the inflammatory reaction can be colonized by microorganisms (Nair *et al*. 1990, Tronstad *et al*. 1990, Sen *et al*. 1995). The degree of this microbial invasion is dependent on

Correspondence: Brenda P. F. A. Gomes, PhD, MSc, BDS, Department of Endodontics, Piracicaba Dental School, UNICAMP Avenue. Limeira 701, B. Areião, Piracicaba, SP 13416-018, Brazil (Tel.: +55 19 3412 5215; fax: +55 19 3412 5218; e-mail: bpgomes@fop.unicamp.br).

the type of microbial species, time, dentine tubule diameter and the third of the root canal involved (Akpata & Blechman 1982, Sen *et al*. 1995).

It has been reported that nonmicrobial factors may be implicated in root canal treatment failure (Nair *et al*. 1990). However, the literature suggests that persistent intraradicular infections because of the anatomical difficulties in cleaning the root canal system even after meticulous chemo-mechanical preparation and intracanal medicaments or to secondary infections caused by coronal microleakage are the major causes (Nair *et al*. 1990, Siqueira & Lopes 2001, Pinheiro *et al*. 2003). Recent studies using anaerobic techniques (Molander *et al*. 1998, Sundqvist *et al*. 1998, Peciuliene *et al*. 2000, Pinheiro *et al*. 2003) have revealed that the composition of the root canal microbiota after treatment failure differs from that normally found in untreated teeth. The necrotic pulp presents a polymicrobial flora characterized by a wide variety of combinations of bacteria, averaging four to seven species per canal, predominantly anaerobic, with approximately equal proportions of Gram-negative and Gram-positive bacteria (Baumgartner & Falker 1991, Sundqvist 1992, Gomes *et al*. 1994; 1996a,b). In contrast, the microbial flora detected in previously root filled teeth with apical periodontitis comprises one or two microbial species, sometimes three bacterial strains, with predominantly Gram-positive microorganisms such as *Enterococcus faecalis*, *Actinomyces* spp. and *Propionibacterium* spp. with approximately equal proportions of facultative and strict anaerobes (Sundqvist *et al*. 1998, Peciuliene *et al*. 2000, Pinheiro *et al*. 2003). Fungal species such as *Candida albicans* have also been described (Sen *et al*. 1995, Waltimo *et al*. 1997).

When the periapical region is colonized by microorganisms, the host attempts to eliminate the infection through the immune system. The wide intercellular spaces allow bacteria to enter or their by-products to leak into the periapex (Nair 1987). Moreover, the resorption lacunae formed in response to the bacterial by-products are able to promote microbial niches (Lomçali *et al*. 1996), where the microorganisms can arrange and organize themselves in associations and produce a polysaccharide layer, which is responsible for their resistance to intracanal medicaments, to host defence and to antibiotics, consolidating the 'periapical bacterial plaque' (Tronstad *et al*. 1990) or 'periapical biofilms' (Siqueira & Lopes 2001). The current definition of biofilm is a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface (Costerton *et al*. 1999), as a result of adhesion, multiplication and development of microorganisms in an aqueous environment (Costerton *et al*. 1987). This insoluble polysaccharide cover seems to be the major resistance mechanism of these associations. According to Lewis (2001), the resistance of infection occurs because of failure of an antimicrobial agent and of components of the immune system to penetrate the full depth of the biofilm (polysaccharide mass), a decrease in the microbial reproduction rate (low metabolism), and the development of tolerance genes that are exchanged amongst microorganisms (Lewis 2001). Thus, the biofilms remain at the site, acting as a continuous irritation, releasing by-products such as endotoxins (Page 1998). The microbial biofilms can only be removed and disrupted physically and not chemically because local and systemic drugs cannot enter them (Page 1998, Lewis 2001).

Several methods have been used to view the microstructure of these biofilms, including light microscopy, transmission electron microscopy, scanning electron microscopy (SEM) and confocal laser scanning microscopy (Molven *et al*. 1991).

Report

A 30-year-old male patient attended a dental surgery complaining of a persistent sinus tract in the second right maxillary premolar region despite two previous attempts of root canal treatment. Clinically, the tooth had an extensive carious lesion and radiographically an extensive periapical radiolucency that was tracked through the discharging fistula with gutta-percha cones. The root canal treatment was inadequate with an incomplete root filling.

A further root canal retreatment was performed using the crown-down technique with 1% sodium hypochlorite irrigation and enlargement of the foramen (patency) for appropriate cleaning of this area. Calcium hydroxide paste was inserted to full working length and left in place for 15 days. The tooth was temporarily sealed with a zinc oxide and zinc sulphate hydrated temporary cement (Cavit, ESPE, Seefeld/Oberbay, Germany). As the canals presented constantly with exudate, the intracanal medication was replaced monthly over a period of 13 months. As the patient preferred to undergo a conventional root canal treatment, the root canals were repeatedly re-instrumented and irrigated with sodium hypochlorite with a final rinse of EDTA before placement of the medicament. During this period, there was continuous drainage through the fistula and through the lingual canal if the file penetrated 1 mm beyond the apical foramen.

Although the fistula did not close, the canals were dry after 13 months of medication, and were filled with gutta-percha and Sealer 26 (Dentsply, Rio de Janeiro, RJ, Brazil) by cold lateral condensation. Sealer 26 includes bismuth oxide and calcium hydroxide, and the liquid is an epoxy resin similar to AH26 but without silver.

After root filling, the fistula continued to drain and endodontic surgery was conducted. Immediately before surgery, the gingiva and mucosa were washed with 0.2% chlorhexidine gluconate for disinfection, followed by a rinse of Tween 80 and soy lecithin to reduce the carry-over effect of chlorhexidine. A microbial sample was collected by means of sterile absorbent paper points inserted into the fistula. The flap was reflected, the periapical tissues was removed with a sterile curette, and a second microbial sample was obtained by rubbing paper points against the root apex.

About 2–3 mm of the root apex was sectioned with a sterile fissure bur in a high-speed handpiece under irrigation with sterile saline. The root tip was then removed with sterile tweezers, rinsed in sterile saline and placed in 0.2% trypsin solution for 24 h for later SEM analysis. The root end cavity was prepared with ultrasound (ENAC, Osada, Japan) and filled with Sealer 26 (Dentsply), which has low toxicity (Barbosa *et al*. 1993) and has been used as root end filling material (Barbosa & Souza-Filho 1999).

Microbiological sampling

The absorbent paper points used for sampling the fistulous tract and root apex were immediately placed in individual vials containing 1 mL of VMGA III transport medium (Möller 1966) and then transported to the anaerobic workstation (Don Whitley Scientific, Bradford, UK) in the microbiology laboratory at the Endodontic Department of the Piracicaba Dental School – UNICAMP. Inside the anaerobic workstation, the transport media, containing 3-mm-diameter glass beads to facilitate mixing and homogenization of the sample, were shaken thoroughly in a mixer for 60 s (vortex MA 162-MARCONI, São Paulo, SP, Brazil). Serial 10-fold dilutions were made up to $1/10^4$ in Fastidious Anaerobe Broth (FAB – Laboratory M, Bury, UK), and 50 μL of each serial dilution was plated onto reduced blood agar plates of Fastidious Anaerobe Agar (FAA – Laboratory M) supplemented with haemin and menadione using sterile plastic spreaders and incubated under anaerobic conditions (atmosphere of 10% H_2, 10% CO_2 and 80% N_2) at 37 °C for 7 days.

Following incubation, each plate was examined and the different colony types were subcultured onto plates to obtain pure cultures. Colony appearance was used to select the colonies for further study. Pure cultures were then initially identified according to their Gram morphology, ability to produce catalase and gaseous requirements.

More detailed biochemical profiling was completed using a commercial diagnostic kit designed for the identification of several microorganisms, including anaerobic and facultative Gram-positive catalase-positive isolates (Rapid ID 32 A, BioMérieux S.A., Marcy-l'Etoile, France).

SEM procedure

The root apex removed during surgery was immediately immersed in 0.2% trypsin solution for periodontal fibre dissolution over a period of 24 h. The apex was fixed in Karnovsky solution (2.5% glutaraldehyde, 4.0% paraformaldehyde and 0.1 M sodium cacodylate, pH 7.02–7.4) for 1 week. The specimen was then dehydrated in an ethanol series, dehydrated in a critical point device (Denton Vacuum DCP-1, USA) and gold sputter coated (Denton Vacuum Desk II, USA). The surface of the root tip was then studied under a scanning electron microscope operated at 10 kV (Jeol JSM – 5600 LV, Japan).

Microbiological results

The sample from the external root surface produced no microbial growth. The sample from the fistulous tract yielded *Propionibacterium acnes,* a facultative Gram-positive, catalase-positive microorganism.

SEM results

The root apex presented a wide lingual foramen and three smaller buccal foramina (Fig. 1). Close to the buccal foramen filled with gutta-percha, there were resorption lacunae without bacteria. Close to the other two buccal foramina, the dentine was normal even though the foramina were not filled.

Extruded gutta-percha was observed in the lingual foramen, surrounded by resorption lacunae (Fig. 2). Higher magnification of this area showed reproducing fungal forms (Fig. 3). Lateral to the gutta-percha, these microorganisms were attached to the filling material (Fig. 4). The surrounding resorptions and dentine of the lingual foramen were totally covered with a substance that resembled extracellular polymeric substance, which linked the microorganisms (Fig. 5). Also, a large number of cocci in division and forming chains were observed neighbouring the fungal forms (Fig. 6), and isolated rods were seen deep inside these microbial aggregates.

Away from the apical foramina, the dentine appeared normal with no evidence of biofilm or matrix.

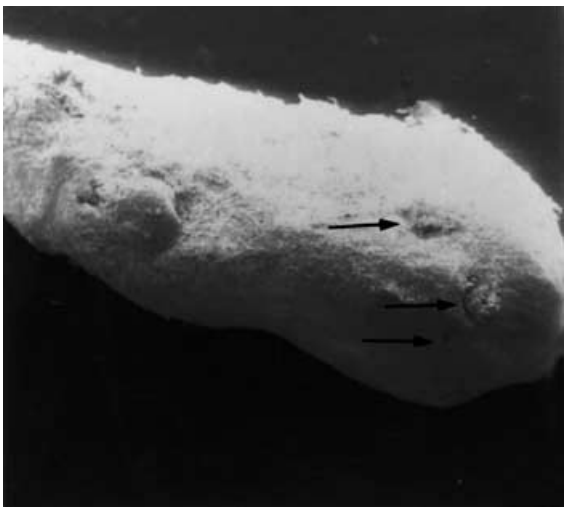

Figure 1 SEM of the removed root apex showing the buccal area (larger) and the lingual area with extruded gutta-percha (20×). The buccal area presented three foramena (arrows), one obturated with gutta-percha and two not filled, but none of them showed bacteria.

Figure 2 The lingual foramen, with extruded gutta-percha surrounded by resorption lacunae (200×).

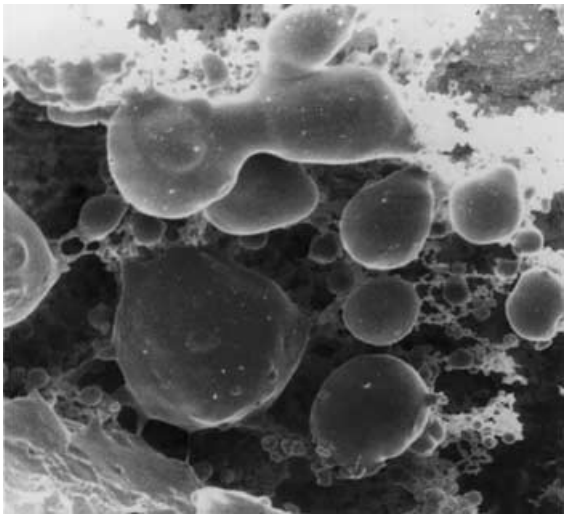

Figure 3 Lingual foramen presenting reproducing fungal forms (1000×).

Postoperative follow-up

Root-end resection and root end filling was performed in May 2001. After 2 months, bony healing was evident radiographically (Fig. 7). Further radiographs were obtained 8 months and 2 years postsurgery, showing successful bony repair. Clinically, the fistulous tract disappeared.

Discussion

During root canal retreatment, all efforts were made to control intracanal infection, including foraminal instrumentation (patency), irrigation with bactericidal substances and intracanal dressings of calcium hydroxide. However, the infection remained active, as it was in a region that was not accessible to chemo-mechanical instrumentation. Only the root end resection proved to be effective in disorganizing or eliminating causative microorganisms.

The present case illustrates how endodontic failure results from persistent infection, although it probably was not because of the difficulties in cleaning and shaping the root

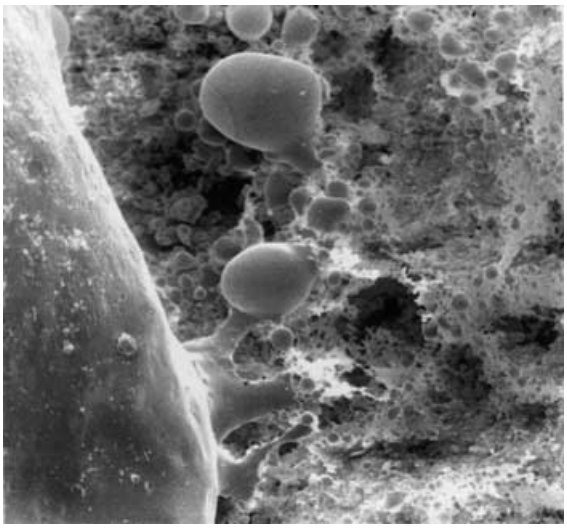

Figure 4 Lateral to the extruded gutta-percha in the lingual foramen, fungal forms were attached to the filling material (500×).

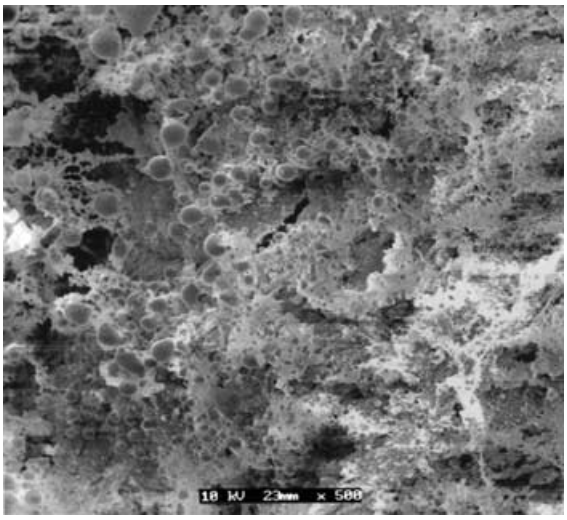

Figure 5 The surrounding resorptions and dentine of the lingual foramen were totally covered with a kind of 'net', probably an extracellular polymeric substance (500×).

canal system (Nair 1987). This lesion was maintained by microbial combinations in extra-radicular resorption lacunae, including fungi, which are naturally resistant microorganisms (Waltimo *et al*. 1997; 2000). These fungi were probably brought into the root canal iatrogenically through ineffective aseptic procedures during the first root filling. After fungi are established, their removal is difficult. Waltimo *et al*. (2000) showed that *C. albicans* was resistant to calcium hydroxide dressings. In the present case, the fungi could have protected the other microorganisms.

Interestingly, SEM examination showed that in the buccal region, there was an apical delta, with three visible foramina, but only one was obturated. All three foramina were free of microorganisms. This confirms the fact that in this case, the anatomical factor was not a major reason for failure in the lingual area. However, the microorganisms were able to

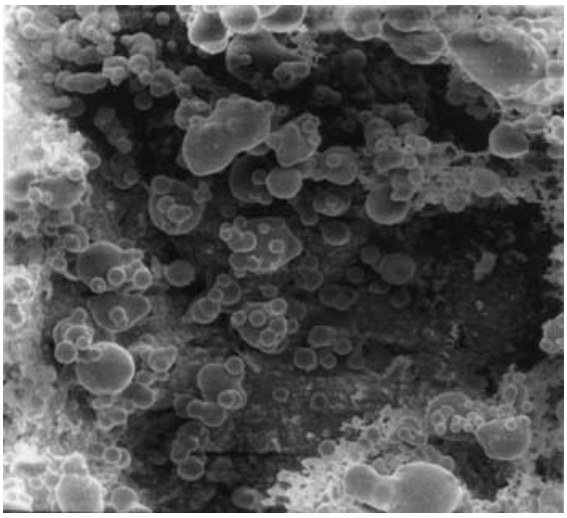

Figure 6 Cocci (small cells) neighbouring fungal forms (larger cells, 1000×).

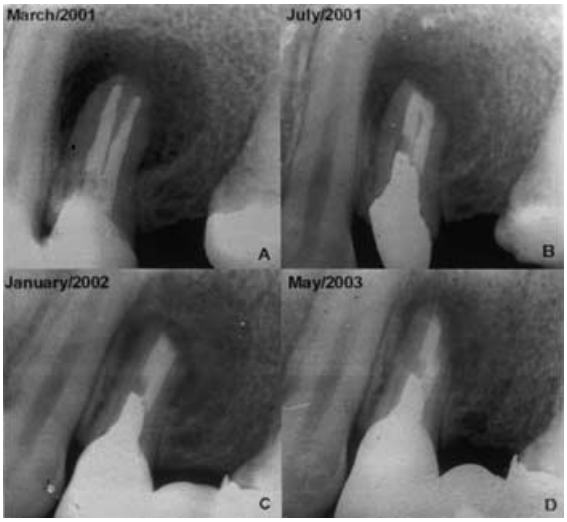

Figure 7 (A) Periapical film after root canal retreatment showing a large periapical lesion; (B) radiographic evidence of healing of the periapical reaction 2 months after endodontic surgery; (C) radiographic review 8 months postsurgery; and (D) radiographic review 24 months postsurgery.

survive and colonize beyond the root canal. In the apical resorption lacunae of the lingual foramen, the microorganisms could also be helped by the wider diameter of this foramen.

In the present case, the tooth was retreated twice. *P. acnes*, isolated from the fistulous tract, is a microorganism associated with endodontic failure (Sjögren *et al*. 1988, Sunde *et al*. 2000), extra-radicular infection (Sunde *et al*. 2000) and bacteraemia (Debelian *et al*. 1992).

A fistula tract is a communication between the internal and external environments. This opening can supply nutrients and more resistant species to the internal microbial niche. Some endodontic failures present communications between the periapical region and the oral mucosa, such as periodontal pockets and sinuses. These communications can be fundamental for the maintenance of periapical infection (Nair 1997).

Trypsin was used on the resected root end specimen to digest and remove the periodontal ligament in order to facilitate the visualization of microbial structures. Because of the

fibrillar nature of the soft tissue covering the root, filamentous organisms may have been overlooked. SEM examination showed fungal shapes, cocci isolated or in chains, and rods, in agreement with the images shown by Sen *et al*. (1995). The present data also agree with the findings of Lomçali *et al*. (1996), especially regarding the presence of a bacterial 'net' (bacterial by-products). This 'net' enveloped the entire lingual foramen and the surrounding region, as part of the microbial biofilm and appeared to be a metabolic product or a polysaccharide providing protection to the microorganisms.

An antibiotic was not administered to the patient in order to eliminate the periapical infection because it has been shown that biofilm-associated microorganisms are 160 times more resistant than their planktonic forms (Wright *et al*. 1997, Chandra *et al*. 2001).

Inevitably, SEM analysis of necrotic teeth with periapical lesions will tend to show microbial shapes near the apical foramina, in the dentine and cement, internally or externally. However, the simple presence of bacteria does not mean that infection will not be eliminated because a number of factors are necessary for their permanence and resistance to endodontic treatment and antibiotic therapy. In the present case, it is impossible to be sure that the microbiological mass was a true biofilm, but all the data confirm an endodontic failure caused by microbial activity.

Conclusions

Persistent extraradicular infections are not affected by the action of antimicrobial agents such as irrigants and medicaments used during root canal treatment. Apical surgery may be the only method for definitive removal of an established extra-radicular infection, promoting repair in therapy-resistant cases. New endodontic procedures able to decompose possible periapical bacteria plaque in persistent periapical infections need further investigation.

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