Histologic Investigation of Root Canal–treated Teeth with Apical Periodontitis: A Retrospective Study from Twenty-four Patients

Domenico Ricucci, MD, DDS,* José F. Siqueira Jr, DDS, MSc, PbD,‡ Anna L. Bate, BDS, MSc,‡ and Thomas R. Pitt Ford, BDS, PbD, FDS§

Abstract
This study intended to examine histologically root canal–treated teeth evincing apical periodontitis lesions and correlate the findings with clinical observations. Specimens were obtained from 24 patients (12 asymptomatic and 12 symptomatic) by extraction or endodontic surgery and consisted of roots or root tips and the associated pathologic lesion. Specimens were processed for histologic analysis, and serial sections were evaluated. Findings were correlated with clinical observations according to the presence or absence of symptoms. The mean period elapsed from treatment to specimen retrieval in the asymptomatic group was 7.5 years, as compared with 2.2 years in the symptomatic group. All specimens exhibited periapical inflammation. Bacteria were visualized in all cases, except for 1 specimen from the asymptomatic group in which a foreign body reaction to overfilled material was the probable reason for emergent disease in a previously vital case. Irrespective of the presence of symptoms, bacteria were always located within the root canal system, although they were also observed in the periapical tissues in 1 asymptomatic and 4 symptomatic teeth. In general, intraradicular bacterial colonization was heavier in symptomatic failed teeth. The present findings support the role of intraradicular infections, usually in the form of biofilms, as the primary cause of endodontic treatment failure. (J Endod 2009;35:493–502)

Key Words
Apical periodontitis, biofilm endodontic treatment failure, extraradicular infection, intraradicular infection

Microbial colonization of the root canal system has been shown to be the prime cause of apical periodontitis (1). Therefore, the ultimate goal of the endodontic treatment is to eliminate microorganisms present in the root canal system, remove disintegrated pulp tissue that could serve as substrate for microbial growth, and fill the endodontic space to preclude bacterial recolonization, so as to prevent apical periodontitis or allow its resolution. The primary cause of post-treatment apical periodontitis is acknowledged to be the continuing presence of bacteria within the root canal system (2–6). However, there are no consistent reports correlating the histopathologic picture with different clinical conditions in teeth with persistent disease.

In recent years, growing importance has been placed on extraradicular infection as a cause of persisting apical periodontitis (7–9). Whereas it has been suggested that bacteria of the genera Actinomyces and Propionibacterium might become established and survive in the inflamed periapical tissues, preventing healing (5, 10, 11), their occurrence as a pathologic entity independent of intraradicular infections has been recently called into question (12). Moreover, the presence of other bacterial species in the periapical tissues and their being a possible cause of persistent apical periodontitis are still a matter of debate. The results of culture studies on pathologic periapical tissues (7, 9) along with molecular techniques (8) and scanning electron microscopy (SEM) studies of external root surfaces of teeth with post-treatment disease (13) have reinforced the view that bacterial occurrence beyond the apex of teeth with chronic apical periodontitis lesions might be more common than previously believed (14). However, it remains to be elucidated whether extraradicular bacteria are actually establishing an extraradicular infection, or they are merely an advanced front of the intraradicular infection, being maintained by the latter. Also, the possibility exists that extraradicular bacteria are more related to symptomatic cases, but it remains to be proved.

It has been suggested that some nonmicrobial endogenous or exogenous factors might cause persistent post-treatment disease (5). Endogenous causes purportedly include cholesterol crystals and true cysts. Among the possible nonmicrobial exogenous causes of persisting apical periodontitis are foreign body reactions caused by extruded endodontic filling materials (15, 16). The successful outcome of root canal treatment is reported to be considerably lower in cases with overfilling, compared with those in which the root filling was confined within the root canal (17, 18). Although this might be related to foreign body reaction, a concomitant microbial infection cannot be disregarded in most cases (19).

The purpose of this retrospective study was to examine histologically root canal–treated teeth presenting with apical periodontitis and correlate the findings with clinical observations.

Material and Methods
The material for this study consisted of sequential biopsies of roots or root tips, together with surrounding pathologic periapical tissues, from root canal–treated teeth in each of 24 patients. Specimens were part of the histologic collection of one of the authors (D.R.). Cases were selected from 12 consecutive patients evincing asymptomatic post-treatment disease and another 12 consecutive patients exhibiting
Symptomatic disease. Hence, an attempt was made to include equal numbers of specimens in each group, and biases were reduced by including consecutive patients for each clinical condition. In the asymptomatic group, teeth had never demonstrated symptoms during the entire postoperative period, and the only sign of disease was a detectable periradicular radiolucency. In the symptomatic group, teeth presented postoperative signs and/or symptoms. These teeth were subjected to a retrospective analysis. All patients gave consent for analysis.

The recall period was at least 1 year for the symptomatic cases and 4 years for the asymptomatic cases, because no case with residual radiolucency should be assessed as failure before a 4-year observation period, unless the lesion increases in size or signs and symptoms of infection arise (20). All selected cases exhibited apical periodontitis diagnoses on the basis of strict clinical and radiographic criteria. There was no filtering mechanism in the selection of cases, except that they represented consecutive cases of each condition that were deemed as treatment failures. Sixteen of the cases were treated by one of the authors (D.R.) in a private endodontic practice during the period from 1983–2006. The remaining 8 cases were treated by 3 experienced endodontists. Complete documentation was available for each case, including initial diagnosis, endodontic procedures, and information on the status of the tooth at review.

At the initial appointment and at subsequent recalls a thorough clinical and radiographic evaluation was made of each case. A record was made of any signs or symptoms that the patient had experienced or was currently experiencing. In the clinical examination, documentation was made as for the presence or absence of swollen lymph nodes, asymmetry or swelling of facial or oral tissues, tenderness to percussion or sensitivity to palpation on the mucosal tissues, sinus tract, grade of mobility, fracture lines, caries, restorative status, periodontal probing depth, discoloration of the tooth, and whenever necessary, electric and thermal pulp sensitivity tests were carried out. Radiographs were taken with periapical films mounted in a beam-aiming device in an effort to obtain projections at a 90-degree angle. Retrospective examination of each radiograph was undertaken by 2 observers jointly in a darkened room with magnification; the radiograph was surrounded by a dark mask. The examiners were blinded to the clinical observations. The presence of a radiolucency and its extent were noted. The presence or absence of unfilled root canal space was also recorded. The apical level of the obturation was recorded as ideal (0–2 mm from the radiographic apex), flush (not ideal), too short (>2.0 mm from the radiographic apex), and too long (Table 1).

Seventeen biopsies consisted of the root tip and surrounding pathologic tissue that had been removed surgically (1 root per tooth). For each of these cases, a mucoperiosteal flap was raised, and cortical bone was removed to access the root apex and the pathologic tissue. The apex was cut by using a thin fissure bur in a handpiece with water coolant. Great care was taken to remove the root tip with the apical periodontitis lesion attached. The other 7 teeth were extracted either because the tooth was unrestorable (as a result of extensive coronal caries or fracture) or because the patient did not wish to undergo further endodontic retreatment. In all these teeth the lesion had remained attached to the apex after extraction. There were 7 multi-rooted teeth showing a lesion on 1 root; 4 of these were extracted, and only the respective root with the lesion was included in the study. The remaining 3 molars were subjected to apicectomy; only the respective root showing a lesion was included in the study.

### Tissue Processing

Immediately after removal (by endodontic surgery or extraction), the biopsy specimen was immersed in 10% neutral buffered formalin for at least 48 hours. Demineralization was carried out in an aqueous solution consisting of a mixture of 22.5% (vol/vol) formic acid and 10% (wt/vol) sodium citrate for 3–4 weeks, with the end point being determined radiographically. All specimens were washed in running tap water for 24–48 hours, dehydrated in ascending grades of ethanol, cleared in xylene, infiltrated, and embedded in paraffin (melting point, 56°C) according to standard procedures. With the microtome set at 4–5 μm, meticulous longitudinal serial sections were taken until each specimen was exhausted. Every fifth slide was stained with hematoxylin-eosin for screening purposes and for assessment of inflammation. A modified Brown and Brenn technique for staining bacteria (21) was used for selected slides. The accuracy of the bacterial staining method was tested by using the protocol described by Ricucci and Bergenholtz (22). Slides were examined under the light microscope by 2 evaluators. Evaluations were performed separately, and whenever disagreement occurred, it was resolved by joint discussion.

The following were specifically looked for in the histologic examination: 1) presence and location of bacteria: none; sparse, planktonic distribution; well-defined biofilm (small colonies); well-defined biofilm (large colonies); and 2) presence and distribution of acute and chronic inflammatory cells: polymorphonuclear leukocytes (PMNs) in contact with biofilm (yes/no); chronic inflammatory cells.

The most severe infection was recorded rather than the mean of a number of observations. In addition, the presence of PMNs was recorded even if the majority of the inflammatory lesion was composed of chronic cells.

### Results

No specimens were lost during histologic processing. Although some occasional sections were lost during the cutting process, neighboring sections allowed the full picture to be seen. Some artifacts were observed in a few cases as a result of partial detachment of soft tissue from the root surface during the surgical procedure. However, these artifacts have not significantly interfered with the overall histologic assessment.

Where microorganisms were demonstrated, various different morphotypes were noted: cocci, rods, and filamentous forms.

### TABLE 1. Summary of Clinical Observations

<table>
<thead>
<tr>
<th>Type of case</th>
<th>N</th>
<th>Initial clinical diagnosis</th>
<th>Preoperative radiographic periradicular status</th>
<th>Radiographic apical limit of fillings</th>
<th>Observation period (y)</th>
<th>Radiographic periradicular status at time of biopsy</th>
<th>Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>12</td>
<td>1 vital, 9 necrotic, 2 root-filled</td>
<td>1 no lesion, 11 lesion</td>
<td>11 ideal, 1 too long</td>
<td>Mean, 7.5; range, 4-19</td>
<td>1 emerged, 3 no change, 8 decreased</td>
<td>1 emerged, 3 no change, 8 decreased</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>12</td>
<td>9 necrotic, 3 root-filled</td>
<td>12 lesion</td>
<td>10 ideal, 2 too long</td>
<td>Mean, 2.2; range, 1-7</td>
<td>9 no change, 3 increased</td>
<td>10 surgery, 2 extraction</td>
</tr>
</tbody>
</table>

494 Ricucci et al. JOE — Volume 35, Number 4, April 2009
Asymptomatic Group

Of the 12 asymptomatic teeth (Table 1), one had been originally diagnosed as having a vital pulp and treated in a single visit, 9 presented necrotic pulps, and the other 2 teeth were retreatment cases. In all these teeth there was radiographic evidence of post-treatment apical periodontitis before biopsy. Of the 11 cases initially with necrotic pulp or treated canals, 2 had been treated in a single visit, and the other 9 had been obturated after 1 or more sessions of intracanal medication. The tooth types included in this group were as follows: 7 incisors, 1 single-rooted premolar, and 4 molars. Patients were aged between 21 and 64 years (mean, 36.4 years).

In 8 of the 12 cases, radiographic lesions had reduced in size during the follow-up period, but radiolucencies were still evident at the time of biopsy-taking. In 3 cases, the radiographic status remained unchanged. In the previously vital case, a lesion had emerged at follow-up.

Bacteria were found in 11 teeth (Table 2). In these cases, they were usually in small numbers and were mainly located intraradicularly, specifically in the main canal (Fig. 1f, g), apical ramifications, lateral canals, isthmi, and/or dental tubules (Fig. 1b) (Table 3). In 3 cases, bacterial colonies were visualized at the foraminal area and were bordered by a concentration of acute inflammatory cells. In one of these teeth, a maxillary lateral incisor, in which the original apical periodontitis lesion had considerably reduced in size after 10 years, a large bacterial biofilm was present at the root canal exit apical to the filling material and against a large accumulation of PMNs (Fig. 2d–f). In one case, bacteria were seen beyond the apical foramen as a small colony in a resorption lacuna of cementum not far from the foraminal area. In this case, bacteria were also present within the root canal and inside dental tubules.

In the root with no detectable bacteria, a large amount of root canal filling material had extruded to the periapical tissues; there was evidence of a foreign body reaction, characterized by severe chronic inflammation with dominance of macrophages, lymphocytes, plasma cells, foam cells, and foreign body cells, surrounding the extruded filling material, dentin chips, and necrotic debris. This particular tooth was the one presenting a vital pulp at the time of treatment.

All cases exhibited periapical inflammation, but a distinct collection of PMNs adjacent to bacterial aggregates was evident only in 3 cases (Table 2) (Fig. 2f).

Symptomatic Group

In the symptomatic group (Table 1), 9 teeth had been originally diagnosed as having necrotic pulps, and the other 3 teeth were treated cases indicated for retreatment. Nine teeth had been treated in multiple visits and 3 in a single visit. At the time of biopsy, 6 teeth presented with spontaneous pain, mobility, and tenderness to percussion, 2 teeth showed an acute abscess with severe swelling and lymphadenopathy, and the other 4 teeth were associated with a sinus tract each. All teeth exhibited apical radiolucencies. The types of teeth included in this group were as follows: 5 incisors, 1 canine, 3 single-rooted premolars, and 3 molars. Patients were aged between 26 and 50 years (mean, 34.8 years). During the follow-up period, in 9 of the 12 cases the size of the radiographic lesion remained unaltered, and in 3 cases the lesion size increased (Table 1).

Bacteria were found in all symptomatic teeth (Table 2). In the majority of cases (8 teeth), they were observed only intraradically, specifically in the main canal, foraminar area (Fig. 3e, f), apical ramifications (Figs. 3d–g and 4d–g), lateral canals, isthmi, and/or dental tubules (Table 4). In contrast to the asymptomatic group, in most cases bacteria were observed in large numbers (Table 2), forming dense biofilms (9 cases) that in some specimens filled the entire lumen of apical ramifications (Fig. 3f, g) and extended beyond the root canal limits (Fig. 3e, f). In one case, a maxillary canine, whose pulp chamber had been exposed to caries for a long period before the root canal treatment was completed 1 year previously, bacteria were seen in the cytoplasm of large vegetable cells that had been packed into the foraminar area during instrumentation. In 4 cases, bacteria were not only found within the root canal system but also extending extraradicularly (ie, beyond the apical or lateral foramen); in 1 tooth, apical ramifications were clogged with bacterial aggregates, which were adhered to the ramification walls, forming biofilms. These bacterial aggregates were contiguous to large colonies located within the periapical tissues, which in turn displayed the typical ray fungus appearance of actinomycotic colonies. A large number of PMNs was surrounding the actinomycotic colonies. This case has been reported separately previously (12). In another tooth, a maxillary premolar with a long-lasting preoperative sinus tract, calculus-like material was found covering the root surface on the palatal aspect; numerous bacterial profiles were seen in the deepest part of this structure. This case has also been described elsewhere (23).

Every tooth from this group was associated with PMN accumulation at the vicinity of the biofilms and chronic inflammatory cells at a distance from these bacterial organizations.

**TABLE 2. Summary of Histologic Findings**

<table>
<thead>
<tr>
<th>Type of case</th>
<th>N</th>
<th>Bacteria located only within root canal system</th>
<th>Bacteria located both within and outside root canal system</th>
<th>Bacterial quantity</th>
<th>PMNs in contact with biofilm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>12</td>
<td>11 yes, 1 no</td>
<td>10</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>12</td>
<td>12 yes</td>
<td>8</td>
<td>4</td>
<td>12</td>
</tr>
</tbody>
</table>

Discussion

This study sought to contribute to the understanding of the etiology of endodontic treatment failures by investigating equal numbers of symptomatic or asymptomatic root canal–treated teeth with post-treatment apical periodontitis. The clinical recording followed established guidelines (20), and 2 experienced observers, both blinded to the clinical and histologic diagnoses, examined the radiographs jointly. Serial sections of the teeth and associated lesions were prepared and also examined by 2 evaluators in order not to miss the presence of bacteria, which were stained by an appropriate technique (21). The reliability of this histologic technique has been reported previously (22, 24). The Brown and Brenn staining method for bacterial detection in tissue sections has a history of being insensitive, particularly with regard to observation of gram-negative bacteria (22, 25). Although this might lead to some oversight of gram-negative bacteria in sections, such...
Figure 1. Asymptomatic case. (A) Tooth 10 in a 38-year-old woman had necrotic pulp and an apical periodontitis lesion. She reported no symptoms, and both percussion and palpation tests were negative. After working length determination, the canal was instrumented manually in the apical third up to a K-file size 60, and with Gates-Glidden burs in the coronal two thirds. Abundant irrigation was performed with 1% NaOCl after each instrument. The tooth was treated during 2 visits and restored with a ceramo-metallic crown after post and core build-up. (B) Follow-up radiograph taken after 4 years showing that the lesion had reduced in size but was still present. Tooth was asymptomatic. (C) Residual radiolucency was still evident after 19 years. Tooth had remained asymptomatic for the whole period. Percussion and palpation always gave negative responses. Clinical and radiographic signs of recurrent distal caries were now present, and a new restoration had become indicated. Apical surgery was scheduled. The root tip was removed with the pathologic periradicular tissue attached, which was located mostly on the palatal side. A root-end cavity was prepared, and a filling was inserted (not shown). (D) Section passing approximately at the center of the root canal. Resorption in the foraminal area is evident. Although on the radiographs the root canal filling seemed confined within root canal limits, it actually extended beyond. There is severe chronic inflammation at the material/tissue contact area (hematoxylin–eosin stain; original magnification ×25, inset ×400). (E) Section passing in the same area as that shown in (D) (Taylor’s modified Brown & Brenn, original magnification ×25). (F) Magnification of the area indicated by the arrow in (E). Small bacterial colonies are visualized between the filling material and the root canal wall (Taylor’s modified Brown & Brenn, original magnification ×400). (G) Another section where small bacterial colonies are apparently “entombed” between the material and the wall (Taylor’s modified Brown & Brenn, original magnification ×1000). (H) Bacterial colonization deep within dentin tubules (Taylor’s modified Brown & Brenn, original magnification ×100, inset ×1000).
limitation might not have been so crucial, because gram-positive bacteria dominate in studies of previously treated root canals (26–28). Bacterial staining in general exhibited excellent performance (Figs. 1–4), because it was even able to discriminate between bacterial cells and sealer particles.

The prevalence of bacteria by using the present technique was higher than that reported previously by correlative light/transmission electron microscopy (2). This is probably because a specific stain for bacteria was used in the present study, and the laboratory approach was performed with particular care not observed in previous studies.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Bacteria in tubules</th>
<th>Bacteria in main canal</th>
<th>Bacteria in lateral canals</th>
<th>Bacteria inside, short of, or at apical foramen</th>
<th>Bacteria outside, beyond the apical foramen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>No</td>
<td>Small colonies</td>
<td>Small colonies</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Large colonies</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>Planktonic</td>
<td>Large colonies</td>
<td>Large colonies</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>Large colonies</td>
<td>No</td>
<td>Large colonies</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>No</td>
<td>Small colonies</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>Small colonies</td>
<td>No</td>
<td>Small colonies</td>
<td>Small colonies</td>
</tr>
<tr>
<td>7</td>
<td>Yes</td>
<td>Small colonies</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>Yes</td>
<td>Small colonies</td>
<td>No</td>
<td>Large colonies</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Large colonies</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>No</td>
<td>Small colonies</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

**Figure 2.** Asymptomatic case. (A) Tooth 10 in a 40-year-old patient with necrotic pulp and apical periodontitis lesion. No symptoms were present. The canal was obturated after 2 weeks of Ca(OH)₂ medication. The tooth was then restored with a cast post and a ceramo-metallic crown. (B) Three-year follow-up radiograph showing a conspicuous reduction in size of the lesion, but a residual radiolucency was evident. Tooth was symptom-free. (C) Patient presented 10 years after completion of treatment, with loss of the coronal restoration. Clinical inspection revealed recurrent caries. A radiograph demonstrated that the apical radiolucency had remained the same size. Although the tooth was still apparently restorable, the patient opted for extraction. (D) Section passing through an oblique foramen, short of the apex. The lesion had remained attached to the apex (Taylor’s modified Brown & Brenn, original magnification ×25). (E, F) Magnifications of the foramen area show large bacterial aggregations present intermixed with filling material and faced against a severe concentration of PMN leukocytes (Taylor’s modified Brown & Brenn, original magnification ×100 and ×400).
Figure 3. Symptomatic case in a 30-year-old patient. (A) A sinus tract had been present for 4 years buccal to tooth 14. The patient’s dentist had carried out root treatment and placed a provisional crown 6 months previously, but a permanent restoration was not placed because a sinus tract was still present. Endodontic retreatment was carried out in 1 visit; a fourth canal was found and accessed. After provisional restoration, instructions were given for the patient to return to the general dentist for placement of post and core. (B) Follow-up radiograph taken after 1 year. Radiolucency was unchanged. Sinus tract was still present, and the tooth was tender to percussion and palpation. (C) Root-end resection was performed of both mesiobuccal and distobuccal roots. Root-end cavities were prepared and filled with mineral trioxide aggregate. (D) Overview of the mesiobuccal root tip. Three ramifications are present. Soft tissue does not appear in close contact with the root tip. This is an artifact caused by partial detachment during surgical procedure (Taylor’s modified Brown & Brenn, original magnification ×25). (E) Magnification of the 2 ramifications on the left in (D) showing bacteria (Taylor’s modified Brown & Brenn, original magnification ×100). (F) The most coronal ramification is filled with a large bacterial biofilm extending to the external root surface (Taylor’s modified Brown & Brenn, original magnification ×200). (G) Ramification on the right side in (D). Its lumen is filled with a large biofilm arranged against inflammatory cells (Taylor’s modified Brown & Brenn, original magnification ×400).
Figure 4. Symptomatic case. (A) A 24-year-old patient complained of spontaneous pain and tenderness to mastication on tooth 14, which exhibited deep carious lesion. The radiograph showed radiolucencies around the buccal root apices. After isolation, caries removal, and access preparation, 3 canals were found and instrumented. Despite meticulous search under operating microscope, the orifice of a possible fourth canal was not found. (B) After a period of Ca(OH)$_2$ medication for 2 weeks, during which symptoms disappeared, the canals were obturated with cold gutta-percha laterally compacted and a sealer. The crown was restored with composite materials. (C) Patient returned 1 year later, complaining of spontaneous pain. Tooth was tender to percussion and palpation. Radiograph showed that the radiolucency around the mesiobuccal root apex had increased. Apicectomy was scheduled, and the root tip was cut and removed together with the periradicular pathologic tissue. (D) Section of the mesiobuccal root apex cut following a buccolingual plane. The overview shows the treated canal and a complex anatomy present instead of the fourth canal. These irregular spaces are clogged with bacteria (Taylor’s modified Brown & Brenn, original magnification $\times$12.5). (E) Detail of the ramifications in (D) (Taylor’s modified Brown & Brenn, original magnification $\times$25). (F) Magnification of the ramifications contents in the area indicated by the arrow in (D). Calcification free in the lumen and a large bacterial biofilm (Taylor’s modified Brown & Brenn, original magnification $\times$100). (G) Detail of the calcification in (F) showing resorption lacunae filled with a bacterial biofilm (Taylor’s modified Brown & Brenn, original magnification $\times$400).
with similar technique. In the light of the results (bacteria found in all cases except one in which a foreign body reaction to a gross overfilling was observed), the alleged superiority of the correlative light/transmission electron microscopy (5) in revealing bacterial presence is so only if compared with old studies in which laboratory procedures were performed according to less than ideal quality standards. However, the correlative technique should be indicated when cell ultrastructure needs to be investigated.

The prevalence of bacteria in root canal–treated teeth with apical periodontitis as revealed by the technique used herein was also higher than that reported by culture techniques (26, 28). It has been well-established that limitations of the culturing technique might underestimate bacterial prevalence and diversity in diverse environments including treated root canals (29). Nevertheless, the present results are in clear agreement with molecular studies that have demonstrated that post-treatment apical periodontitis is almost always associated with intraradicular polymicrobial infection (29, 30).

Bacteria were not visualized in only 1 asymptomatic tooth. This case presented a vital pulp at the time of initial treatment, and an apical periodontitis lesion emerged to become visible 1 year after treatment. After 4 years it was unchanged, and apicoectomy was performed. Although bacterial presence might have been overlooked because of the method’s limitations discussed above, accumulations of PMNs typical of bacterial infections were not observed, and the treatment apparently failed because of a foreign body reaction associated with large PMNs in 3 cases. In a maxillary lateral incisor in which the apical foramen was filled with similar technique, in the light of the results (bacteria found in all cases except one in which a foreign body reaction was observed), the alleged superiority of the correlative light/transmission electron microscopy (5) in revealing bacterial presence is so only if compared with old studies in which laboratory procedures were performed according to less than ideal quality standards. However, the correlative technique should be indicated when cell ultrastructure needs to be investigated.

The prevalence of bacteria in root canal–treated teeth with apical periodontitis as revealed by the technique used herein was also higher than that reported by culture techniques (26, 28). It has been well-established that limitations of the culturing technique might underestimate bacterial prevalence and diversity in diverse environments including treated root canals (29). Nevertheless, the present results are in clear agreement with molecular studies that have demonstrated that post-treatment apical periodontitis is almost always associated with intraradicular polymicrobial infection (29, 30).

Bacteria were not visualized in only 1 asymptomatic tooth. This case presented a vital pulp at the time of initial treatment, and an apical periodontitis lesion emerged to become visible 1 year after treatment. After 4 years it was unchanged, and apicoectomy was performed. Although bacterial presence might have been overlooked because of the method’s limitations discussed above, accumulations of PMNs typical of bacterial infections were not observed, and the treatment apparently failed because of a foreign body reaction associated with large PMNs in 3 cases. In a maxillary lateral incisor in which the apical foramen was filled with similar technique.

### TABLE 4. Bacterial Distribution in Symptomatic Cases

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Bacteria in tubules</th>
<th>Bacteria in main canal</th>
<th>Bacteria in lateral canals</th>
<th>Bacteria inside, short of, or at the apical foramen</th>
<th>Bacteria outside, beyond the apical foramen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>Large colonies</td>
<td>Large colonies</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>No</td>
<td>Small colonies</td>
<td>Large colonies</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>Small colonies</td>
<td>Small colonies</td>
<td>Large colonies</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Small colonies</td>
<td>Small colonies</td>
<td>Large colonies</td>
<td>Large colonies</td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>No</td>
<td>Large colonies</td>
<td>Large colonies</td>
<td>Large colonies</td>
</tr>
<tr>
<td>6</td>
<td>No</td>
<td>No</td>
<td>Large colonies</td>
<td>Large colonies</td>
<td>Large colonies</td>
</tr>
<tr>
<td>7</td>
<td>No</td>
<td>No</td>
<td>Large colonies</td>
<td>Large colonies</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>Yes</td>
<td>Large colonies</td>
<td>No</td>
<td>Large colonies</td>
<td>Large colonies</td>
</tr>
<tr>
<td>9</td>
<td>Yes</td>
<td>No</td>
<td>Large colonies</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>No</td>
<td>Small colonies</td>
<td>Large colonies</td>
<td>Large colonies</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>Yes</td>
<td>No</td>
<td>Large colonies</td>
<td>Large colonies</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>No</td>
<td>Small colonies</td>
<td>No</td>
<td>Large colonies</td>
<td>No</td>
</tr>
</tbody>
</table>

It is important to point out that the fact that a lesion decreases in size during the first 6–12 months after treatment does not automatically mean that the lesion is going to heal completely at longer follow-up periods. International guidelines establish that root canal treatment outcome is interpreted as unfavorable when an apical periodontitis lesion has remained the same size or has only diminished in size during a 4-year assessment period (20). In the present study, several lesions showed diminished diameters, but even after a long follow-up period (4–19 years), they were still radiographically detectable. Because the size of apical periodontitis lesion is correlated to the diversity and density of bacteria in the root canal (1), the probable reason for initial decrease in size was reduction in the load of bacterial irritants within the canal by treatment procedures. A short-term follow-up evaluation would erroneously interpret those lesions as healing. However, the present study indicates that persistence and stabilization of the lesion after long-term evaluation indicate that bacteria were not completely eradicated or at least reduced to levels compatible with complete peri-radicular healing (31).
The symptomatic cases all evinced lesions before root canal treatment and still had them, of the same size or larger, at the time of biopsy. Intervention on all these teeth was undertaken because of ongoing symptoms. The follow-up period ranged from 1–7 years, with a mean of 2.2 years, which is relatively short in comparison with the average life expectancy of endodontically treated teeth. All teeth in this group were shown to harbor intraradicular bacteria. Bacterial numbers were clearly larger when compared with asymptomatic cases. In 4 of these teeth, bacteria were also found beyond the boundaries of the apical foramen, ie, within the periradicular tissues. Symptomatic cases are generally associated with periodic exacerbations, and therefore it comes as no surprise that bacteria were seen in all symptomatic cases, including outside the root canal in some cases. Indeed, a higher frequency of extraradicular bacteria should be expected, but the results that they were not so commonly detected beyond the canal limits might be explained by the fact that no case was operated or extracted during the acute phase of the disease, and that antibiotics were prescribed in some cases until symptoms subsided. Five cases (1 in the asymptomatic group and 4 in the symptomatic group) showed the extraradicular occurrence of bacteria in addition to the intracanal infection. Bacteria outside the confines of the root canal system were seen on the external root surface in 4 cases and within an abscess cavity in the other. All these situations in which bacteria were visualized extraradicularly were clearly distinct from possible bacterial contamination that might occur during biopsy-taking procedures. Bacteria were present in large numbers in calculus-like deposits adhered to the external root surface (23), in an abscess cavity contiguous to apical ramifications clogged with bacteria (12), and in resorption lacunae, always faced with PMNs. At the same time, it was easy to distinguish between bacterial colonies actually located within the tissue and surrounded by a severe concentration of inflammatory cells (Fig. 2d–f) and bacteria merely contaminating the external surface of an apical periodontitis lesion, because the latter appear as scattered cells not related to inflammation (24). Among the teeth that were extracted because they were deemed unrestorable, caries had in some instances exposed the root filling, or the latter had been exposed for some time to oral biofilms (Fig. 2c). A possible objection could be that bacteria might have penetrated into the canal through the root filling, reaching the apical third. Although histologic sections demonstrated large quantities of bacteria in associated caries lesions, they were not observed in the middle third of the root canal, suggesting that bacteria in the apical third probably did not come from coronal leakage. A recent histologic study demonstrated that optimally prepared and filled root canals held up well against bacterial penetration, even on frank and long-standing oral exposure by caries, fracture, or loss of restoration (22). Histologic examination cannot identify bacterial species or determine their virulence, but the technique’s strength resides in revealing spatial location. In a number of cases from both groups, bacterial colonies were present within apical ramifications, which could not be reached by instruments, and where irrigants or medicaments would have restricted access. These findings are in agreement with Nair et al (32), who showed that 14 of 16 root canal–treated teeth revealed residual intracanal infection in inaccessible areas after instrumentation, antimicrobial irrigation, and obturation. The fact that virtually all failed cases harbored an intraradicular infection underlines the difficulty of adequately disinfecting root canals with current techniques and substances before obturation. These findings also demonstrated that root canal obturation fails to entomb residual bacteria in the root canal system and then prevent their access to the periradicular tissues to induce or maintain disease. The present results indicate that bacterial persistence in the root canal in areas not affected by treatment is the major cause of post-treatment apical periodontitis. Nevertheless, it must be pointed out that bacteria persisting in the root canals after instrumentation or intracanal medication will not always cause failures, because some lesions can heal even when bacteria are found in the canal at the time of filling (33, 34). Explanations for this might be that residual bacteria 1) die after filling because of the antibacterial activity of filling materials, access denied to nutrients, or disruption of bacterial interactions; 2) are present in subcritical counts to maintain inflammation; or 3) are located in areas with no access to the periradicular tissues (35). Actually, bacteria that endure endodontic procedures can influence the treatment outcome provided they 1) withstand periods of famine, 2) resist to disturbances in the community ecology, 3) reach sufficient numbers to wreak havoc on the host, 4) have frank access to the periradicular tissues, and 5) produce and release virulence factors at enough concentrations to harm the periradicular tissues (35).

Conclusions

Of the 24 root canal–treated teeth with apical periodontitis investigated in this study, bacteria were not found only in one. This strongly confirms the essential role of intraradicular infections as the primary cause of post-treatment disease. The intraradicular occurrence of bacteria was basically similar in both asymptomatic and symptomatic treated teeth, but larger numbers were evident in symptomatic teeth. Although not very common, extraradicular occurrence of bacteria was more frequent in the symptomatic group.

References


Clinical Research

Histologic Investigation of Endodontic Failures 501