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Residual bacteria in root apices removed by a diagonal root-end resection: a histopathological evaluation

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Abstract

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Aim To assess bacteria in the apical portion of the root end after 45° root-end resection in teeth with persistent periapical lesions.

Methodology The study included 27 apical root segments from patients with persistent periapical lesions. Histological sections of the coronal part of the amputated root segment were stained with Brown and Brenn to detect the presence of bacteria in the main root canal and/or in irregular root spaces and dentinal tubules. The quality of each root canal filling was evaluated using preoperative radiographs of filling, length of root filling as assessed from the distance between its apical end and the radiographic apex, diameter of apical preparation, and presence of apical perforations or deviations from the root canal. Two endodontists, blinded to the bacteriological results, independently evaluated the radiographs.

Results Bacteria were present in 23 (85.2%) specimens: five in only the main canal (21.7%), 10 in only the dentinal tubules and irregular spaces (43.5%), and eight in both irregular spaces and dentinal tubules and in the main root canal (34.8%). No correlation was found between the technical quality of the root filling assessed radiographically and bacterial presence in the central canal or irregular areas.

Conclusions Infected irregular areas were found in the root tips of teeth with persistent periapical lesions. This was found regardless of the radiographic quality of the root filling. Diagonal, 45° root-end resection may expose such contaminated irregularities to the periapical tissue.

Keywords: brenn stain, brown, dentinal tubules, root-end resection.

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Introduction

The relationship between bacteria in the root canal and periapical pathosis has been established (Kakehashi *et al.* 1966, Sundqvist 1992). In an attempt to eliminate bacteria from the root canal system, mechanical instrumentation, irrigatant solutions and medicaments are used. As mechanical preparation is limited to the central main canal, bacteria cannot be eliminated from either dentinal tubules or root canal irregularities, such as isthmuses, apical delta or lateral canals. The use of intracanal medicament substantially decreases the amount of bacteria in canal irregularities after mechanical preparation and irrigation (Haapasalo & Ørstavik 1987, Fuss *et al.* 2002, Lin *et al.* 2003, 2005). Nevertheless, residual infection in the root canal and in irregular areas may be present and thus prevent healing (Sjögren *et al.* 1997, Nair *et al.* 1999).

Surgical endodontic treatment is recommended for teeth with persistent lesions when nonsurgical root canal treatment is impossible or would not achieve better open ended results (Gutmann 1984, Gutmann &

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Harrison 1985). Treatment includes root resection to remove the apical part of the root that may contain infected irregular areas not disinfected during nonsurgical root canal treatment and sealing of the apical root end. Nevertheless, resection of the root apex may expose to the periapical tissues infected dentinal tubules and canal irregularities included in this part of the root, which may cause endodontic surgery to fail.

Root-end resection was traditionally performed with a bevel of 45°, to allow visualization of the main canal, followed by a root-end cavity preparation of the canal with a round bur. An approximate 60% success rate has been reported (Allen *et al.* 1989, Friedman 1998). With the introduction of the intraoral microscope, root-end resection at 90° replaced the traditional method and root-end apical preparation is performed using an ultrasonic tip to the depth of 3–4 mm. This allowed success rates of over 90% (Rubinstein & Kim 1999, 2002, Tsesis *et al.* 2006).

Even though the traditional 45° resection is not practiced by most endodontists, it is still a common practice in many oral surgery clinics. Furthermore, in many places around the world apical surgery is still commonly performed by oral surgeons with no operating microscope (Rahbaran *et al.* 2001, Schwartz-Arad *et al.* 2003, Tsesis *et al.* 2006).

The purpose of this study was to highlight one of the main drawbacks of this approach by demonstrating bacterial presence in the apical portion of the root end after a 45° root-end resection in teeth with persistent periapical lesions.

Materials and methods

The study consisted of 27 apical segments obtained from a Department of Oral and Maxillofacial Surgery. These samples were taken from 27 patients referred for surgical intervention after clinical diagnosis of a persistent periapical lesion. All patients gave their consent for the procedures. Teeth with pathosis associated with vertical root fractures, coronal perforations or deep (>7 mm) periodontal pockets were excluded. The study included six maxillary and six mandibular molars, five maxillary premolars and 10 maxillary incisors.

The quality of root fillings was evaluated from preoperative radiographs according to radiographic density of filling, length of root filling as assessed from the distance between its apical end and the radiographic apex, diameter of apical preparation, and presence of apical perforations or deviations from the root canal. Each criterion received a score of 1 (adequate) or 0 (inadequate). A total score was calculated for each tooth as maximum of 4 points and minimal 0 points (Table 1). Two endodontists, blinded to the bacteriological results, independently evaluated the radiographs. The case was discussed and defined by the two observers when disagreement occurred.

Surgical procedure

The surgical procedure was undertaken by oral surgeons and was performed as usual. Preoperatively, patients rinsed with 0.2% chlorhexidine for 2 min, and the mucosa was then wiped with a sponge saturated in 0.2% chlorhexidine. A gingival full-thickness flap was raised with one releasing incision. A high-speed bur was used to amputate the exposed 2–3 mm of the apical portion of the root at approximately 45° angle from the long axis of the root. The apex and surrounding tissues were curetted and immediately placed in a sterile test tube containing 10% formaldehyde and preserved for histological evaluation.

Surgical procedures were completed with preparation of root-end cavities using bur and retrograde filling with intermediate restorative material (Caulk Dentsply, Milford, DE, USA), flap re-approximation and sutures. An operating microscope was not used in any of these cases.

Histological procedure

Histological sections were taken in parallel to the resection plane. To orient the sectioning plane and select the optimal sections for identification of bacteria, the initial sections were stained with haematoxylin–eosin and observed under a light microscope (Fig. 1(a,b)). Based on this orientation, the next 4 μm slices were prepared and stained with Brown and Brenn to allow bacterial detection. From each specimen, four of the most coronal sections were selected for examination.

A light microscope, at ×200 and ×1000 magnification, was used to detect bacterial presence in the irregular areas (lateral/accessory canals, fins, isthmuses and dentinal tubules) and in the main root canal.

Statistical analysis

The percentage of specimens in which bacteria were present in the central canal and in irregular areas was calculated. Fisher's exact test was used to determine a

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Table 1 Root-filling quality

Sample number	Tooth (jaw)	Root-filling quality				
		Length	Width	Condensation quality	Apical deviations	Total score
1	M (mand)	1	0	1	1	3
2	M (mand)	1	0	1	1	3
3	M (max)	1	1	1	1	4
4	M (mand)	1	0	1	1	3
5	P (max)	1	1	1	1	4
6	M (max)	1	1	1	1	4
7	M (mand)	0	0	1	1	2
8	I (max)	1	1	0	1	3
9	I (max)	1	0	1	1	3
10	I (max)	1	1	0	1	3
11	M (mand)	1	1	0	0	2
12	M (max)	0	0	0	0	0
13	M (max)	1	0	0	1	2
14	M (max)	1	1	1	1	4
15	P (max)	1	1	1	1	4
16	P (max)	1	1	1	1	4
17	I (max)	1	1	1	1	4
18	I (max)	1	1	1	1	4
19	P (max)	1	0	1	1	3
20	I (max)	1	1	0	1	3
21	M (mand)	1	1	1	1	4
22	I (max)	1	1	1	1	4
23	I (max)	1	1	1	1	4
24	M (max)	1	1	1	1	4
25	P (max)	1	1	1	1	4
26	I (max)	1	1	1	0	3
27	I (max)	1	1	1	1	4

M, molar; P, premolar; I, incisor; mand, mandibular teeth; max, maxillary teeth.

possible relation between bacterial presence in the central canal and their presence in the dentinal tubules. Logistic regression determined whether bacterial presence in the histological specimen, in either the central canal or dentinal tubules, could be related to the quality of the root filling.

Results

Bacteria were present in either the main root canal, or in dentinal tubules and irregular areas in 23 of the 27 specimens (85.2%): five (21.7%) in only the main canal, 10 (43.5%) in only dentinal tubules and irregular areas, and eight (34.8%) in both irregular areas and dentinal tubules as well as in the main root canal (Table 2; Fig. 1(c–e)).

No statistical correlation was found between bacteria in the dentinal tubules and their presence in the central canal ($P = 0.695$).

The average root-filling quality had a score of 3.29 (of 4). According to the logistic regression, no correlation was found between the quality of the root canal filling and bacterial presence in the central canal or irregular areas (Table 3).

Discussion

The objective of root canal treatment is to allow healing of periapical tissues by removing residual necrotic pulp tissue from the canal and disinfecting the central canal and any irregular areas. Teeth with periapical lesions have bacteria present in the root canals (Sundqvist 1992). In chronic apical periodontitis or abscesses, bacteria cause a destructive periapical tissue response because of communication between the periapical tissue and bacterial presence in the main canal or in irregular areas.

The Brown and Brenn stain, widely used to demonstrate bacteria in dentinal tubules, is a modification of Gram staining and allows for clearer bacterial identification. Similar to the Gram stain, bacteria are stained either blue (Gram+) or red (Gram−), with a yellow

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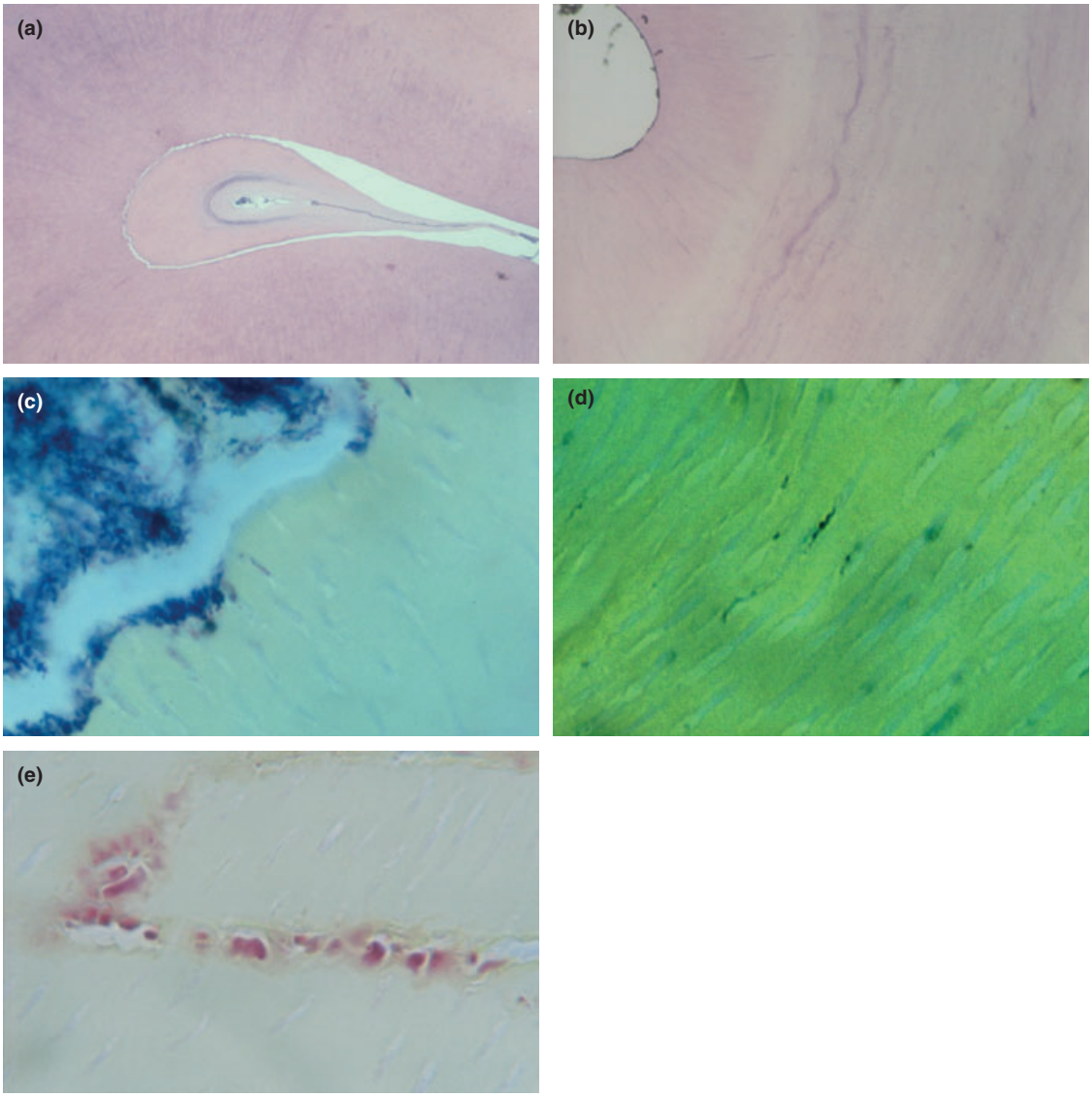

Figure 1 (a) Slice stained with haematoxylin–eosin (H&E) shows the main canal, isthmus, dentin and cementum (×50). (b) Main canal, tubuli in the dentin and the circumferential layers of the cementum can be observed (H&E ×100). (c) Bacteria stained blue in the main canal and in the tubuli orifice facing the main canal [Brown and Bren (B&B) ×400]. (d) Bacteria stained blue in the tubuli orifice facing the periapical tissue after root-end resection (B&B ×400). (e) Bacteria stained red in the tubuli orifice facing the periapical tissue after root-end resection (B&B ×1000).

background to facilitate identifying bacteria in the tissues, whereas in Gram staining, the background is in hues of blue and red. Brown and Brenn stain has detected bacteria in dentinal tubules of extracted bovine and human teeth (Perez *et al.* 1993, Berkiten *et al.* 2000).

Root-end resection is aimed to eliminate residual infection that could be present in the apical part of the root. Nevertheless, this resection may expose infected irregular areas of the apical root canal system and dentinal tubules to the surrounding periapical tissue. The more the beveled resection, the more the dentinal tubules are exposed (Tidmarsh & Arrowsmith 1989). It is well established that resection at a plane perpendicular to the long axis of the root has many advantages and is thus the common procedure amongst

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Table 2 Bacteria present in the main canal or in irregular areas

Sample number	Tooth	Bacteria present in the main canal	Bacteria present in the irregular areas
1	M (mand)	–	+
2	M (mand)	–	+
3	M (mand)	–	+
4	M (mand)	–	+
5	P (max)	–	+
6	M (max)	–	+
7	M (mand)	+	+
8	I (max)	–	–
9	I (max)	+	+
10	I (max)	–	–
11	M (mand)	+	–
12	M (max)	+	+
13	M (mand)	+	–
14	M (max)	–	+
15	P (max)	+	+
16	P (max)	+	+
17	I (max)	–	–
18	I (max)	+	–
19	P (max)	+	–
20	I (max)	+	+
21	M (mand)	+	+
22	I (max)	–	+
23	I (max)	+	+
24	M (max)	+	–
25	P (max)	–	+
26	I (max)	–	+
27	I (max)	–	–

M, molar; P, premolar; I, incisor; mand, mandibular teeth; max, maxillary teeth.

endodontists (Tsesis *et al.* 2006b). An *ex vivo* study has demonstrated that the resection angle of 30° or 45° exposes many more dentinal tubules than a flat cut without any bevel (90-degree cut), there was also a significant increase in leakage as the amount of bevel increased (Gilheany *et al.* 1994). Nevertheless, it is believed that the use of beveled resection is still widely practiced amongst oral surgeons and is the common procedure in many oral surgery departments. The material for the present study was obtained from an oral surgery department in which the traditional oblique bevel is used. A further, parallel, study, using perpendicularly resected root ends is warranted.

The present study revealed that bacteria remained in the dentinal tubules and irregular areas of the resected root apices. It seems plausible that the remaining apical area of the resected root also contained bacteria inside the dentinal tubules and irregular areas of the canal. Bacteria in dentinal tubules and/or irregular areas of dentine in the apical root were obvious in 85.2% of these root-filled human teeth. This high bacterial presence could indicate a possible source of periapical re-infection. Bacteria remaining in either lateral canals or dentinal tubules at the diagonal resection plane probably caused a perpetuation of periapical irritation.

In specimens where bacteria were not identified, the cause for periapical tissue irritation was probably either bacterial presence apically or coronally to the slide level as well as presence of extra-radicular infection (Hirshberg *et al.* 2003, Lin *et al.* 2007). True cystic lesions or the presence of scar tissue may also explain bacterial absence in resected root apices (Nair 2006). Furthermore, bacterial contamination from the exposure of the root tip to the oral environment or the surgical equipment should be taken in consideration as the source of the present bacteria (Hogg & Morrison 2005).

The method used in the present study cannot fully preclude that some of the bacteria found in the sections were introduced during the surgical procedure or the handling of the specimens. However, this is unlikely because of (i) their morphological distribution, as seen in the sections and (ii) they were detected in serial sections and not on the surface of the specimens.

Most of the roots that were included in the present study had radiographically adequate root fillings but also had persistent periapical lesions. This is consistent with the finding that most of these cases had bacteria present in the apical part of their root canal system. There was no correlation between the root-filling quality as seen on the radiographs and bacterial presence in the central canal irregularities and dentinal tubules, which is similar to other findings (Rahbaran

Table 3 Correlation between filling quality and bacterial presence (logistic regression)

Location of bacteria	Root-filling quality effect (β)	Standard deviation (β)	Effect *Pv*	Model *Pv*
Root canal (entire sample)	–0.79	0.54	0.143	0.097
Dentinal tubules (entire sample)	0.12	0.43	0.774	0.773
Only in root canal (out of all samples exhibiting bacteria)	–0.31	0.47	0.513	0.521
Only in dentinal tubules (out of all samples exhibiting bacteria)	0.80	0.60	0.178	0.121
Dentinal tubules and root canal as opposed to all other specimens	–0.399	0.45	0.372	0.362

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et al. 2001, Tsesis *et al.* 2006). This indicates that a radiographically acceptable filling does not necessarily imply thorough disinfection in which bacteria were eliminated from the canal (Kersten *et al.* 1987). It is noteworthy that although impossible to accurately evaluate the adequacy of the canal filling from the two-dimensional radiograph, this is still an acceptable clinical method to asses the quality of root canal treatment (Consensus Report of the European Society of Endodontology 2006).

Conclusions

1. Infected irregular areas were found in the root tips of teeth with persistent periapical lesions. This was found regardless of the quality of the root canal filling, as evaluated from radiographs.
2. Diagonal, 45° root-end resection may expose such contaminated irregularities to the periapical tissue.
3. To further support the above, a similar study with root tips removed by a perpendicular resection is warranted.

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