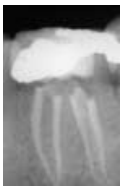

CLINICAL ARTICLE

Bacterial status in root-filled teeth exposed to the oral environment by loss of restoration and fracture or caries – a histobacteriological study of treated cases

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Abstract

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Aim To describe histological and microbiological findings in teeth where root fillings had been exposed to caries and the oral environment for a prolonged period.
Methodology For inclusion in the study, only teeth with a follow-up period of 3 years or more and those that had been without proper restoration for at least a period of 3 months were considered. Some root fillings had been without restoration for several years. In all, 39 roots representing 32 teeth were examined by histology.
Results The majority of the specimens were without a discernible periapical bone lesion as assessed by radiography. Osteolytic lesions were seen with five roots. Longitudinal tissue sections stained with a modified Brown/Brenn staining technique revealed presence of stainable bacteria in abundance at the canal entrance and in dentinal tubules but were absent mid-root and apically in all but two specimens. Soft tissue attached to the root tip and in apical ramifications displayed distinct inflammatory cell infiltrates, suggesting microbial exposure in 7 of the 39 roots examined. In all other specimens, inflammatory cell infiltrates were either nonexistent or sparse and then associated with extruded sealer material.
Conclusions Well-prepared and filled root canals resist bacterial penetration even upon frank and long-standing oral exposure by caries, fracture or loss of restoration.

Keywords: clinical follow up, coronal leakage, endodontics, periapical disease, root-canal treatment.

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Introduction

Numerous reports give support to the view that leakage of bacteria and bacterial elements along margins of restorations and fillings of root canals (e.g. coronal leakage) may lead to failure of root-canal treatment (Saunders & Saunders 1994). Because evidence has been derived primarily from studies in extracted teeth (Marshall & Massler 1961, Swanson & Madison 1987, Madison *et al*. 1987, Madison & Wilcox 1988, Torabinejad *et al*. 1990, Khayat *et al*. 1993, Trope *et al*. 1995, Chailertvanitkul *et al*. 1996, 1997a,b, Alves *et al*. 1998, Barthel *et al*. 1999, Fan *et al*. 2000, Gilbert *et al*. 2001) and in some animal experiments (Friedman *et al*. 1997, 2000), translation to clinical conditions has so far been tentative (Ricucci *et al*. 2000). To shed light on the real importance of the problem, clinical observations are required. In a retrospective cohort analysis involving 55 cases with optimally filled root canals that were exposed either to caries or directly to the oral microflora for an extended period, Ricucci *et al*. (2000) observed few periapical lesions at follow-up, while in most of the cases, the periapical status remained radiographically normal. Although the odds ratio for a lesion to occur was 3.0 in comparison to a control group of properly restored teeth matched with regard to relevant parameters, coronal leakage did not appear to be a significant problem in more than a few cases.

The result of root-canal treatment depends on a multitude of factors of which a bacteria-tight seal of the root canal by filling is considered critical to prevent re-infection of the canal system. Yet, a large number of *in vitro* studies have indicated that this may be a far-fetched goal, and even under the most ideal conditions, such as in extracted teeth, root fillings may not be performed such that leakage of dyes, bacterial elements and cells are prevented (Wu & Wesselink 1993, Saunders & Saunders 1994). It must be kept in mind that for bacterial organisms to grow and multiply, there has to be space and a reasonable nutritional supply. In spaces along root-canal walls or in between gutta-percha cones coated with sealer, the nutritional conditions are normally sparse. It is therefore possible that in the *in vitro* models so far employed, where bacterial organisms were used as tracers of leakage, bacterial penetration was favoured by the percolation of the culture medium in a way that may not occur *in vivo*. In order to further address the clinical significance of coronal leakage, a series of cases were analysed by histology combined with a bacterial-staining technique, where root fillings had been exposed to the oral environment by caries, crown fracture or loss of restoration for an extended period of time prior to extraction.

Materials and methods

Criteria for inclusion of cases

Teeth included in this survey were from patients (one tooth per patient) who had received root-canal treatment and restorative treatment in a private dental clinic operated by one of the authors (D.R.). The patients were treated between 1983 and 1997. To be included, root fillings should have been exposed to either extensive caries or a direct oral exposure because of crown fracture or loss of the coronal restoration for at least a period of 3 months. While the exact time for exposure to the bacterial challenge could not be determined in each individual case, records, radiographs and clinical inspection at the time of extraction were used to ensure that the stipulated time period was exceeded. Teeth were extracted because of nonrestorability or according to the patients' preference of not having the tooth restored (Figs 1A,B, 3E,F and 4E,F). To prevent inclusion of periapical lesions, where healing was in progress, teeth were accepted only if they had been root filled 3 years or more prior to extraction.

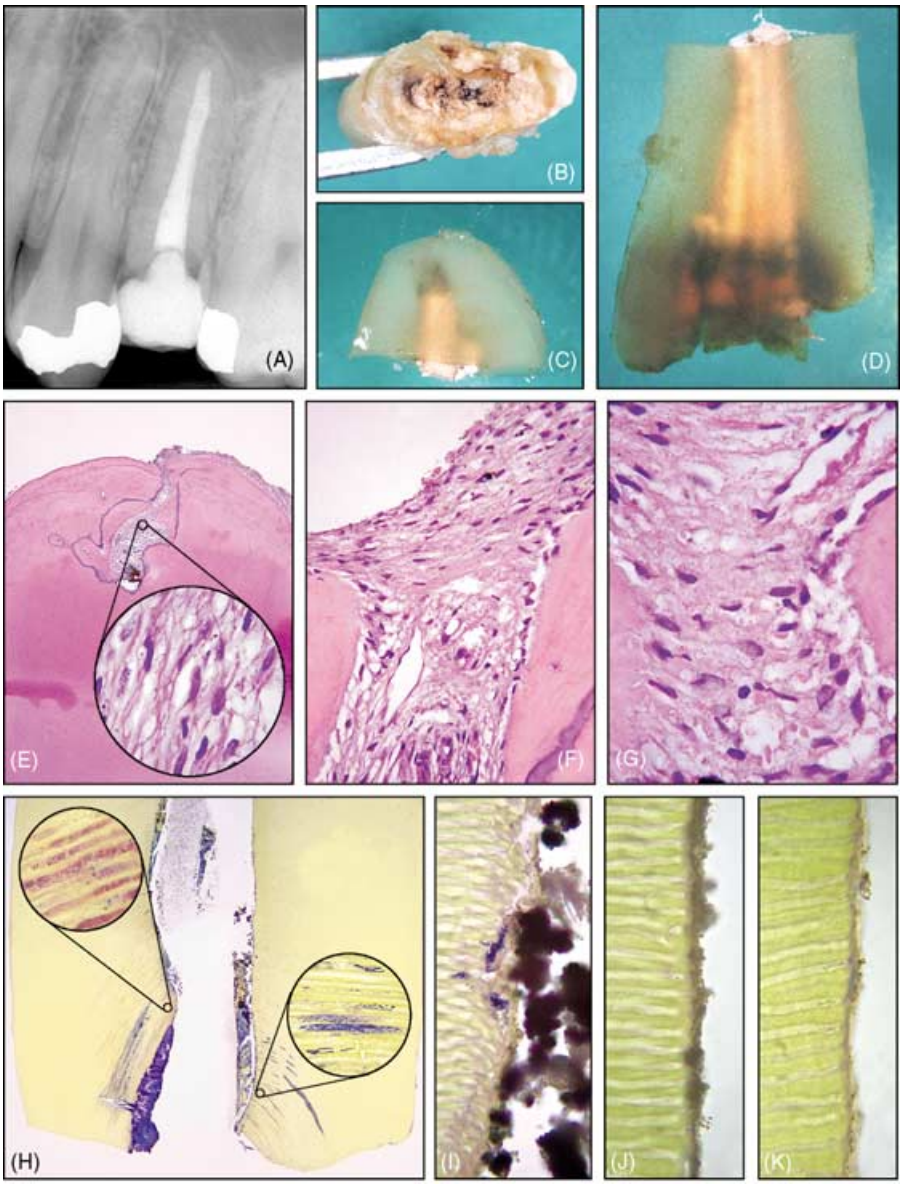

Figure 1 Specimen (tooth 25) of a 29-year-old man. The tooth had been treated for a vital pulp condition in one visit and restored with resin composite. One and a half years later the patient presented with a deep fracture of the palatal cusp and received a provisional restoration. The patient never returned for final treatment and was not seen until 3 years later when the tooth presented with extensive caries, although some temporary cement was still in place (A). The tooth was deemed nonrestorable and was extracted. The radiograph in (A), taken in conjunction with the extraction 4 years and 6 months post to the initial endodontic treatment, shows a normal periapical bone structure. Specimen after the extraction in (B) shows carious tissue and dental plaque in contact with the root filling. After demineralization, the apical third was separated, and the two pieces were embedded separately (C, D). Histological examination of the soft tissue in the apical portions of the root demonstrated noninfiltrated connective tissue and some hard tissue repair of previous resorptive defects (E–G). Examining the specimen for stainable bacteria (H–K) revealed bacterial plaque linings of the root-canal walls at the entrance of the canal (H). Two insets demonstrate dentinal tubules invaded by bacterial profiles of different morphology. Apical to the area in (H), stainable bacteria were reduced and were seen mixed with sealer material (black clumps; I). In an area yet closer to the middle third of the root (J) and apically to (K), no stainable bacteria were observed.

To be included in the study, root fillings had to meet high technical standards. This meant that root fillings had to end within 0–2 mm from the root tip and be radiodense without visible spaces between the root-filling material and the root-canal wall (Fig. 2C–E).

Only those cases were accepted where the histological processing had been successful. Hence, it was required that the longitudinal plane of sectioning of individual roots became oriented such that it displayed the apical two-thirds of the root canal and that sections included the main apical foramen.

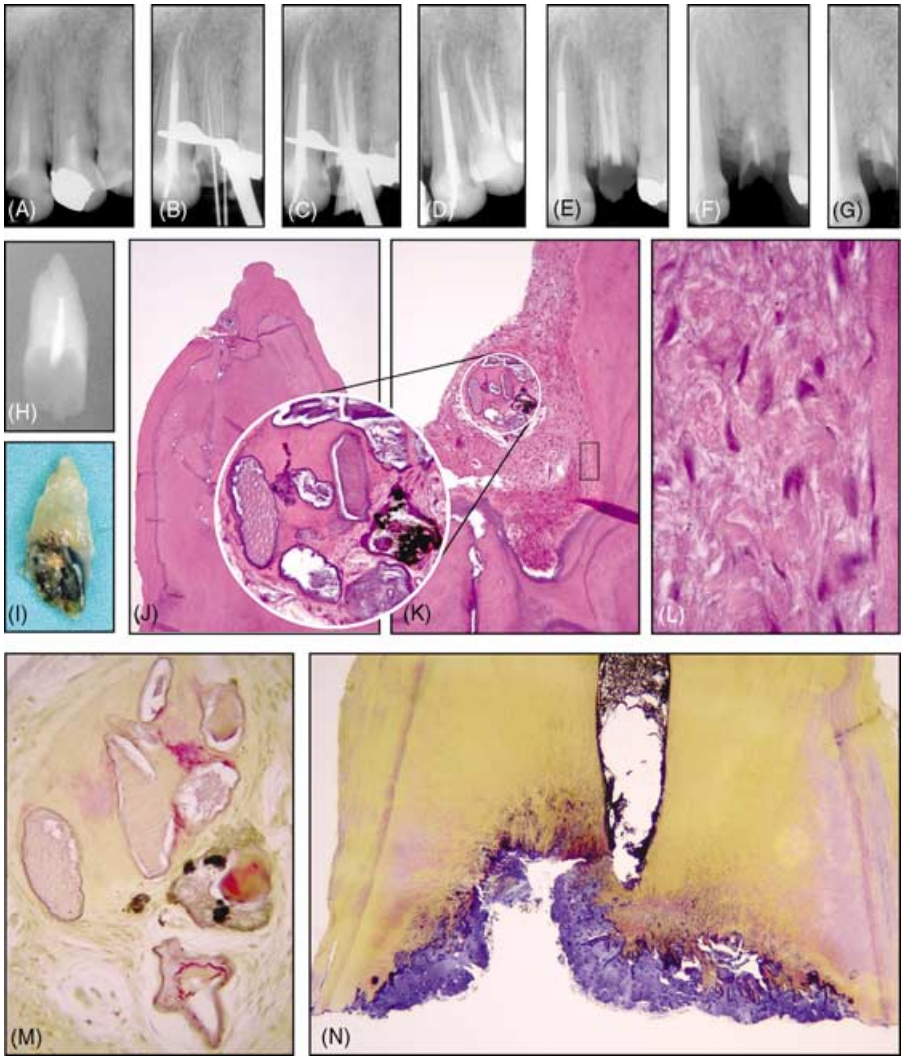

Figure 2 Specimen (tooth 24) of a 31-year-old woman. Tooth had been endodontically retreated (B, C) because of an incomplete root-canal filling (A). Calcium hydroxide was used as an inter-appointment dressing. The 1-year follow-up radiograph showed normal periapical conditions (D). The restoration was missing and destructive caries was present at the 7 years and 2 months follow-up (E). The patient refused to have the tooth restored, and 11 years and 7 months postoperatively, little tooth structure remained (F, G). Note the absence of osteolytic lesion apically. The root fragments were now extracted with the buccal fragment shown in (H, I). Examination of apical soft tissue showed dentine debris and sealer material extruded in what appears to be a noninflamed connective tissue (J, K). High magnification of area demarcated by rectangle in (K) demonstrates only fibroblasts and fibres. Overview of the coronal region of the root fragment (N) shows bacterial masses while absent in apical soft tissue remnants (M).

Cases included

A total of 32 teeth fulfilling the inclusion criteria were collected. Twenty of the cases were part of a previous report by Ricucci *et al.* (2000). Complete records of initial diagnosis, endodontic procedures employed and those on follow-up checks carried out over the years were available for all these patients. At the onset of endodontic treatment, 17 of the teeth had been diagnosed with a vital pulp condition, and 14 teeth had originally a necrotic pulp of which 10 teeth had presented radiographic evidence of apical periodontitis. The majority of the teeth were premolars ($n = 19$). Six were molars and seven incisors or canines. Patients were between 10 and 63 years of age (mean 38 years). Three of the patients were 16 years or younger at the time of the root-canal treatment. The gender distribution was 20 females and 12 males.

Radiographs and clinical procedures

For all cases, a similar radiographic technique was used (see Ricucci *et al.* 2000). Before extraction, a radiograph of each tooth was taken for an evaluation of the periapical status including presence or absence of an osteolytic lesion. The assessments for the 20 cases included in the study by Ricucci *et al.* (2000) were used; the 12 additional cases were analysed according to the same radiographic criteria.

The details of the endodontic treatments performed have been described previously by Ricucci *et al.* (2000). Briefly, a standardized protocol was followed, including instrumentation and filling to appropriate length in one or two appointments depending on the preoperative pulpal diagnosis (vital pulp condition vs. infected pulp necrosis). No attempt was undertaken to remove the smear layer. Different sealers in combination with laterally compacted gutta-percha were used. Teeth were restored, when appropriate, with amalgam or resin composites or full crowns with post and core build-ups. Two of the patients received only a temporary restoration and did not attend for a permanent restoration.

Tissue processing

Immediately after extraction, teeth were immersed in 10% neutral buffered formalin for a minimum of 48 h. Demineralization was carried out in formic acid/sodium citrate (22.5%) for 20–30 days with the end-point being determined radiographically. Specimens were washed in running tap water for 12–24 h, dehydrated in ascending grades of ethanol, cleared in xylene, infiltrated and embedded in paraffin (melting point 56 °C), according to standard procedures.

To produce sections parallel to the long axis of the root canal, special precautions were undertaken. Roots in multi-rooted teeth were dissected free and processed separately. If the roots were curved, they were cut into two pieces, one part incorporating the coronal two-thirds and one including the apical one-third. These two pieces were embedded separately.

With the microtome set at 4–5 μM, consecutive sections were taken in a longitudinal plane until each specimen was exhausted. Particular care was taken in sectioning the apical third to obtain sections including the apical foraminal tissue in direct continuity with any connective tissue attached to the root tip. During sectioning, a magnifying device was used to establish the proper plane of sectioning.

Every fifth slide was stained with haematoxylin and eosin for screening purposes and for assessment of inflammatory status. A modified Brown/Brenn technique for staining of bacteria (Taylor 1966) was used for selected slides. Slides were then covered with cover glasses and examined by light microscopy.

To test the accuracy of the bacterial-staining method, the following procedures were undertaken. Ten untreated teeth with necrotic pulps and radiographic evidence of apical periodontitis were extracted and processed in a manner identical to the procedures described above. Samples of plaque were collected from two patients and placed on glass slabs. A drop of saline was added, and the plaque was smeared with the help of a cover slip, fixed in an open flame and exposed to the staining protocol. Two different brands of root-canal sealers used in conjunction with the root fillings (Apexit: Vivadent Ets., Schaan, Liechtenstein; Kerr Pulp Canal Sealer: Kerr Manufacturing Co., Romulus, MI, USA) were mixed according to the manufacturers' directions, smeared on glass slabs and put aside to set for 3 days and then subjected to the staining protocol.

Analytic procedure

In two- and three-rooted teeth, all roots were included in the analysis and reported separately, provided they were sectioned properly and were available after the extraction. This means that the data analysis was based on the root as the unit. For each molar or premolar root included in the analysis, the radiograph taken at the time of extraction was re-examined to ascertain the periapical status of that particular root.

Sections were scanned, when possible, from the canal entrance to the apical end for the presence of bacterial profiles. The examination comprised the root-canal space and its ramifications and involved dentinal tubules. A bluish stain of clearly identifiable cell bodies was regarded as a positive staining response (Figs 1H and 2N). Greyish to black particles that were most likely sealer elements were designated a negative finding (Figs 1J and 5V).

To categorize the specimens in terms of the extent of bacterial penetration of the root canals, the following descriptors were set:

1 Bacterial presence along the entire root-canal space.

2 Bacterial presence only within the coronal one-third (Figs 1H,I, 2N, 3K–M, 4J,K and 5T–V).

3 Bacterial presence in the apical one-third only (Fig. 6).

4 Evaluation of the coronal one-third of the root canal not possible because of loss of tissue by the extraction procedure, but no visible bacteria in the apical one-third.

5 Evaluation of the coronal one-third of the root canal not possible because of loss of tissue by the extraction procedure, but visible bacteria in the apical one-third.

The haematoxylin- and eosin-stained sections were examined with regard to organization and inflammatory status of any soft tissue contained in the apical portion of the main canal, in lateral canals and apical ramifications and of attached periapical tissue (Figs 1–5). To categorize the specimens in this regard, the following descriptors were applied:

1 Dominance of a mixed inflammatory cell infiltrate by polymorphonuclear leucocytes (PMNs) and mononuclear leucocytes (MNLs, e.g. macrophages, lymphocytes and plasma cells) in a disorganized connective tissue (Figs 4G–I and 5Q,R).

2 Noninfiltrated, apparently necrotic tissue and dentine debris in contact with the root-filling material. Dispersion of sealer particles within a distinguishable connective tissue infiltrated by MNLs of varying intensity. Infiltrates sometimes tapering off in an apical direction and occasionally ending up in a noninfiltrated connective tissue at the apical foramen (Fig. 3I,J).

3 Virtual absence of inflammatory cell infiltrates in a well-organized connective tissue. Necrotic tissue and dentine debris may or may not separate the noninflamed connective tissue from the root-filling material (Fig. 1E–G).

The two authors carried out the assessments independently. Sections with discordant assessments were re-examined jointly and discussed until a consensus was reached.

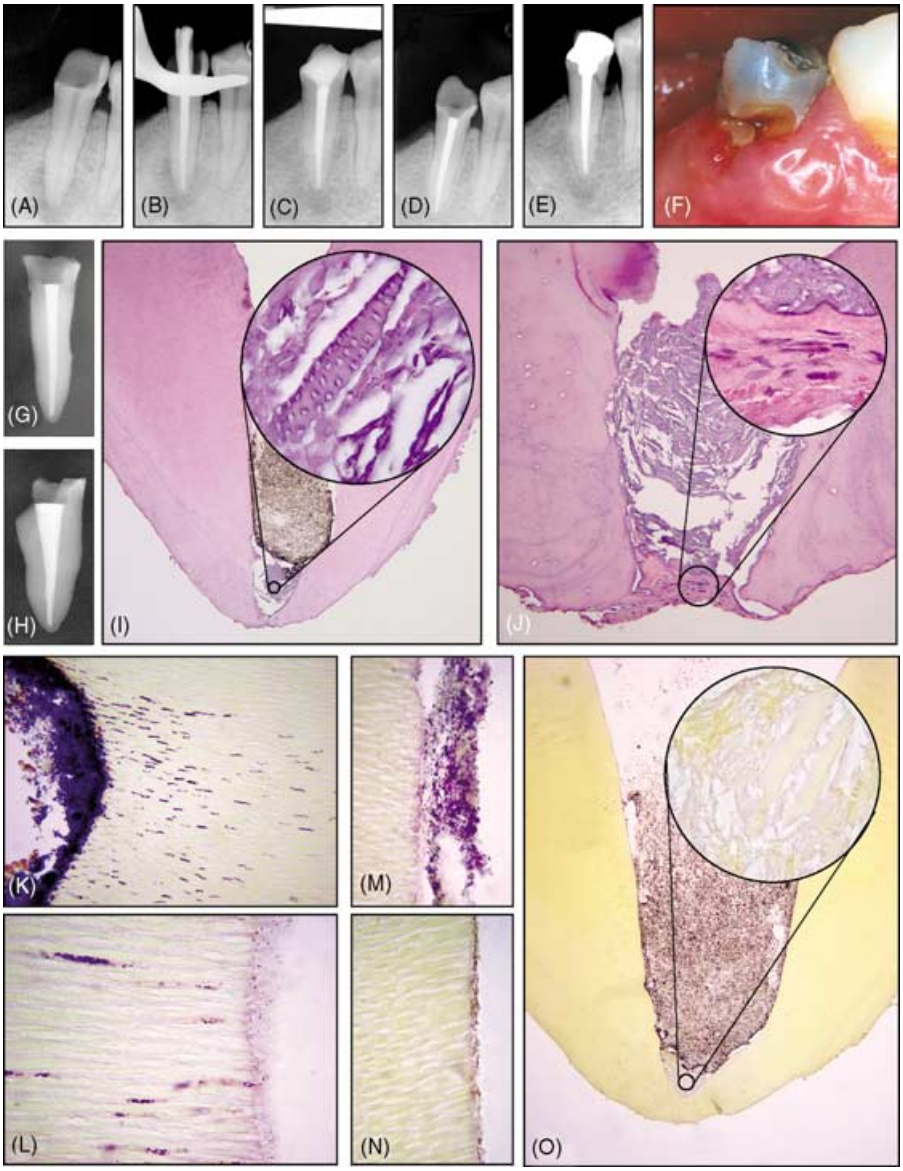

Figure 3 Case of a 45-year-old woman. Tooth 45 exhibited at the initiation of endodontic treatment penetrating caries, necrotic pulp and a periapical osteolytic lesion (A). Following completion (B), a restoration with composite resin was placed. At the 2-year follow-up bone regeneration was evident (C). Three years and 9 months postoperatively, the composite restoration was lost (D). As the tooth showed no signs of apical periodontitis, an amalgam restoration was performed following the placement of a base with zinc-phosphate cement over the root filling. After 8 years and 9 months from the root-canal treatment, the patient came back with a gingival swelling and a carious lesion on the buccal aspect of the tooth (F). The radiograph taken showed an oblique fracture to the bone level (E). The tooth was extracted. Radiographs of the extracted tooth taken at 90° degree angles (G, H) demonstrate an optimally conducted root-canal filling. Histological examination of the apical third (I, J) showed filling in contact with debris packed apically. Debris was separated from noninflamed periodontal connective tissue attached to the root tip (J). Bacterial profiles were obvious in the coronal portion of the root canal (K–M) while absent in a section taken a few millimetres below the root canal orifice (N) and in debris packed apical to the root filling (O).

Results

On testing the bacterial-staining method, typically stained bacterial profiles were seen in the dental plaque samples as well as in the untreated root canals. No such staining reaction was seen with any of the sealer materials. Hence, the ability of the method to allow the

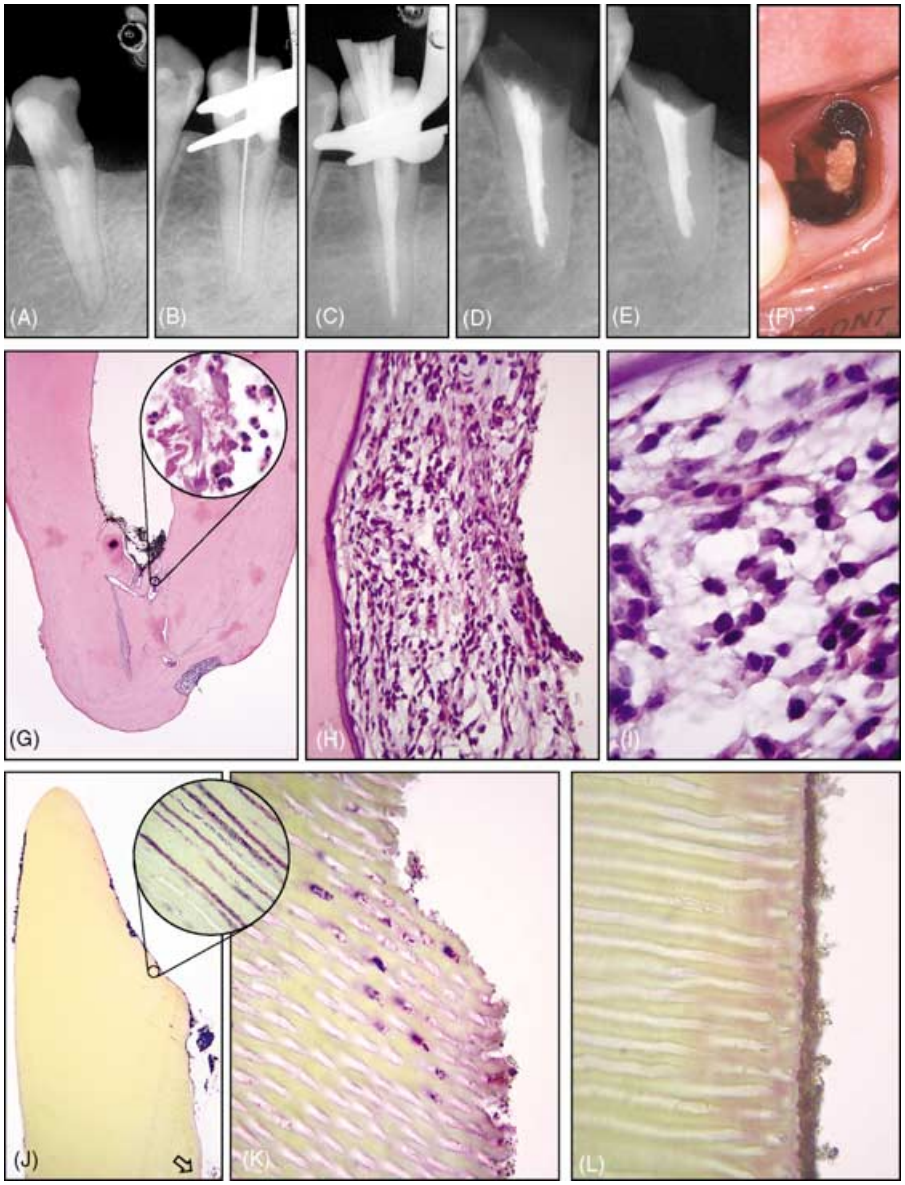

Figure 4 Case of 26-year-old female with signs of inflammation in soft tissue of apical ramifications and root tip. Tooth 35 was a retreatment case (A–C) restored permanently after root filling with resin composite. Four years later (D), the patient returned following loss of the restoration. Radiograph demonstrated no signs of apical periodontitis. Further treatment was refused. Patient was seen 5.5 years later, e.g. 9.5 years after the retreatment. While there was an extensive caries in contact with the gutta-percha root filling, the radiograph showed no signs of apical periodontitis (E). The tooth was extracted. Tissue section (G) shows apical ramifications with presence of PMNs (see also insert). Tissue at the root tip displayed numerous MNLs (H, I). In the coronal third of the root, there was extensive bacterial penetration of dentinal tubules (J, K), whereas a few millimetres apically to the section in (K), no bacteria were detected on the root-canal wall (L).

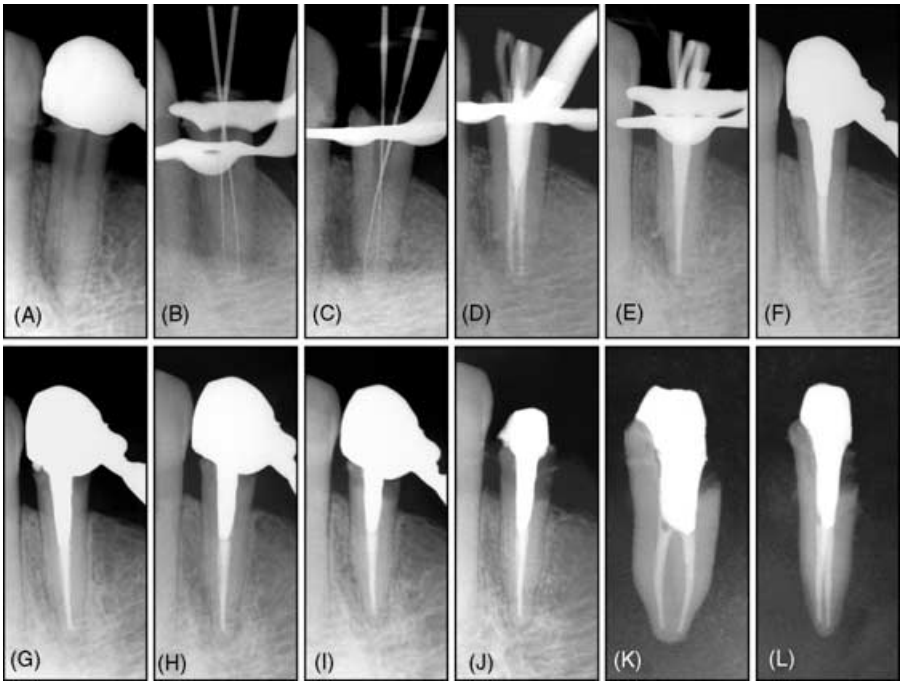

Figure 5 Case of a 56-year-old woman. Tooth 34 was treated because of an abscess associated with a necrotic pulp (A–E). There was one canal in the coronal third, which was broken up in two canals at the middle third. After $Ca(OH)_2$ medication, the canals were filled with cold gutta-percha laterally condensed and a sealer whereupon a lateral canal appears 'filled' (D, E). A cast post and core was prepared and the old bridge re-cemented. At the 1-year check, new caries appear to emerge. Periapical tissue was normal (F). Follow-ups were undertaken at 5 years and 2 months (G), 7 years (H), and 8 years and 7 months (I). Caries was not treated as per the patient's instruction. When the bridge became loose 10 years and 9 months post treatment (J), the tooth was deemed nonrestorable. Radiograph indicated no signs of apical periodontitis. Two radiographs of the extracted tooth show the extent of the carious destruction (K, L). Photographs taken after clearing the specimen in xylene, before embedding, showed several filled ramifications (M, N). Tissue section in (O) demonstrates the outer end of the most apical ramification seen in (N) where connective tissue was noninflammed. Magnification of the foraminal area in (O) demonstrates haemorrhage likely to be caused by the extraction and sealer material (P) adjacent to which there were some inflammatory cells. Section in (Q) includes the major lateral canal seen mid-root in (N). Mixed with sealer material in what appeared to be an overfilled canal were necrotic tissue, PMNs and MNLs. (R) shows a higher magnification of the canal content. Magnification of the area indicated by the arrow in (Q) demonstrates multinucleated foreign body cell. The partly necrotic tissue in the lateral canal did not display bacterial presence (T). Apically to the post space, bacterial colonies were present on the root-canal walls (U) but were absent a few millimetres apically to the point where the canal bifurcated (V).

examiners to distinguish bacterial elements from extraneous material was deemed satisfactory.

Clinical features of the material

Of the 32 teeth included in the study, 23 teeth had caries in contact with root filling (Figs 1B,D, 2E–I,N and 4D–F). Four teeth had been fractured and showed signs of caries (Fig. 3D,F), and four other were fractured without signs of caries. In one case, the restoration was lost without caries being present. Five teeth were diagnosed with osteolytic lesions. At the time of extraction, none of the patients had presented with symptoms of acute apical periodontitis.

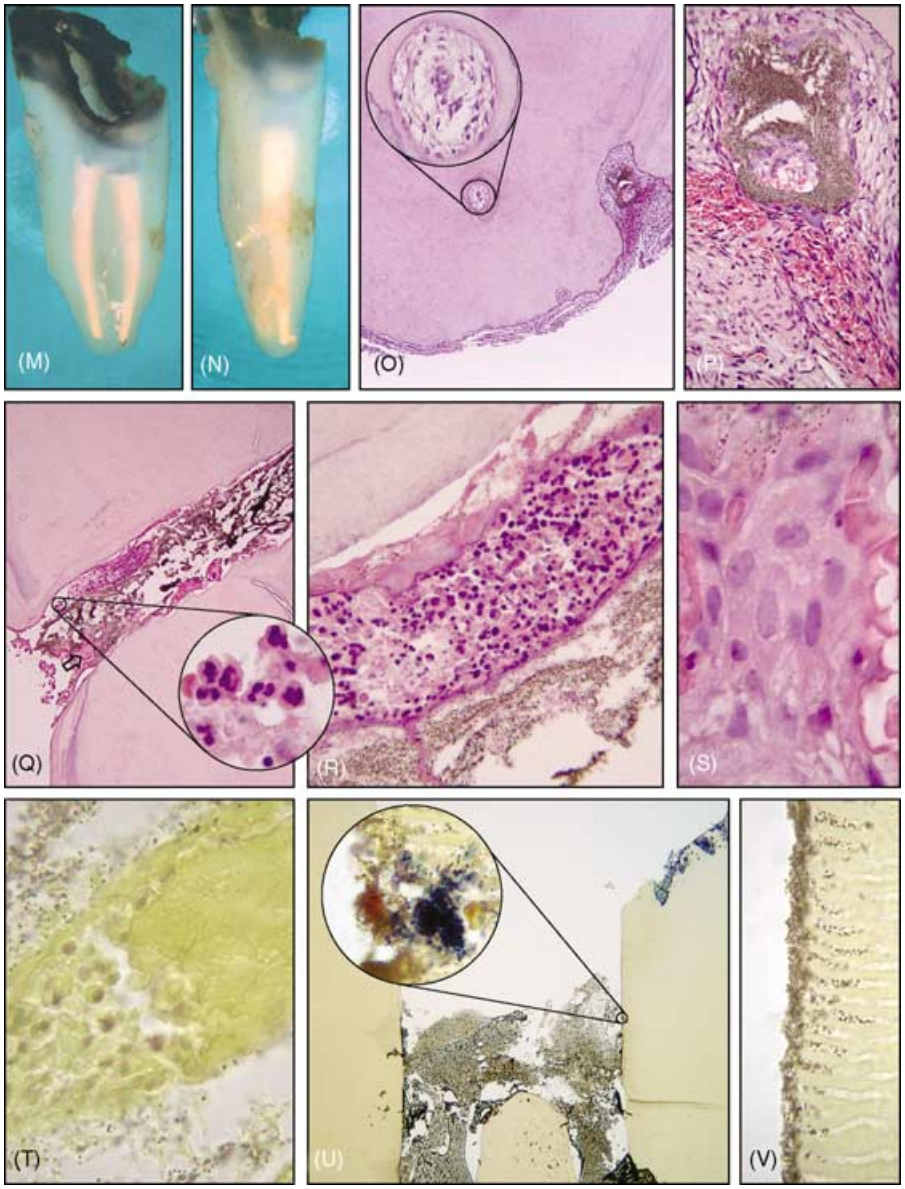

Figure 5 continued

Histological findings

In all, 39 roots became available for histological analysis, of which five showed discernible periapical bone lesions in the radiographs taken immediately prior to extraction (Table 1). In 28 of 29 roots (96%), where the entire length of the root canal could be viewed, bacteria were limited to the coronal one-third only and were not present in the apical portion of the canal (Table 2). In a set of nine other roots where the coronal end had been lost during extraction, the same conditions prevailed apically. In one root, bacteria were noted in the apical end while not being seen either in the mid-root portion or coronally (Fig. 6). This particular case derived from the previous report (Ricucci *et al*. 2000) had been judged to be without an osteolytic lesion when in fact there was an inflammatory lesion as indicated by

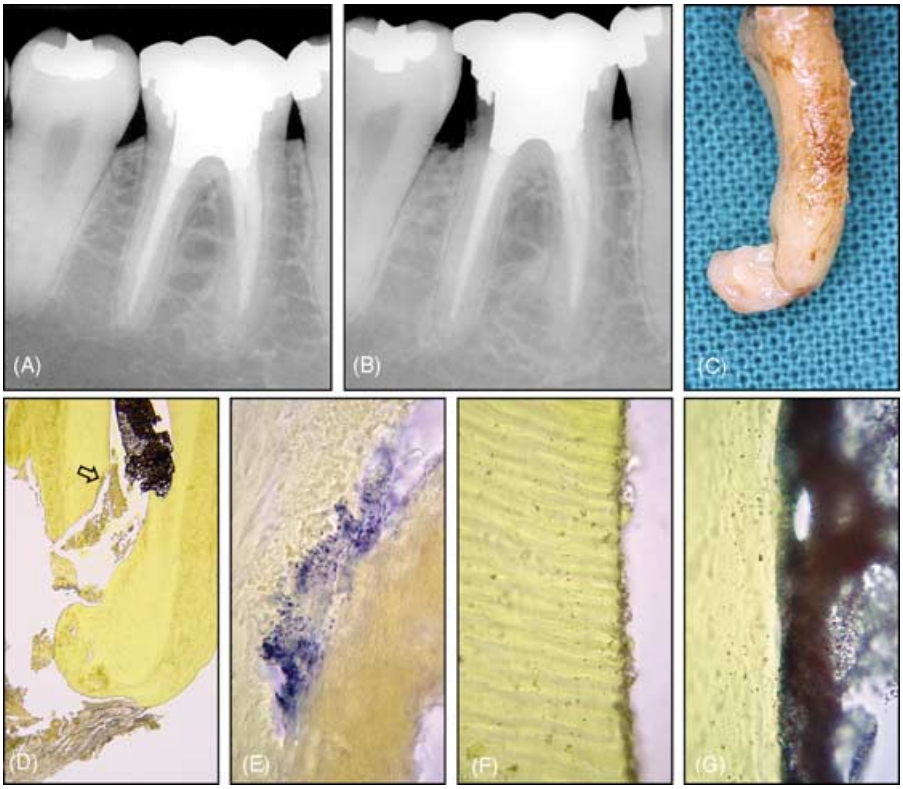

Figure 6 Case of endodontic treatment of tooth 46 in a young female at the age of 16. The crown was restored with full amalgam. The 5-year follow-up radiograph is shown in (A). The periapical tissue structures appeared normal. At 13-year follow-up, an extensive carious lesion had developed on the distal aspect (B). When the case was examined in the study by Ricucci *et al.* (2000), the periapical structures were deemed to be within normal limits. The patient did refuse further treatment and opted to have the tooth extracted. Upon extraction, soft tissue was attached to the mesial root. Sections stained for bacteria in (D) show an overview of the apical third of the mesial root. Note that the foramen ended on the distal aspect of the root. Magnification of the area indicated by arrow in (D) demonstrates bacterial colonies in necrotic debris (E). In the coronal third of the root, no stainable bacteria were discernible (F, G).

the sac of inflamed tissue that accompanied the root upon extraction (Fig. 6C). In yet another root, bacterial profiles were identified apically, but in this case, the coronal root portion was not available for observation. The finding of bacteria correlated with radiographic observation of osteolytic lesion.

Soft tissue attached to the root tip or inside the root was available in all samples to serve as areas for evaluation of inflammatory involvement. Most of these specimens displayed either a noninfiltrated connective tissue (Fig. 1E–G) or small tissue infiltrates of MNLs

Table 1 Periapical status at the time of extraction by radiographic examination of individual roots available for the survey

	Osteolytic lesion	Unclear	No lesion
Previous report (Ricucci *et al.* 2000) (20 teeth)			
$n = 24$ roots	2	1	21
New cases (12 teeth)			
$n = 15$ roots	3	0	12
Total	5	1	33

Table 2 Bacterial presence at different levels of the examined root canals

	Category				
	1	2	3	4	5
Previous report	–	18	1	5	–
New cases	–	10	–	4	1
Total	–	28	1	9	1

To categorize the specimens the following criteria were applied:
1. Bacterial presence along the entire root canal space.
2. Bacterial presence only within the coronal one-third (Figs 1H,I, 2N, 3K–M, 4J–L and 5T–V).
3. Bacterial presence in the apical one-third only (Fig. 6).
4. Evaluation of the coronal one-third of the root canal not possible because of loss of tissue by the extraction procedure, but no visible bacteria in the apical one-third.
5. Evaluation of the coronal one-third of the root canal not possible because of loss of tissue by the extraction procedure, but visible bacteria in the apical one-third.

Table 3 Condition of the soft tissue attached to the root tip, in apical portion of main canal and in lateral and apical ramifications

	Soft tissue status		
	1	2	3
Previous report	4	11	9
New cases	3	7	5
Total	7	18	14

For the assessment of soft tissue status, the following descriptors were used:
1. Dominance of a mixed inflammatory cell infiltrate by PMNs and MNLs, for example, macrophages, lymphocytes and plasma cells, in a disorganized connective tissue.
2. Noninfiltrated, apparently necrotic tissue and dentine debris in contact with the root-filling material. Dispersion of sealer particles within a distinguishable connective tissue infiltrated by MNLs of varying intensity. Infiltrates sometimes tapering off in an apical direction and occasionally ending up in a noninfiltrated connective tissue at the apical foramen.
3. Virtual absence of inflammatory cell infiltrates in a well-organized connective tissue. Necrotic tissue and dentine debris may or may not separate the noninflamed connective tissue from the root-filling material.

associated with dispersed sealer particles (Table 3; Fig. 2J,K). In seven roots, there was an overt inflammatory cell infiltrate consisting of a mixture of PMNs and MNLs in a poorly organized connective tissue environment (Fig. 4G–I; Table 3). Two of these specimens correlated with the radiographic finding of an osteolytic lesion. Three other specimens with osteolytic lesions had all inflammatory cell infiltrates in the soft tissue attached to the root. Cell infiltrates were mononuclear in nature and associated with sealer material, thus, fitting descriptor 2.

Discussion

The analysis of the root-filled teeth observed in this report indicated that, despite exposure to bacterial plaques and caries for a prolonged period, bacterial penetration of the root fillings appeared limited to the coronal portion of the canals. In all but two cases, the apical portion harboured no stainable bacteria. In one of the cases with presence of bacteria in the apical third, there were no bacterial stains in the middle or coronal thirds of the root canal, suggesting that in this particular case, organisms had survived the initial endodontic treatment and were not the result of so-called coronal leakage. Indeed, these findings are at odds with numerous observations in *in vitro* models (Madison *et al*. 1987, Swanson & Madison 1987, Torabinejad *et al*. 1990, Khayat *et al*. 1993, Chailertvanitkul *et al*. 1996,

1997a,b, Trope *et al*. 1995, Alves *et al*. 1998, Barthel *et al*. 1999, Siqueira *et al*. 2000, Gilbert *et al*. 2001). They also challenge the frequently quoted observations in the analysis by Ray & Trope (1995) on full-mouth radiographs taken of patients seeking treatment at a US dental school. In their odds-ratio computations, adequate coronal restoration came out as a much stronger predictor for successful outcome than did adequate endodontic filling. Although their findings have received only moderate support in other similar studies (Kirkevang *et al*. 2000, Tronstad *et al*. 2000, Hommez *et al*. 2002), it is clear from these surveys that in cases where root fillings are of suboptimal quality, improper coronal restoration may be crucial to the emergence or continuance of periapical inflammatory lesions in endodontically treated teeth. Therefore, the present set of findings should not be construed to suggest that the quality of the coronal restoration is insignificant to the outcome of root-canal treatment. But the observations here and those of a previous report, based partly on the identical cases (Ricucci *et al*. 2000), do lend support to the long-held concept that proper instrumentation and optimal filling of the instrumented root-canal space is to be regarded a highly desirable feature of root-canal treatment. The present findings further imply that this is all the more critical if the restoration, for any reason, is lost or caries have penetrated to the vicinity of the root filling.

The Brown/Brenn staining method for identification of bacterial organisms in tissue sections is known to be rather insensitive. The validity of the present findings may therefore be questioned. Gram-negative organisms may especially escape identification. While this does not mean that Gram-negatives are not stained, the bluish-stained Gram-positives may overwhelm the normally red-stained Gram-negatives, if they occur together. This shortcoming may not be all that crucial as Gram-positives indeed dominate in cultures of previously treated root canals (Molander *et al*. 1998, Sundqvist *et al*. 1998, Chávez de Paz *et al*. 2003). Yet, the possibility does exist that both kinds of organisms escaped discovery in critical areas, that is, in the apical portion of the root canals. Furthermore, stainability is weakened by demineralization procedures thus giving the risk of false-negative findings (Wijnbergen & van Mullem 1987). This risk is particularly great when the number of organisms is small. Therefore, it is possible that in those areas where the space was limited and where only a few organisms were detected, for example, where sealer separates the gutta-percha from the root-canal walls or in apical ramifications, bacterial organisms may have gone unnoticed.

Nonetheless, these shortcomings of the staining method are not important for the interpretation of the results of the current study. First, stainability in general was deemed excellent (Figs 1–6). Indeed, the modification used was able to clearly discriminate between bacterial profiles and sealer particles. Second, the soft tissue rarely displayed overt inflammatory cell infiltrates except for a limited number of cases, hence strengthening the perception that penetration of bacterial organisms and their elements was a rare entity in the cases observed. Yet, in some specimens, the inflammatory status did not correlate with the absence of bacterial profiles, for example, in the case shown in Fig. 4. The findings of PMNs in lateral and apical ramifications in this and a few other similar cases may be related to penetration of bacterial byproducts. Unfortunately, it is not possible to recognize such elements by the staining method and these may have played a role in causing inflammation. Given this possibility, it is intriguing nonetheless that few of the cases analysed appeared with radiographic signs of apical periodontitis.

Admittedly, it would have been desirable to have employed a more sensitive method than the histobacteriological staining technique. However, considerable method development would have been necessary to make use of, for example, *in situ* hybridization techniques with probes identifying ribosomal RNA universal for prokaryotic cells. Methods based on sampling for amplification by culture or PCR would not have been useful because of the obvious risk for oral contamination and the lack of opportunity to gain information on the location of the organisms.

Although it was not the purpose of the present study to assess the reliability of the term 'radiographic success' in root-canal treatment, it is noteworthy that in a number of cases, histological finding of inflammatory infiltrates in the apical tissue did not correlate with the presence of osteolytic lesion. This is likely to be explained by the fact that these infiltrates were small and prominent more often near the exit and within the confines of the root canal than in the attached soft tissue. Thus, the findings confirm the observation by Brynolf (1967) that the sensitivity of conventional radiographs to detect mild inflammatory processes, caused by irritants released from root canals, is low. By contrast, all osteolytic lesions recorded at extraction correlated with overt inflammatory cell infiltrates of the attached soft tissue. However, in one case, radiographs failed to reveal a large inflammatory process (see Fig. 6). Consequently, radiographic assessment of endodontic success must be thought of as a crude estimation of whether or not bacterial elements are released into the periapical tissue environment. In the present study, this was demonstrated by the observation that only two of the seven roots with inflammatory score 1 (dominance of PMN and MNL infiltrates) correlated with radiographic lesion. These infiltrates are likely to be caused by leakage of bacterial byproducts, while those designated score 2 (dominance of macrophages and other MNLs) correlated with a scattering of sealer particles.

The limitations of the case descriptions presented here are appreciated as they represent a select group of patients and therefore may not allow wide generalizations. The observation that bacterial penetration had been limited despite extremely long times of oral exposure times in some cases and that the apical tissue was virtually unaffected suggests that the threat of coronal leakage in well-performed root-canal treatments may be an overrated issue. Thus, *in vitro* observations on sealability of root fillings appear to provide limited clinical value, if any at all (Wu & Wesselink 1993). Clinical extrapolations of such findings should therefore be undertaken with care. On the basis of *in vitro* results, misleading statements are occasionally made and oral exposures of root fillings even for a relatively short period have been considered an indication for endodontic retreatment (Siqueira *et al*. 2000). Although it is not to be questioned that bacterial infection plays a decisive role in the failure of endodontic treatments, retreatment of sound root fillings after short period of exposure to the oral environment does not seem justified.

Conclusion

Optimally prepared and filled root canals resist bacterial penetration upon challenge by direct oral exposure, caries and fracture.

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Disclaimer

Whilst this clinical article has been subjected to Editorial review, the opinions expressed, unless specifically indicated, are those of the author. The views expressed do not necessarily represent best practice, or the view of the IEJ Editorial Board, or of its affiliated Specialist Societies.

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