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Wound healing of apical tissues after root canal therapy: a long-term clinical, radiographic, and histopathologic observation study

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Objective. The purpose of this study was to evaluate the pulp healing process and the dentin-cementum complex in 51 endodontically treated human teeth after long observation periods and to correlate histologic observations with conventional periapical radiographic findings.
Study design. Specimens were obtained from the extraction of 77 treated teeth, which were deemed to be unrestorable, with no evidence of periapical bone lesion at the follow-up. After stringent evaluation of the radiographs, 51 cases that 3 independent evaluators assessed as having normal periapical conditions were selected. The specimens were histologically evaluated using serial sections.
Results. In the majority of the cases, complete healing was observed, with no signs of acute or chronic inflammatory processes in the remaining apical tissue or periodontal tissue fragments. Some cases showed moderate inflammation in the root canal tissue. Narrowing of the apical root canal by cementum was a common finding in most cases, but total closure was not observed. Debris intermixed with necrotic tissue and sealer particles was a common finding in the pulp stump. Bacteria were present in the coronal portion of the root in almost all cases, but in only 1 case could bacteria be demonstrated in the coronal and apical portions of the root.
Conclusions. Apical tissue of properly treated teeth with no signs of periapical changes is only rarely significantly inflamed. When the tissue is inflamed, microbial causes can always be demonstrated. Despite the presence of microorganisms coronally in nearly all cases, apical tissue is seldom affected. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:609-621)**

Traditionally, diagnosis of disease of tissues or organs is dependent on clinical and histologic findings. Clinical diagnosis is a provisional diagnosis and uses objective and subjective signs and/or symptoms and all available tests in clinical dental medicine as well as imaging systems, such as conventional radiography, digital radiography, and computerized tomography. Radiography is designed to detect pathologic changes at the tissue or organ level and not at the cellular level. Histologic examination provides information that makes a definitive and final diagnosis possible at a cellular level. To increase the sensitivity and optimize the chances of detecting the presence of a few inflammatory cells in the periapical tissues, meticulous techniques must be applied. This includes serial sectioning or step-serial sectioning of the entire specimen in an optimal direction. Random and/or selective sections are never sufficient for optimal histologic diagnosis. Furthermore, tissue fixation and preparation must be optimal. Substandard histologic information also makes attempts to correlate histologic and radiographic information questionable.[1-4] Because histologic examination cannot be routinely used in clinical practice to diagnose pathologic changes of tissues or organs at the cellular level, radiographic examination is indispensable as a screening tool to diagnose pathologic changes of tissues or organs. Therefore it is desirable to increase the sensitivity and specificity of radiographic examinations of the periapical region of teeth.[4] Much reliance has been placed on results from

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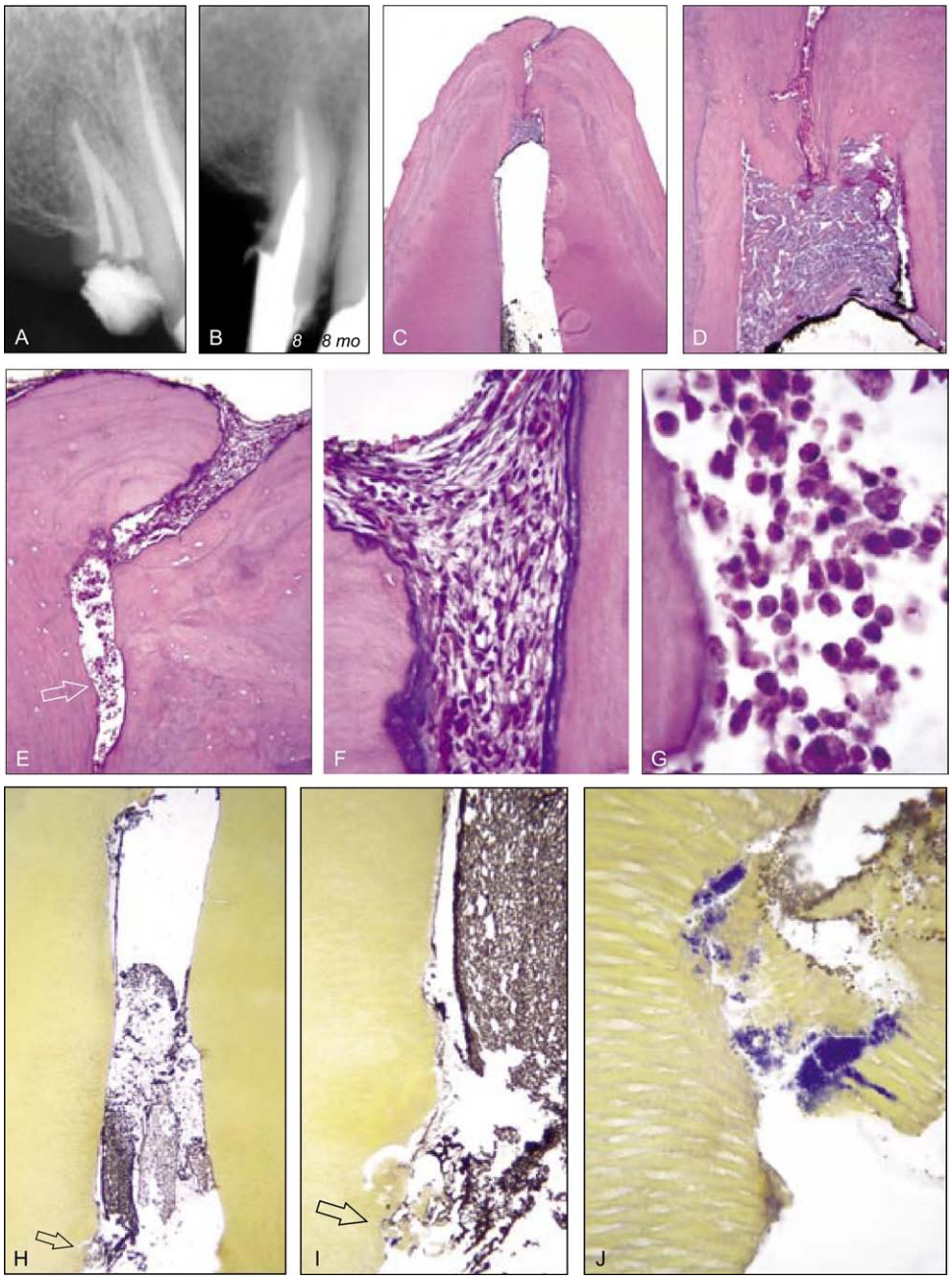

Fig. 1. Case V22. **A,** Maxillary first premolar with vital pulp. Endodontic treatment was performed in 1 visit. Apexit was used as a sealer. The tooth has 2 canals that are merging in the apical third. Cast post and core was fabricated and an acrylic temporary crown adapted. The patient never wanted a final ceramometalic crown. **B,** After 8 years 8 months, recurrent caries is present. Periapical conditions were normal. The tooth was extracted to accommodate a prosthetic treatment plan. **C,** Overview showing the apical limit of the instrumentation. Debris of the root canal instrumentation are packed at the apical preparation limit (hematoxylin and eosin [H-E], original magnification ×25). **D,** Detail of the apical stop. Large amount of debris can be seen apical to the root filling material. More apically, the root canal can be followed, narrowed by calcified tissue and containing inflamed tissue (H-E, original magnification ×100). **E,** Detail of the canal terminus in C (H-E, original magnification ×100). **F,** Magnification of the foramen. Connective tissue with moderate accumulation of chronic inflammatory cells can be observed (H-E, original magnification ×400). **G,** Magnification of the area more coronally, indicated by the *arrow* in *E*. Severe accumulation of polymorphonuclear leukocytes at this level (H-E, original magnification ×1,000). **H,** Apical portion of the instrumented root canal (Taylor's modified Brown & Brenn, original magnification ×25). **I,** Magnification of the area indicated by the arrow in H. Irregularity of the root canal wall containing debris (Taylor's modified Brown and Brenn stain, original magnification ×100). **J,** Magnification of the area indicated by the *arrow* in *I*. Dentin chips colonized by bacteria (Taylor's modified Brown and Brenn

a single study by Brynolf[1] on the interpretation of periapical radiographs from autopsy material. The sample used in that study consisted of only central and lateral maxillary incisors. Furthermore, the teeth were retrieved and fixed after a day or more, resulting in compromised tissue preservation. Based on her findings, Brynolf suggested that periapical inflammation can be observed histologically in about 93% of root-filled teeth.[1] Despite the methodologic shortcomings, the results of the Brynolf study have been used as a basis for the periapical index disease scoring system applied to all teeth.[5] A similar study on cadaver material was presented by Barthel et al.[4] They reported a finding of >30% periapical inflammation in teeth with no apical radiographic lesions.

During routine histopathologic studies of endodontically treated teeth, we have observed a higher degree of normal periapical tissues, calling into question the very low degree of complete healing reported after well executed endodontic treatment.[1-4]

Much interest has been focused on the effect of coronal bacterial leakage and its relationship to periapical inflammation.[6] Though often implicated, there is little factual evidence in the literature that periapical lesions and predictable treatment failure will follow coronal exposure of root canal fillings.

The purpose of the present case series study was to:

1. Correlate conventional periapical radiographic findings of teeth with normal periapical tissues and no clinical signs and/or symptoms of disease with histologic findings of the apical tissues of the same endodontically treated teeth after a long-term follow-up period.
2. Evaluate the wound healing process of pulp wound and the dentin-cementum complex subsequent to endodontic treatment and later loss of coronal integrity of the pulp space.
3. Discuss whether it is practical to expect complete absence of inflammatory cells in tissue present in and around apical foramina to consider the endodontic treatment to be clinically successful.

MATERIALS AND METHODS

Inclusion criteria

Teeth included in this study were from patients who had received root canal therapy and restorative treatment in a private dental practice operated by 1 of the authors (D.R.). Only 1 tooth per patient was included (the first one treated). The patients were treated in the practice from 1983 to 2003 and subjected to periodic follow-up. Recall rate in the practice was approximately 60%. All patients gave consent for treatment and subsequent analysis.

Seventy-seven endodontically treated teeth without clinical signs and/or symptoms as well as no evidence of periapical bone lesion at the follow-up were extracted in the period from 1999 to 2007. The teeth were extracted either because the tooth was unrestorable due to extensive caries or fracture or because the root fillings had been exposed to oral environment for some time and the patient did not wish to undergo further treatment.

To be included in the study, the root filling in each tooth had to meet high technical standards. This meant that the root filling should end within 0-2 mm from the radiographic root apex and be homogeneous without visible voids between the root filling material and the root canal walls (Fig. 1). The visible integrity of the coronal seal was recorded for all cases. In each case, the distance between the radiographic apex and the apical terminal of the root fillings was measured and recorded.

Two intact teeth without endodontic therapy, extracted for orthodontic reason, were used as negative control samples for radiographic and histologic evaluation.

Radiographic evaluation

The final radiographs of the 77 cases, exposed immediately before extraction, were digitized and subjected to a blinded evaluation of the periapical status. One or more periapical radiographs were exposed before extraction. Blinding was carried out by masking the coronal portion of the tooth, so that the examiners had no information on the presence or absence of possible factors associated with coronal leakage. The periapical conditions of the teeth were radiographically evaluated using the classic assessment system described by Strindberg.[7] To avoid bias in evaluating periapical status of endodontically treated teeth by only 1 examiner, radiographs of the 77 cases were subjected to stringent evaluation by 3 experienced endodontists. Using radiographs of control teeth as reference, all 3 endodontists completely agreed on the criteria of what

stain, original magnification ×1,000). **Summary:** The presence of severe inflammation in the apical tissue adjacent to the debris accumulation might suggest an irritating effect of necrotic material. However, this conflicts with what has been observed in other cases where noninflamed tissue was in direct contact with necrotic tissue and debris. The most likely explanation for the severe inflammatory response is the bacterial colonization in the post space. Bacteria gained access to the root canal space owing to recurrent caries and poor coronal restoration.

normal periapical tissues should look like before evaluating experimental teeth. Radiographically, normal periapical tissues require: 1) uniform width of the periodontal ligament space; 2) no obvious breakdown of lamina dura; and 3) well organized bone trabeculae.[7] The radiographs were projected on a screen in a dark room without any other light interference. The radiologic examination was performed without prior knowledge of the results of the histologic evaluation. Three examiners evaluated the radiographs independently. In case of disagreement, no attempts were made to come to a consensus and the case was excluded. Only cases where all 3 evaluators completely agreed were included. Out of 77 cases, 51 cases without clinical signs and/or symptoms, as well as normal radiographic appearance of the periapical tissue, were included.[7]

Case history

Complete records of initial diagnosis, endodontic procedures used, and follow-up examinations were available for all 51 cases. At the beginning of the endodontic treatment, 27 teeth were diagnosed as having irreversible pulpitis, 10 teeth had a necrotic pulp without apical periodontitis, 12 teeth presented with pulp necrosis and radiographic evidence of apical periodontitis, and 2 teeth had earlier root fillings of which 1 showed apical periodontitis. The majority of the teeth were premolars (n = 28). Six teeth were molars, 11 were incisors, and 6 were canines. Patients were between 18 and 69 years of age (mean 42 years). The gender distribution was 30 women and 21 men.

Observation periods ranged from 2 years to 22 years 4 months (mean 10 years 3 months).

Endodontic treatment procedures

All endodontic treatments were performed following a standardized protocol. Before the initiation of treatment at least 1 diagnostic radiograph was exposed. This radiograph and subsequent radiographs were obtained using a film holder (Rinn Corp., Elgin, IL) to permit paralleling technique projection. A long cone (Explor-X 65 KV; Fiad, Trezzano, Italy) and Kodak Ultraspeed film 31 × 41 (DF58) or 22 × 35 (DF54; Eastman Kodak Co., Rochester, NY) were used. Radiographs were processed manually in a dark room following the recommendation of the manufacturer.

A pulpal and periapical diagnosis was established after obtaining objective and subjective symptoms, pulp tests, percussion, and palpation as well as radiographic findings. Endodontic treatments were performed using a strict aseptic technique. Plaque and calculus were removed from the tooth surfaces using ultrasound scaling and/or curettes followed by polishing with a prophylaxis paste in a rubber cup. After rubber dam isolation, the field (tooth, rubber dam, and clamp) was disinfected with 30% H_2O_2 and 5% tincture of iodine.[8] In case of substantial loss of tooth substance, build-ups were carried out with either a copper ring, composite material, or reinforced glass ionomer cement to exclude bacterial contamination during the operation. After the working length was established, an effort was undertaken to limit the instrumentation to the confines of the root canals. Abundant amounts of 1% sodium hypochlorite solution were used for irrigation. After adequate preflaring of the coronal two-thirds of the root canals and apical instrumentation, the root canals were filled with cold laterally compacted guttapercha and a sealer. Different sealers were used over the years, including AH 26 (De Trey Frères, Zurich, Switzerland), Bio Seal (Ogna, Milano, Italy), Endomethasone (Septodont, Saint-Maur-des-Fossés, France), Pulp Canal Sealer (Kerr Manufacturing Co., Romulus, MI), Tubli-Seal (Kerr Manufacturing Co.), Apexit (Vivadent, Schaan, Liechtenstein), and AH Plus (De Trey, Konstanz, Germany). The treatments were completed in either 1 visit (usually teeth with vital pulps) or ≥2 visits (teeth with necrotic pulp) after placement of $Ca(OH)_2$ or metacresylacetate (Cresatina; Ogna, Milano, Italy) as an intracanal dressing.

Tissue processing

Immediately after extraction, the teeth were fixed 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% (v/v) formic acid and 10% (w/v) sodium citrate for 3-4 weeks with the end point being determined radiographically. All specimens were then washed in running tap water for 24-48 hours, dehydrated in ascending grades of ethanol, cleared in xylene, and 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 multirooted teeth were dissected free and processed separately. If curved, roots were separated into 2 pieces, 1 encompassing the coronal two-thirds and 1 including the apical third. These 2 pieces were embedded separately. With the microtome set at 4-5 μm, meticulous longitudinal serial sections were taken until each specimen was exhausted. Particular care was taken in sectioning the apical third to obtain sections passing through the apical foramen in direct continuity with the periapical tissues. Longitudinal or cross-cut sections were obtained from the coronal two-thirds. Every fifth section was stained with hematoxylin and eosin for screening purposes and for assessment of inflammation. A modified Brown and Brenn technique

for staining bacteria was used for selected slides.[9] Additional slides were stained as needed. The accuracy of the bacterial staining method was tested, using the protocol described by Ricucci and Bergenholtz.[10] Cover slips were then placed on the slides, which were examined under the light microscope.

Histologic evaluation criteria

The following semiquantitative criteria were used for the recording of observations during the microscopic evaluation of the tissue sections.

1. Presence of debris packed against the wound of remaining vital pulp stump:
   None (Figs. 3 and E1; see the on-line version of this article for Figs. E1-E9)
   Sparse (Fig. E2)
   Abundant (Fig. 1)
2. Presence of necrotic tissue in the apical root canal:
   None (Fig. 3)
   Superficial (limited to the material/tissue contact surface) (Fig. E1)
   Partial necrosis of pulp stump (Figs. 2 and E2)
   Complete necrosis of pulp stump (Fig. E3)
3. Histologic status of any vital tissue contained in the apical portion of the main canal, in lateral canals, and apical ramifications:
   Severe. Presence of severe inflammatory cell infiltrate with polymorphonuclear leukocytes, and mononuclear leukocytes (e.g., macrophages, foam cells, lymphocytes, and plasma cells) tapering off at the foramen in moderate or absence of inflammation (Figs. 1 and E4).
   Moderate. Moderate to mild chronic inflammation immediately below the wound surface. Dispersion of sealer particles within a distinguishable connective tissue. Infiltrates tapering off in an apical direction ending up in a connective tissue without inflammatory cell infiltrates at the apical foramen (Fig. 4).
   None. Virtual absence of inflammatory cell infiltrates in a well organized connective tissue. Only few scattered chronic inflammatory cells could sometimes be seen in the tissue. Necrotic tissue and dentin debris may or may not separate the noninflamed connective tissue from the root filling material (Figs. 3, E5).
4. Histologic status of any periodontal tissue fragment attached to the apical portion close to the foramen (when present):
   No inflammation (Fig. 2)
   Mild to moderate inflammation
   Severe inflammation (Fig. E4)
5. Presence of cementum formation in the apical root canal:
   None (Figs. 4 and E1)
   Minimal (Fig. E3)
   Diffuse (abundant cementum formation was observed restricting concentrically the apical lumen, in a way that serial sections disclosed only strands of connective tissue) (Figs. 3 and E6-8)
   Complete calcification of the foramen and apical root canal
6. Presence of bacteria in the apical root canal/ramifications:
   Yes
   No
7. Presence of bacteria in the coronal portion of the canal:
   Yes (Fig. 2)
   No

Allocation of teeth

To correlate clinical diagnosis and histologic findings at extraction, all teeth were divided into 3 groups based on the initial diagnosis: 1) teeth with an irreversible pulpitis (27 teeth); 2) teeth with a necrotic pulp with or without an apical periodontitis (22 teeth); and 3) teeth that were retreated (2 teeth).

RESULTS

The 2 control teeth did not show any radiographic or histologic changes in the apical tissues. The results of the 51 experimental teeth are summarized in Tables I-III.

Bacteria could be demonstrated in the coronal part of 47 out of 49 roots where coronal tissue was available for analysis.

Only 2 cases showed severe inflammation in the attached periodontal tissues (V25 and R2). In only 1 case (V25, #30) could bacteria be demonstrated in both the coronal and the apical portion of the root when using Taylor's modified Brown and Brenn stain. The observation period for this case was 13 years. There were no signs of cementum closure of the apical portion of the root canal. The tissue in the apical portion of the root canal was completely necrotic with severe inflammation in the attached periodontal tissues. The other case with severe apical inflammation was a retreatment case (R2, #20). The observation period was 9.5 years. In neither of these 2 cases were there any clinical symptoms or radiographic signs of periapical disease.

There were many cases of complete healing with no signs of chronic inflammatory processes. Case N5 (tooth #11) illustrates this group (Fig. 3). Tooth #11 in a 48-year-old patient was endodontically treated owing to painful pulp necrosis. The root canal was filled with gutta-percha and Tubli-Seal after 1 week dressing with calcium hydroxide. The tooth was restored with resin

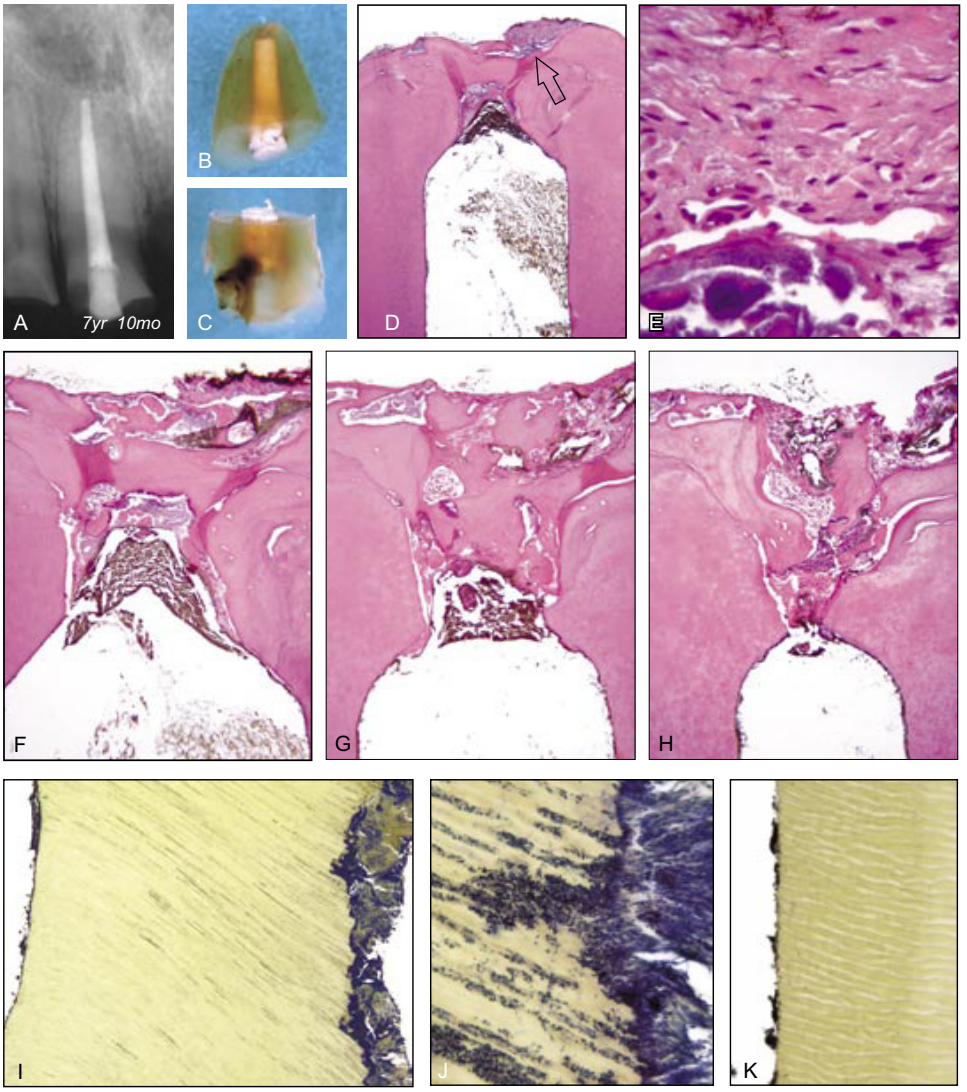

Fig. 2. Case N8. **A,** This maxillary central incisor of a 68-year-old woman was treated endodontically owing to pulp necrosis. No periapical lesion was present. Bio-Seal was used as a sealer. This 7 years 10 months follow-up shows normal periapical conditions. The patient had now lost all teeth except the central and a lateral incisor. Note the advanced attrition and the deep distal caries. The treatment plan was now a complete maxillary denture, and the 2 incisors were extracted. **B,** Apical portion of the root after clearing in xylene. **C,** Cleared coronal portion. It is evident that caries (*dark area*) had reached the root canal filling. **D,** The overview shows that endodontic instrumentation and root filling were performed at the correct apical level. Last apical instrument was a #80 K-file. Despite generous preparation in the dentin the pulp wound surface is small. Newly formed cementum is visible at the foramen (hematoxylin and eosin [H-E], original magnification ×50). **E,** Magnification of the area of the foramen indicated by the *arrow* in *D*. Limited amounts of necrotic tissue beyond the calcified barrier are bordered by noninflamed fibrous connective tissue (H-E, original magnification ×400). **F-H,** Sequence of sections passing through the apical foramen area. There are 60 histologic sections (approximatively 300 μm) between *F* and *G* and between *G* and *H*. The calcified tissue is irregular with lacunae containing vital or necrotic tissue. This calcified tissue seems to fill the foramen in some sections, whereas in others it appears interrupted (H-E, original magnification ×100). **I,** Coronal carious dentin. The root canal is on the left. The surface of the carious cavity is covered by a dense biofilm. Subjacent dentinal tubules are clogged with bacteria (Taylor's modified Brown and Brenn stain, original magnification ×50). **J,** High magnification of the surface of caries (Taylor's modified Brown and Brenn stain, original magnification ×400). **K,** Magnification of the root canal wall about 2 mm apical of the root canal orifice. No biofilm is present on the wall, and the tubules appear free of bacteria (Taylor's modified Brown and Brenn stain, original magnification ×400). **Summary:** Apical healing has progressed through a calcification process, which after nearly 8 years is still incomplete. Independent from the level of calcification, the important feature is the presence of connective tissue free from inflammation. This process does not appear to be influenced by the massive presence of bacteria in contact with the endodontic filling material at the root canal entrance. This case demonstrates that optimal obturation is a valid barrier against bacterial penetration for a long time.

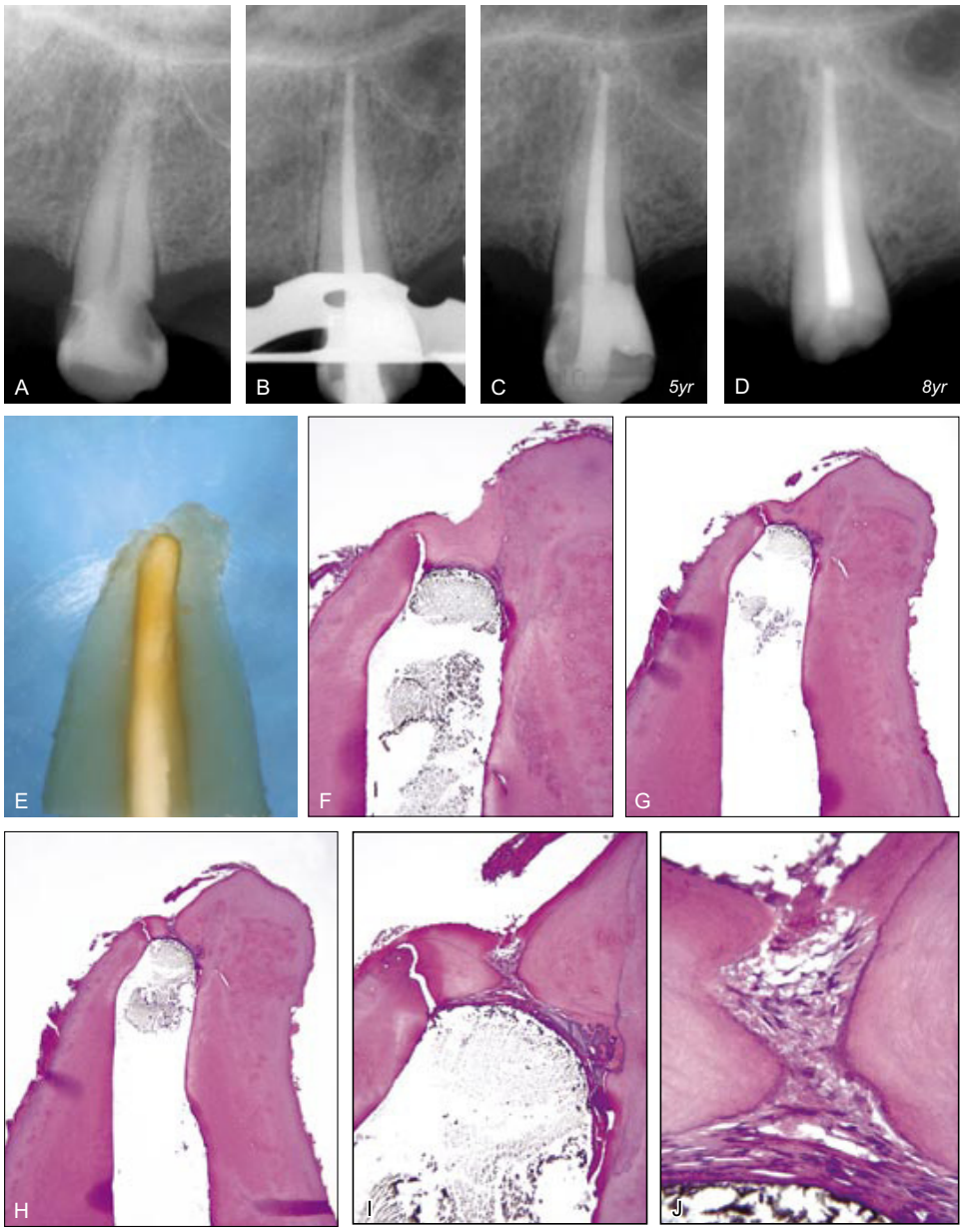

Fig. 3. Case N5. **A,** Maxillary canine with necrotic pulp in a 48-year-old woman. There was spontaneous pain. **B,** The root canal was filled after 1 week of medication with calcium hydroxide. Tubli-Seal was used as a sealer. The crown is restored with resin composite materials. **C,** The 5-year follow-up shows normal periapical structures. **D,** The patient presented with the loss of the restoration 8 years after treatment. Caries was present in the coronal part of the root. Although the tooth was restorable, the patient opted for extraction. **E,** Cleared tooth before paraffin infiltration. Note that the root filling is at a certain distance from the apex, which deviates in a buccal direction. **F,** Section passing approximately at the center of the canal. Calcified tissue is present apically to the root filling, which in this section seems to close the foramen (hematoxylin and eosin [H-E], original magnification ×50). **G,** After 50 sections (about 250 μm) through the apical calcification soft tissue appears. The empty space on the left between calcified tissue and radicular dentin is a shrinkage artifact (H-E, original magnification ×25). **H,** After an additional 50 sections it is evident that the newly formed hard tissue is incomplete and crossed by soft tissue (H-E, original magnification ×25). **I,** Magnification of the foraminal area in *H*. Connective tissue is present in contact with the root-filling material and continuous with the tissue entrapped in calcified tissue (H-E, original magnification ×100). **J,** The connective tissue is free from inflammation (H-E, original magnification ×400). **Summary:** This case illustrates the importance of serial sectioning when evaluating healing in the apical portion of the root canal.

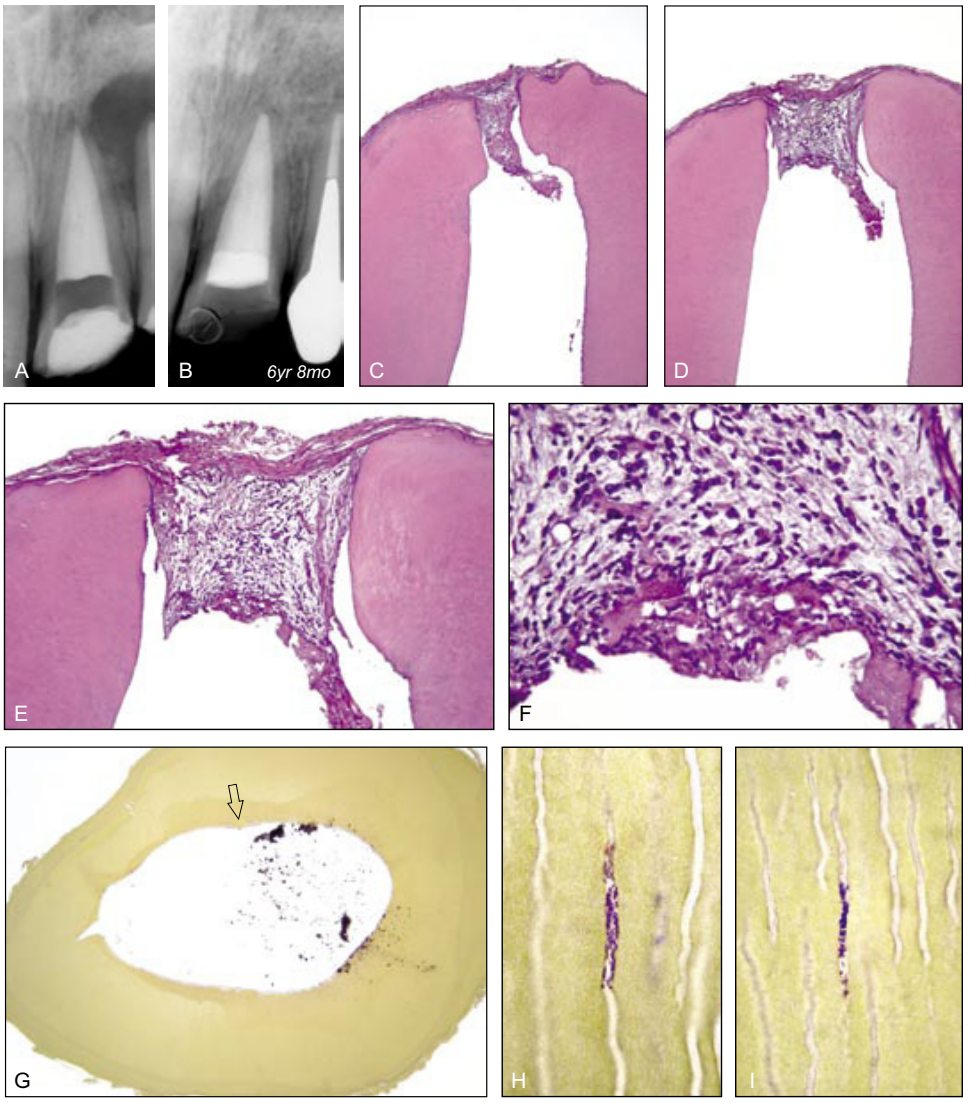

Fig. 4. **A,** Case N9. Maxillary central incisor with necrotic pulp in a 22-year-old man. The canal was filled after 2 weeks of medication with calcium hydroxide. Tubli-Seal was used as a sealer. The coronal two-thirds of the root canal was flared generously. The last apical instrument was a #90 K-file. The crown was restored with resin composite. **B,** The patient returned after 6 years 8 months complaining of mobility of the coronal restoration. Inspection shows an oblique fracture, which extended lingually to the periodontal bone level. Normal periapical conditions. The root filling appears shortened compared with the postoperative radiograph. **C,** Sections are cut longitudinally. The apical stop is visible at about 1 mm from the radiographic apex. Vital tissue fills the most apical part of the root canal (hematoxylin and eosin [H-E], original magnification ×25). **D,** After about 80 sections (about 400 μm), approximately in the center of the root canal, the prepared stop is visible only on 1 wall. Empty spaces between soft tissue and dentin are shrinkage artifacts (H-E, original magnification ×25). **E,** Magnification of the foraminal area in *D*. The tissue, filling the foramen area, is bordered externally by fibrous connective tissue (H-E, original magnification ×100). **F,** Magnification of the material-tissue contact area. Small area of necrotic debris is present. Subjacent tissue shows a moderate accumulation of chronic inflammatory cells, mainly plasma cells (H-E, original magnification ×400). **G,** The middle third of the root has been cross-cut. The overview shows the width of the preparation (Taylor's modified Brown and Brenn stain, original magnification ×25). **H, I,** High-power view of the point of the root canal wall indicated by the *arrow* in *G*. Bacteria can be seen in some tubules deep in the dentin (Taylor's modified Brown and Brenn stain, original magnification ×1,000). **Summary:** Despite the long observation period, no cementum formation has taken place in the foramen. Normal radiographic conditions were observed despite a chronic inflammation in the apical tissue remnants. It was not possible to establish whether the inflamed connective tissue in foramen area was preexisting or the result of proliferation from the periodontal ligament.

Table I. Summary of clinical and histologic observations (initial diagnosis vital pulp)

Case no.	*Tooth/ root*	*Follow-up*	*Radiographic apical limit of filling*	*Sealer*	*Debris in apical root canal*	*Necrosis in apical root canal*	*Histologic status of tissue in apical root canal*	*Histologic status of periodontal fragments*	*Cementum formation in apical root canal*	*Bacteria in apical canal or ramifications*	*Bacteria in coronal portion of root canal*
V1	11	5 yr 4 mo	short	Pulp Canal Sealer	sparse	superficial	none	—	diffuse	no	yes
V2	5/b	15 yr 6 mo	short	Bio Seal	abundant	none	none	none	diffuse	no	yes
V3*	5/p	2 yr	short	AH Plus	sparse	superficial	none	none	diffuse	no	yes
V4	5	1 yr	short	Apexit	abundant	superficial	none	—	diffuse	no	yes
V5	20	3 yr 7 mo	short	Pulp Canal Sealer	sparse	partial	none	none	diffuse	no	yes
V6*	20	5 yr 4 mo	short	Pulp Canal Sealer	sparse	superficial	none	none	diffuse	no	yes
V7	13	4 yr 6 mo	short	Pulp Canal Sealer	sparse	superficial	moderate	none	diffuse	no	yes
V8	4/p	15 yr	short	Pulp Canal Sealer	abundant	total	—	none	diffuse	no	yes
V9*	29	13 yr	short	Pulp Canal Sealer	none	none	none	none	diffuse	no	yes
V10	29	18 yr	short	Pulp Canal Sealer	abundant	partial	none	—	diffuse	no	yes
V11	8	20 yr	short	Pulp Canal Sealer	none	none	none	none	diffuse	no	yes
V12	31/m	18 yr	short	Endomethasone	abundant	partial	moderate	none	diffuse	no	yes
V13	28	6 yr	short	Apexit	sparse	none	none	none	diffuse	no	yes
V14	29	14 yr	short	Endomethasone	sparse	none	moderate	moderate	diffuse	no	yes
V15	13	14 yr 8 mo	short	Pulp Canal Sealer	abundant	partial	moderate	moderate	diffuse	no	yes
V16*	20	4 yr 7 mo	short	Apexit	sparse	superficial	none	none	diffuse	no	yes
V17	8	13 yr	short	Bio Seal	none	none	none	none	diffuse	no	yes
V18	11	13 yr 6 mo	short	Bio Seal	abundant	complete	—	none	diffuse	no	no
V19	15/p	12 yr	short	Bio Seal	none	none	moderate	none	minimal	no	yes
V20	8	19 yr 3 mo	short†	Pulp Canal Sealer	abundant	superficial	none	—	diffuse	no	yes
V21	11	14 yr 7 mo	short	Apexit	abundant	superficial	none	—		no	yes
V22	5	8 yr 8 mo	short	Apexit	abundant	partial	severe	—	diffuse	no	yes
V23*	7	9 yr	short	Apexit	abundant	partial	severe	severe	diffuse	no	yes
V24	22	3 yr	short	Apexit	none	superficial	none	none	diffuse	no	—
V25	30	13 yr	short	Pulp Canal Sealer	abundant	complete	—	severe	none	yes	yes
V26	20	4 yr 4 mo	short	Apexit	sparse	superficial	moderate	—	minimal	no	yes
V27	12/p	11 yr	short	Apexit	abundant	partial	none	none	diffuse	no	yes

*Lateral canals are present at a certain distance from the apical constriction. Their evaluation is not reported.
†Obturation material resorbed at follow-up. Obturation appears short at the moment of extraction.

composite materials. After 8 years the patient presented with loss of the restoration. Caries was present in the cervical root area. Although the tooth was restorable, the patient opted for extraction. The root filling was short of the apex with no residual debris or necrotic tissue present. The residual root canal space was nearly completely closed by cementum. Vital tissue in the residual pulp space with no signs of inflammation was in direct contact with the root filling material. This tissue contacted the periodontal tissue.

More residual debris and partial necrosis of the residual root canal content can be seen in case N8 (tooth #8), which was treated in a 68-year-old patient owing to pulp necrosis (Fig. 2). The root canal was filled with gutta-percha and Bio Seal in 1 visit. The tooth was restored with resin composite. After 7 years 10 months the patient presented with loss of the restoration. Caries was present in the coronal area with massive concentration of bacteria in the coronal part of the root canal. The patient had lost all teeth except the central and a lateral incisor, which were extracted. The root filling was short of the apex. There was sparse amount of debris between the root filling material and the residual tissue. Cementum was partially closing the root canal, including necrotic and vital tissue without inflammation. Multinucleated phagocytes were resorbing sealer fragments. There were no clinical or radiographic signs of periapical disease.

Several cases showed moderate inflammation in the root canal tissue (N20, tooth #14). There was pulp necrosis and periapical lesion at the time of endodontic treatment (Fig. 5). The root canals were filled after 2 weeks of medication with calcium hydroxide with gutta-percha laterally compacted with Bio Seal as a sealer. The tooth was used as a bridge abutment. It was extracted after 14 years. In the palatal root, the root filling was 1.5 mm from the radiographic apex, with vital tissue in the apical part of the root canal. Irregular calcifications were visible in the soft tissue. There was sparse amount of debris between root filling and the tissue, which had a superficial necrosis with minor level of inflammation. There was extensive deposit of cementum closing up the apical root canal and vital tissue without inflammation in the area. There were no clinical or radiographic signs of periapical disease.

Severe inflammation in the apical residual tissues can be seen in case V22 (tooth #5; Fig. 1). The pulp diagnosis

Table II. Summary of clinical and histologic observations (initial diagnosis necrotic pulp)

Case no.	Tooth/ root	Follow-up	Radiographic apical limit of filling	Sealer	Debris in apical root canal	Necrosis in apical root canal	Histologic status of tissue in apical root canal	Histologic status of periodontal fragments	Cementum formation in apical root canal	Bacteria in apical canal or ramifications or dentinal tubules	Bacteria in coronal portion of root canal
N1	5/p	2 yr 2 mo	short	AH Plus	abundant	complete	—	none	diffuse	no	yes
N2	22	5 yr	short	Pulp Canal Sealer	abundant	partial	moderate	none	minimal	no	yes
N3	29	8 yr 9 mo	short	Bio Seal	abundant	complete	—	none	minimal	no	yes
N4*	8	8 yr 5 mo	short	Pulp Canal Sealer	sparse	complete	none	—	diffuse	no	yes
N5	11	8 yr	short	Tubli-Seal	none	none	none	none	diffuse	no	yes
N6	18	15 yr	short	Endomethasone	none	complete	—	none	minimal	no	yes
N7	20	5 yr	overfilling	Apexit	none	partial	none	none	minimal	no	yes
N8	8	7 yr 10 mo	short	Bio Seal	sparse	partial	none	none	diffuse	no	yes
N9	9	6 yr 8 mo	Rad. Apex†	Tubli-Seal	none	superficial	moderate	none	none	no	yes
N10*	21	10 yr 9 mo	short	Bio Seal	sparse	superficial	moderate	none	minimal	no	yes
N11	7	2 yr	short	Apexit	sparse	superficial	moderate	none	none	no	yes
N12	13	17 yr 8 mo	Rad. Apex†	Endomethasone	abundant	partial	none	none	diffuse	no	yes
N13	29	6 yr 6 mo	Rad. Apex†	Pulp Canal Sealer	—	—	—	moderate	minimal	no	yes
N14	30/d	18 yr 9 mo	short	Endomethasone	abundant	—	—	moderate	minimal	no	yes
N15*	9	1 yr 7 mo	short	Apexit	abundant	partial	none	—	minimal	no	yes
N16	5	18 yr 8 mo	short	Pulp Canal Sealer	abundant	complete	—	—	diffuse	no	yes
N17	9	3 yr	short	Pulp Canal Sealer	sparse	superficial	moderate	none	diffuse	no	—
N18*	20	22 yr 4 mo	short	Endomethasone	abundant	complete	—	none	none	no	yes
N19	20	7 yr 10 mo	short	Bio Seal	none	none	severe	none	minimal	no	yes
N20	14/p	14 yr	short	Bio Seal	sparse	superficial	moderate	none	diffuse	no	yes
N21	21	9 yr	short	Apexit	sparse	superficial	none	none	diffuse	no	no
N22	20	17 yr	overfilling	Pulp Canal Sealer	abundant	—	—	none	minimal	no	yes

*Lateral canals are present at a certain distance from the apical constriction. Their evaluation not reported.
†Obturation material resorbed at follow-up. Obturation appears short at the moment of extraction.

Table III. Summary of clinical and histologic observations (retreatment cases)

Case no.	Tooth/ root	Follow-up	Radiographic apical limit of filling	Sealer	Debris in apical root canal	Necrosis in apical root canal	Histologic status of tissue in apical root canal	Histologic status of periodontal fragments	Cementum formation in apical root canal	Bacteria in apical canal or ramifications	Bacteria in coronal portion of root canal
R1	9	19 yr	short	Pulp Canal Sealer	abundant	superficial	moderate	moderate	diffuse	no	yes
R2	20	9 yr 5 mo	short	Bio Seal	sparse	superficial	severe	severe	diffuse	no	yes

at treatment was pulpitis and the tooth was treated in 1 visit with gutta-percha and Apexit. Cast post and core was placed with a temporary crown that was never replaced. The tooth was extracted after 8 years 8 months owing to caries. The pulp space along the post was heavily infected but could not be followed in the apical part of the root canal. Large amount of debris could be observed apical of the root filling materials. The root canal, which was narrowed by calcified tissue, contained connective tissue with moderate accumulation of chronic inflammatory cells. Some areas contained severe accumulation of polymorphonuclear leukocytes. There were no clinical or radiographic signs of periapical disease.

Some cases showed large amounts of necrotic debris in the apical area of the root canal (case N6, #18; Fig. E3). The tooth was endodontically treated owing to pulp necrosis. The tooth was extracted 15 years later owing to extensive caries making it unrestorable. The root filling was placed 1.5 mm from the radiographic apex. The space between the root filling material and the periodontal tissues was filled with necrotic debris. There was a transition from necrotic tissue to vital and noninflamed connective tissue. This tissue was composed of fibroblasts, collagen fibers, and vessels. There was no sign of microorganisms in the apical area. Midroot, there were microorganisms in the canal intermixed with the root filling material.

In most cases significant narrowing of the apical root canal could be observed. A few cases, however, failed to heal with cementum formation. In case N9, tooth #9 (Fig. 4), the pulp was necrotic and the root canal was filled with gutta-percha and Tubli-Seal after 2 weeks of calcium hydroxide medication. The tooth was restored with resin composite. The patient returned after 6 years 8 months

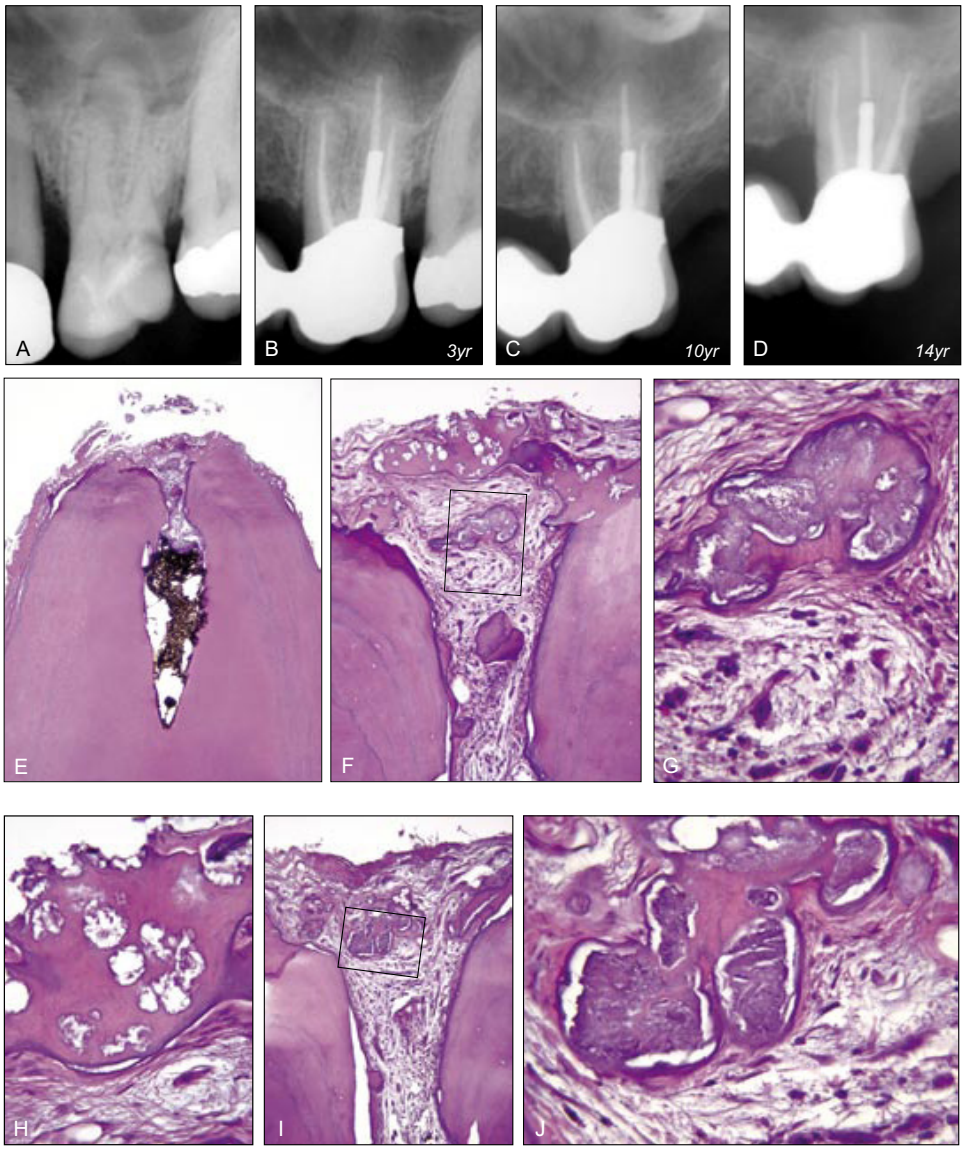

Fig. 5. Case N20. **A,** Palatal root of a maxillary first molar in a 54-year-old woman with necrotic pulp and periapical lesions. The root canals were filled after 2 weeks of medication with calcium hydroxide. Laterally compacted gutta-percha with Bio Seal. The tooth was then used as an abutment for a bridge. **B,** Three-year follow-up radiograph. Periapical healing is nearly complete. **C,** After 10 years, periapical conditions are radiographically normal. **D,** The patient returned after 14 years for emerging symptoms. Swelling was present buccally owing to an oblique crown fracture. The radiograph confirms the fracture line starting from the mesial aspect. Periapical conditions are normal. The tooth was extracted. **E,** Section passing through the foramen of the palatal root. The root filling ends about 1.5 mm from the apex. Vital tissue is present in the apical portion of the root canal (hematoxylin and eosin [H-E], original magnification ×25). **F,** Detail of the foraminal area. Nuclei of irregular calcification are present in the connective tissue (H-E, original magnification ×100). **G,** Magnification of the area demarcated by the *rectangle* in *F*. Amorphous material, partly calcified, is surrounded by fibrous tissue. Few chronic inflammatory cells at bottom (H-E, original magnification ×400). **H,** Magnification of the structure located out of the foramen in *F*. This appears as a calcified mass with irregular lacunae containing amorphous material (H-E, original magnification ×400). **I,** Other section, taken not far from that shown in *F*. Unusual structures are present in and outside the foramen (H-E, original magnification ×100). **J,** Magnification of the area out of the foramen demarcated by the *rectangle* in *I*. Multilocular structures, partly calcified within inflammation-free connective tissue (H-E, original magnification ×400).

with an oblique fracture extending lingually to the periodontal bone. Vital tissue filled most of the apical canal space, but a small amount of necrotic debris was present. The tissue showed a moderate accumulation of chronic inflammatory cells, mainly plasma cells. The tissue filling the foramen was free of inflammatory cells and was bor-

dered externally by a fibrous connective tissue. No bacteria could be seen in the apical region, but in the midroot area bacteria could be seen in the dentin tubules. Despite normal radiographic conditions, chronic inflammation was present in the pulp stump.

It was not unusual to find reactive calcified tissue deposits in the apical tissues surrounded by normal connective tissue (N1, tooth #5).

DISCUSSION

Unlike the examination materials in the studies by Brynolf, Green et al., and Barthel et al.,[1,3,4] the teeth in the present study were collected from living patients, which allowed optimal tissue handling, fixation, and preservation. All endodontic treatment was performed by 1 operator during a 20-year period, and the quality of all root canal therapy was carefully controlled. Therefore, the complete case history, such as clinical signs and/or symptoms, endodontic and restorative treatment details, status of coronal restorations, as well as period of follow-up time, was available. This is in contrast to cadaver material, where most such information, including the operator, is unavailable.

Much reliance has been afforded the results from the study by Brynolf[1] on cadaver teeth and the proposed true status of periapical tissues associated with endodontically treated teeth. Her study inferred the notion that in most cases of endodontic treatment there is residual inflammation in the apical/periapical tissues which can be visualized radiographically.[1,5]

Previously, the descriptions of the periapical tissues after endodontic treatment was often known as described by Kronfeld[11]: "Most roots are slightly underfilled, the length of the unfilled portion of the main canal varying between 0.5 and 2 mm [and] containing fibrous connective tissue, which may be either a remnant of the original pulp tissue or periodontal connective tissue that proliferated into the open apical portion of the root canal. The connective tissue has a tendency to form cementum, which is deposited in layers on the wall of the pulp canal." Then he describes several cases where "The apical portion of the root canal contains well vascularized connective tissue that is entirely free from inflammation; cementum has been deposited over all of the walls of the root canal."

Nygaard-Østby[12] stated: "To leave the apical and foraminal part of the pulp tissue and to retain its vitality will play a decisive role for the success in the treatment of the vital pulp . . . . In cases where the pulp is vital before treatment, no matter whether the diagnosis is clinically intact pulp [or] acute or chronic pulpitis, partial extirpation seems to give the most favorable prognosis. By appropriate treatment, in the majority of cases, the vitality of the residual pulp may be conserved, the result being a normal apical periodontal ligament and fibrous connective tissue in the apical portion of the root canal."

In random observations from extracted root filled teeth, our observations have been more in agreement with the description by Kronfeld[11] than with the description by Brynolf.[1]

Another issue of great interest has been the concept of coronal leakage, which was emphasized by Ray and Trope.[6] Careful study of this issue, however, suggested that coronal microbial ingress throughout the root canal of root-filled teeth, although possible, may be a random process.[10] In optimally endodontically treated teeth, prolonged exposure of the endodontic filling to the oral environment may evoke a localized histologic inflammation in the apical connective tissue, but development of a frank periapical lesion was not a common occurrence.

Histologic observation of the healing after endodontic treatment requires a biopsy of the tissue to be studied. In some earlier studies on human clinical cases, such biopsy involved the surgical removal of a substantial amount of periapical bone together with the apical root portion.[13,14] This invasive methodology restricts the number of cases available to study owing to ethical limitations. However, when the endodontic treatment is systematically confined within the root canal system it is possible to obtain substantial and valuable information on extraction material by observing the tissue remaining on the root surface and within the pulp space.[15] The endodontic technique used in the present study always limited the root canal preparation and filling to be short of the apex.

The material presented here suggests that the apical tissue of carefully root-filled teeth, with no conventional radiographic signs of periapical changes, such as thickening of periodontal ligament space, loss of lamina dura, or breakdown of apical alveolar bone, is only rarely significantly inflamed. When the tissue is inflamed, microbial causes can always be demonstrated. These findings suggest that moderate inflammation in the apical connective tissue is compatible with normal conventional periapical radiography.

In this case series study, we were able to demonstrate the presence of microorganisms in the coronal portion of the root canal in 47 of the 49 cases with coronal tissue. This is compatible with the fact that all root canals were lacking coronal closure. The coronal presence of bacteria, however, did not necessarily result in the presence of microorganisms in the apical portion of the root canal. Only 1 case (V25) had bacteria in both the coronal and the apical portions of the root canal. That case was originally diagnosed with pulpitis. At the time of extraction, 13 years after original treatment, the

residual tissue in the apical pulp space was necrotic with severe inflammation in the apical tissues. Despite this, there were no clinical symptoms or periapical pathologic disease visible on the radiograph.

The presence of debris and partially necrotic tissue was a common observation in the apical pulp space. In most cases this was not associated with significant inflammation in the tissues. We conclude from these observations that unless infected, this necrotic tissue is not able by itself to sustain tissue inflammation, because uninfected autologous necrotic tissue cannot indefinitely release inflammatory mediators and proinflammatory cytokines.[16]

Various sealers were used in a random fashion during this study (Tables I-III). Most commercially available sealers become practically inert after some time. Because the observation periods in this study were very long, it is very unlikely that any material toxicity would be discernible as tissue changes. No clinical association with outcome could be observed when comparing the long-term treatment results of the sealers used here (Fig. E9).[17]

The root fillings, which were well performed, appear to have effectively prevented the complete penetration of bacteria to the apical foramen. All root canals included in this study were severely infected at the coronal end, because the teeth were not protected by permanent restoration and often had catastrophic caries lesions (Fig. 2). Despite these conditions, bacteria could be seen in the apical part of the root canal in only 1 case. However, in one-third of the cases we could observe a mild inflammatory response in the apical tissues remnants. This is most likely the result of the penetration of nonantigenic and antigenic active bacterial byproducts inducing a mild inflammatory response. This microleakage, however, appeared to be unable to sustain a frank apical bone resorption.

It was common to find significant but incomplete apical closures of the apical part of the root canal with cementum. Despite the long observation time, no case with complete cementum closure could be observed.

All teeth in the present study were clinically free of symptoms and were radiographically without any discernible disease. This suggests that good diagnostic tools to assess the state of health of endodontically treated teeth are lacking. The ultimate goal of endodontic treatment must be not only radiographically normal periapical conditions, but also absence of inflammation in the periodontal ligament space and in remaining connective tissue in the apical foramen and apical pulp tissue ramifications.

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SUPPLEMENTARY DATA

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