
Cone-beam computerized tomographic, radiographic, and histologic evaluation of periapical repair in dogs' post-endodontic treatment

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Objective. To evaluate the periapical repair after root canal treatment in the teeth of dogs using CT and conventional radiography and to compare these findings with the gold standard microscopic evaluation.

Study design. The animals were divided into three groups according to endodontic treatment performed: Group 1, single-visit endodontic treatment in teeth without apical periodontitis; Group 2, single-visit endodontic treatment in teeth with apical periodontitis; and Group 3, endodontic treatment in teeth with apical periodontitis using calcium hydroxide as a root canal dressing. Group 4 consisted of teeth with apical periodontitis not submitted to root canal treatment and Group 5 consisted of healthy teeth without periapical disease. Radiographic, tomographic, and microscopic evaluations were performed by blind examiners. At 180 days experimental time, CT and radiographic measurements of periapical disease were compared with the gold standard microscopic measurement using intraclass correlation coefficient. Intergroup comparisons considering different methods of periapical lesions measurement or different clinical protocols of root canal treatment were performed by Kruskal Wallis test followed by Dunn. Integrity of lamina dura, presence of radiolucent areas, and presence of root resorption were analyzed by Fisher's exact test.

Results. There was discontinuity of the lamina dura and CPD in all teeth from Groups 2, 3, and 4 evaluated by tomography and radiography 45 days after CPD induction. Radiographically, 180 days after root canal treatment, there was no periapical lesion in teeth from Groups 1 and 3, different from groups 2 and 4 ($p < .05$). The highest reduction in the CPD size was observed on Group 3 ($p < .05$). According to the tomographic results, there was decrease of the size of the CPD on Group 3 but not on Groups 2 or 4. However, in all groups the periapical lesions presented larger mesio-distal extension if compared with radiography, both 45 days after CPD induction and 180 days after root canal treatment. At 180 days, CT measurements were closely related to microscopic results (ICC = 0.95) differently from radiographic evaluation (ICC = 0.86).

Conclusion. CT Scan evaluation of periapical repair following root canal treatment provided similar information than that obtained by microscopic analysis, whereas radiographic evaluation underestimated the size do periapical lesion. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:796-805)

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Periapical radiolucence is an important signal for detection of apical periodontitis which occurs due to bone resorption as a result of the host defense against bacteria inside the root canal system. Root canal therapy is accepted as the most effective conservative method for treating apical periodontitis. The treatment outcome should be determined at follow-up examinations for at least 4 years, when it is established whether the preexisting periapical radiolucence has completely disappeared.¹⁻³

Conventional and digital periapical radiographs have been widely used for root canal treatment follow-up. However, in teeth with apical periodontitis, microscopic findings and radiographic examinations are often divergent.⁴ These outcomes suggest that, in many cases, chronic periapical inflammation may persist for years after root canal filling, even in the absence of clinical symptoms and radiographic alterations.^{5,6} Ra-

diographs provide bidimensional images that do not permit the evaluation of bone thickness and determination of the size and localization of periapical lesions.^{7,8} Also, structures placed along the X-ray beam are projected in the same position on the radiographic film, and vestibular and lingual root canals in multirrooted teeth cannot be differentiated in the radiograph, because they appear superimposed. The mandibular canal can be projected over the tooth apex or over periapical lesion, even if they are not nearly located.⁸⁻¹⁰

Computerized tomography (CT) scan has been gradually introduced in endodontics.^{11,12} The cone-beam CT (CBCT) scan is a technique that exposes an object to multiple cone-shaped beams to acquire the volume of the object, and later serial section images are obtained making possible the 3-dimensional (3D) interpretation.^{13,14} Thus, the clinician can visualize morphologic features and pathologies from different 3D perspectives.¹¹ Current publications have demonstrated that CT scans permit 3D evaluation of bone structures and the relationship of bone with maxillary sinus and mandibular canal.^{8,10,15-23}

Because it has been demonstrated previously that detection of periapical lesions following root canal treatment using CT scan technology is more accurate than radiographic evaluation,^{18,24,25} the aim of the present study was to compare the radiographic and tomographic findings after root canal treatment in teeth experimentally induced apical periodontitis with the gold standard microscopic evaluation.

MATERIAL AND METHODS

Animal procedures conformed to protocols reviewed and approved by the Animal Care Committee of the University of Sao Paulo (no. 2007.1.192.53.6). Fifteen mongrel dogs (12 months old, weighing 10-15 kg) were selected for treatment. Third and fourth mandibular premolars of the dogs were used, thereby yielding a total of 120 roots, assigned to 5 experimental groups according to the treatment performed.

The animals were anesthetized intravenously with sodium thiopental (30 mg/kg body weight; Thionembutal; Abbott Laboratories, Sao Paulo, Brazil), and standardized periapical radiographs were taken. After isolation of the teeth with rubber dam and disinfection with 2% chlorhexidine gluconate, accesses to the pulp chambers were made.

In group 1 (vital pulpectomy; n = 24), the working length was determined to be 1.5 mm short of the radiographic apex. The pulp tissue was extirpated and the root canals irrigated with 2.5% sodium hypochlorite

solution. The apical cementum layer characteristic of dogs' teeth was then perforated with the sequential use of size #15 to #30 K-files, thus creating standardized apical openings. The root canals were instrumented to the working length up to a size #60 K-file using a step-back technique. A size #30 K-file was taken to the total root length to ensure apical patency. After final irrigation with saline solution, the root canals were dried with sterile paper points and then filled with 14.3% buffered EDTA, pH 7.4 (Odaçan-Herpo Produtos Dentários, Rio de Janeiro, Brazil), for 3 minutes. Saline solution was used to rinse out the EDTA, and the canals were then dried with sterile paper points. The root canals were filled with gutta percha points (Dentsply Indústria e Comércio, Petrópolis, Brazil) and AH Plus Jet Mix sealer (Dentsply/De Trey, Konstanz, Germany) according to the manufacturer's instructions using the lateral condensation technique. The coronal access cavities were restored with a glass ionomer cement base (Vitremer; 3M/Espe, St. Paul, MN) followed by silver amalgam (Velvalloy; SS White, Rio de Janeiro, Brazil).

In groups 2 and 3, the dental pulps were removed and the root canals exposed to the oral cavity for 7 days to allow microbial contamination. After that, access openings were sealed with zinc oxide-eugenol cement (IRM; Dentsply Indústria e Comércio). Radiographs were taken at 15-day intervals until radiolucent images indicating apical periodontitis were obtained, which occurred after 45 days. Neutralization was carried out in a crown-down direction using 2.5% sodium hypochlorite irrigation at each file change. After that, instrumentation was performed as described for group 1. In group 2 (single visit; n = 24), the root canals were filled after instrumentation. In group 3 (two visits; n = 24), a Ca(OH)₂-based paste was used as root canal dressing (Calen [composition: 2.5 g Ca(OH)₂, 0.5 g zinc oxide p.a., 0.05 g colophony, 1.75 mL polyethylene glycol 400]; SS White Artigos Dentários). Sterile cotton pledgets were placed in the pulp chamber, and the access cavity was filled with IRM. Fifteen days later, intracanal dressing was removed and the canals were dried, filled with EDTA for 3 minutes, irrigated with saline, and redried. Root canal filling was performed with gutta-percha cones and AH Plus Jet Mix sealer.

In group 4 (n = 24), apical periodontitis was induced as described above, except the root canal treatment was not performed. Healthy teeth (group 5; n = 24) were used as control samples to compare a healthy microscopic periapical area with the findings obtained after different root canal treatment protocols (groups 1, 2, and 3).

Radiographic and CT scan evaluations

The animals were followed for 180 days, and standardized periapical radiographs were taken before intervention, 45 days after root canal contamination, and 180 days after root canal filling. A custom-made film-holding device was used for standardization of the radiographic technique according to parallel radiographic technique.²⁶ The radiographs were taken with size 2 periapical films (Ultraspeed; Eastman Kodak Company, Rochester, NY) and X-ray equipment (Heliolent; Siemens, New York, NY) operating at 60 kVp and 10 mA with 1-second exposure time. The exposed films were processed manually by the time/temperature method. The images were digitized through an optical scanning process (Scanjet 7450C; Hewlett-Packard; Palo Alto, CA) with a resolution of 1,200 dpi.

A CT scan was performed using a CBCT scan apparatus (NewTom 3G; QR, Verona, Italy) operating at 120 kV and 3.6 mA with field of view 9 inches and exposure time 36 seconds. This apparatus captures 360 images with 1-degree interval, resolution of 512×512 pixels, and 12 bits per pixel (4,096 levels of gray). In the present study we used sagittal view, with slices of 1 mm thickness and 0.5 mm between slices. This view was selected to perform direct comparisons with radiographs, which provide images in sagittal view.

Radiographic and tomographic evaluations were performed by 3 calibrated examiners ($\kappa = 0.96$) who evaluated the following parameters: integrity of the lamina dura, presence of areas of periapical bone rarefaction, and external root resorption. Radiolucent images suggestive of periapical lesions were measured using Image J 1.28 u software (National Institutes of Health, Bethesda, MD) as previously described.²⁷

Microscopic evaluation

After 180 days following root canal treatment, the animals were killed with an intravenous overdose of sodium pentobarbital. The maxillas and mandibles were dissected and sectioned to obtain individual roots. Teeth were fixed in 10% buffered formalin for 72 hours, demineralized in EDTA, and embedded in paraffin. Sagittal 5- μ m serial sections were obtained, and the sections were stained with hematoxylin and eosin (HE) and examined under light microscopy by skilled observers blinded to the treatment groups ($\kappa = 0.87$).

The histomorphologic parameters evaluated in this study were based on criteria previously described.²⁸⁻³⁰ Morphometric analysis of periapical lesion size was performed in representative HE-stained slides with the microscope operating in fluorescence mode (excitation

at 460-500 nm and emission at 512-542 nm). Measurements were restricted to histologic sections representing the maximum size of the lesion at $\times 4$ magnification. The extension of the inflammatory reaction, presence of tooth resorption, and presence of apical sealing by mineralized tissue were qualitatively scored according to the intensity of the event ($\times 20$ and $\times 40$ magnification).

Statistical analysis

At 180 days' experimental time, CT scan and radiographic measurements of periapical disease were compared with the gold standard microscopic measurement using the intraclass correlation coefficient (ICC). Data distribution were non-normal; therefore, intergroup comparisons considering different methods of periapical lesions measurement or different clinical protocols of root canal treatment were performed by Kruskal-Wallis followed by Dunn test. Integrity of lamina dura, presence of periapical radiolucent areas, and presence of root resorption were analyzed by Fisher exact test. The significance level was set at $\alpha = .05$.

RESULTS

Lamina dura disruption, periapical radiolucent area, and external root resorption evaluation by CBCT scan and radiography after periapical disease induction and root canal treatment

The lamina dura was present in all teeth before root canal treatment, and there were no periapical radiolucent area or external root resorption in none of the groups. Forty-five days after root canal contamination, there were discontinuity of the lamina dura and periapical disease formation on specimens from groups 2, 3, and 4 (Fig. 1). External root resorption could be observed in about 60% of the specimens in groups 2, 3, and 4 without difference among them ($P > .05$; Table I). There was no difference between CT scan and radiography evaluation of these parameters ($P > .05$).

After root canal treatment (180 days), CT scan evaluation detected periapical radiolucent area suggestive of periapical disease and lamina dura discontinuity in 45.8% of the specimens from group 1 versus 37.5% detected by radiography ($P > .05$; Fig. 2). In group 2, all specimens presented periapical disease and discontinuity of lamina dura 180 days after root canal treatment detected by either CT scan or radiography, without difference between the methods used ($P > .05$). External root resorption was detected in 88.8% of the specimens of group 2, different from groups 1 and 3, which presented lower incidence of external root resorption ($P < .0001$). In group 3, periapical lesions and discontinuity of lamina dura were detected in 57.1% of the cases using radiography, different from CT scan, which

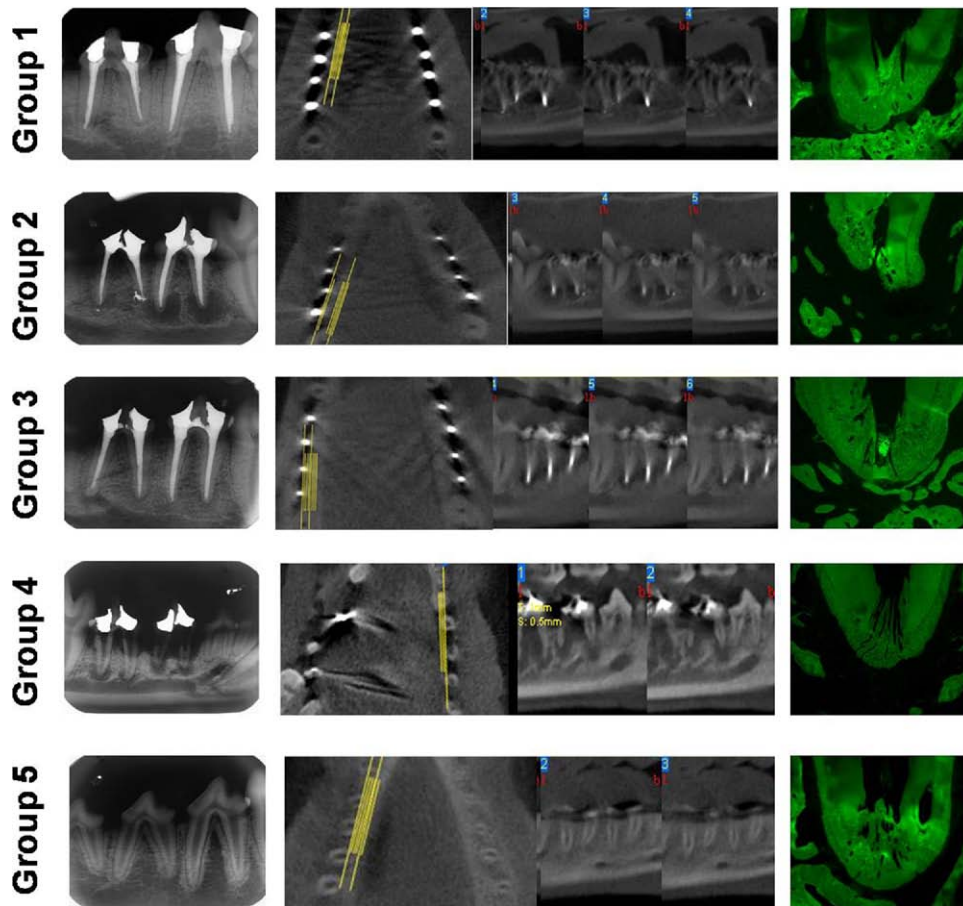


Fig. 2. Representative conventional radiographs (*left*), computerized tomography (CT) scans (*center*), and microscopic sections (*right*) of specimens from group 1 (root canal treatment in teeth without periapical disease), group 2 (single-visit root canal treatment), group 3 (calcium hydroxide as a root canal dressing), group 4 (periapical lesion without root canal treatment), and group 5 (healthy teeth). The CT scan pictures are presented in axial and sagittal views. Yellow lines on axial view depict the sequence and angle of the sections in vestibulolingual orientation. Numbers inside blue box on sagittal views indicate the position of the section in the sequence presented on axial view.

lesions over time ($P < .0001$). To evaluate the reduction of the lesion after root canal treatment using different methods (CT scan vs. X-ray), the percentage of reduction of the lesion was calculated based on the size of the lesion before root canal treatment. We observed that in group 3, CT scan revealed lower reduction of the size of the lesion (43.62%) compared with X-ray (61.91%; $P = .0479$). On the other hand, in group 2 no difference was observed regarding the percentage of reduction of the size of the lesion by radiography using either CT scan or X-ray ($P > .05$; 6.5% vs. 10.19%, respectively).

Comparison of conventional radiography and CT scan with microscopy to measure periapical disease after root canal treatment

At 180 days after root canal treatment, remaining periapical diseases were measured using conventional radiog-

raphy, CT scan, and microscopy. The sizes of the lesions are expressed on Fig. 4. ICC was calculated to compare the measurements obtained using CT scan and conventional radiographs with the gold standard microscopic evaluation and indicated a higher correlation between CT scan with microscopic measurement (ICC = 0.95) and a lower correlation between conventional radiograph and microscopic measurement (ICC = 0.86).

Microscopic findings after root canal treatment using different clinical protocols

In most of the specimens from groups 1 and 3, the inflammatory infiltrate was located up to one-half of the apical periodontal ligament extension, different from groups 2 and 4, where >75% of the teeth presented inflammatory infiltrate beyond one-half of the periodontal ligament ($P < .0001$). In roots from group 2, tooth resorption involving cementum and dentin were

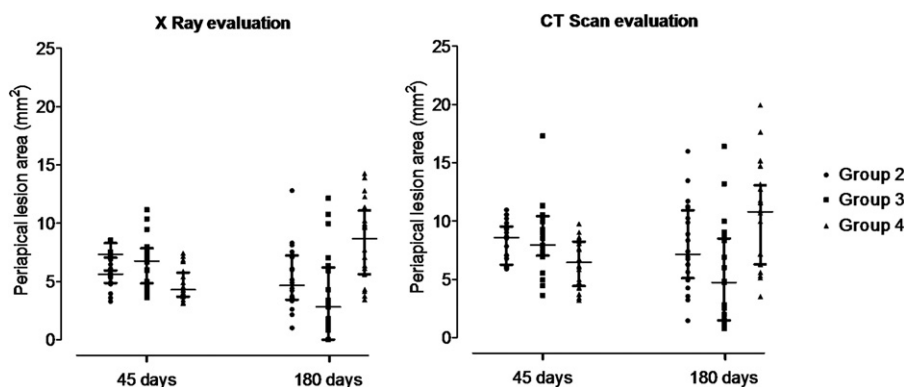


Fig. 3. Size of periapical disease evaluated at 45 days after root canal contamination and 180 days after root canal treatment using X-ray and computerized tomography scan for all specimens from group 2 (circle—single-visit root canal treatment), group 3 (square—calcium hydroxide as a root canal dressing), and group 4 (triangle—periapical lesion without root canal treatment). Values are expressed in mm²; points represent each specimen, and bars depict median and interquartile ranges.

observed, whereas in groups 1 and 3 tooth resorption involved cementum alone. Root resorption was more intense in teeth with periapical disease without root canal treatment ($P < .0001$), followed by teeth from groups 2 and 3 without difference between them ($P > .05$). In group 1, root resorption was present in a small amount of samples and overall not different from healthy teeth ($P > .05$). Apical sealing with a cementum-like tissue could be observed on specimens from groups 1 and 3, different from groups 2 and 4 ($P < .0001$; Table II; Fig. 5).

DISCUSSION

Two radiographic methods are currently described in the literature to evaluate periapical repair after root canal treatment in teeth with chronic periapical disease. In the first one, the periapical repair is evaluated subjectively regarding the characteristics of the radiolucent area, and the second one involves the measurement of the lesion in different time intervals after root canal treatment and comparison of the percentage of periapical disease reduction. The latter presents the advantage of being standardized and more accurate.³¹⁻³⁴ In the present study, we used this method to compare the size of the periapical disease between the radiographs and CT scans.

The most important finding in this paper was the observation that using CT scans, the periapical disease area presented larger mesiodistal extension compared with conventional radiographs, either 45 days after root canal contamination or 180 days after root canal filling. This divergence between CT scan and radiography could be related to the superimposition of structures in the periapical radiograph which can mask the real size of the lesion. In contrast, using CT scan it is possible to

perform thin serial sections in order to access different areas of the lesion. Based on that, we considered as periapical disease measurement the larger mesiodistal extension among the sagittal slices obtained from each root. We believe that this is the most adequate measure of the lesion to be considered, because it represents the maximum diameter of the lesion when it is possible to work without superimposition of images.

We could observe that in group 3, some periapical lesions were detected using CT scan which were not detected using periapical radiographs, which is in agreement with earlier reports that demonstrate that CT scan is better than conventional radiographs to detect periapical disease.^{35,36} Periapical lesions with mean mesiodistal diameter of 2.8 mm not identified by radiographs could be detected using CT scan.³⁵

Different methods of acquisition of images by tomographic techniques have been used for diagnosis in endodontics. Tammisalo et al.¹⁷ used conventional spiral tomography (Scanora) and observed that tomography is adequate to detect periapical lesions on premolar and molar teeth, but they reported that the difference in accuracy to detect periapical lesions did not surpass conventional radiographs. However, they used thicker slices (8 mm), which increases the chances of superimposition of structures. Cone-beam CT scan technology makes possible work with thin slices (1 mm), and it is important to use this tool to detect small-sized lesions.

In a recent paper, Estrela et al.²² demonstrated the accuracy of CBCT compared with panoramic and periapical radiographs for detection of apical periodontitis. They showed that apical periodontitis is correctly identified with conventional radiographic methods when disease is in advanced stage according to periapical index. Also, CBCT tends to provide greater scores than

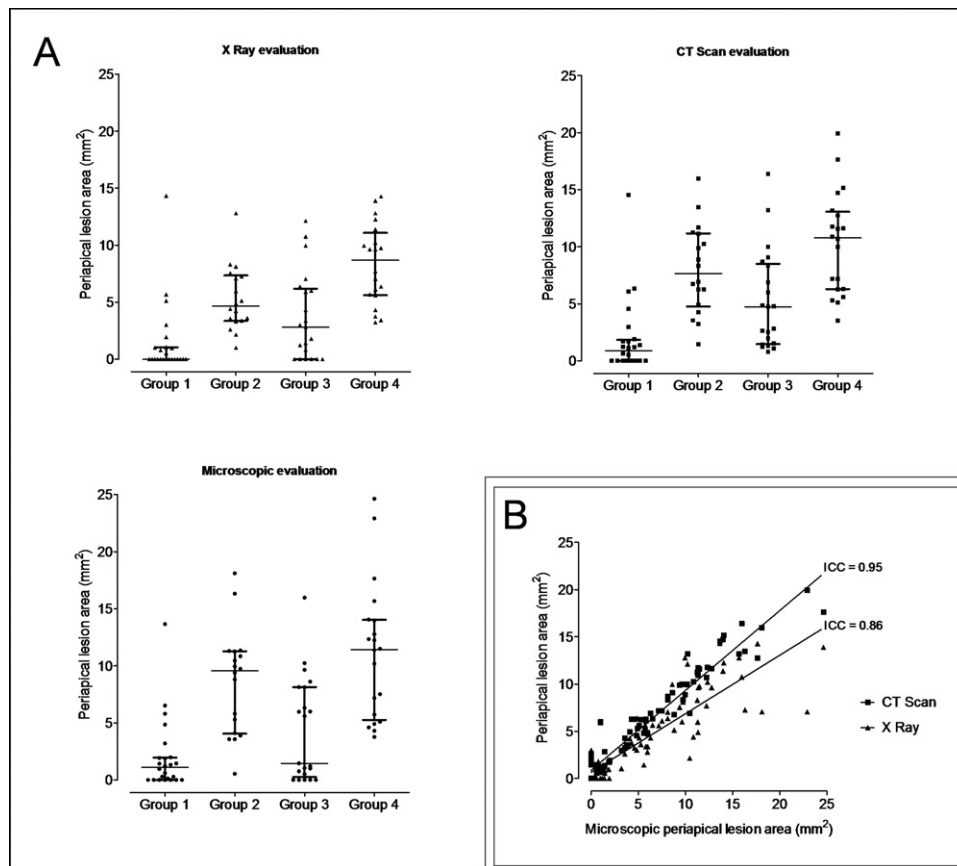


Fig. 4. Size of periapical disease evaluated 180 days after root canal treatment using X-ray, computerized tomography (CT) scan, or microscopy for specimens from group 1 (root canal treatment in teeth without periapical disease), group 2 (single-visit root canal treatment), group 3 (calcium hydroxide as a root canal dressing), and group 4 (periapical lesion without root canal treatment). **A**, Values are expressed in mm²; points represent each specimen, and bars depict median and interquartile ranges. **B**, ICC was calculated to compare the measurements obtained using CT scan and conventional radiographs with the gold standard microscopic evaluation.

Table II. Microscopic evaluation of apical and periapical areas 180 days after root canal treatment

Parameter	Group 1 (n = 24)	Group 2 (n = 18)	Group 3 (n = 21)	Group 4 (n = 20)	Group 5 (n = 24)
Extension of the inflammatory reaction					
1 Absent	6 (25%)	0	0	0	20 (100%)
2 Restricted to the apical foramen	9 (37.5%)	0	7 (33.3%)	0	0
3 Up to half of the apical periodontal ligament	9 (37.5%)	4 (22.2%)	8 (38.1%)	0	0
4 Beyond half of the periodontal ligament	0	14 (77.8%)	6 (28.6%)	20 (100%)	0
Tooth resorption					
1 Absent	20 (83.4%)	3 (16.7%)	9 (42.9%)	0	24 (100%)
2 Cementum resorption	4 (16.6%)	12 (66.6%)	12 (57.1%)	15 (75%)	0
3 Dentin resorption	0	3 (16.7%)	0	5 (25%)	0
Apical opening sealed with mineralized tissue					
1 Complete sealing	10 (41.6%)	1 (5.5%)	15 (71.4%)	0	—
2 Sealing beyond half	2 (8.4%)	2 (11.1%)	2 (9.5%)	0	
3 Sealing up to half	8 (33.4%)	0	0	0	
4 Absence of sealing	4 (16.6%)	15 (83.4%)	4 (19.1%)	20 (100%)	

periapical and panoramic radiographs, which is in agreement with the larger periapical disease mesiodistal extension detected in the present study using CT scan

compared with periapical radiographs, both at 45 days after root canal contamination and 180 days after root canal filling.

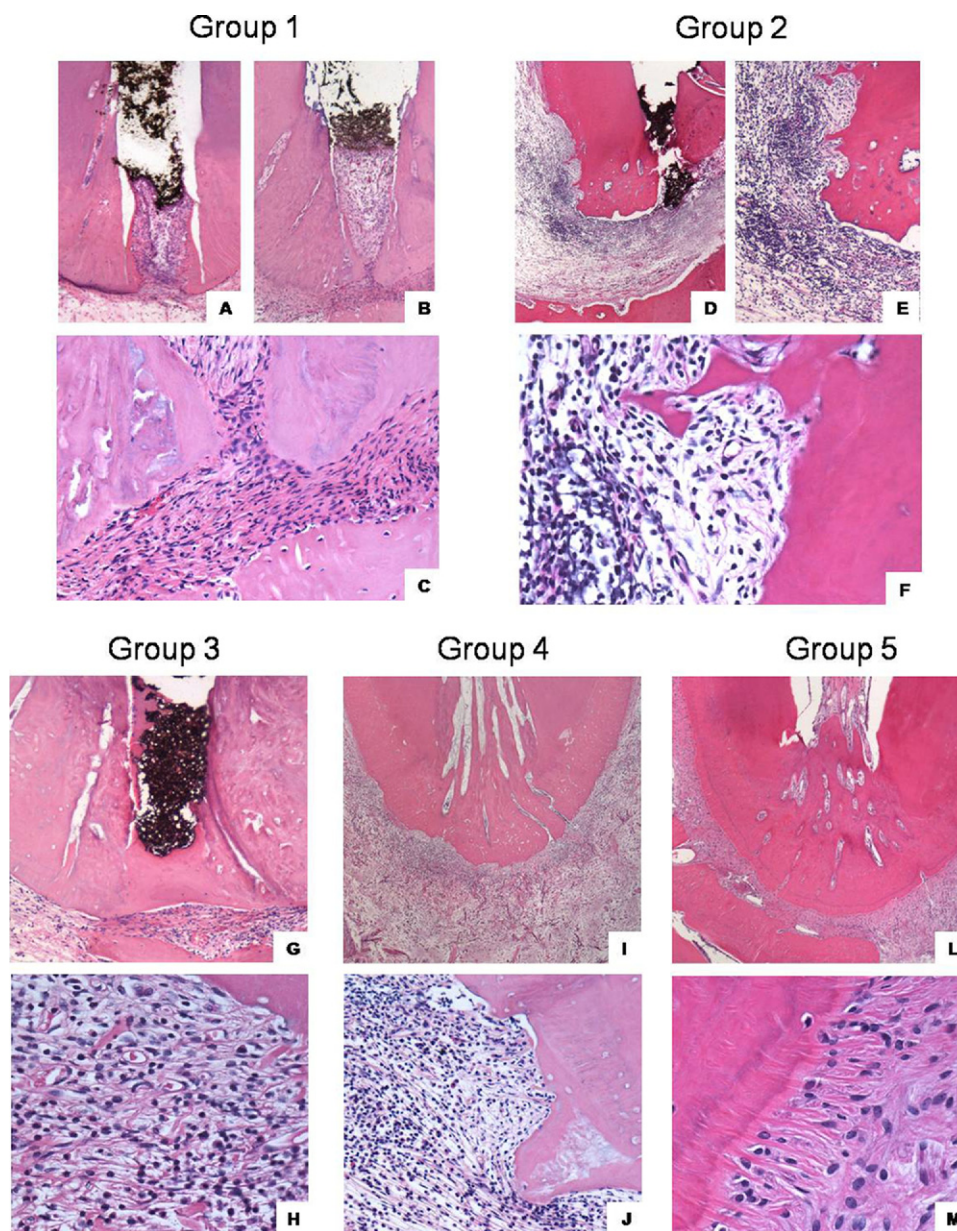


Fig. 5. Microscopic sections obtained 180 days after root canal treatment. **A, B, C**, Group 1 (root canal treatment in teeth without periapical disease): presence of mineralized tissue partially sealing the apical opening. Higher magnification shows integrity of the apical periodontal ligament area and bone tissue. **D, E, F**, Group 2 (single-visit root canal treatment): presence of an intense inflammatory infiltrate in periapical area concomitant to bone and cementum resorption areas; absence of apical sealing by mineralized tissue. **G, H**, Group 3 (calcium hydroxide as a root canal dressing): presence of mineralized tissue completely sealing the apical opening and absence of periapical inflammatory reaction. Lateral to the apex, small areas of root resorption could be observed concomitant to a moderate inflammatory infiltrate. **I, J**, Group 4 (periapical lesion without root canal treatment): presence of inflammatory periapical reaction characterized by bone and tooth resorption. Presence of small newly formed blood vessels and tissue fibers disorganization. **L, M**, Group 5 (healthy teeth): healthy periapical area characterized by absence of inflammatory cells and presence of periodontal ligament fibroblasts, cementoblasts, and Sharpey fibers inserted into the cementum. Hematoxylin-eosin staining; magnification $\times 40$ (A, D, G, I, L), $\times 100$ (B, E, J), and $\times 400$ (C, F, H, M).

The major advantage of CBCT is the elimination of the superimposition of anatomic structures, such as cortical plates and other structures.^{11,35} We could con-

firm that statement, because when we obtained thin slices of cancellous bone we could detect periapical lesions of higher mesiodistal extension compared with periapical

radiographs. Information provided here is innovative, because it could be demonstrated that using the sagittal view, periapical radiographs fail to show the real dimension of the periapical disease, probably owing to superimposition of images. The images obtained by CT scan represent the real maximum size of the lesion, which could not be accessed by periapical radiography.

The most important finding in the present paper was the observation that using CT scans and periapical conventional radiographs, the mean area of periapical disease presented larger mesiodistal extension when evaluated using CT scans compared with conventional radiographs, either 45 days after root canal contamination or 180 days after root canal filling. The underestimation of the size of periapical lesion by periapical radiographs compared with CT scan highlights the importance of future human clinical trials to determinate the mean time needed for periapical healing using CT scan, especially because recent reports have suggested that persistence of periapical disease can have an impact on both oral and general health.

Because the usefulness of periapical radiography in determining the absence of apical periodontitis has been challenged, the viability and cost-effectiveness of CBCT images in clinical routine should be weighed. The effective radiation dose for a full mouth series has been reported to range from 33 to 84 microsieverts (μSv), depending on different variables.³⁷ The effective dose of radiation for CBCT ranges from 36.9 to 50.3 μSv .³⁸ Therefore, the amount of radiation exposure to the patient from CBCT scanning is actually similar to that received from routine diagnostic imaging.^{11,37,38}

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