Acta Odontologica Scandinavica, 2005; 63: 143–152

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ORIGINAL ARTICLE

Effect of root canal dressings on the regeneration of inflamed periapical tissue

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Abstract
The aim of this study was to evaluate the effects of chlorhexidine and calcium hydroxide on apical periodontitis in rats. Experimentally induced apical periodontitis was established on the mesial roots of maxillary molars of Wistar rats by leaving the root canals exposed to the oral cavity for 14 d. In the positive control group ($n = 10$ teeth), the root canals were not further treated, but the coronal access openings were filled with composite. In the negative control group ($n = 10$ teeth), partial pulpotomies were performed aseptically and the coronal access openings were sealed immediately. In a third control group ($n = 10$ teeth) the canals were instrumented, left empty, and the coronal access openings were sealed. In the experimental groups, the root canals were instrumented and filled with either 2% chlorhexidine gel or calcium hydroxide paste ($n = 10$ teeth per group). After 7 d all rats were killed and the histological sections were stained for microscopic analysis of periapical regeneration. The data of the subjective evaluation were analyzed with the Kruskal-Wallis test. Lesion sizes were measured and statistically analyzed using the ANOVA and post-hoc Scheffé test. The two treatment groups showed significantly lower average inflammatory scores and smaller lesion sizes than the positive and third control group ($p < 0.05$). No statistically significant differences were obtained between the two treatment groups ($p > 0.05$). Chlorhexidine used as an intracanal medicament showed good periapical regeneration, suggesting that this may be an alternative to calcium hydroxide root canal dressing.

Key Words: *Apical periodontitis, calcium hydroxide, chlorhexidine, rats*

Introduction

An infection of the pulp, if present for a considerable time, can result in microbial colonization of the entire root canal system together with the dentinal tubules adjacent to the root canal. Microorganisms and their toxic metabolic products are responsible for the development and persistence of apical periodontitis of endodontal origin. The elimination of microorganisms from the endodontium is therefore crucial in treatment of the infected root canal [1]. It has often been shown that vital microorganisms remain in the root canal and can subsequently multiply despite chemomechanical root canal treatment [2]. Insertion of an antimicrobial root canal dressing after preparation is therefore generally recommended in treatment of the infected root canal [1–3].

Such medications must have the greatest possible and most long-lasting antimicrobial efficacy against the various bacterial species in the infected root canal without causing irritation of the periapical tissue [4]. The most desirable dressing would thus be one that combines maximal antimicrobial effect with minimal toxicity. Chlorhexidine digluconate solutions of varying concentrations have recently been recommended as endodontic irrigants [5,6] and as root canal dressings [3,7]. Early reports demonstrated a marked antimicrobial effect of 2% chlorhexidine digluconate [5,7,8]. Since intracanal medicaments can contact vital apical and periapical tissues, biocompatibility with these tissues is important. Although the toxicity of chlorhexidine has been investigated in animal studies [9,10], specific usage tests with histologic control of the reactions of the periapical tissues are necessary for

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(Received 11 November 2004; accepted 4 February 2005)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.1080/00016350510019801

evaluation of root canal medicaments [11]. Tanomaru Filho et al. [12] have conducted an animal study on the regeneration of periapical tissues of teeth with periapical lesions when 2% chlorhexidine was used as an irrigating solution during root canal preparation. According to their results, the use of chlorhexidine during biomechanical preparation resulted in good periapical regeneration [12]. On the contrary, the effect of chlorhexidine on the regeneration of periapical tissue of teeth with periapical lesions when used as a root canal dressing for several days has not been studied.

In addition to liquid medications, pastes have also been used to reduce microorganisms in infected root canals. Among dressings for the treatment of teeth with infected root canals, aqueous calcium hydroxide suspensions in paste form have been shown to possess a prolonged and marked antimicrobial action [11,13] and to produce excellent histopathological results in teeth with pulp necrosis and a periapical lesion [11,14].

The objective of this study was to compare, histologically, the effects of 2% chlorhexidine gel and calcium hydroxide paste on experimentally induced apical periodontitis in rat molars.

Material and methods

Animal model

The first maxillary molars of male and female Wistar rats, aged 3 months, weighing 250 g to 300 g were selected for this study. The animal research was licensed by the regional government of Münster (Germany) under no. G 53/2001.

The animals were anesthetized by intramuscular injection (0.1 ml per 50 g body weight) with a combination of 1 ml ketamine (10%), 0.2 ml xylazine (2%), and 3.8 ml isotonic saline solution. Experimentally induced apical periodontitis was established on the mesial roots of first maxillary molars, corresponding to a previously reported method [11,12].

For infection control, the use of rubberdam was not possible on rat molars. Before the treatments, all teeth were mechanically cleaned using a small brush with polishing paste (Hawe Cleanic; Kerr Hawe, Bioggio, Switzerland) and a handpiece. The teeth and the surrounding gingiva were swapped with NaOCl (2.5%) and a chlorhexidine solution (2%; Engelhard Arzneimittel, Niederdorfelden, Germany) for disinfection. Sterile paperpoints (Roeko, Langenau, Germany) served as cotton rolls and were placed in the mucobuccal fold. For root canal preparation on every tooth, new sterile root canal instruments (VDW, Munich, Germany) were used. All other instruments were disinfected before use on a different animal.

Using prism loupes (magnification $\times 4.5$; Zeiss, Aalen, Germany), crown access was prepared in the first maxillary, caries-free right and left molar using a micromotor handpiece with a cylindrical diamond (ISO 008, NTI, Kahla, Germany) running at max. 3000 rpm under permanent cooling with water spray. A new bur was used on every rat. After pulp removal and irrigation with saline, the mesio-buccal root canal was left exposed to the oral cavity for 14 d. Five animals (10 molars) were used to analyze the chronic periapical lesions and served as positive controls. After 14 d the root canals were left empty in these rats and not further treated, but the coronal access openings were filled with a flowable composite (see below). Five rats (10 molars) served as negative controls. In these teeth, partial pulpotomies were performed aseptically and an aqueous suspension of calcium hydroxide was placed over the exposure. Excess water was removed with a sterile cotton pellet and the coronal access openings were sealed with a resin composite, as described in the following. A third control group (10 molars) was established to investigate solely the effect of mechanical instrumentation and irrigation of the canals. Therefore, the root canals were instrumented after 14 d as below, rinsed with NaOCl and left empty without any medication. The coronal access openings were sealed with a flowable resin composite as described in the following. Identically to the experimental groups, the animals of all groups were killed 21 d after the first operation (preparation of the coronal access openings).

Fourteen days after preparation of the coronal access openings and pulp removal, the root canals in the experimental groups were instrumented up to ISO size 20 using Hedström files (VDW, Munich, Germany) up to 0.5 mm from the apex. Working length was established using an electronic apex locator (Root ZX; J. Morita, Irvine, Calif., USA). Teeth in which the mesiobuccal root canal was not instrumented up to at least 0.5 mm from the apex were excluded from the study. This was the case in two teeth. These animals were replaced and not further treated. The root canals were rinsed with sodium hypochlorite (NaOCl) 2.5% and dried with sterile, absorbent paperpoints (Roeko, Langenau, Germany). Two experimental groups were tested in each animal, with the left and right molar receiving one of the treatments. Group A: In 10 teeth ($=5$ rats) the root canals were filled with 2% chlorhexidine digluconate gel (Speiko, Münster, Germany). Group B: The canals of 10 teeth were filled with an aqueous suspension of calcium hydroxide. The calcium hydroxide was prepared freshly from pure calcium hydroxide (Merck, Darmstadt, Germany) and isotonic saline solution. The dressings were placed in the root canals using a lentulo spiral (VDW, Munich, Germany). The coronal access openings were sealed with a flowable resin composite (Tetric flow, Vivadent, Ellwangen, Germany) using a dentin bonding material (Resulcin AquaPrime + MonoBond (RAPMB); Merz Dental, Lütjenburg, Germany) in accordance with the manufacturer's instructions.

Histological assessment

Seven days after placement of the different medications the animals were killed by CO_2 inhalation. The maxillary molars and the surrounding bone were dissected out of the upper jaw, fixed by immersion in glutaraldehyde (2.5% in phosphate buffer, pH 7.4), demineralized for 4 d with trichloroacetic acid (TCA, 5%), and embedded in epoxy resin (Epon 812; Serva, Heidelberg, Germany). All specimens were sectioned using a microtome (Ultracut; Reichert-Jung, Vienna, Austria). For light microscopic investigation, the epoxy resin embedded specimens were prepared by routine histological methods and 1 μm serial sections were cut throughout the complete extent of the apical lesion. Only the mesial roots were evaluated. The specimens were stained with toluidine blue to estimate the 3-dimensional extension of the apical lesions. Sections were evaluated using a light microscope (Zeiss, Oberkochen, Germany) at 10–330 magnification.

Six representative sections from the mesial root of each tooth were randomly selected. Analysis of the histological specimens was performed by two experienced oral anatomists independently and via collective consultation on those specimens upon which agreement was not achieved initially. A final score was then assigned to the specimens. Before histological examination, both examiners underwent a training process with reference to the scoring system. They were blinded to the treatment groups and evaluated the histological sections according to the following predetermined criteria: (a) periapical infiltrate with bacteria: 1 = absent, 2 = slight, 3 = moderate, 4 = severe; (b) periapical infiltrate with inflammatory cells (i.e. neutrophil granulocytes): 1 = absent, 2 = slight, 3 = moderate, 4 = severe; (c) cementum-dentin resorption: 1 = absent, 2 = present (cementum), 3 = present (cementum and dentin); (d) bone resorption: 1 = absent, 2 = present; (e) apical periodontal ligament space: 1 = normal, 2 = slightly increased, 3 = moderately increased, 4 = severely increased.

Statistical analysis

The data established for scoring the five different criteria were recorded separately. For all scores, a non-parametric Kruskal-Wallis test was performed initially to assess whether there were significant differences between the two independent examiners' mean scores. Because no statistically significant differences between the two examiners were found ($p > 0.05$), their scores were combined in each criterion and were substituted by their numeric mean. Subsequent statistical analysis was performed on these scores.

Owing to the ordinal nature of the scores, the data were subjected to the non-parametric Kruskal-Wallis test. *P*-values were computed and compared to the $p = 0.05$ level.

Moreover, lesion sizes of the two treatment and the positive and third control groups were measured in mm^2 (error: $< 1\%$) using Lucia software (Nikon, Kingston Surrey, UK). Because the data of the lesion sizes were distributed normally according to the Kolmogorov-Smirnoff test, statistical comparison between the two treated groups and the positive control group was done using ANOVA and the post-hoc Scheffé test ($p < 0.05$).

Leakage test

To ensure that the resin temporaries were adequate and did not leak in the 7 d medication period, the sealing ability of the composite resin restorations was evaluated in a pilot study using a leakage test. Access cavities were prepared in four maxillary molars of one rat until pulpal exposure occurred. The coronal access openings were then sealed with resin composite as described above. After 7 days the rat was sacrificed and the molars and the surrounding bone were dissected out of the upper jaw. Thereafter, the teeth were immersed in new fuchsine for 24 h at 37°C. The teeth were then washed in water and sectioned longitudinally with a diamond saw in a bucco-palatal direction. The sections were positioned in the middle of the restoration and were evaluated for leakage under a stereomicroscope (Wild M400; Wild Heerbrugg, Heerbrugg, Switzerland) at 32× magnification.

This technique is a common procedure for evaluating the sealing ability of such restorations; it allows observation of dye that penetrates into gaps between dental substrates and restoration [15]. It is commonly accepted that if no dye penetration occurred the restoration prevents microleakage of microorganisms between the cavity walls and the restorative material applied to it [16]. Marginal gaps allowing the leakage of bacteria would be expected to be in the region of 0.5–1.0 μm or larger [16], and it seems reasonable that the dye would have gained access to such gaps. Thus, the present results confirmed the ability of the composite resin material to prevent the penetration of bacteria into the root canals. Admittedly, a root canal bacterial culture taken 21 d from the negative controls would have been a more convincing method to ensure that the temporaries were bacteria-tight. But, since bacterial cultures of root canals in rats are difficult to control, time-consuming and expensive, and due to the favorable results obtained in the leakage tests, we conducted this study without any further bacterial culture.

All specimens revealed no dye penetration and no voids or porosities.

Results

Negative controls

The periapical tissue of the mesial root remained healthy in all specimens. A representative section of the healthy periapical tissue is shown in Figure 1. Microorganisms could not been detected in any of these specimens.

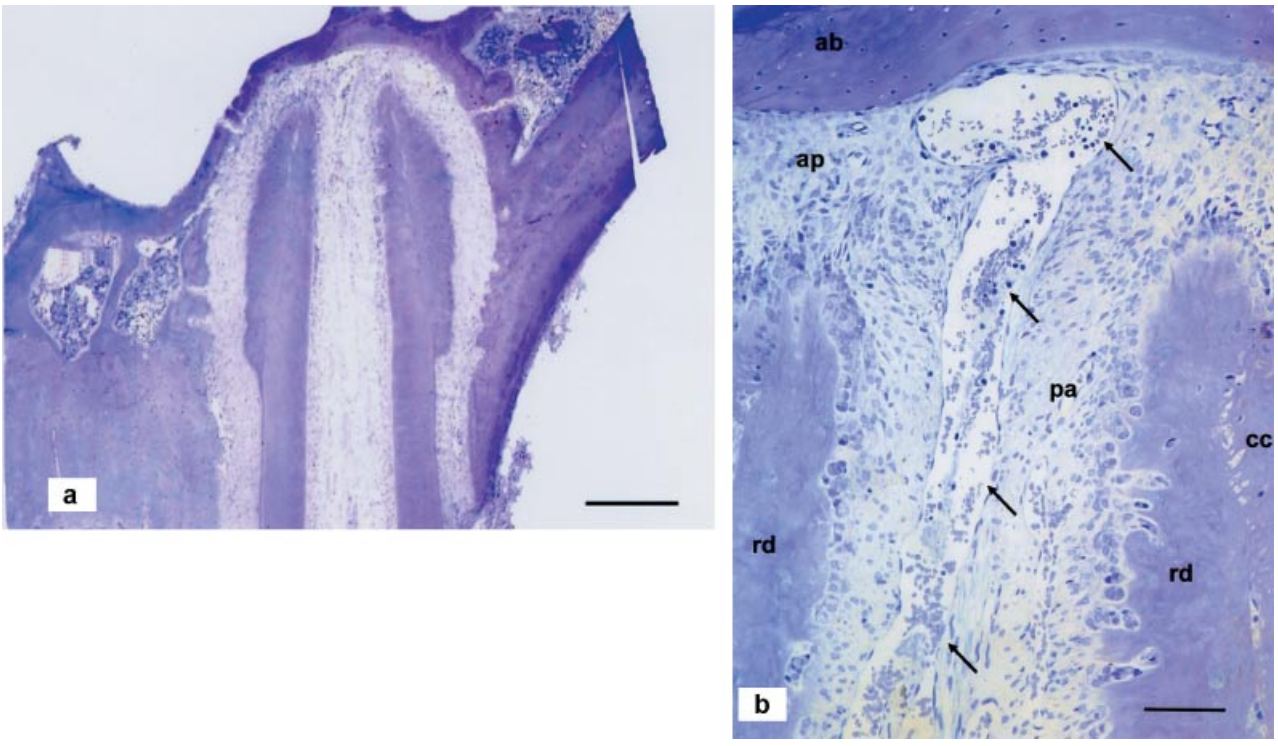

Figure 1. (a) Healthy periapical periodontium of a mesial root of a Wistar rat's maxillary 1st molar (negative control) (bar represents 500 μm, original magnification ×36). (b) Detail of the periapical tissue from (a) (bar represents 50 μm, original magnification ×210) (ab = alveolar bone, ap = apical periodontium, cc = cellular cementum, rd = root dentin, arrows = pulp vein).

Positive controls

A representative section of the periapical tissue after removal of the pulp and leaving the root canal open for 14 d (experimentally induced periapical lesion; positive control group) is shown in Figure 2. The size of the lesion was about the same in all teeth and in all animals. The root canal was mostly filled with food impactions and free erythrocytes (Figure 2a). Figure 2b depicts the typical histological features of

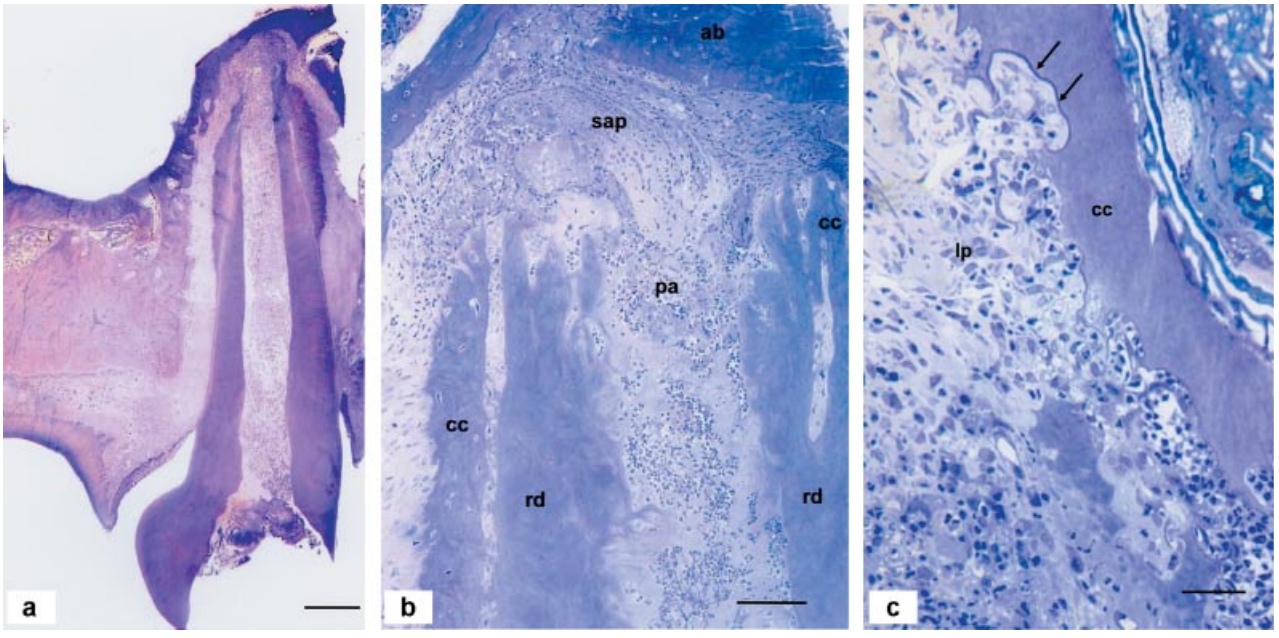

Figure 2. (a) Experimentally induced apical periodontitis 14 d after the pulp has been removed. The tooth was left open during this time (positive control) (bar represents 500 μm, original magnification ×36). (b) Detail of the subacute apical periodontitis from (a) (bar represents 50 μm, original magnification ×210) (ab = alveolar bone, sap = subacute apical periodontitis, cc = celluar cementum, rd = root dentin). (c) Detail of the periodontal ligament showing a subacute periodontitis and cementum and dentin resorption (bar represents 10 μm, original magnification ×330) (cc = cellular cementum, lp = lateral periodontium, arrows = resorption of the cellular cementum).

Table I. Overall results for the groups and statistical analysis (Kruskal-Wallis test) of the scores of the histopathological evaluation in the control groups and the two treatment groups

Treatment	No. of observations	Periapical infiltrate with bacteria		Periapical infiltrate with inflammatory cells		Cementum-dentin resorption		Bone resorption		Apical periodontal ligament space	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
(+)–Control	60	2.58	0.57	3.13	0.77	1.89	0.81	1.40	0.59	2.33	0.46
Third control	60	2.48	0.75	2.88	0.78	1.87	0.60	1.38	0.51	2.31	0.51
Chlorhexidine	60	1.28§	0.61	1.22§	0.44	1.41*	0.52	1.43*	0.50	1.73†	0.45
$Ca(OH)_2$	60	1.20§	0.40	1.13§	0.32	1.37*	0.49	1.30*	0.46	1.60‡	0.50

* Not significantly different from the controls ($p > 0.05$). † Significantly different from the controls ($p < 0.05$). ‡ Significantly different from the controls ($p < 0.01$), § Significantly different from the controls ($p < 0.001$).

an inflamed periodontal tissue in the region of the major apical foramen. The light microscopic appearance was dominated by numerous defense or immune cells. Among these mobile cells that had transmigrated through inter-endothelial gaps of dilated blood capillaries, the fraction of neutrophilic granulocytes comprised the major portion. Because defense cells such as lymphocytes, plasma cells, and macrophages appeared only sporadically or were completely absent, and due to the absence of a fibrous granulation tissue that is regularly found in apical granuloma, we defined the inflammatory state to be subacute rather than chronic. Between the granulocytes, multiple clusters of bacteria were visible under high magnification. The bacterial cells were morphologically classified as cocci with a predominant portion of staphylococci and a lower portion of streptococcus chains. Besides these cellular and microbiological signs of inflammation, also amorphous necrotic tissue areas, deposits, and empty spaces indicating microabscesses could be observed. Moreover, leukocytes had also immigrated into the root canal (Figure 2b), which was densely infiltrated with bacteria up to the crown cavity. Regarding the occurrence of inflammation-mediated resorptive activity at alveolar bone, osteoclasts could be identified in some of the specimens. In half of the specimens, resorptive activity of cementoclasts and dentinoclasts was clearly identified in the apical part of the root extending throughout the total cementum layer up to the outer layers of the root dentin (Figure 2c).

Third control group

In the third control group, no microscopical features that revealed noticeable deviations from the above described positive controls were observed.

Group A: 2% chlorhexidine gel

The scores of the histopathological evaluation are presented in detail in Table I. Figure 3a is representative of 80% of the specimens in which the experimentally induced apical periodontitis resolved. In detail, the signs of acute inflammation (i.e. granulocytes, bacteria, and necrotic tissue or microabscesses) were no longer visible. Ingrowths of granulation tissue consisting of metabolically active fibroblasts and capillaries that had filled spaces of resorbed necrotic tissue can be seen. In contrast to these indications of soft tissue regeneration, the formation of reparative hard tissue filling the lacunae of resorbed alveolar bone or cementum has not been observed (Table I). In some specimens, a noticeable feature was the presence of a sharp demarcation line towards the root canal, whereby the granulation tissue was separated from neutrophilic granulocytes persisting in the apical region of the root canal (Figure 3a). No accumulation of bacteria between these defense cells was observed. The histological appearance of peripheral regions near to the apical foramen showed a corresponding microstructure indicating a resolving of the inflammation (Figure 3b). However, in about 20% of the specimens, persistent defense cells and sporadic bacteria accumulations in the apical and more peripheral regions of the periodontal ligament were identified.

Group B: Calcium hydroxide paste

The scores of the histopathological evaluation are detailed in Table I. Analysis of the histological specimens (Figure 4) revealed the same microscopical features as described for the chlorhexidine treatment group. Thus, the calcium hydroxide treatment had resulted in complete regeneration of both the apical and the more peripheral regions of the periodontal ligament inflammation in all investigated specimens.

Statistical results

The use of both chlorhexidine and calcium hydroxide resulted in statistically significant differences from the negative control group. Statistical analysis (Table I) revealed that with the exception of the scores for 'cementum-dentin resorption' and 'bone resorption' the two treatment groups showed significantly lower average scores than the positive and the third control groups ($p < 0.05$). For all five criteria, no statistically significant differences were obtained between the chlorhexidine and calcium hydroxide treatment groups ($p > 0.05$). Also, no statistically significant differences

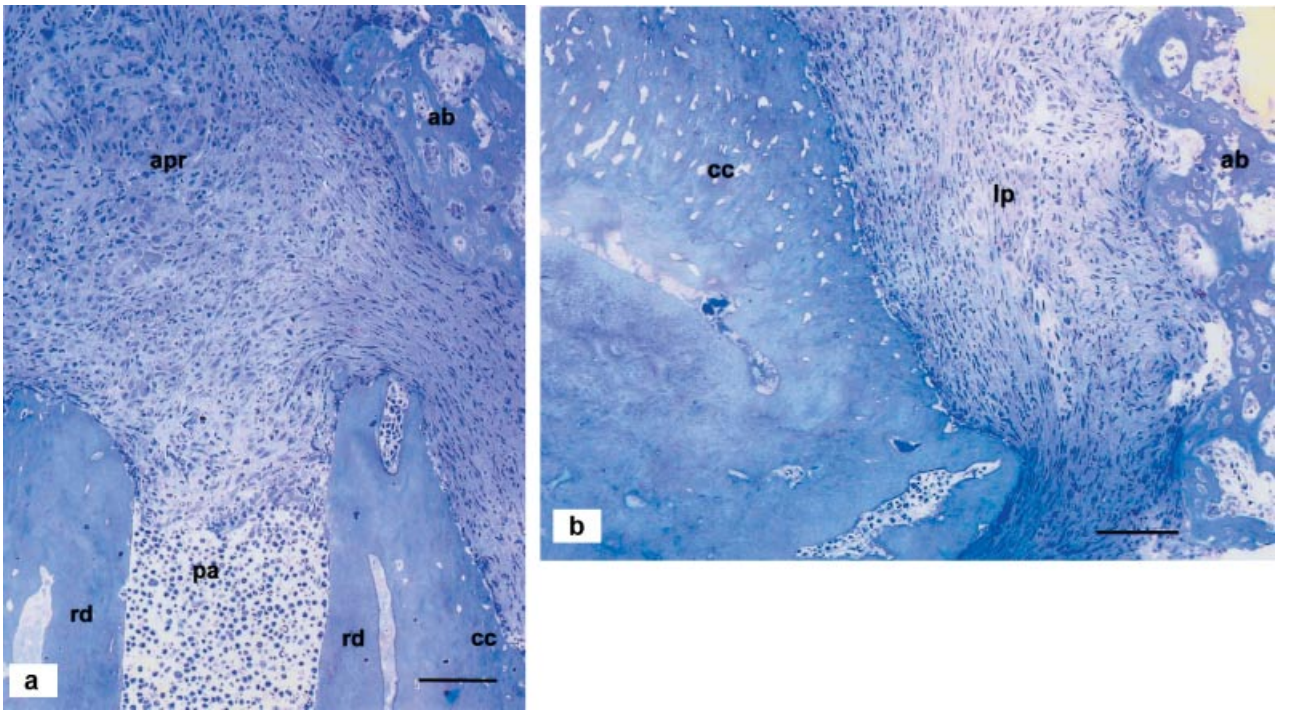

Figure 3. (a) Apical region of the mesial root of a maxillary 1st molar after intracanal medication with 2% chlorhexidine gel for 7 d (bar represents 50 μm, original magnification ×210). The experimentally induced apical periodontitis was resolved (ab = alveolar bone, apr = apical periodontitis resolving, cc = cellular cementum, pa = pulp apex, rd = root dentin). (b) Detail of the periodontal ligament adjacent to the apical region from (a) (bar represents 50 μm, original magnification ×210). The histological appearance showed a microstructure indicative of a regeneration of the inflammation (ab = alveolar bone, cc = cellular cementum, lp = lateral periodontium).

were obtained between the positive group and the third control group ($p > 0.05$).

Statistical analysis of the lesion sizes (Table II) displayed that lesion sizes in the two treatment groups were significantly smaller compared to the positive and third control group ($p < 0.01$). There were no statistical significant differences between the two different root canal dressings and between the positive and third control group with respect to lesion sizes ($p > 0.05$).

Discussion

Evaluation of a new root canal dressing involves two distinct properties: biocompatibility and antimicrobial effectiveness. Numerous *in vitro* studies have shown a marked antimicrobial efficiency of 2% chlorhexidine [5,7,8]. Moreover, in order to assess the biocompatibility of chlorhexidine, *in vivo* non-specific tissue reactions caused by this medicament were investigated

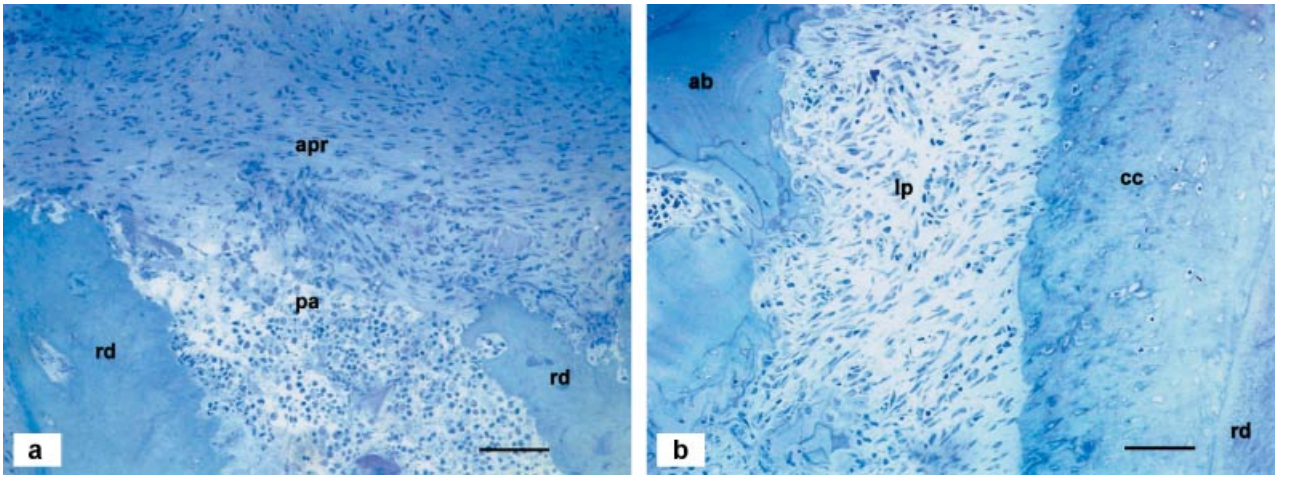

Figure 4. (a) Apical region of the mesial root of a maxillary 1st molar after intracanal medication with calcium hydroxide for 7 d (bar represents 25 μm, original magnification ×270). The experimentally induced apical periodontitis was resolved (apr = apical periodontitis resolving, pa = pulp apex, rd = root dentin). (b) Detail of the periodontal ligament neighboring the apical region from (a) (bar represents 25 μm, original magnification ×270) (ab = alveolar bone, cc = cellular cementum, lp = lateral periodontium, rd = root dentin).

Table II. Mean, SD (mm^2), and statistical analysis (ANOVA and post-hoc Scheffé) of the lesion sizes in the control groups and the two treatment groups

Treatment	No. of teeth	Mean	SD
(+)–Control	10	0.492*	0.071
Chlorhexidine	10	0.375†	0.040
$Ca(OH)_2$	10	0.311†	0.074
Third control	10	0.471*	0.075

Means with the same symbol (*, †) were not statistically different from the $p < 0.05$ level.

in two histological studies following the implantation of chlorhexidine into various tissues of animals [9,10]. But it has to be kept in mind that tests of *in vitro* cytotoxicity of intracanal medicaments cannot be transferred to the *in vivo* situation unrestrictedly. Differences in uptake of medicaments, in their solubility and chemical changes when brought into contact with tissue and tissue components might influence their effect on the periapical tissue [4,11]. Moreover, specific environmental factors of the periapical situation, such as the infection of the root canal and the specific reaction patterns of periapical tissues, have a crucial influence on the success of endodontic treatment. Therefore, in this present study an application test was used to investigate the antimicrobial effectiveness of two different root canal dressings when used in infected root canals in rats. The outcome measure for the present antimicrobial testing was histological evaluation of the regeneration of inflamed periapical tissue. According to previous reports, rats are an appropriate model for this type of study [11,17–19]. Anatomically, the molars of rats are comparable to small human molars [20]. Rat molars develop analogously to humans and possess the same structural characteristics of the pulp chamber, the root canal, and the apical delta with foramina apicalia minores [21]. The inflammatory reactions of the periapical tissues of the rat are similar to those of humans, dogs, hamsters, and other animal species [22]. With respect to root canal microbiology, the rat model is closely similar to humans and primates [23]. During the active phase of apical lesion development the root canal flora present is similar in rat and human models [24]. In chronic periapical lesions, too, the cell infiltrates are similar to those found in chronic human tissue [23]. Muruzábal et al. [25] showed that the periapical tissue in the rat reacts in many respects similarly to the overfilling of gutta percha and sealer material compared to humans and dogs.

The time course of lesion development in rats has been described in detail in a previous study [26]. The authors reported that periapical lesions developed rapidly after pulp exposure, with a maximum of bone loss occurring approximately between days 1 and 15. This period is the most active phase of periapical lesion pathogenesis, thus the initial acute phase. The period afterwards (day 15 onward) is a phase of relative size stability and can be considered as a chronic phase [26]. It can be assumed that in this chronic phase there is a reduction in the number of acute inflammatory cells; a slight reduction of the lesion size might even occur. Therefore, in the present study the controls were of the same time period as the two experimental groups in order to avoid a bias in subject interpretation of the histological data. Moreover, since bone deposition is the ultimate determination of regeneration, lesion sizes of the positive control and the two treatment groups were measured for objective comparison.

In the present study, a third control group was established in which the infected root canals were instrumented and irrigated. The canals were left empty without any medication and the coronal access cavities were tightly sealed in order to investigate the effect of a sole instrumentation on regeneration of the inflamed periapical tissue. No statistically significant differences between the positive controls and the third control group were obtained ($p > 0.05$). This finding clearly indicates that sole instrumentation and occlusal obturation had no significant effect on apical regeneration. Moreover, this observation is in good agreement with previously published studies: It has been reported that infected root canals rinsed with saline after preparation, medicated with $Ca(OH)_2$ for 1 week and obturated with gutta percha showed statistically better histological results than root canals directly filled after preparation without any medication [14]. Moreover, root canals infected with bacterial endotoxin (lipopolysaccharides) and rinsed with NaOCl after preparation were evaluated histologically to display statistically lower results concerning regeneration than root canals medicated with $Ca(OH)_2$ [27]. It can therefore be concluded that sole instrumentation and occlusal obturation seems to have no significant effect on apical regeneration. In accordance with the local ethics committee for animal research we did not sacrifice rats to evaluate well-known facts. In order to avoid a possibility of contamination of the mesio-buccal root canals during the medication period, all root canals of the rat molars were instrumented and filled with the particular dressing. Otherwise, uninstrumented canals would undoubtedly harbor some bacteria and contaminate the mesio-buccal canal.

Although histobacteriology is usually done using specific stains, such as Brown and Brenn or Brown and Hopps performed on paraffin sections, toluidine blue staining of Epon sections was used in the present study. This was done because pilot studies revealed that the bacteria detection rate in semi-thin sections (thickness 1 μm) after toluidine blue staining was clearly superior to that in paraffin sections due to superposition of image planes lying out of the focus in paraffin sections with a thickness of 8 μm. Besides, the gram staining technique of Brown and Brenn or Brown and Hopps does not allow distinguishing between staphylococci

and streptococci, since both bacteria specimens are gram-positive [28].

In the present study, working length was established using an electronic apex locator. The accuracy of apex locators in rat molars has not been reported. On the other hand, because of the very small anatomical structures in rats, radiographic determination of the working length was impossible. The use of an apex locator thus seems justified, although it is conceivable that the small rat molar root canals have constrictions short of the apical foramen. This could result in a short working length and would undoubtedly have influenced the histological results. Therefore, at the beginning of the histological evaluation of the sections, the working length was examined in all cases.

During the instrumentation, all root canals were irrigated with 2.5% sodium hypochlorite in order to simulate clinical conditions. Certainly, the antimicrobial effectiveness of sodium hypochlorite on its own was not considered in this present investigation, although it has been shown in several studies that sodium hypochlorite displays a marked antimicrobial effect when used as a root canal irrigant [29,30]. Nevertheless, considering the major objective of the present study (solely to compare the effects of the two medicaments on the regeneration of inflamed periapical tissue), a clinically accepted irrigation technique was used. Since sodium hypochlorite was used in both experimental groups, it can be assumed that use of this irrigant did not result in a bias in interpretation of the histological data of the two experimental groups.

In the present study, histological regeneration was evaluated using subjective criteria, since Trope et al. [31] have shown the outcomes of subjective and objective methods to be similar. As previously discussed by Katebzadeh et al. [14], this is probably due to the fact that a truly objective method of counting inflammatory cells does not exist.

Of the two intracanal medicaments tested in the present study, an aqueous suspension of calcium hydroxide clearly supported periapical regeneration (Figure 4, Table II). This finding corroborates the results of previous histopathological studies on periapical regeneration in rats [11] and in dogs [14,27,32]. Calcium hydroxide was used in this study because the scientifically documented procedure for the best results in treatment of teeth with an infected root canal and an apical periodontitis is based on complete debridement and irrigation of the root canal, followed by the application of a calcium hydroxide dressing for 7 d [14]. The periapical regeneration produced by a calcium hydroxide dressing therefore constitutes the basis for histopathological evaluation of periapical regeneration when using 2% chlorhexidine gel as a root canal dressing.

Although there were no statistically significant differences in periapical regeneration between the 2% chlorhexidine and the calcium hydroxide treatment group, persistent defense cells and bacteria were still visible in 20% of the specimens in the chlorhexidine group, whereas in the $Ca(OH)_2$ group a regeneration could be observed in all specimens (Tables I, II). Nevertheless, under clinical situations it can be concluded that the elimination of bacteria by chlorhexidine seems to be sufficient to induce regeneration of the periapical defect. Thus 2% chlorhexidine is a suitable intracanal interappointment dressing (Figure 3), but under the conditions of the present study it was slightly less effective than calcium hydroxide, although this difference was not statistically significant.

A previous study has shown that 2% chlorhexidine shows residual antimicrobial activity that remains for at least 72 h [6]. The present investigation indicates that chlorhexidine can be effectively used as an intracanal medicament for at least 7 d. Moreover, according to the present results, irritation of the periapical tissue is highly unlikely when 2% chlorhexidine is used within the root canal. These results are in good agreement with those of a previously published histopathological study in dogs, evaluating the periapical regeneration using 2% chlorhexidine as root canal irrigation [12] and corroborate the findings of another recent study on the biocompatibility of a 2% chlorhexidine digluconate solution [10]. Chlorhexidine was injected into the peritoneal cavity of mice and the inflammatory response was evaluated. The authors reported that 2% chlorhexidine was biocompatible, since this solution did not induce a significant inflammatory response [10].

Apart from the discussed positive characteristics of chlorhexidine, it is of importance that 0.1–2% chlorhexidine solutions are classified as toxicologically safe, both in implant experiments [9] and in determining the lethal dose [33], and also with regard to its acute oral toxicity [34,35]. Furthermore, it has been shown that chlorhexidine does not display a genotoxic effect [36]. A 2% chlorhexidine solution has also been used as subgingival irrigant without adverse effects [37]. To the contrary, other studies have shown that chlorhexidine reduces cellular proliferation and prevents collagen production of human gingival fibroblasts [38], and that it was cytotoxic to human fibroblasts via inhibition of protein synthesis [39] and to human PDL cells by inhibiting double-stranded nucleic acid content, protein synthesis, and mitochondrial activity at various concentrations [40]. The authors of the latter study speculated that the detrimental effects of chlorhexidine irrigating solution on vital tissues might impair the reparative and regenerative potential of periapical tissues [40]. Chang et al. [40] reported their findings with the reservation that the clinical significance of these results needs to be further evaluated. The present histopathological evaluation produced no evidence that chlorhexidine might decrease the regenerative potential of apical and periapical tissues.

Comparing the effects of $Ca(OH)_2$ and chlorhexidine as a root canal medication *in vitro*, it was found

that after coronally sealing of extracted teeth with IRM there were no statistically significant differences between the two substances concerning the time needed by bacteria for recontamination of the root canal [41]. $Ca(OH)_2$ and chlorhexidine used as medication for 14 d had no effect on the apical seal of the root canal system after obturation with gutta percha and AH plus for 60 d [42]. On the other hand, Barthel et al. [43] have shown that a group of root canals ($n=20$) medicated with $Ca(OH)_2$ for 1 week and sealed with AH 26 and gutta percha for 1 year had fewer leaking samples than the chlorhexidine group.

Since the results of *in vivo* and *in vitro* studies are still different concerning the effects of chlorhexidine compared with $Ca(OH)_2$, more research is necessary, especially on higher animals like dogs or monkeys.

Conclusion

In conclusion, 2% chlorhexidine used as an intracanal medicament for 7 d showed good periapical regeneration, suggesting that it can be an alternative to calcium hydroxide root canal dressing.

References

[1] Tronstad L. Clinical endodontics. Stuttgart: Thieme; 1991.

[2] Barnett F, Trope M, Khoja M, Tronstad L. Bacteriologic status of the root canal after sonic, ultrasonic and hand instrumentation. Endod Dent Traumatol 1985;1:228–31.

[3] Barbosa CAM, Gonçalves RB, Siqueira JF, de Uzada M. Evaluation of the antibacterial activities of calcium hydroxide, chlorhexidine, and camphorated paramonochlorphenol as intracanal medicament. A clinical and laboratory study. J Endod 1997;23:297–300.

[4] Spångberg L, Engström B, Langeland K. Biologic effects of dental materials. 3. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. Oral Surg Oral Med Oral Pathol 1973;36:856–71.

[5] Jeansonne MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. J Endod 1994;20:276–8.

[6] White RR, Janer LR, Hays GL. Residual antimicrobial activity associated with a chlorhexidine endodontic irrigant used with sodium hypochlorite. Am J Dent 1999;12:148–50.

[7] Delany GM, Patterson SS, Miller CH, Newton CW. The effect of chlorhexidine gluconate irrigation on the root canal flora of freshly extracted necrotic teeth. Oral Surg Oral Med Oral Pathol 1982;53:518–23.

[8] Schäfer E, Bößmann K. Antimicrobial efficacy of chloroxylenol and chlorhexidine in the treatment of infected root canals. Am J Dent 2001;14:233–7.

[9] Yesilsoy C, Whitaker E, Cleveland D, Phillips E, Trope M. Antimicrobial and toxic effects of established and potential root canal irrigants. J Endod 1995;21:513–5.

[10] Tanomaru Filho M, Leonardo MR, Silva LAB, Anibal FF, Faccioli LH. Inflammatory response to different endodontic irrigation solutions. Int Endod J 2002;35:735–9.

[11] Tepel J, Darwisch M, Hoppe W. Reaction of inflamed periapical tissue to intracanal medicaments and root canal sealers. Endod Dent Traumatol 1994;10:233–8.

[12] Tanomaru Filho M, Leonardo MR, da Silva LAB. Effect of irrigating solution and calcium hydroxide root canal dressing on the repair of apical and periapical tissues of teeth with periapical lesion. J Endod 2002;28:295–9.

[13] Estrela C, Pimenta FC, Ito Y, Mammann LL. In vitro determination of direct antimicrobial effect of calcium hydroxide. J Endod 1998;24:15–17.

[14] Katebzadeh N, Hupp J, Trope M. Histological periapical repair after obturation of infected root canals in dogs. J Endod 1999;25:364–8.

[15] Fabianelli A, Kugel G, Ferrari M. Efficacy of self-etching primer on sealing margins of class II restorations. Am J Dent 2003;16:37–41.

[16] Taylor MJ, Lynch E. Microleakage. J Dent 1992;20:3–10.

[17] Muruzábal M, Erausquin J, Devoto F. A study of periapical overfilling in root canal treatment in the molar of the rat. Arch Oral Biol 1966;11:373–83.

[18] Erausquin J, Muruzábal M. A method for root canal treatment in the molar of the rat. Oral Surg Oral Med Oral Pathol 1967;24:540–6.

[19] Muruzábal M, Erausquin J. Discussion of: methods and criteria in evaluation of periapical response. Int Dent J 1970;20: 539–54.

[20] de Terra P. Vergleichende Anatomie des menschlichen Gebisses und der Zähne der Vertebraten. Jena: Fischer; 1911. p 295.

[21] Stratmann U. Personal communications; 2005.

[22] Muruzábal M, Erausquin J. Processos apicales experimentales en el molar de rata. Rev Asoc Odont Argent 1963;51:3–12.

[23] Stashenko P, Wang CY, Tani-Ishii N, Yu SM. Pathogenesis of induced rat periapical lesions. Oral Surg Oral Med Oral Pathol 1994;78:494–502.

[24] Tani-Ishii N, Wang CY, Tanner A, Stashenko P. Changes in root canal flora during the development of rat periapical lesions. Oral Microbiol Immunol 1994;9:129–35.

[25] Muruzábal M, Erausquin J, Devoto FH. A study of periapical overfilling root canal treatment in the molar of the rat. Arch Oral Biol 1966;11:373–83.

[26] Yu SM, Stashenko P. Identification of inflammatory cells in developing rat periapical lesions. J Endod 1987;13:535–40.

[27] Tanomaru JMG, Leonardo MR, Tanomaru Filho M, Bonetti Filho I, Silva LAB. Effect of different irrigation solutions and calcium hydroxide on bacterial LPS. Int Endod J 2003;36: 733–9.

[28] Marsh P, Martin M. Orale Mikrobiologie. Stuttgart, New York: Thieme; 2003. p 20–37.

[29] Yesilsoy C, Whitaker E, Cleveland D, Philips E, Trope M. Antimicrobial and toxic effects of established and potential root canal irrigants. J Endod 1995;21:513–5.

[30] Siqueira JF, Machado AG, Silveira RM, Lopes HP, de Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal in vitro. Int Endod J 1997;30: 279–82.

[31] Trope M, Löst C, Schmitz H. Healing of apical periodontitis in dogs after apicoectomy and retrofilling with various filling materials. Oral Surg Oral Med Oral Pathol 1996;81:221–8.

[32] Holland R, Otoboni JA, de Souza V, Nery MJ, Bernabé PFE, Dezan E. A comparison of one versus two appointment endodontic therapy in dogs' teeth with apical periodontitis. J Endod 2003;29:121–4.

[33] Klimm W, Krause L, Krause P, Wenzel J. Tierexperimentelle Untersuchungen zur Toxizität verschiedener Wurzelkanalantiseptika. Stomatol DDR 1989;39:153–5.

[34] Cannell JS. The use of antimicrobials in the mouth. J Int Med Res 1981;9:277–82.

[35] Foulkes DM. Some toxicological observations on chlorhexidine. J Periodontol 1973;8 Suppl 12:55–7.

[36] Klimm W, Janz S, Gabert A. Experimentelle Untersuchung zur Genotoxizität verschiedener Wurzelkanalantiseptika im SOS-Chromotest. Zahn Mund Kieferheilkd 1989;77: 128–30.

[37] Southard SR, Drisko CL, Killoy WJ, Cobb CM, Tira DE. The effect of 2.0% chlorhexidine digluconate irrigation on clinical

parameters and the level of *Bacteroides gingivalis* in periodontal pockets. J Periodontol 1989;60:302–9.

[38] Mariotti AJ, Rumpf DAH. Chlorhexidine-induced changes to human gingival fibroblast collagen and non-collagen protein production. J Periodontol 1999;70:1443–8.

[39] Pucher JJ, Daniel JC. The effects of chlorhexidine digluconate on human fibroblasts in vitro. J Periodontol 1993;62:526–32.

[40] Chang YC, Huang FM, Tai KW, Chou MY. The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells. Oral Surg Oral Med Oral Pathol 2001;92:446–50.

[41] Gomes BPFA, Sato E, Ferraz CCR, Teixeira FB, Zaia AA, Souza-Filho FJ. Evaluation of the time required for recontamination of coronally sealed canals medicated with calcium hydroxide and chlorhexidine. Int Endod J 2003;36:604–9.

[42] Wuerch RMW, Apicella MJ, Mines P, Yancich J, Pashley DH. Effect of 2% chlorhexidine gel as an intracanal medication on the apical seal of the root-canal system. J Endod 2004; 30:788–91.

[43] Barthel CR, Zimmer S, West G, Roulet J-F. Bacterial leakage in obturated root canals following the use of different intracanal medicaments. Endod Dent Traumatol 2000;16:282–6.