
Periapical healing of endodontically treated teeth in one and two visits obturated in the presence or absence of detectable microorganisms

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Abstract

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Aim The purpose of the present study is to evaluate the healing of periapical lesions of teeth with positive and negative canal cultures at the time of obturation, and to evaluate the periapical healing of teeth treated in one visit (without) or in two visits with an interappointment dressing of calcium hydroxide.

Methodology Thirty-nine patients received root-canal treatment. In the first visit, teeth were instrumented, and 18 of these teeth were filled (after microbiological sampling) with calcium hydroxide in sterile saline. The other 21 teeth were obturated with gutta-percha and AH-26 sealer after microbiological sampling. Four weeks later, the teeth with calcium hydroxide were accessed again and after microbiological sampling they were obturated with gutta-percha and AH-26 sealer. Healing of periapical radiolucency was recorded over a period up to 4.5 years.

Results In both the treatment groups, the size of the periapical lesions reduced significantly during the follow-up period. Complete radiographic healing was observed in 81% of the cases in the one-visit group, and in 71% of the cases in the two-visit group. The probability of success increased continuously over time for both treatment groups. Seven out of eight cases (87.5%) that showed a positive root-canal culture at the time of obturation healed. The number of colony forming units (CFU) in six out of eight positive canals was $<10^2$ CFU mL⁻¹.

Conclusions Within the limitations of this study, no significant differences in healing of periapical radiolucency was observed between teeth that were treated in one visit (without) and two visits with inclusion of calcium hydroxide for 4 weeks. The presence of a positive bacterial culture (CFU $< 10^2$) at the time of filling did not influence the outcome of treatment.

Keywords: calcium hydroxide, endodontology, microbiology, periapical healing.

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Introduction

Root-canal treatment aims to eliminate bacteria from the infected root-canal system to create an environment that is most favourable for healing. Several studies have shown that it is impossible to achieve a bacteria-free root-canal space in all cases, even after thorough

cleaning, shaping and irrigation with disinfectants or antiseptics (Byström & Sundqvist 1981, Byström *et al.* 1985, Ørstavik *et al.* 1991, Sjögren *et al.* 1997, Peters *et al.* 2002). Therefore, concern exists as to the fate and consequences of the remaining microorganisms in the canal. They may multiply rapidly, in some cases, to almost the initial numbers in 2–4 days, if the canal is left empty (Byström & Sundqvist 1981).

It is generally believed that the remaining bacteria can be eliminated or be prevented from repopulating the root-canal space by introducing an interappointment dressing such as calcium hydroxide in the root canal

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(Byström *et al.* 1985, Chong & Pitt Ford 1992). However, it has been shown that calcium hydroxide fails to consistently produce sterile root canals and even allows regrowth in some cases (Reit & Dahlén 1988, Ørstavik *et al.* 1991, Peters *et al.* 2002). It is unclear in which cases failure occurs, because the root-canal treatment with an interappointment calcium hydroxide dressing or a negative culture before obturation gives no guarantee of healing in all cases (Sjögren *et al.* 1990, Nair *et al.* 1990, Sjögren *et al.* 1997, Trope *et al.* 1999, Weiger *et al.* 2000).

Another approach is to eliminate the remaining microorganisms or to render them harmless by entombing them by complete obturation immediately after preparing and irrigating the canal space at the same visit. This way, the remaining microorganisms may be killed by the antimicrobial activity of the sealer or the Zn²⁺ ions of gutta-percha (Moorer & Genet 1982, Kaplan *et al.* 1999, Fuss *et al.* 2000, Siqueira *et al.* 2000) or may be deprived of nutrition and space to multiply (Soltanoff 1978, Oliet 1983, Weiger *et al.* 2000).

The healing potential for teeth that are treated in one or two visits with placement of an intracanal disinfectant appears similar (Trope *et al.* 1999, Weiger *et al.* 2000). Some studies report the presence of remaining microorganisms after cleaning and shaping and dressing with calcium hydroxide, but do not relate this to healing (Byström 1986, Yared & Bou Dagher 1994, Shuping *et al.* 2000). One study (Sjögren *et al.* 1997) has compared the healing rate (after 5 years) between the canals that were obturated in the presence or absence of cultivable microorganisms after instrumentation and irrigation. A lower healing rate was found for teeth that harboured microorganisms at the time of root-canal obturation compared to those that had a negative culture at the time of obturation (68% vs. 94%).

The purpose of this study is to evaluate the healing of periapical lesions of teeth with positive and negative canal cultures at the time of obturation and to evaluate periapical healing of teeth treated in one visit (without) or two visits with an interappointment dressing of calcium hydroxide.

Materials and methods

Patient selection

Thirty-nine, patients with a non-contributory medical history, referred to the endodontic clinic of the Academic Centre for Dentistry in Amsterdam for root-canal treatment, were selected according to the following criteria.

All the selected roots (17 incisors, 6 canines, 5 premolars and 11 distal roots of mandibular molars) had one canal, were asymptomatic, did not respond to sensitivity testing, had not received any endodontic treatment previously, and showed radiographic evidence of periapical bone loss. Maxillary molars were not included, because of radiographic overlaps hindering reproducible observation of changes in the periapical lesion size.

The mean age of the participants (19 females and 20 males) was 40 years and ranged from 19 to 86 years. The teeth were randomly divided into two treatment groups, every second patient was assigned to group 2. Group 1 teeth ($n = 21$) were treated in one visit, group 2 teeth ($n = 18$) were treated in two visits with intracanal disinfection by calcium hydroxide for 4 weeks. The size of the periapical lesions was determined from the preoperative radiograph on a light box by measuring the largest diameter in millimetres with a ruler to an accuracy of 0.5 mm.

Microbial procedure

All the canals were cultured at the start of treatment (S1, $n = 39$); after instrumentation (S2, $n = 39$); after removal of calcium hydroxide (S3, $n = 18$); and before obturation with gutta-percha (S4, $n = 18$) as described previously (Peters *et al.* 2002). After cleaning the tooth with pumice and isolation with rubber dam, the crown and the surrounding rubber dam were disinfected with 80% ethanol for 2 min. An access cavity was prepared with sterile high-speed diamond burs under irrigation with sterile saline. Before entering into the pulp chamber, the access cavity was disinfected again for 2 min with 80% ethanol. Sterility was checked by sampling with a cotton swab over the cavity surface and streaked on blood agar plates. All subsequent procedures were performed aseptically. The pulp chamber was accessed with burs and rinsed with Reduced Transport Fluid (RTF) (Syed & Loesche 1972) which was aspirated with suction tips. RTF was then introduced to the root canal with a syringe and 27-gauge needle. Care was taken not to overfill the canal. The canal was enlarged to a size 20 Hedström file to the estimated working length as calculated from the preoperative radiograph. Five sterile paper points were consecutively placed in the canal and left for 10 s (sample 1, S1). Then, these were placed in sterile tubes containing 1 mL RTF and transferred to the laboratory within 15 min for microbiological processing.

Ten-fold serial dilutions of the samples were prepared and 100 µL of each dilution was inoculated on blood agar

plates supplemented with 5% horse blood, 5 mg L⁻¹ haemin and 1 mg L⁻¹ menadione. Plates were incubated anaerobically (80% N₂, 10% H₂, 10% CO₂) at 37 °C for 7 days. After incubation, the total colony forming units (CFU) and the different colony types were counted using a stereomicroscope at 16× magnification (Zeiss, Oberkochen, Germany).

All the colony types were streaked to purity and incubated aerobically in air with 5% CO₂ (BBL Gaspak CO₂ systems, Becton Dickinson & Co., Cockeysville, MD, USA) as well as anaerobically to determine strict anaerobic and facultative anaerobic growth. Identification of the isolates was made on the basis of Gram stain, catalase activity and a commercially available identification kit – ATB rapid ID32A (Biomerieux SA, Lyon, France), for strict anaerobes and ATB rapid ID32Strep for facultative anaerobic cocci (Biomerieux SA).

In order to allow the slow-growing species to develop the blood agar plates with the total samples were kept under anaerobic conditions up to 14 days. The newly emerging colonies were also streaked to purity and were identified.

Root-canal procedure

All procedures were performed by one endodontist. The working length (1 mm from the radiographic apex) was checked with a radiograph after inserting a size 15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) in the canal to the estimated working length, or shorter if the attached electronic apex locator (Apit, Osada, Japan) indicated that the apical foramen had been reached. After the first microbiologic sample (S1), the canal was enlarged using Flexofiles (Dentsply Maillefer) with the modified double flare technique (Saunders & Saunders 1992), to a master apical file of at least size 35 (range 35–60). Each file was followed by irrigation of the canal with 2 mL sodium hypochlorite (2%) in a syringe with a 27-gauge needle. After preparation, the canal was irrigated with 5 mL sodium hypochlorite (2%). Then, inactivation of the sodium hypochlorite was accomplished with 5 mL sterile sodium thiosulphate, before a second microbiological sample (S2) was taken from the root canal in the same manner as the first sample.

After drying the canal with paper points, the teeth in group 1 (*n* = 21) were obturated using the warm lateral-compaction technique with gutta-percha and AH-26 sealer (Dentsply, Konstanz, Germany). At the end of the first visit all these teeth were restored. Teeth that did not receive a permanent restoration were restored

with a temporary filling of two layers of Cavit (ESPE, Seefeld, Germany) and a glass ionomer restoration (Fuji-II, GC Corporation, Tokyo, Japan).

After drying the canal, the teeth in group 2 (*n* = 18) were dressed with a thick mix of calcium hydroxide (Merck, Darmstadt, Germany) in sterile saline. The calcium hydroxide slurry was inserted in the canal with a size 30 lentulo spiral (Dentsply Maillefer) and packed with the blunt end of a paper point. The access cavities in group 2 were filled with two layers of Cavit and a glass ionomer restoration. In the mandibular molars, the entrance of the distal canal was isolated with Cavit from the remaining pulp chamber in order to prevent contamination by microorganisms from the mesial canals (that had been instrumented but were not included in the study). A radiograph was taken to ensure proper placement of the calcium hydroxide in the canal.

The patients in group 2 returned after 4 weeks. The canal was aseptically accessed under rubber dam isolation and the calcium hydroxide removed with RTF and careful filing of the canal with the master apical file. Removal of calcium hydroxide from the canal was checked with an operating microscope at 16× (Zeiss, Oberkochen, Germany). A third bacteriological sample (S3) was taken as described previously. After sampling, the canal was rinsed with 5 mL of sodium hypochlorite (2%) and gently instrumented with the master apical file. After inactivation of the sodium hypochlorite with sodium thiosulphate, a fourth sample (S4) was taken from the root canal. The canal was dried and obturated with gutta-percha and AH-26 sealer using the warm-lateral compaction technique. After obturation of the canal, the tooth was restored in the same manner as the teeth in group 1. A final radiograph was taken using the paralleling technique with the aid of a beam guiding device (X-Act, Oral Diagnostic Systems, Amsterdam, the Netherlands), followed by control radiographs at 3, 6, 12 and 24 months. If complete healing had not taken place at 24 months the patients returned annually up to 4.5 years. At the first follow-up appointment, all temporary restorations were replaced by a permanent restoration.

Evaluation

Clinically, all the patients were free of symptoms and periodontal disease. The coronal restorations were of good quality during the entire follow-up period.

Three experienced endodontists who had not been involved in the treatment or follow-up appointments were asked to analyse the radiographs. Thirty

Table 1 Definitions of periapical scores and treatment outcome

| Periapical score | |
|-------------------|---|
| 1 | Periapical destruction of bone definitely not present |
| 2 | Periapical destruction of bone probably not present |
| 3 | Unsure |
| 4 | Periapical destruction of bone probably present |
| 5 | Periapical destruction of bone definitely present |
| Treatment outcome | |
| (A) Success | The width and contour of the periodontal ligament is normal, or there is a slight radiolucent zone around excess filling material |
| (B) Uncertain | The radiolucency is clearly decreased but additional follow-up is not available |
| (C) Failure | There is an unchanged, increased or new periradicular radiolucency |

radiographs (not included in the study) were used for calibration of the evaluators.

One set of radiographs consisted of four to six radiographs from one patient, taken at different follow-up appointments and projected in an at-random sequence. Each radiograph only showed the root and apical bone structure, the rest of the radiograph was masked. The 39 sets (one set of radiographs per patient) were projected on a screen in a dark room. The evaluators were asked to indicate the largest periapical radiolucency and the smallest periapical radiolucency of the set that was projected. Both images were given a periapical score from 1 to 5 (Table 1) (Reit & Gröndahl 1983). The evaluators also indicated a score for the treatment outcome according to the criteria presented in Table 1 (A, B or C). After each evaluator had given his individual periapical scores and treatment outcome, a joint evaluation was made to reach a consensus.

Statistics

A Student's *t*-test for independent samples or chi-square test (when appropriate) was performed for differences between patient groups related to gender, age and the clinical parameters, such as the tooth type, size of radiolucency, preparation length, master apical file size and apical extent of the root-canal filling.

Differences amongst evaluators and consensus for periapical scores and treatment outcome were tested using the Friedman's test for ordinal data.

The indicated largest and smallest periapical consensus scores were compared using the Wilcoxon's signed ranks test to indicate if there was a significant reduction of the periapical bone lesions over time.

The time needed by each tooth included in the study to be assigned to the 'success' group was of interest. An ana-

lysis of event times that also accounts for the observation period of teeth associated with 'failure' was applied as described by Weiger *et al.* (1998). This approach also considers the individual time span within which the tooth under observation that is scored as B may show complete healing (A), although this time is cut off before the event occurs. The distribution of the event times for both treatment groups were separately calculated on the basis of the Kaplan–Meier method (Kaplan & Meier 1958) and presented as step functions. The log-rank test was applied for comparison of the two treatment groups.

A chi-squared test with Yate's correction was performed for healing results of canals that showed bacterial growth at the time of obturation and canals that showed a negative culture.

For all tests, the *P*-values <0.05 were considered statistically significant. When no differences were found power statistics (power set at 80%) were conducted to determine the numbers required to find significant differences (*P* = 0.05) between healing of teeth obturated with a positive canal culture and teeth obturated with a negative canal culture as well as differences in healing results of teeth treated in one and two visits.

Results

During the follow-up period of 4.5 years, none of the patients had any discomfort and all teeth were functional. All patients returned for follow-up. One series of radiographs was excluded because of imperfections of radiographic technique.

There were no significant differences between patient groups related to gender, age and (the clinical parameters) tooth type, size of radiolucency, preparation length, master apical file size and the apical extent of the root-canal filling (*P* > 0.05).

Kappa scores between observers were 0.7–0.9. The agreement between treatment outcome scores of the individual observers and consensus score was at least 94% with kappa scores of 0.8–0.9.

The consensus on periapical scores and treatment outcome are presented in Tables 2 and 3. Before the treatment, all teeth had a periapical score 4 or 5. At the end of the follow-up period 18% still had a radiolucency (periapical score 4 and 5), in 82% a clear radiolucency was not present (periapical score 1 and 2). The periapical scores after the follow-up period were significantly lower (*P* < 0.05) than the scores before the start of treatment, indicating that the lesions had reduced significantly over the time.

Table 2 Consensus periapical score

| Periapical score ^a | Largest radiolucency | Smallest radiolucency |
|-------------------------------|----------------------|-----------------------|
| 1 | 0 | 22 (58) |
| 2 | 0 | 9 (24) |
| 3 | 0 | 0 |
| 4 | 4 (10.5) | 2 (5) |
| 5 | 34 (89.5) | 5 (13) |
| Total | 38 (100) | 38 (100) |

^aScore 1–5, see Table 1.

The values in parentheses are in per cent.

Table 3 Consensus treatment outcome

| | |
|----------------|----------|
| A ^a | 29 (76) |
| B | 8 (21) |
| C | 1 (3) |
| Total | 38 (100) |

^aScore A, B, C see Table 1.

The values in parentheses are in per cent.

Consensus treatment outcome A or B was assigned in 97% of the cases. One case was judged as a failure (Table 3). Upon extraction a vertical root fracture was diagnosed in this case.

Of the 21 teeth treated in one visit (group 1), 17 showed complete radiographic healing (score A, 81%), and 4 teeth a reduction in lesion size (score B, 19%). In group 2, 12 teeth showed complete radiographic healing (score A, 71%), and 4 had a reduction in the lesion size (score B, 23%) whilst one tooth had failed (score C, 6%) (Table 4).

Table 4 Treatment outcome scores related to one- and two-visit endodontic treatment

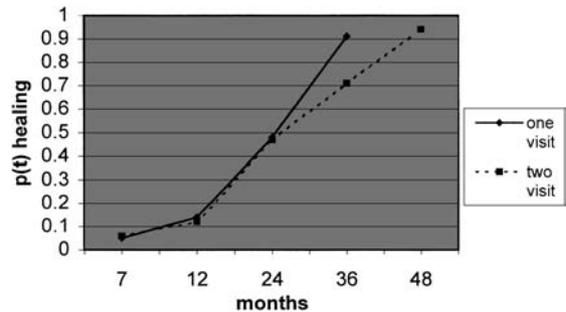
| | Score A ^a | Score B | Score C |
|----------------------------------|----------------------|---------|---------|
| One-visit group (<i>n</i> = 21) | 17 (81) | 4 (19) | 0 |
| Two-visit group (<i>n</i> = 17) | 12 (71) | 4 (23) | 1 (1) |
| Total (<i>n</i> = 38) | 29 (76) | 8 (21) | 1 (3) |

^aScore A, B, C see Table 1.

The values in parentheses are in per cent.

Table 5 Probability of success (95% confidence interval)

| | 1 years | 2 years | 3 years | 4 years |
|----------------------------------|---------------|------------------|------------------|------------------|
| One-visit group (<i>n</i> = 21) | 0.14 (0–0.29) | 0.48 (0.27–0.69) | 0.91 (0.77–1.00) | |
| Two-visit group (<i>n</i> = 17) | 0.12 (0–0.27) | 0.47 (0.23–0.71) | 0.77 (0.57–0.97) | 0.94 (0.83–1.00) |

**Figure 1** Estimation of the fraction of teeth [p(t)] that are successful following one- or two-visit root-canal treatment within 4 years.

The time necessary to complete the periapical-healing used as basis for calculation ranged from 7 to 55 months. The individual observation times varied between 12 and 35 months for those teeth associated with incomplete healing or failure. The probability that complete healing occurred within a certain time span increased continuously with the length of the observation period (Table 5, Fig. 1) The log-rank test did not reveal any significant difference between the one- and two-visit treatment groups ($P > 0.05$). Power statistics showed that the presented differences (91% vs. 94%) between one- and two-visit root-canal treatment could be significant if one experimental group would comprise of 1275 participants (power set at 80%).

Prior to the final obturation, there were eight teeth (seven in group 1, one in group 2) with a positive root-canal culture (Table 6). Six positive root canals contained $<10^2$ CFU mL⁻¹, one canal contained 2×10^3 and one canal harboured 8×10^4 CFU mL⁻¹. The latter case scored a treatment outcome B. Of the 30 cases that were filled with a negative culture prior to obturation, 22 (74%) healed (11 from group 1 and 11 from group 2) whereas this was the case for seven out of eight cases (six from group 1 and one from group 2) with a positive root-canal culture prior to obturation (87.5%). The chi-squared analysis showed no significant difference in healing whether cultivable bacteria were present or not prior to filling. Power statistics showed that the presented differences (74% vs. 87%) between negative and

Table 6 Positive and negative root-canal cultures related to treatment outcome

| | Group 1 (A) | Group 1 (B) | Group 2 (A) | Group 2 (B) | Group 2 (C) |
|--------------------------------------|----------------|----------------|----------------|----------------|----------------|
| Positive culture (<i>n</i> = 8) | 6 | 1 | 1 | 0 | 0 |
| Negative culture (<i>n</i> = 30) | 11 | 3 | 11 | 4 | 1 |

positive cultures at the time of obturation can be significant if one experimental group would comprise 160 patients (power set at 80%, $P < 0.05$).

Discussion

The presence of bacteria in the root canal results in the development of apical periodontitis. In the present study, no correlation was seen between the healing of endodontic lesions and the presence or absence of a positive canal culture after proper cleaning and shaping, and no correlation was seen between healing of endodontic lesions and treatment in one or two visits with intracanal dressing of calcium hydroxide. It is widely accepted that inclusion of calcium hydroxide renders the canal sterile and ready for obturation (Tronstad 1991, Friedman 1998). However, studies have shown that calcium hydroxide is not always effective and that its action is unreliable (Reit & Dahlén 1988, Ørstavik *et al.* 1991, Yared & Bou Dagher 1994, Peters *et al.* 2002). Comparisons of the success rate between the two-visit endodontics with calcium hydroxide as an intracanal interappointment dressing and the one-visit endodontic treatment of teeth with necrotic pulps in this and other prospective studies do not show significant differences (Friedman *et al.* 1995, Trope *et al.* 1999, Weiger *et al.* 2000). Other studies evaluating the healing of teeth with necrotic pulps after either one or two-visits report success rates between 75 and 90% for both treatment options (Byström *et al.* 1987, Murphy *et al.* 1991, Jurcak *et al.* 1993, Calişkan & Sen 1996).

It seems more reliable to evaluate infection at the time of root filling. Studies by Zeldow & Ingle (1963) and Engström *et al.* (1964) showed inferior results for teeth with a positive culture at the time of root filling. On the other hand, Seltzer *et al.* (1963) and Matsumoto *et al.* (1987) could not find any significant differences in healing results. All these studies are limited in bacteriological technique, because no strict anaerobic techniques were used and contamination cannot be ruled out in some reports owing to a lack of information about

the treatment procedures. Sjögren *et al.* (1997) using strict anaerobic techniques, related infection at the time of obturation and success. They showed inferior healing results for root canals with a positive culture at the time of obturation. Our results do not indicate such differences. It is stressed that, in both studies, the number of CFU left in the canal at the time of obturation was very low. In our study, six out of the eight positive root canals contained $<10^2$ CFU mL⁻¹, one canal contained 2×10^3 and one canal harboured 8×10^4 CFU mL⁻¹. The latter case scored a treatment outcome B. In the study by Sjögren *et al.* (1997), 22 (40%) root canals contained bacteria at the time of obturation. In eight cases, bacteria could only be detected after the enrichment growth in fluid media, nine samples contained 10^2 – 10^3 CFU, three samples contained $>10^4$ CFU, and in two samples, no data were available. Of these 22 positive samples 7 failed. The authors did not relate the failed cases to the number of CFU found. If the failures are related to higher number of CFU ($>10^3$) in the canal, it could provide an explanation for the reason why cases with a positive culture appeared successful in both studies, 15/22 (Sjögren *et al.* 1997) and 7/8 in our study.

Comments have been made previously about the uncertainty of the bacteriological sampling procedure immediately after the removal of a calcium hydroxide dressing (Reit & Dahlén 1988). It has been suggested (Reit *et al.* 1999) that the microbiological samples should be taken after filling the canal with a sampling fluid (after removal of the calcium hydroxide) for 7 days. However, when the authors applied this procedure, culture reversals were seen in both directions. Thus, Reit *et al.* (1999) reported seven canals that turned from a negative to a positive culture after 1 week, but also the seven canals that changed from a positive culture to a negative culture over the same period. It cannot be ruled out completely that some negative canals in the present study after calcium hydroxide removal (S3), may have become positive if evaluated 1 week later. The studies of Reit & Dahlen (1988) and Reit *et al.* (1999) demonstrated the limitations of microbiological root-canal sampling, and this should be taken into account when evaluating all root-canal procedures. Because it was found that removal of calcium hydroxide from the root canal with the aid of the operating microscope was enhanced, and because it has been shown previously that a second culture taken 7 days later did not result in more reliable data (Reit & Dahlen 1988, Molander *et al.* 1990, Reit *et al.* 1999), cultures were taken immediately after removal of the calcium hydroxide. This process was also less demanding

for the patients, as it reduced the number of appointments.

Differences in healing can also be created because the size of the preoperative radiolucency, between canals with detectable and non-detectable microorganisms, is different in both groups. In the present study, these differences were not found.

Sjögren *et al.* (1997) found 60% negative root-canal samples after similar preparation of the root canals. The difference with our findings (79% negative) could be a result of the concentrations of sodium hypochlorite (0.5% vs. 2%) and different delivery systems used for irrigation. In addition, the sampling techniques and transport media used may have also created differences.

A question that remains to be answered is the reason for failure in cases of root canals from which no bacteria could be cultured. In Sjögren's study (1997), two of those cases failed. In one of these, bacteria were found at the time of surgery in a lateral canal. This points to the inability of current cleaning and shaping techniques to reach microorganisms present in lateral canals and dentinal tubules (Peters *et al.* 2001). These bacteria cannot be identified using current endodontic sampling procedures and seem inaccessible to an intracanal disinfectant like calcium hydroxide (Siqueira & Lopes 1999).

The number of patients available in our study as well as most other prospective studies is limited. Given the high-success rates of both treatment options, the sample size per group required to detect a difference is very high. Weiger *et al.* (2000) showed that the probability for complete periapical repair over 5 years was 93% for two-visit root-canal treatment and 92% for one-visit root-canal treatment. When we calculate power statistics (power set at 80%) with these proportions, the numbers per group would have to exceed 10 000. Trope *et al.* (1999) calculated the need for a group size of 354 (power set at 80%) to show significance for the differences he found (74 vs. 64%) in comparing one- versus two-visit root-canal treatment. However, the short observation period of 52 weeks in that study may lead to an underestimation of periapical healing over longer observation periods, as was shown by Weiger *et al.* (1998). In the present study, the probability for healing increased gradually to 91 and 94% over a period of 4 years, indicating the need for a group size of 1275 teeth to show significance at $P = 0.05$. However, the cases that were selected in these studies, all present a relatively simple anatomy resulting in high success rates. The numbers that are needed in a prospective study with more complex cases may be much lower because of lower success rates.

Conclusions

Within the limitations of this study, using teeth with a relatively simple anatomy, no significant differences in healing results occurred when small numbers of bacteria ($CFU < 10^2$) could be cultured or not cultured at obturation. There was no significant difference in healing after root-canal treatment in one and two visits (with interappointment calcium hydroxide dressing). In order to quantitate the effects of treatment on healing in a similar prospective study, a very large group size is needed for definite conclusions.

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References

- Byström A (1986) Evaluation of Endodontic Treatment of Teeth with Apical Periodontitis. Odontological Dissertations (Abstract 27). Umeå University, Umeå, Sweden.
- Byström A, Claesson R, Sundqvist G (1985) The antibacterial effect of camphorated *para*-monochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. *Endodontics and Dental Traumatology* **1**, 170–5.
- Byström A, Happonen RP, Sjögren U, Sundqvist G (1987) Healing of periapical lesions of pulpless teeth after endodontic treatment with controlled asepsis. *Endodontics and Dental Traumatology* **3**, 58–63.
- Byström A, Sundqvist G (1981) Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *Scandinavian Journal of Dental Research* **89**, 321–8.
- Calışkan MK, Sen BH (1996) Endodontic treatment of teeth with apical periodontitis using calcium hydroxide: a long-term study. *Endodontics and Dental Traumatology* **12**, 215–21.
- Chong BS, Pitt Ford TR (1992) The role of intracanal medication in root canal treatment. *International Endodontic Journal* **25**, 97–106.
- Engström B, Hard AF, Segerstad L, Ramström G, Frostell G (1964) Correlation of positive cultures with the prognosis for root canal treatment. *Odontologisk Revy* **15**, 257–70.
- Friedman S (1998) Treatment outcome and prognosis of endodontic therapy. In: Ørstavik D, Pitt Ford TR, eds. *Essential Endodontology*, p. 387. London, UK: Blackwell Science.

- Friedman S, Löst C, Zarrabian M, Trope M (1995) Evaluation of success and failure after endodontic therapy using a glass ionomer cement sealer. *Journal of Endodontics* **21**, 384–90.
- Fuss Z, Charniaque O, Pilo R, Weiss E (2000) Effect of various mixing ratios on antibacterial properties and hardness of endodontic sealers. *Journal of Endodontics* **26**, 519–22.
- Jurcak JJ, Bellizzi R, Loushine RJ (1993) Successful single-visit endodontics during Operation Desert Shield. *Journal of Endodontics* **19**, 412–3.
- Kaplan EL, Meier P (1958) Nonparametric estimations from incomplete observations. *Journal of the American Statistical Association* **53**, 457–81.
- Kaplan AE, Picca M, Gonzalez MI, Macchi RL, Molgolini SL (1999) Antimicrobial effect of six endodontic sealers: an *in vitro* evaluation. *Endodontics and Dental Traumatology* **15**, 42–5.
- Matsumoto T, Nagai T, Ida K, Ito M, Kawai Y, Horiba N, Sato R, Nakamura H (1987) Factors affecting successful prognosis of root canal treatment. *Journal of Endodontics* **13**, 239–42.
- Molander A, Reit C, Dahlén G (1990) Microbial evaluation of clindamycin as a root canal dressing in teeth with apical periodontitis. *International Endodontic Journal* **23**, 113–8.
- Moorer WR, Genet JM (1982) Evidence for antibacterial activity of endodontic gutta-percha cones. *Oral Surgery, Oral Medicine and Oral Pathology* **53**, 503–7.
- Murphy WK, Kaugars GE, Collett WK, Dodds RN (1991) Healing of periapical radiolucencies after nonsurgical endodontic therapy. *Oral Surgery, Oral Medicine and Oral Pathology* **71**, 620–4.
- Nair PN, Sjögren U, Krey G, Kahnberg KE, Sundqvist G (1990) Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. *Journal of Endodontics* **16**, 580–8.
- Oliet S (1983) Single-visit endodontics: a clinical study. *Journal of Endodontics* **9**, 147–52.
- Ørstavik D, Kerekes K, Molven O (1991) Effects of extensive apical reaming and calcium hydroxide dressing on bacterial infection during treatment of apical periodontitis: a pilot study. *International Endodontic Journal* **24**, 1–7.
- Peters LB, van Winkelhoff AJ, Buijs JF, Wesselink PR (2002) Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. *International Endodontic Journal* **35**, 13–26.
- Peters LB, Wesselink PR, Buijs JF, van Winkelhoff AJ (2001) Viable bacteria in root dentinal tubules of teeth with apical periodontitis. *Journal of Endodontics* **27**, 76–81.
- Reit C, Dahlén G (1988) Decision making analysis of endodontic treatment strategies in teeth with apical periodontitis. *International Endodontic Journal* **21**, 291–9.
- Reit C, Gröndahl H-G (1983) Application of statistical decision theory to radiographic diagnosis of endodontically treated teeth. *Scandinavian Journal of Dental Research* **91**, 213–8.
- Reit C, Molander A, Dahlén G (1999) The diagnostic accuracy of microbiologic root canal sampling and the influence of antimicrobial dressings. *Endodontics and Dental Traumatology* **15**, 278–83.
- Saunders WP, Saunders EM (1992) Effect of noncutting tipped instruments on the quality of root canal preparation using a modified double-flared technique. *Journal of Endodontics* **18**, 32–6.
- Seltzer S, Bender I, Turkenkopf S (1963) Factors affecting successful repair after root canal therapy. *Journal of the American Dental Association* **67**, 651–62.
- Shuping GB, Ørstavik D, Sigurdsson A, Trope M (2000) Reduction of intracanal bacteria using Nickel-Titanium rotary instrumentation and various medications. *Journal of Endodontics* **26**, 751–5.
- Siqueira JF, Jr, Favieri A, Gahyva SM, Moraes SR, Lima KC, Lopes HP (2000) Antimicrobial activity and flow rate of newer and established root canal sealers. *Journal of Endodontics* **26**, 274–7.
- Siqueira JF, Jr, Lopes HP (1999) Mechanisms of antibacterial activity of calcium hydroxide: a critical review. *International Endodontic Journal* **32**, 361–9.
- Sjögren U, Figdor D, Persson S, Sundqvist G (1997) Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *International Endodontic Journal* **30**, 297–306.
- Sjögren U, Haggglund B, Sundqvist G, Wing K (1990) Factors affecting the long-term results of endodontic treatment. *Journal of Endodontics* **16**, 498–504.
- Soltanoff W (1978) A comparative study of the single-visit and the multiple-visit endodontic procedure. *Journal of Endodontics* **4**, 278–81.
- Syed SA, Loesche WJ (1972) Survival of human dental plaque flora in various transport media. *Applied Microbiology* **24**, 638–44.
- Tronstad L (1991) *Clinical Endodontics*, 1st edn. Stuttgart, Germany: Georg Thieme Verlag, p. 101.
- Trope M, Delano EO, Ørstavik D (1999) Endodontic treatment of teeth with apical periodontitis: single vs. multivisit treatment. *Journal of Endodontics* **25**, 345–50.
- Weiger R, Axmann-Krcmar D, Löst C (1998) Prognosis of conventional root canal treatment reconsidered. *Endodontics and Dental Traumatology* **14**, 1–9.
- Weiger R, Rosendahl R, Löst C (2000) Influence of calcium hydroxide intracanal dressings on the prognosis of teeth with endodontically induced periapical lesions. *International Endodontic Journal* **33**, 219–26.
- Yared GM, Bou Dagher FE (1994) Influence of apical enlargement on bacterial infection during treatment of apical periodontitis. *Journal of Endodontics* **20**, 535–7.
- Zeldow BJ, Ingle JL (1963) Correlation of the positive cultures to the prognosis of endodontically treated teeth: a clinical study. *Journal of the American Dental Association* **66**, 9–13.