

# Reduction of Intracanal Bacteria Using GT Rotary Instrumentation, 5.25% NaOCl, EDTA, and Ca(OH)<sub>2</sub>

Robin McGurkin-Smith, DDS, MS, Martin Trope, DMD, Daniel Caplan, DDS, PhD, Asgeir Sigurdsson, DDS, MS

## Abstract

This study was conducted to determine the bacterial reduction using Profile GT® files and a strict irrigation protocol utilizing 5.25% NaOCl and EDTA. The additive antibacterial effect of Ca(OH)<sub>2</sub> was also evaluated. In addition, the study compared the bacterial reduction with the GT protocol versus larger instrumentation. Thirty-one subjects with apical periodontitis were recruited. Bacterial samples were taken upon access (S1), after instrumentation and a strict irrigation protocol (S2), and following >1 wk of Ca(OH)<sub>2</sub> (SC). A log<sub>10</sub> transformation of colony forming units was done since sample bacterial counts are not normally distributed. At S1, 93.55% of canals sampled bacteria. At S2, 52.72% of the cases sampled bacteria. At SC, 14% of the cases cultured bacteria. The McNemar test showed a significant reduction ( $p < 0.0009$ ) in bacteria between S1 and S2. This was also true between S2 and SC ( $p < 0.0019$ ). It was concluded the GT protocol significantly reduced the number of bacteria in the canal but failed to render the canal bacteria free in more than half of the cases. Ca(OH)<sub>2</sub> application significantly further reduced bacteria. Lastly, large apical instrumentation removed more bacteria than small apical instrumentation.

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Dr. McGurkin-Smith is a former resident; Dr. Trope is the J.B. Freeland Professor; and Dr. Sigurdsson is associate professor and graduate program director, Department of Endodontics, Dr. Caplan is associate professor, Department of Dental Ecology, The University of North Carolina, Chapel Hill, NC.

Address requests for reprints to Dr. Martin Trope, Department of Endodontics, The University of North Carolina, School of Dentistry, CB#7450, Chapel Hill, NC 27599; E-mail address: martin\_trope@dentistry.unc.edu.

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Endodontics is the prevention or treatment of apical periodontitis. Endodontic research has shown a cause and effect relationship between bacteria and apical periodontitis (1–4).

Because bacteria are the cause of apical periodontitis, it is logical that the elimination of bacteria would cure apical periodontitis. A tooth without periapical radiolucency has a higher endodontic success rate upon treatment than a tooth with a radiolucency (5–8). Likewise, teeth that are obturated after a negative culture have a better prognosis than those obturated following a positive culture (9–11). Although there is substantial evidence that a negative culture does not equate to bacteria free (12–14), there is a threshold level of bacteria below that gives a negative sample and a success rate similar to teeth treated without apical periodontitis (11).

The current technique for bacterial reduction includes instrumentation, irrigation with NaOCl and an intracanal antimicrobial medication. Bystrom et al. (15–18) showed that each step leads to less bacteria and eventually a canal that does not sample bacteria. In a 5 yr follow-up study, root canal samples that gave a negative culture following these steps were recalled to evaluate healing. Ninety-five percent of these cases showed complete radiographic healing or a decrease in the radiolucency size (10).

A recent series of studies have been carried out where NiTi instruments were used in place of stainless steel files (19–21). Similar to the Bystrom and Sunqvist (15) findings, Dalton et al. (19) found that while instrumentation decreased the number of canals that cultured bacteria, instrumentation alone does not predictably result in canals that did not culture bacteria. Shuping et al. (20) evaluated the bacterial reduction following the addition of 1.25% NaOCl irrigation and Ca(OH)<sub>2</sub> to NiTi instrumentation. Similar to previous studies, Shuping et al. (20) found that instrumentation and irrigation with NaOCl significantly reduced the number of canal samples that cultured bacteria. The number of canal samples that cultured bacteria was further reduced with the addition of intracanal Ca(OH)<sub>2</sub>. In Card et al. (21), teeth were instrumented to apical sizes much larger than seen in previous studies (16, 20). Following large apical instrumentation with NaOCl, 100% of the bicuspid samples rendered bacteria free when cultured. In addition, 89% of mesial canals in molars cultured bacteria free following the larger instrumentation protocol.

A line of rotary files has gained great popularity among general dentists and endodontists. The file system is known as the ProFile GT (Dentsply/Tulsa Dental, Tulsa, OK) files. The instrumentation using ProFile GT files focuses on the sequential increase in the taper of the files compared to other file systems that focus more on increasing the end size of the files and thereby the apical size of the preparation. The greater taper files differ primarily from other instrumentation systems evaluated with respect to the size of instrumentation at the apex of the tooth. When using ProFile GT files, roots are divided into small, medium and large roots (22) (Table 1). The category a tooth falls into dictates the final apical instrumentation. The size chosen to instrument at the apex with ProFile GT files is smaller than in past studies (17, 20). This concept was largely based on the school of thought where the canal is shaped to obturate. To achieve this objective, the apical opening is kept as small as possible so that the preparation is wider as it is prepared coronally (23). The thought is that a “tapered funnel preparation” increases the action of an irrigant and facilitates obturation (23). The proponents of the GT files feel that most root termini in small root canals are between 0.15 to 0.25 mm in diameter and 0.20 to 0.35 mm in large root canals (24). The protocol therefore advocates instrumenting a small root canal to a 0.20 mm tip with a 0.06 to 0.08 taper. The files are

**TABLE 1.** ProFile GT size corresponding to tooth type

Small Roots (mandibular incisors, 2 and 3 canal premolars, mesial root of lower molars, buccal root of upper molars)	Medium Roots (distal roots of lower molars, palatal roots of upper molars)	Large Roots (lower cuspids, upper anteriors, one canal premolars)
20/.04	20/.10	20/.10
20/.06	30/.10	30/.10
20/.08	30/.04–30/.08	40/.04–40/.1
30/.04	35/.12	35/.12
30/.06		50/.12
30/.08		70/.12

carried to the canal terminus as determined by the apex locator. The proponents of the ProFile GT file contend that the increasing taper with the ProFile GT file allows better irrigation with less instrumentation at the apex. The ProFile GT system is based on cleaning and shaping the root canals while removing minimal apical dentin. To do this, the system advocates the use of ProFile GT files, EDTA to remove the smear layer and 30 min of 5.25% NaOCl irrigation replenished every 5 min before obturation.

The purpose of this study was to determine the ability of the ProFile GT file system with repeated application of 5.25% NaOCl and EDTA over 30 min to reduce or render root canals bacteria free. In addition, Ca (OH)<sub>2</sub> was placed in the canals at the end of instrumentation to see if the medicament had any additional effect on bacteria remaining in the canals after the first appointment.

## Materials and Methods

### Subject Recruitment and Qualification

Approval for this project was obtained from the University Of North Carolina School Of Dentistry Committee on Investigation Involving Human Subjects. Patients presenting to the University of North Carolina School of Dentistry endodontic clinic for evaluation and treatment of apical periodontitis were considered for this study. The primary investigator (RM) conducted all clinical and sampling procedures. The study and associated risks were explained to the patients and consent obtained. Each patient was assigned a reference number only the primary investigator knew. References to individual treatment were by reference number to maintain confidentiality.

Only mandibular teeth were included in the study. When treating a molar, only the mesiobuccal canals of the first and second molars were sampled, but both mesiobuccal and mesiolingual canals were fully instrumented in accordance with the study guidelines.

The other tooth requirements included:

- Radiographic evidence of a periapical radiolucent lesion associated with the tooth
- No response to thermal or electric pulp testing
- Enough crown structure for adequate isolation
- No history of previous endodontic treatment of the tooth

### Treatment Group Assignment

Qualified subjects were accepted into the study in a nonrandom consecutive sample and treated with nickel-titanium ProFile GT files. After instrumentation, all teeth were irrigated with 5.25% NaOCl and EDTA in accordance with study guidelines.

### Bacterial Sampling

The patient was anesthetized and the tooth isolated with a rubber dam. Dental floss was securely tied around the neck of the tooth and the tooth and adjacent rubber dam were cleaned with 30% hydrogen peroxide until no further bubbling of the peroxide occurred. If difficulty

occurred in attaining a bubble-free status, Oraseal Putty (Ultradent Products Inc.) was placed around the neck of the tooth and the process repeated. All surfaces were then coated with tincture of iodine and allowed to dry. Gross caries removal and initial access form was accomplished with sterile high speed and low speed burs. The rubber dam and surrounding tooth structure were disinfected with iodine tincture before completing the access with another sterile bur. Sterile saline was used to flush debris within the chamber. The method for molar treatment varied slightly from the method for the single rooted teeth because of the need to block off the distal canal in the molar before sampling, and the need to instrument both mesial roots during treatment. Sterile orifice openers (Dentsply/Tulsa Dental) were used to open the orifice of the distal canal before sealing the distal canal orifice with Cavit (ESPE, Norristown, PA). This file was set aside and a set of sterile 20-.10 GT files were used to initiate access into the two mesial canals. With the single rooted teeth only one 20-.10 GT file was needed. Sterile saline was again used to flush any debris from the chamber. The chamber was then dried with sterile cotton pellets and/or paper points before placement of 0.02 ml of Liquid Dental Transport Media (LDT) (Anaerobic Systems, Morgan Hill, CA) into the sampled mesiobuccal or single canal with a sterile tuberculin syringe. The canal was instrumented to a 15 to 20 file size with sterile stainless steel Hedstrom and/or K-file that was placed to within 1 mm of the estimated working length. Once each instrument was removed from the canal, the fluted part of the file was cut off with a sterile wire cutter and allowed to fall into the opened bottle of LDT. The LDT remaining in the canal was soaked up and transferred to the LDT bottle with sterile xx-fine to fine paper points (Mynol, Block Drug Corp., Jersey City, NJ) placed as close to working length as possible. This constituted the first sample (S1).

Working length was established to the root terminus using an apex locator (Root ZX, Endex Plus) and confirmed radiographically. The canals were then instrumented with a predetermined final ProFile GT file size. All Profile GT rotary instrumentation utilized the Aseptico Electric Moter (Dentsply/Tulsa Dental) rotating at 150 or 300 rpm. During instrumentation, the canal was irrigated with 5.25% NaOCl utilizing the 28 gauge Double D needle (Beutlich Pharmaceuticals LP, Waukegan, IL). A radiograph was taken to confirm the ProFile GT file reached the working length. A K-file corresponding to the apical diameter of the last ProFile GT used was taken to length. If that file bound at length, a K-file the apical diameter size larger was placed in the canal to verify a tapered preparation. Lastly, a K-file two apical diameter sizes larger than the last GT file was placed in the canal. If that file bound 0.5 mm short of the full length of the canal, instrumentation was complete. After instrumentation was completed, the canal was irrigated for one minute with EDTA. The canal was then irrigated for 30 min with 5.25% NaOCl changing to a fresh solution of NaOCl every 5 min. After this time, each canal was flushed with 2 ml of 5% sodium thiosulfate to neutralize the NaOCl. The canals were then flushed with sterile saline and dried with sterile paper points. Approximately 0.02 ml of LDT was placed into the canal with a sterile tuberculin syringe. A sterile file identical to the final file was

placed to length in the canal and pumped five times with minimal reaming motion to disrupt the canal contents. The remaining LDT was absorbed with sterile paper points and transferred to the LDT sample bottle. This constituted the second sample (S2).

The canal was rinsed and dried with paper points.  $\text{Ca}(\text{OH})_2$  was placed in the canal with a Lentulo spiral filler (Caulk, Milford, DE) and the tooth was temporized with IRM (Dentsply Int. Inc., York, PA). After at least 1 wk of calcium hydroxide therapy, the patient was brought back and anesthetized. Under rubber dam isolation the tooth was accessed with the strict aseptic protocol adhered to earlier in the study. Calcium hydroxide was removed with a K-file and sterile saline irrigation. Next 0.5% citric acid (25) was introduced into each canal to neutralize the calcium hydroxide. The canal was irrigated again with sterile saline and dried. LDT was placed as previously described and a final sample (SC) was taken. The canal was further instrumented to a larger apical diameter and obturated at this appointment if the patient was asymptomatic. If the patient was symptomatic, the instrumentation was completed and  $\text{Ca}(\text{OH})_2$  placed for at least one more week.

The sampling technique used in this study was the PMR (pumping maximum removal) technique developed by Moller (25) modified using LDT as both the sampling and transport media. LDT is a buffered salt transport media that sustains the bacteria life but does not allow bacteria to multiply. LDT is purported to keep anaerobic bacteria viable for 72 h.

Additional teeth diagnosed as irreversible pulpitis served as negative controls to detect contamination potential. Each control was treated in the same manner as the study teeth with apical periodontitis. The laboratory procedures were performed at the University of North Carolina Dental Microbiology Laboratory.

### Microbial Examination

The laboratory procedures were performed at the University of North Carolina Dental Microbiology Laboratory (a CLIA certified laboratory). The vials with the paper point samples were agitated with a vortex for 30 s at a power setting of 4. A model D spiral plater (Microbiology International) delivered 49  $\mu\text{l}$  of sample to each agar plate. The Model D spiral plater delivered a 2.3 log dilution of the sample across each plate. Each sample was plated in duplicate on aerobic plates, anaerobic plates and chocolate plates. The anaerobic gas consisted of 5%  $\text{CO}_2$ , 85%  $\text{N}_2$ , and 10%  $\text{H}_2$ . The chocolate plates were grown aerobically in a carbon dioxide enriched environment. This was to support the growth of Haemophilus and Neisseria species that normally will not grow on sheep blood agar. Bacteria growth was measured by direct counting of colonies and grid specific calculations. The spiral plater deposited a known volume of the sample to areas of the plate or grid. Once the colonies were counted in each grid, a dilution factor (determined by the manufacturer) was used to translate the grid calculations to the original bacterial count in the sample.

### Statistical Analysis

The differences in bacterial colonies were assessed at three time points (S1, S2, and SC). A  $\log_{10}$  transformation of colony forming units was done since sample bacterial counts are not normally distributed. A paired *t* test was used to determine the difference in mean bacterial numbers between the samples. A McNemar test was done to compare the proportion of cases that cultured bacteria between samples. It was also of interest to compare data from this study to data of past studies using other instrumentation techniques in similar teeth. A two sample *t* test was used to compare the different studies' results. The significance level was set at 0.05 for all tests.

### Results

Thirty-one test teeth were qualified and accepted into the study. Four teeth diagnosed as irreversible pulpitis served as controls.

The bacterial profile during treatment for each patient is seen in Table 2.

Bacteria were initially found in 29 of 31 (93.55%) diagnosed necrotic teeth and in no control teeth. The mean bacteria count at the initial sample (S1) was  $1.01 \times 10^7$  with a range between 0 to more than could be counted ( $>10^9$ ). The median number was  $5.60 \times 10^4$ . At S2 (after complete instrumentation with ProFile GT files, EDTA, and 30 min of irrigation with NaOCl), 52.72% of samples cultured bacteria. The mean count in S2 samples was  $6.60 \times 10^6$  with a range from 0 to too many to count ( $>10^9$ ). The median bacteria count at S2 was  $8.00 \times 10^1$ . The number of teeth that culture anaerobic bacteria at SC was 14%. The mean count in the SC sample was  $8.25 \times 10^1$  and the median was 0.0.

The paired *t* test showed a highly significant difference in mean bacterial count between S1 and S2 ( $p < 0.0001$ ) and between S1 and SC ( $p < 0.0001$ ). A significant difference was found between S2 and SC ( $p < 0.05$ ). The McNemar test showed a significant difference ( $p < 0.0009$ ) between the proportion of canals that sampled bacteria in S1 compared to P2. There was also a significant difference ( $p < 0.0019$ ) in canals that sampled bacteria from S2 to SC with regard to percent reduction.

The average number of days in calcium hydroxide therapy was 40 days with a range of 7 to 110 days. The median number of days for  $\text{Ca}(\text{OH})_2$  treatment was 37 days. Five cases had calcium hydroxide for over 60 days. This was because of lack of patient compliance rather than the design of the study.

TABLE 2. Patient Data—Bacterial sampling

Patient #	Tooth Type	P1	P2	PC
1	i	$2.40 \times 10^5$	$3.80 \times 10^3$	0
2	p	$>10^9$	$>10^9$	$1.20 \times 10^3$
3	p	$1.20 \times 10^4$	0	$1.89 \times 10^2$
4	p	$8.60 \times 10^6$	$4.50 \times 10^6$	NC
5	m	$3.70 \times 10^4$	0	$3.35 \times 10^{2*}$
6	m	$2.90 \times 10^5$	$2.10 \times 10^3$	0
7	m	0	0	0
8	m	$1.30 \times 10^6$	$3.60 \times 10^4$	0
9	m	$2.40 \times 10^2$	0	0
10	m	$6.67 \times 10^5$	0	0
11	m	$8.10 \times 10^2$	0	0
12	c	$7.70 \times 10^4$	$3.50 \times 10^4$	0
13	m	$2.00 \times 10^5$	0	0
14	m	0	0	0
15	m	$>10^9$	$>10^9$	0
16	m	$2.90 \times 10^5$	$2.10 \times 10^3$	0
17	m	$2.03 \times 10^2$	0	0
18	m	$5.60 \times 10^4$	$2.20 \times 10^2$	0
19	m	$1.90 \times 10^5$	$1.00 \times 10^2$	$8.53 \times 10^2$
20	i	$5.10 \times 10^3$	0	NC
21	i	$>10^9$	$4.00 \times 10^2$	0
22	i	$1.30 \times 10^5$	$2.00 \times 10^3$	0
23	p	$1.50 \times 10^5$	$1.60 \times 10^2$	$6.00 \times 10^1$
24	i	$7.00 \times 10$	0	0
25	m	$2.20 \times 10^2$	$8.00 \times 10^1$	NC
26	m	$4.06 \times 10^3$	$2.84 \times 10^2$	0
27	m	$2.70 \times 10^4$	0	0
28	i	$1.70 \times 10^4$	0	0
29	m	$1.14 \times 10^4$	0	NC
30	p	$8.10 \times 10^1$	0	0
31	m	$6.20 \times 10^4$	$6.99 \times 10^3$	0

NC = Patient did not return to complete treatment.

\* = contaminated sample.

Discussion

The correlation between bacteria and apical periodontitis was supported in this study. Twenty-nine of the 31 (94%) test teeth that showed clinical and radiographic signs of apical periodontitis cultured bacteria at the first sample. This supports earlier studies that show a correlation between bacteria and apical periodontitis (3, 19–21, 26).

Our group has performed a number of similar studies using different clinical protocols to evaluate if any protocol advocated by its proponents resulted in more negative cultures, and therefore, more (assumed) success. Shuping et al. (20) is such a study where another file system was used with 1.25% sodium hypochlorite used in conjunction with filing but not leaving the irrigant to soak in the canal. It is interesting to compare the results of these two studies with similar research protocols. Shuping et al. obtained negative cultures in 61.9% of samples compared to 47.3% in this study after final instrumentation. When the teeth were compared after instrumented to comparable apical sizes, 71% of teeth in the Shuping study sampled positive compared to 52.7% in the present study. The results following Ca(OH)<sub>2</sub> treatment show that 14% of the teeth cultured positive in the present study versus 7.5% in the Shuping study.

Statistical analysis was done to compare means between the two studies at different points. A two-sample *t*-Test for equal means was used to compare the different points. A common application of this is to test if the new treatment is superior to the gold standard. The tests indicate that there was a significant difference in mean bacterial count at the final instrumentation between the present study (S2) and the Shuping study (S4). A comparison was made between the S2 sample in the present study and the S3 sample in the Shuping study because of the similar apical instrumentation size. This comparison indicates no significant difference between the two studies with regard to mean bacteria count. There was a significant difference between the two studies following Ca(OH)<sub>2</sub> treatment.

In this study, 25 of the original 29 patients that had cultured bacteria in the canal at the first appointment returned for the second appointment. The percent of negative cultures went from 47.3% at S2 to 86.7% at SC. Current molecular endodontic research has shown that a canal that does not culture bacteria is not sterile and there is always the possibility of regrowth (18, 28–33). Nevertheless, when a canal does not culture bacteria before obturation the prognosis increases greatly (11, 33). The interappointment placement of Ca(OH)<sub>2</sub> significantly reduced the number of canals that cultured bacteria and may therefore increase the prognosis of teeth with apical periodontitis.

Shuping et al. (20) found that 92.5% of canals did not sample bacteria following a larger apical preparation and at least 1 wk of Ca(OH)<sub>2</sub>. The canals that sampled bacteria in the present study following Ca(OH)<sub>2</sub> had high initial counts at S1. Ando and Hashino (34) showed that bacteria invade deep into the dentin. They found that bacteria extended into dentin 2 mm from the dentin/canal border. Haapasalo et al. (35), in an in vitro experiment, showed that dentin powder has an inhibitory effect on Ca(OH)<sub>2</sub>. There was a significant difference in the mean bacterial count following Ca(OH)<sub>2</sub> in the present study versus the larger instrumented Shuping et al. (20) study. The combination of more infected dentin left following instrumentation in this study plus the decrease in pH seen the further a dentin sample is from the Ca(OH)<sub>2</sub> may explain this difference.

In the study we found that the number of cases that sampled bacteria was reduced significantly following Ca(OH)<sub>2</sub> treatment. This study did not have any teeth without Ca(OH)<sub>2</sub> placed between the second and third appointment. Therefore, it cannot be conclusively stated that Ca(OH)<sub>2</sub> was the reason there was a decrease in bacteria between the two samples from this study alone. Past studies have shown that if the

canal is left empty but sealed the remaining bacteria in the canal multiply (15, 16). These historical results serve as a proxy for a control group in the current study.

The basic premise of this paper is that a negative culture after the microbial control phase of endodontics will result in a high level of elimination of disease (10, 11). Therefore any instrument or clinical procedure that falls into the microbial control phase of endodontics should be evaluated as to its ability to reduce bacteria in the root canal. Thus an evidence based clinical approach would lead to the endodontist using a technique that would result in the most (experimental) negative cultures. This approach allows quick evaluations that enable us to evaluate current techniques rather than wait for 5 yr follow-up radiographs and report on a technique that may not still exist.

The results of this study suggest that an intra-canal application of calcium hydroxide is necessary to get a high incidence of negative cultures before filling and refutes the notion that small apical sizes can be overcome by a 30 min soak with 5.25% sodium hypochlorite. The use of calcium hydroxide should not be confused when one reads recent in vitro studies showing that *Enterococcus faecalis* is a predominant bacteria in failed endodontic cases and that calcium hydroxide is relatively ineffective against this bacterium (36, 37). These studies only relate to those failed cases and are more relevant to re-treatment protocols. Hopefully if a protocol is used with microbial control in mind, those failed cases will be very few.

In summary, a statistically significant reduction in bacteria, cultured from infected teeth with apical periodontitis, was found following the instrumentation and irrigation protocol used in this study. Of the 29 teeth that cultured bacteria before treatment, 47% did not culture bacteria following instrumentation with Profile GT files and irrigation with EDTA and 5.25% NaOCl. An interappointment dressing of calcium hydroxide further significantly reduced the number of teeth that cultured bacteria. Following Ca(OH)<sub>2</sub> treatment, 86% of these teeth did not culture bacteria. There was a significant decrease in the mean number of bacteria sampled in teeth instrumented to large apical sizes versus the small apical sizes when the results of the present study were compared to historical studies. There was also a significant difference in mean bacterial count following Ca(OH)<sub>2</sub> treatment in teeth instrumented to larger versus smaller apical diameters. Endodontic treatment is carried out to eliminate or prevent apical periodontitis. The use of EDTA and 5.25% NaOCl with minimal apical instrumentation does not predictably render canals bacteria free. The present study supports past studies that show the importance of adequate apical instrumentation to eliminate or reduce bacteria in a case with apical periodontitis. In addition to adequate instrumentation and irrigation, an intracanal medicament may be needed to increase long term success.

References

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germfree and conventional laboratory rats. *Oral Surg Oral Med Oral Pathol* 1965;20:340–9.
2. Bergenholtz G. Micro-organisms from necrotic pulp of traumatized teeth. *Odontologisk Revy* 1974;25:347–58.
3. Sundqvist G. Bacteriological studies of necrotic dental pulps [master's thesis]. Umeå, Sweden: University of Umea, 1976.
4. Möller AJ, Fabricius L, Dahlén G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. *Scand J Dent Res* 1981;89:475–84.
5. Strindberg LZ. The dependence of the results of pulp therapy on certain factors. An analytic study based on radiographic and clinical follow-up examination. *Acta Odontol Scand* 1956;14(Suppl. 21):1.
6. Grahnén H, Hansson L. The prognosis of the pulp and root canal treatment. A clinical and radiographic follow-up examination. *Odontol Revy* 1961;12:146–65.
7. Kerekes K, Tronstad L. Long-term results of endodontic treatment performed with a standardized technique. *J Endod* 1979;3:83–90.
8. Sjögren U, Hagglund B, Sundqvist G, Wing K. Factors affecting the long-term results of

- endodontic treatment. *J Endod* 1990;16:498–504.
9. Engström B, Hard AF, Segerstad L, Ramström G, Frostell G. Correlation of positive cultures with the prognosis for root canal treatment. *Odontol Revy* 1964;15:257–70.
  10. Byström A, Happonen RP, Sjögren U, Sundqvist G. Healing of periapical lesions of pulpless teeth after endodontic treatment with controlled asepsis. *Endod Dent Traumatol* 1987;3:58–63.
  11. Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. *Int Endod J* 1997;30:297–306.
  12. Loesche WJ, Lopatin DE, Stoll J, van Poperin N, Hujuel PP. Comparison of various detection methods for periodontopathic bacteria: can culture be considered the primary reference standard? *Clin Microbiol* 1992;30:418–26.
  13. Conrads G, Gharbia SE, Gulabivala K, Lampert F, Shah HN. The use of a 16s rDNA directed PCR for the detection of endodontopathogenic bacteria. *J Endod* 1997;23:433–8.
  14. Rolph HJ, Lennon A, Riggio MP, Saunders WP, MacKenzie D, Coldero L, Bagg J. Molecular identification of microorganisms from endodontic infections. *J Clin Microbiol* 2001;39:3282–9.
  15. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *Scand J Dent Res* 1981;89:321–8.
  16. Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. *Oral Surg Oral Med Oral Pathol* 1983;55:307–12.
  17. Byström A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *Int Endod J* 1985;18:35–40.
  18. Byström A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. *Endod Dent Traumatol* 1985;1:170–5.
  19. Dalton BC, Ørstavik D, Phillips C, Pettiette M, Trope M. Bacterial reduction with nickel-titanium rotary instrumentation. *J Endod* 1998;24:763–7.
  20. Shuping GB, Ørstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. *J Endod* 2000;26:751–5.
  21. Card SJ, Sigurdsson A, Ørstavik D, Trope M. The effectiveness of increased apical enlargement in reducing intracanal bacteria. *J Endod* 2002;28:779–83.
  22. Buchanan LS. The standardized-taper root canal preparation, part I: Concepts for variably tapered shaping instruments. *Dent Today* 1999;18:78–86.
  23. Schilder H. Cleaning and shaping the root canal. *Dent Clin North Am* 1974;18:269–96.
  24. Buchanan LS. The standardized-taper root canal preparation, part II: GT file selection and safe handpiece-driven file use. *Dent Today* 1999;18:68–70, 72–6.
  25. Möller AJ. Microbiological examination of root canals and periapical tissues of human teeth. *Methodological studies. Odontologisk Tidskrift* 1966;74(Suppl.):1–380.
  26. Sundqvist G. Associations between microbial species in dental root canal infections. *Oral Microbiol Immunol* 1992;7:257–62.
  27. Ørstavik D, Kerekes K, Molven O. Effects of extensive apical reaming and calcium hydroxide dressing on bacterial infection during treatment of apical periodontitis: a pilot study. *Int Endod J* 1991;24:1–7.
  28. Loesche WJ, Lopatin DE, Stoll J, van Poperin N, Hujuel PP. Comparison of various detection methods for periodontopathic bacteria: can culture be considered the primary reference standard? *J Clin Microbiol* 1992;30:418–26.
  29. Conrads G, Gharbia SE, Gulabivala K, Lampert F, Shah HN. The use of a 16s rDNA directed PCR for the detection of endodontopathogenic bacteria. *J Endod* 1997;23:433–8.
  30. Rolph HJ, Lennon A, Riggio MP, Saunders WP, MacKenzie D, Coldero L, Bagg J. Molecular identification of microorganisms from endodontic infections. *J Clin Microbiol* 2001;39:3282–9.
  31. Ingle JL, Zeldow BJ. An evaluation of mechanical instrumentation and negative culture in endodontic therapy. *J Am Dent Assoc* 1958;57:471–6.
  32. Reit C, Dahlen G. Decision making analysis of endodontic treatment strategies in teeth with apical periodontitis. *Int Endod J* 1988;21:291–9.
  33. Waltimo TM, Trope M, Haapasalo MPP, Ørstavik D. Clinical efficacy of treatment procedures in endodontic infection control and one year follow-up of periapical healing. 2004. In press.
  34. Ando N, Hoshino E. Predominant obligate anaerobes invading the deep layers of root canal dentin. *Int Endod J* 1990;23:20–7.
  35. Haapasalo HK, Siren EK, Waltimo TM, Ørstavik D, Haapasalo MP. Inactivation of local root canal medicaments by dentine: an in vitro study. *Int Endod J* 2000;33:126–31.
  36. Han GY, Park SH, Yoon TC. Antimicrobial activity of Ca(OH)<sub>2</sub> containing pastes with *Enterococcus faecalis* in vitro. *J Endod* 2001;27:328–32.
  37. Sukawat C, Srisuwan T. A comparison of the antimicrobial efficacy of three calcium hydroxide formulations on human dentin infected with *Enterococcus faecalis*. *J Endod* 2002;28:102–4.