Reduction in intracanal bacteria during root canal preparation with and without apical enlargement

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Abstract

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Aim To compare *in vitro* intracanal bacterial reduction using nickel–titanium rotary instruments with and without apical enlargement.

Methodology Thirty-eight palatal roots of maxillary molar teeth, with mature apices were subdivided according to lengths and then randomly assigned to two experimental and one control groups. The roots were sterilized and then reinfected with *Enterococcus faecalis*, which served as a bacteriological marker. All roots in the experimental groups were prepared in a step-down sequence with engine-driven GT rotary files at 350 rpm. In experimental group A ($n = 16$) additional apical enlargement to ISO size 35 was performed. In group B ($n = 16$) a serial step-back technique was followed with no apical enlargement. This was combined in groups A and B with irrigation with NaOCl and EDTA. In the control group (group C, $n = 6$) irrigation only was carried out, with no mechanical preparation. Samples were then taken from the root canals to determine the numbers of remaining bacteria.

Results In groups A and B, 15 (94%) and 13 (81%) specimens were rendered bacteria-free, respectively. In the control group C none of the specimens were bacteria-free. There was a significant difference ($P < 0.001$) in the antibacterial effects of experimental and control regimens. There was, however, no significant difference ($P = 0.276$) between the preparation methods used in the experimental groups.

Conclusions There was no significant difference in intracanal bacterial reduction when Ni–Ti GT rotary preparation with NaOCl and EDTA irrigation was used with or without apical enlargement preparation technique. It may therefore not be necessary to remove dentine in the apical part of the root canal when a suitable coronal taper is achieved to allow satisfactory irrigation of the root canal system with antimicrobial agents.

Keywords: apical size, bacteria, GT rotary, nickel–titanium, root canal preparation.

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Introduction

The role of bacteria and their products in the initiation, propagation and persistence of periradicular periodontitis has been established (Kakehashi *et al.* 1965, Sundqvist 1976, Moller *et al.* 1981). These microorganisms obtain their nutritional supply from vital, degenerating and necrotic pulpal tissue, saliva from the mouth, serum protein from the periradicular tissues and metabolites from other bacteria. The number of microorganisms within an infected root canal system may vary anywhere from 10^2 to more than 10^8 (Sjogren *et al.* 1991). Microbes are present in all parts of the root canal system, including fins, and anastomoses and may be found at varying depths of up to 300 μm within the dentinal tubules, from the pulpal end (Horiba *et al.* 1990). There also appears to be a regional variation in the extent to which dentine is invaded; cervical tubules are invaded to a greater extent than the midroot tubules, which are, in turn invaded more than those in the apical region (Love 1996). Any method used to disinfect the root canal should be capable of accessing and eliminating microbes as much as possible from all parts of the system.

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The aims of root canal treatment are to disinfect the root canal system thoroughly, completely obturate the space created so as to entomb any microbes that have escaped elimination and to prevent reinfection; without causing any iatrogenic damage (Saunders & Saunders 1997, Sundqvist & Figdor 1998). A chemomechanical preparation technique is advocated to disinfect root canals (Bystrom *et al.* 1985) because it allows a greater number of root canals to be rendered bacteria free; between 20% (without the use of an antimicrobial irrigant) to around 80% (with the use of an antimicrobial irrigant) of canals can be disinfected (Bystrom & Sundqvist 1981, Bystrom & Sundqvist 1983).

The latest generation of root canal instruments include engine-driven nickel–titanium (Ni–Ti) instruments. These have been found to produce good shaping (Glossen *et al.* 1995) by virtue of their increased flexibility (Walia *et al.* 1988). This superelasticity together with the development of specially configured cutting blades means that these engine-driven instruments can be efficiently and safely used in even narrow, curved root canals (Short *et al.* 1997).

Investigators have evaluated the efficacy of different preparation techniques in cleaning the apical region of the root canal (Wu & Wesselink 1995, Siqueira *et al.* 1997). Wu & Wesselink (1995) compared the efficiency of three techniques, step-back, crown-down pressureless and the balanced-forced techniques using K-files, in cleaning the apical portion of curved root canals. The remaining surface debris was used as the evaluation criteria and they concluded that the apical portion of the canal was cleaned less than the middle and the coronal portions, regardless of the technique used. The balanced-force technique produced a cleaner apical portion of the canal when compared to the other two techniques. Siqueira *et al.* (1997) compared the effectiveness of five instrumentation techniques for cleaning the apical third of root canals using a histological evaluation method. Canals were prepared with the step-back technique using stainless steel or Ni–Ti files, an ultrasonic technique using a ISO size 15 ultrasonic file, balanced-force using Flex-R-files and Canal Master U instruments. They concluded that none of the five instrumentation techniques tested were effective at completely debriding the root canal system. Apical enlargement during canal cleaning and shaping procedures has the potential to eliminate more bacteria from the root canal system (Parris *et al.* 1994, Yared & Bou Dagher 1994, Siqueira *et al.* 1999). However, there still remains some controversy as to whether apical enlargement is necessary. Buchanan (1993a,b, 1996, 1998) has advocated minimal apical preparation on the grounds that it minimizes the potential for the creation of apical zips and provides better control of filling materials.

The aim of this study was to compare the intracanal bacterial reduction during root canal preparation using Ni–Ti GT rotary files and an antimicrobial irrigant with and without apical enlargement to test the null hypothesis that there was no difference between the two techniques.

Materials and methods

The method used was based on previously published protocols (Orstavik & Haapasalo 1990, Dalton *et al.* 1998, Siqueira *et al.* 1999). Forty-five extracted permanent human maxillary first and second molar teeth were obtained so that their palatal roots could be used in the study. These teeth were stored in normal saline with a few crystals of thymol. In order to be included in the study the palatal root had to satisfy the following criteria:

1 Long, minimally curved, narrow and caries free.

2 Mature root apex with no evidence of external root resorption.

3 Sufficiently separated from the buccal roots to allow easy palatal root resection.

A slow speed diamond disc under saline irrigation was used to separate the palatal root from each tooth. A barbed broach (ISO size 20) was used to remove any pulp remnants from the root canal ensuring that the canal wall was not instrumented. The patency of each canal was established by gently inserting an ISO size 10 K-Flexofile (Dentsply Maillefer, Ballaigues, Switzerland) until the tip emerged from the apical foramen. This length was noted and the working length (WL) of each root specimen was calculated by subtracting 1 mm. After WL determination, the apical 3 mm, including the foramen, of each specimen was covered with Dyract AP (DeTrey, Dentsply, UK) to prevent any extrusion or leakage of material during root canal preparation and sample collection.

Sterilization of specimens

Aliquots of 5 mL of sterile Tryptic Soy Broth (TSB) solution (Bioconnections, Leeds, UK) were added to each universal container with the specimen. Agitation for about 30 s on a vortex mixer (McQuilkin, Glasgow, UK) was performed to aid the penetration of TSB into the root canal of the specimen. The 45 universal containers with the specimens were placed on an aluminium stack tray and autoclaved at 121°C for 15 min. The specimens were

then stored in an aerobic incubator at 37°C. Frequent visual checks were made to determine if the TSB in any of the containers became turbid, indicating that bacteria had survived the autoclaving. Each container and specimen were vortexed for 30 s after each observation and before returning to the incubator. The TSB in all the containers remained clear.

Maintenance of viable *Enterococcus faecalis*, preparation of infecting broth, test for viability of infectious TSB and infection of samples

A vial of frozen *Enterococcus faecalis* NCTC 29212 was the source of bacteria and a sample on a sterile wire loop was used to inoculate a blood agar plate. The plate was incubated aerobically at 37°C overnight. A viable growth of *E. faecalis* was maintained by subculturing two colonies from the previous plate to blood agar, every other day. Every 3–4 days, four bottles containing 100 mL sterile TSB were each inoculated with two colonies of *E. faecalis* and after overnight incubation 0.5 mL aliquots from these were used to infect and maintain infection of the samples for the duration of the experiment. At around 10 AM on the days following the TSB inoculation (i.e. on every Tuesday and Friday for the duration of the experiment), the liquid content of each specimen container was decanted and replaced with 5 mL of infected TSB. These were vortexed for 30–40 s to enhance the penetration of the infected TSB into the root canal of each specimen. The purity of the *E. faecalis* that each specimen was being exposed to was checked on a regular basis by plating samples taken randomly from several universal containers. Initially, an infecting period of 2 weeks was designated for infecting the specimens, based on the study by Orstavik & Haapasalo (1990). After this initial 2 weeks, the infected TSB was replaced for the remaining samples at the same frequency for the total duration of the experiment.

Verification of specimen reinfection

Evidence proving that the specimens were successfully reinfected with *E. faecalis* was obtained in two ways. First, visual evidence using scanning electron microscopy was performed on two randomly selected specimens. The two additional specimens were prepared for SEM examination (Philips SEM 500, Eindhoven, The Netherlands). The specimens were first freeze-fractured in liquid nitrogen, fixed, completely dehydrated, attached to glass slides and sputter coated with gold before being examined in the SEM (Fig. 1). Secondly, the specimens

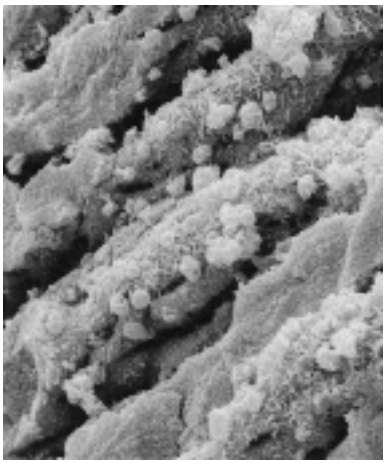

Figure 1 Representative SEM appearance of root canal wall in the apical one-third after reinfecting with *E. faecalis* showing the bacteria occupying dentinal tubules (original magnification ×6400).

used in the experimental groups were each tested for the presence of viable *E. faecalis* within their canals. A sample was obtained from the canal of each specimen before they were prepared and the concentration in $CFUmL^{-1}$ was later determined (*vide infra*).

Specimen grouping

Five specimens from the initial 45 were used for a pilot study of the experiment protocol. Two specimens were randomly chosen for SEM examination.

The 38 remaining specimens were first grouped according to their working lengths (WL). Based on this WL grouping, specimens were then randomly assigned to three study groups; two experimental groups contained 16 specimens each and the third, the control group, contained six specimens. A preliminary statistical evaluation was performed to verify that the specimens were evenly distributed with respect to their working lengths.

Determination of *E. faecalis* sensitivity to materials used in study

The sensitivity of *E. faecalis* to the materials (at their specific concentration or state) used in the study was investigated. This was deemed necessary as a result of the pilot study. The materials tested were Dyract AP (DeTrey Dentsply, UK), 4.4% NaOCl (Tesco, Cheshunt, UK), 15% EDTA (McQuilkin, Glasgow, UK), sodium thiosulphate (McQuilkin, Glasgow, UK), 0.85% NaCl (McQuilkin, Glasgow, UK), paper points (Dentsply Maillefer, Ballaigues,

Switzerland). Seven labelled blood agar plates were inoculated with infected tryptic soya broth (TSB) using a sterile cotton swab for each plate. Absorbent paper disks were impregnated with each liquid, the paper points were presoaked in sterile TSB and the Dyract AP was light cured with its primer on a clean glass slide before placing on the surface of the inoculated plates. Two samples of each material were used on each plate. On the final plate the disks were first dipped in NaOCl and then in sodium thiosulphate ($Na_2S_2O_3$). This was to determine if there was complete neutralization of the NaOCl by the $Na_2S_2O_3$ concentration used. All plates were incubated over night at 37°C and were then examined for the presence or absence of zones of inhibited *E. faecalis* growth.

Prepreparation sampling and root canal preparation sequence applied to experimental groups

A single operator, using aseptic techniques, carried out the preparation and sampling procedures on each specimen under a class I laminar air flow cabinet (Fischer *et al.* 1998) to prevent airborne bacterial contamination. The following procedures were applied to each specimen regardless of their grouping. The infected broth was carefully decanted from the universal container and the specimen removed with sterile cotton pliers. An ISO size 20 sterile paper point was used to obtain a broth sample from the root canal of the specimen. The paper point was kept in the canal for about 30 s and then transferred to a prelabelled bijou bottle (Inverclyde Biological, Bellshill, UK) containing 1 mL of sterile TSB. This sample was called the prepreparation (preprep.) sample and the concentration of bacteria was determined. The external surface of the root specimen was washed with 3 mL of sodium hypochlorite (NaOCl). The entire specimen, except for the coronal 2–3 mm, was then carefully wrapped in a presterilized 5 cm^2 sheet of aluminium foil. The specimen and foil were then held firmly in the jaws of a mini-vice; the jaws of the vice were first lined with a sheet of barrier plastic.

Group A: Crown-down chemomechanical preparation with apical enlargement to a GT rotary file size 35/.04 taper file to the WL

The nickel–titanium GT rotary and 04 Profile file series (Dentsply Maillefer) were used for root canal preparation. In group A, this was initiated with a GT rotary file size 20/.12 taper used in a crown-down manner. The file was rotated at 350 r.p.m. in an 18 : 1 handpiece driven by an electric motor (Analytic Endodontics, Orange, CA, USA). The GT rotary files, sizes 20/.10, 20/.08 and 20/.06, were all used serially in a crown-down technique toward the apex. Each file went deeper into the canal but was not allowed to go further than 1 mm short of the WL. Irrigation was performed between each instrument using 1 mL of 4.4% NaOCl delivered in a luer-lock syringe with a 27-gauge endodontic needle (QED, Peterborough, UK). The apical preparation for specimens in group A was initiated by placing the Profile size 20/.04 taper to the WL. This was followed sequentially by the size 25/.04 file, size 30/.04 file and then the 35/.04 file, all to the WL. Again, the root canal was irrigated with 1 mL of 4.4% NaOCl after each instrument. Finally, the GT rotary file size 20/.10 taper was placed to the WL followed by 1 mL of NaOCl irrigation. The root canal system was irrigated with 1 mL of 15% EDTA solution to remove the smear layer. This solution was kept in the canal for 3 min before being washed out with 1 mL of NaOCl. A total of 10 mL of 4.4% NaOCl was used for irrigation in each specimen. On average, the NaOCl was kept in contact with the root canal contents for about 15 min (Senia *et al.* 1971, Bystrom & Sundqvist 1983).

Group B: Crown-down chemomechanical preparation with 0.25 mm step-back apical preparation to a size 35/.04 taper 1 mm short of the WL, using GT rotary files

In this group, the same preparation regimen that was applied in group A with the first four GT rotary files (i.e. sizes 20/.12–20/.06 tapers). However, the Profile .04 tapered files were applied using a 0.25-mm incremental step-back technique, starting with the size 20/.04 file at the WL and ending with the size 35/.04 file at 1 mm short of the WL. The root canal was irrigated with 1 mL of 4.4% NaOCl after each file. As with the preparation regimen for group A, the final GT file used was the size 20/.10 taper to the WL and this was followed with another 1 mL 4.4% NaOCl irrigation. The specimens in group B were also each irrigated with 1 mL of 15% EDTA solution, which was then left in the root canal for 3 min before being irrigated with another 1 mL of 4.4% NaOCl solution. Each specimen was again exposed to a total of 10 mL of 4.4% NaOCl for about 15 min.

Group C: Positive control group with irrigation without mechanical preparation

In this group, each of the six specimens were set up the same as those in groups A and B. However, no mechanical preparation with GT rotary files was performed. Instead, irrigation with 4.4% NaOCl and 15% EDTA

solutions was performed so that the specimens were exposed to the same volume of irrigants for the same length of time. The tip of the needle was able to penetrate only to a depth of approximately 5 mm into each root canal.

Following the irrigation regime for each group, root canals were then irrigated with 3 mL sterile NaCl solution to dilute the NaOCl within the canal. The NaOCl was then neutralized with 3 mL sodium thiosulphate ($Na_2S_2O_3$) solution. A sterile syringe with luer-lock connection and 27-gauge endodontic needle was used with each irrigant. The same syringe that was used to deliver the $Na_2S_2O_3$ was used to aspirate the majority of the solution from the canal and each specimen was then ready for sampling.

Postpreparation specimen sampling method

A sample was collected from each specimen after preparation and this was used to calculate the numbers of bacteria surviving the preparation process. After the $Na_2S_2O_3$ solution was aspirated from the canal of each specimen, 1 mL of sterile TSB was used to flush the canal. With the canal filled with TSB, a sterile cotton roll was used to remove excess TSB from the coronal surface of each specimen. The contents within the canal were then mixed using a sterile file (Dalton *et al.* 1998). The files used were GT rotary files 35/.04, 20/.04 and K-Flexofile size 15/.02 for groups A, B and C, respectively. Each sterile instrument was inserted manually into the root canal of each specimen to the WL and pumped five times vertically with minimal reaming action. A sterile paper point was then used to absorb the fluid content from the canal with care being taken not to touch the outer surface of the specimen. In groups B and C, ISO size 20 paper points were used and size 35 points were used in group A. The points were left in each canal for about 30 s before they were each transferred to a sterile bijou bottle with a 1-mL aliquot of TSB. This constituted the postpreparation (postprep.) sample. The bijou bottle with the postprep. paper point sample and the other with the preprep. sample taken earlier, were vortexed for 30 s. Serial 10-fold dilutions were produced from each sample and were used to inoculate blood agar plates using an automatic spiral inoculator. The labelled plates were incubated for 24 h at 37°C and the concentration of each preprep. and postprep. sample was determined in CFU mL^{-1}; these were called the preprep. and the postprep. concentrations, respectively. The bijou bottle with the neat sample was also incubated for 24 h and was examined the following day for any sign of turbidity.

Statistical evaluation

A one-way analysis of variance was used to compare the preprep. concentrations and the working lengths for the groups. Generalized linear model and stepwise logistic regression tests were used to determine if there were differences amongst the methods of preparation. A Bonferroni adjustment, with a 95% confidence interval, was used to make paired comparisons between the three groups.

Results

Prepreparation findings and values

The autoclave sterilization of the specimens proved to be 100% effective as subsequent checks made for evidence of microbial growth were negative. The average concentration of the inoculate used for reinfecting the specimens was 8.24×10^{12} CFUmL^{-1} (range 4.27×10^{12}–1.47×10^{13} CFUmL^{-1}, respectively. The SEM examination revealed that *E. faecalis* occupied the main root canals in the randomly selected specimens and had penetrated the dentinal tubules to varying extents. Figure 1 is an example of the penetration of dentinal tubules by the bacteria in the apical one-third of a root canal.

Since no other morphological types of bacteria were evident, the results from the SEM suggested that the reinfection procedure was successful.

The results of the *E. faecalis* sensitivity tests showed that only NaOCl and EDTA exhibited antimicrobial activities as expected. Neutralization of the NaOCl by $Na_2S_2O_3$ was complete, as the corresponding plate showed no signs of inhibited microbial growth.

Prepreparation results

There were no statistically significant differences amongst the groups for the prepreparation concentration of microorganisms or working lengths prior to treatment ($P = 0.865$ and 0.961, respectively) (Table 1).

Table 1 Means (standard deviations) of prepreparation concentration and working length by group

Treatment group	Mean $_{(SD)}$ Preprep. concentration (CFU mL^{-1})	Mean $_{(SD)}$ Working length (mm)
a (n = 16)	5.76 E + 7 $_{(9.16\ E + 7)}$	15.63 $_{(1.06)}$
b (n = 16)	4.61 E + 7 $_{(9.78\ E + 7)}$	15.53 $_{(1.01)}$
c (n = 6)	3.56 E + 7 $_{(5.59\ E + 7)}$	15.50 $_{(1.55)}$

Table 2 Specimen number, specimen group number, group, pre- and postpreparation concentrations, percentage reduction, presence (1) or absence (2) of *E. faecalis* after preparation and 24 h postpreparation neat appearance

Specimen no.	Specimen group no.	Group	Preprep. concentration (CFU mL^{-1})	Postprep. concentration (CFU mL^{-1})	% reduction	Presence or absence of *E. faecalis*	24 h neat appearance
1	1	a	1.31E + 06	0	100.00000	2	–ve
2	2	a	6.00E + 07	0	100.00000	2	–ve
3	3	a	7.50E + 06	0	100.00000	2	–ve
4	4	a	5.90E + 06	0	100.00000	2	–ve
5	5	a	1.94E + 06	0	100.00000	2	–ve
6	6	a	3.60E + 07	0	100.00000	2	–ve
7	7	a	3.20E + 07	0	100.00000	2	–ve
8	8	a	2.94E + 08	0	100.00000	2	–ve
9	9	a	5.00E + 05	0	100.00000	2	–ve
10	10	a	7.60E + 06	0	100.00000	2	–ve
11	11	a	7.10E + 06	0	100.00000	2	–ve
12	12	a	9.80E + 07	8.00E + 01	99.99992	1	Turbid
13	13	a	3.60E + 06	0	100.00000	2	–ve
14	14	a	1.03E + 08	0	100.00000	2	–ve
15	15	a	2.57E + 08	0	100.00000	2	–ve
16	16	a	6.30E + 06	0	100.00000	2	–ve
17	1	b	2.00E + 07	0	100.00000	2	–ve
18	2	b	4.04E + 08	0	100.00000	2	–ve
19	3	b	3.20E + 07	4.40E + 02	99.99863	1	Turbid
20	4	b	9.40E + 06	0	100.00000	2	–ve
21	5	b	2.67E + 07	0	100.00000	2	–ve
22	6	b	1.48E + 07	0	100.00000	2	–ve
23	7	b	1.39E + 07	0	100.00000	2	–ve
24	8	b	1.12E + 07	0	100.00000	2	–ve
25	9	b	7.20E + 07	0	100.00000	2	–ve
26	10	b	7.80E + 06	0	100.00000	2	–ve
27	11	b	1.18E + 07	0	100.00000	2	–ve
28	12	b	9.00E + 06	0	100.00000	2	–ve
29	13	b	7.00E + 07	0	100.00000	2	–ve
30	14	b	3.00E + 07	1.20E + 02	99.99960	1	Turbid
31	15	b	4.50E + 06	8.40E + 02	99.98133	1	Turbid
32	16	b	1.21E + 06	0	100.00000	2	–ve
33	1	c	1.65E + 06	1.20E + 04	99.27273	1	Turbid
34	2	c	1.47E + 08	1.06E + 03	99.99928	1	Turbid
35	3	c	1.20E + 07	1.80E + 03	99.98500	1	Turbid
36	4	c	1.40E + 07	2.02E + 03	99.98557	1	Turbid
37	5	c	3.60E + 06	6.20E + 02	99.98278	1	Turbid
38	6	c	3.54E + 07	9.64E + 04	99.72768	1	Turbid

Postpreparation results

Table 2 shows the postpreparation results obtained for each specimen from the three study groups showing the concentrations of *E. faecalis*, as a percentage of bacterial reduction (calculated using the pre- and postpreparation concentrations), the presence or absence of bacteria after preparation (another interpretation of the latter) and the results of the 24 h appearance.

In group A, 15 specimens (i.e. all but one specimen) were rendered bacteria free after preparation. In Table 2, column 6, the results from group A show 15 specimens with 100% bacterial reduction and one specimen with 99.99% bacterial reduction.

In group B, 13 specimens were rendered bacteria free after preparation with less than 100% reduction in three specimens.

In group C, the positive control group, all six specimens showed less than 100% reduction in *E. faecalis* after irrigation.

Ten of the neat samples appeared turbid after 24 h incubation, indicating the presence of *E. faecalis*. The 10 neat samples were from the same specimens (from the three groups) that gave positive results for the presence of

Table 3 Means (standard deviations) of percentage reductions in concentration by group

Treatment group	Mean $_{(SD)}$% reduction in concentration
a (n = 16)	100.00% $_{(0.00)}$
b (n = 16)	100.00% $_{(0.00)}$
c (n = 6)	99.83% $_{(0.29)}$

Table 4 Bonferroni-adjusted intervals for differences between groups for percentage reduction in concentration

Group–group	(Joint) 95% confidence intervals for difference in percentage reduction
a–b	(–0.09, 0.10)%
a–c	(0.05, 0.30)%
b–c	(0.04, 0.31)%

E. faecalis after they were prepared (i.e. one specimen from group A, three from group B and six from group C).

Statistical analysis of the results

Table 3 shows the mean percentage reduction for the three groups. All three groups showed high percentage reductions, with group C showing a slightly lower value. There were two other variables that may have also been linked to the percentage reduction in concentration, namely the working length and the prepreparation concentration. A generalized linear model (used for continuous data), was used to identify which, if any, of the variables might have had a significant effect on the percentage reduction in concentration. The prepreparation concentration and the working length were not significant ($P = 0.656$ and $P = 0.616$, respectively), whilst the group effect (i.e. the preparation regimen applied to each group) was highly significant ($P = 0.007$). Thus, the only variable that had a significant effect on the percentage reduction in concentration was the preparation method of the group. Table 4 shows the results of the follow-up multiple paired comparisons, using the Bonferroni Correction (Bland & Altman 1995), which was used to identify between which groups the differences exist. The interval estimates confirm that there was a significant difference between groups A and C (group A had on average a significantly greater percentage reduction in concentration by between 0.05% and 0.30% than group C). There was also a significant difference between groups B and C (group B had on average a significantly greater percentage reduction in concentration, by between about 0.04% and 0.31% than group C). The interval estimate however, showed that there was no significant difference in percentage reduction between groups A and B (interval ranged from –0.09% to 0.10%). Thus, the GT rotary preparation with antimicrobial irrigation gave a significantly greater mean percentage reduction in *E. faecalis* concentration in both experimental groups (A and B), regardless of the size to which the apical region of each specimen was prepared, compared to the control group (C). In addition, there was no significant difference in the percentage reduction in concentration of bacteria between the two experimental groups. The data in Table 2 however, show that one sample in group A was not rendered bacteria free compared with three in group B. Thus, another method was used to consider a second 'outcome of interest'.

Table 5 Percentage of 'absence' per group (see Table 2, column 7)

Group	Percentage of 'absence'
a	93.75%
b	81.25%
c	0%

This 'outcome of interest' was considered to be either the presence (1) or absence (2) (Table 2, column 7) of *E. faecalis* in the sample after preparation. Therefore:

- presence was defined as postprep. concentration > 0 and was arbitrarily designated 1;
- absence was defined as postprep. concentration = 0 and was arbitrarily designated 2.

In this statistical method, the determination to be made was whether the percentage of 'absence' was the same across the three study groups. The percentages of 'absence' in the three groups were 93.75% for group A, 81.25% for group B and 0% for group C as shown in Table 5. The percentages clearly suggested that there was a difference between the experimental and the control groups. A stepwise logistic regression (used for binary data) was used to identify whether any of the explanatory variables (i.e. group preparation regimen, prepreparation concentration and working length) had a significant effect on whether *E. faecalis* was absent after preparation. In this instance, both the prepreparation concentration and working length were again not significant ($P = 0.626$ and $P = 0.529$, respectively), whilst the group effect was highly significant ($P < 0.001$). Therefore the only significant variable was the preparation method. Intergroup analysis of the results indicated that groups A and C were significantly different, as were groups B and C (i.e. the percentage of 'absences' significantly different in both instances). However, the outcome from groups A and B showed that the preparation effect was not significant ($P = 0.276$). Therefore, there is

insufficient evidence to reject the null hypothesis that the percentage of 'absence' in the two experimental groups is the same.

Discussion

The methodology used in this study was similar to those used by Orstavik & Haapasalo (1990), Dalton *et al.* (1998) and Siqueira *et al.* (1999). However the use of a chemomechanical preparation technique meant that it was unique when compared to these other studies. Dalton *et al.* (1998) and Siqueira *et al.* (1999) omitted the use of an antimicrobial irrigant, on the grounds that only the mechanical effects of instrumentation and irrigation were to be assessed. A bacteriological assessment was chosen for the present study because of the importance of canal disinfection in the successful treatment of apical periodontitis.

Root sections were used instead of whole teeth because it was thought that sample retrieval would be more convenient. Palatal roots were chosen because they are usually long and contain relatively gently curving root canals. The decision to select roots with canals of this configuration was made because curved canals are associated with a more complicated curvature determination and distribution process (Schneider 1971) and with an increased risk of intracanal procedural accidents such as zipping, ledging and root perforation (Roane *et al.* 1985). No canal possessed a curvature greater than five degrees as assessed using the Schneider model (Schneider 1971).

Enterococcus faecalis was chosen as the bacteriological marker in the study. It is a non-fastidious, easy-to-grow aerobic bacterium of significant clinical importance, that could be used in a study applying a bacteriological assessment method. Other bacteria commonly associated with endodontic infections may require symbiotic support from other bacteria, but *E. faecalis* has been reported to survive and successfully thrive alone (Dahlen & Haapasalo 1998). The SEM evaluation used in the present study confirmed that the specimens were successfully reinfected with the organism (Fig. 1).

An empirical method was used to establish the working length of each specimen. This method has also been used by a number of other workers (Al-Omari & Dummer 1995, Wu & Wesselink 1995, Siqueira *et al.* 1997). The point at which the file just emerged from the canal (visualized under magnification for improved accuracy) was considered to be the apical foramen and the point 1 mm short of the latter was designated the apical constriction. The latter is the ideal biological limit of root canal instrumentation and obturation (Ricucci & Langeland 1998). In the study, apical enlargement at the working length referred to the sequential widening of the apical region of the root canal at the apical constriction, without its destruction.

The results of the present study demonstrated that both chemomechanical preparation techniques (experimental groups A and B) were more effective at eliminating *E. faecalis* from the root canals when compared to the technique which only employed the antimicrobial irrigation regimen (control group C). Siqueira *et al.* (1999) showed that instrumentation and irrigation could remove more than 90% of bacterial cells from root canals. However, they used 0.85% saline solution as the irrigant (which had no antimicrobial effect on the *E. faecalis* used). Dalton *et al.* (1998) compared two preparation techniques *in vivo* using different instruments, but they also used a saline irrigant. Although they obtained considerable bacterial reduction after preparation, none of the test subjects were rendered free of bacteria. Yared & Bou Dagher (1994) also obtained sizeable bacterial reductions after chemomechanical root canal preparation *in vivo*. However, none of their subjects was rendered free of bacteria after chemomechanical preparation.

The difference between the two experimental techniques used in the present study was in the way the apical portion of the root canal was prepared. This part of the root canal is important, as it is the region most likely to harbour intraradicular bacteria associated with root canal treatment failure (Nair *et al.* 1990). The basis for apical enlargement was in its potential ability to directly or indirectly eliminate bacteria in this area of the root canal system. By widening the canal apically, infected dentine can be removed physically. A wider canal may also mean that the needle delivering the antimicrobial irrigant could penetrate deeper into the root canal, thereby providing a more effective flushing action (Chow 1983).

Buchanan (1993a,b,c, 1998) described the use of the preparation technique applied to the specimens in group B. In his description he referred to the regimen as 'the tapered preparation'. Buchanan's concepts on cleaning and shaping of the root canal were based on descriptions by Schilder (1974) and Weine *et al.* (1975). The proposed tapered preparation, with serial step-back, was designed to prevent unwanted effects such as zipping, gouging and perforation. Therefore, a tapered preparation would result in a more ideally shaped root canal with minimal iatrogenic damage. Buchanan (1998) incorporated coronal flaring in his technique and so delivery of the antimicrobial irrigant was thought to be sufficient. He indicated that a tapered preparation would facilitate a better root canal filling and any remaining bacteria would be entombed, and eventually starved, within the

root canal system. Interestingly, Buchanan did not provide any evidence to support this claim but Peters *et al.* (1995), in a review article, expressed a similar opinion regarding the fate of microorganisms in the root canal.

The use of Ni–Ti rotary instruments means there is no need to compromise on canal disinfection and a reliance on the root filling to 'seal-in' any remaining bacteria, because larger instruments can now be used safely to carefully enlarge the canal and so directly eliminate more infected dentine. The concept of a weakened root subsequent to apical enlargement still remains a valid concern and so indiscriminate use of root canal widening should be avoided.

Another key reason given by Buchanan (1993c) justifying the serial step-back technique was that it is a safeguard against changes to the working length during preparation. Schilder (1974) reported that as the root canal is widened, a straightening effect was produced that eventually shortened the distance between the coronal reference point and the apical limit of preparation. If the same working length (which was usually determined early, before any canal preparation was done) was consistently used, overpreparation of the canal may result. Application of a step-back method compensated for this relative decrease in canal length. Modern endodontics however, advocates that the working length be determined at a later stage during preparation (Saunders & Saunders 1997). It is advised that the coronal two-thirds of the canal be flared before the working length is established. Thus, any further apical preparation after the working length is found would produce little or no change to the latter.

It is possible to extrapolate the results of the present study to the clinical setting but the latter is significantly more complex. First, only a few instances of failed root canal treatment are associated with a single species bacterial infection (Dahlen & Haapasalo 1998) and the vast majority of endodontic infections resulting in apical periodontitis are associated with a polymicrobial flora (Stashenko *et al.* 1998). Secondly, whereas the present study used straight or only slightly curved root sections, narrow and curved canals are frequently encountered clinically and are more difficult to clean and shape (Walton 1976, Siqueira *et al.* 1997). The consequence that the latter could have on the results of a study was exemplified in the study by Yared & Bou Dagher (1994), where chemomechanical preparation failed to completely disinfect the canals in clinical test subjects. However, coronal flaring was not undertaken in the same controlled way as with the Ni–Ti instruments used in the present study.

Conclusion

Within the limitations of this study, chemomechanical preparation using GT rotary and Profile instruments and either a serial step-back or apical enlargement technique is effective at eliminating *E. faecalis* from the root canal system. The coronal flare generated by these instruments is sufficient to allow access to the apical part of the root canal with antimicrobial irrigant without the need to remove dentine at the working length.

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