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Comparison of ultrasonic and mechanical cleaning of primary root canals using a novel radiometric method

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Abstract

Although ultrasound is employed increasingly as an adjunct to biomechanical preparation in clinical endodontics for adult teeth, there have been no previous investigations of this technique for primary teeth. This investigation studied the efficacy of ultrasonication compared with mechanical cleaning in primary root canals using a novel radiometric method. The results indicated that in multiple-rooted teeth, ultrasonication with an endosonic file for 3 min was able to remove 81.1% of inoculated bacteria, compared with only 65.2% using conventional filing. A combination of mechanical filing followed by ultrasonication produced the best results, with > 95% bacteria removed. The results show that ultrasonication may be useful for primary teeth endodontics.

Introduction

In recent years, the use of ultrasound as an adjunct to biomechanical preparation of root canals has gained increasing popularity in clinical endodontics (Cunningham et al. 1982a; Barnett et al. 1985; Martin and Cunningham 1985; Stamos et al. 1985; Walmsley 1987; DeNunzio et al. 1989).

This technique, also known as "endosonics" (Martin and Cunningham 1985) utilizes ultrasound energy (above 20 kHz) to activate endodontic files and flow-through irrigation to effectively debride, cleanse, disinfect, and shape the root canals (Fig 1 and 2). Endodontic endosonics has been found to be superior to conventional methods in terms of debridement and irrigation (Cunningham and Martin 1982; Cunningham et al. 1982b; Krell et al. 1988), filing action (Martin et al. 1980a, b) as well as speed of action (Barnett et al. 1985; Nehammer and Stock 1985). Furthermore, less incidence of postoperative pain was reported following endosonic instrumentation compared to conventional therapy (Martin and Cunningham 1982). However other studies have found no advantages in the use of ultrasound in endodontics. For example, no difference in the efficacy of either hand or ultrasonic instrumentation was detected in the investigation of Weller et al. (1980) and the shapes of root canals filed by ultrasonication were found to be not significantly different from those treated by conventional methods (Rodrigues and Biffi 1989; Cymerman et al. 1983).

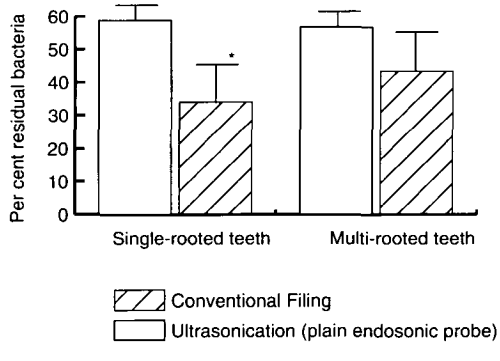

Fig 1. Comparison of the efficacy of root canal cleaning using three different techniques. * $P < 0.05$, ** $P < 0.01$.

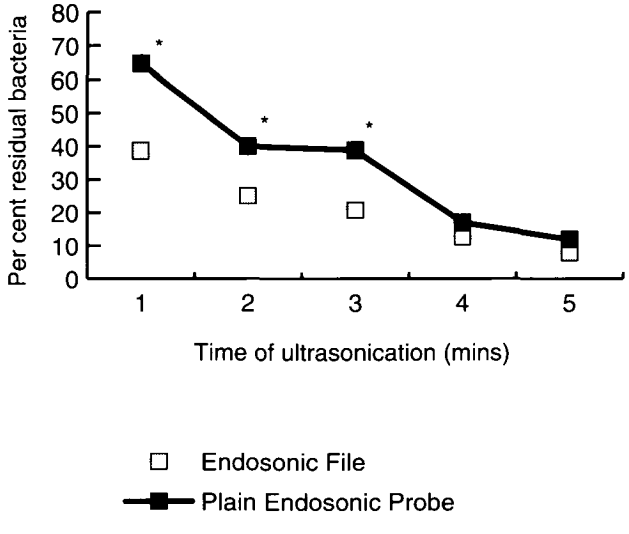

Fig 2. Per cent residual bacteria with increasing time of ultrsonication.
* $P < 0.01$ compared with corresponding result using the endosonic file.

The efficacy of bacterial removal from infected root canals is an important consideration in the comparison of instrumentation techniques in endodontics. In this regard, previous studies comparing ultrasonication with conventional methods have shown equivocal results. An early investigation by Martin (1976) showed that ultrasonication alone was effective in decreasing the bacterial counts in root canals by several thousandfold, particularly when coupled with antimicrobial agents. Later, Cunningham et al. (1982b) showed that endosonic techniques were more effective in reducing the number of inoculated *Bacillus subtilis* spores compared to conventional hand-filing. However, more recent in vivo studies on dogs by Barnett et al. (1985) as well as DeNunzio et al. (1989) have shown no significant differences in the number of recoverable bacteria from the root canals of ultrasonicated or conventionally filed teeth. Furthermore, a histobacteriological study by Biffi and Rodrigues (1989) confirmed the presence of residual microorganisms and compacted debris in nonvital human teeth after endosonic instrumentation.

The conflicting results obtained from previous microbiological studies are most probably methodological, and indicate the need for further comparison studies of ultrasonication and conventional endodontic techniques. In addition, previous studies evaluating the ultrasonic technique all have been done on permanent teeth. Major differences in root canal morphology between primary and permanent teeth (Goerig and Camp 1983) suggest that the efficacy of endosonics needs to be established in the primary dentition. Hence, this study was conducted to determine if ultrasonication is more efficient in the removable of bacteria compared to conventional hand filing in primary teeth. A novel radiometric method is employed to overcome some of the technical difficulties associated with previous microbiological studies.

Materials and Methods

Bacteria

The organism selected for study was *Streptococcus sanguis* (UQM 2263) which is one of the organisms commonly isolated from infected root canals (MacFarlane and Samaranayake 1989; Seow 1989). Furthermore, this organism has an ability to adhere to tooth surfaces (Gibbons 1989), a property which may affect its removal during endodontic cleaning. The bacteria were grown from frozen stock cultures on blood agar plates at 37°C for 1 day and purity ascertained by Gram-staining, colony morphology, and biochemical criteria (Seow et al. 1987, 1989; Seow 1990). Approximately 1 x 10^{10} bacteria were subcultured into 100 ml of Todd-Hewitt Broth (Oxoid, Basingstoke, Harts, England) containing 20% sucrose and 25 µl of a solution containing 1 µCi/ml ^{3}H-thymidine (Amersham, England). After incubation of 18 hr at 37°C, the bacteria were harvested by centrifugation at 2500 g for 15 min, washed twice with phosphate-buffered saline, and concentration adjusted to 3 x 10^{10} bacteria/ml prior to inoculation into the root canals.

Teeth

Extracted single and multiple-rooted human primary teeth with at least two-thirds of the roots intact were selected randomly for the study. One hundred and eighty teeth were used for the entire study. These teeth were extracted because of pulpal abscesses or for orthodontic reasons. The teeth were assigned randomly into subgroups of single-rooted or multiple-rooted groups of nine teeth each (Table 1, next page). In the groups using multiple-rooted teeth, all the molars used in any one experiment were of the same series, i.e. either mandibular primary second molars or mandibular primary first molars. In the groups using single-rooted teeth, maxillary primary canines or incisors were included at random.

The total numbers of teeth used in the test and control groups in the individual sets of experiments are shown in Table 1. Each tooth was used only once, since it was dissolved completely in hydrochloric acid at the end of the experiment.

Preparation

The teeth which had been stored in formalin were rinsed thoroughly in tap water, standard endodontic access cavities were cut on the occlusal surfaces using high speed diamond burs, and the pulps removed using barb broaches.

The teeth were soaked in 3% hydrogen peroxide for 24 hr, after which they were rinsed twice in distilled water. The teeth then were placed in a solution of 5% sodium hypochlorite solution for 1 hr (Martin 1976), then rinsed thoroughly with sterile distilled water.

The teeth were dried in an incubator at 37°C for 18 hr. The apical half of the roots and furcation areas of the multiple-rooted teeth were sealed with red wax (Ringlestein and Seow 1989) to prevent leakage of the bacterial suspensions.

Inoculation With Labelled Bacteria

In single-rooted teeth, 10 µl of a 3 x 10^{10}/ml (average disintegration per min, DPM = 10,000) labelled bacterial suspension was carefully inserted in the root canal with a micropipette tip. In multiple-rooted teeth, 40 µl (average DPM = 40,000) of the bacteria suspension was used, ensuring that all the root canals of the tooth were filled. These volumes of bacteria suspension had been found to be accommodated easily in the respective teeth.

The inoculated teeth were incubated at 37°C for 18 hr

to allow the bacteria to adhere sufficiently to the root canal walls. The incubator contained 100% humidity to prevent drying of the bacterial suspension.

At the end of the incubation period, endodontic treatment began using either conventional filing methods or ultrasonication.

Conventional Hand Filing

Mechanical hand filing was performed in the standard clinical manner, using K-files, sizes #15, 20, 25. Filing began with the smallest file inserted to approximately 1.5 mm short of the anatomical apex (Goerig and Camp 1983) and the root canal was filed circumferentially. The procedure was repeated sequentially with files of the next two larger sizes, the total filing time being 3 min. Irrigation of the canals was achieved with sterile 0.9% sodium chloride dispensed through a 19-gauge needle in a hypodermic syringe. A total of 27 ml of irrigant was used for single-rooted and 70 ml for multiple-rooted teeth. These volumes corresponded to the amounts dispensed through the endosonic filing unit for 3 and 9 min respectively, to ensure that equal volumes of irrigant were used in both techniques.

Endosonic Cleaning

An endosonic insert (Endosonic®, P-105, Dentsply International Inc., York, PA) coupled to a Cavitron® ultrasound generator (Dentsply International Inc., York, PA) was used. The tooth was irrigated during ultrasonication by connecting the inlet of the endosonic insert with the flexible tubing of a butterfly intravenous infusion set (Travenol Laboratories, NSW, Australia, Figs 1 and 2). The needle end of the intravenous set was inserted into the rubber bung of a 200 ml bag of sterile 0.9% sodium chloride solution (Fig 2). This irrigation unit supplied solution at the rate of approximately 9 ml/min directly to the endosonic file.

Either a plain #15 endosonic probe or a #15 endosonic K-file was used for the experiments. The plain endosonic probe was used approximately 1.5 mm short of the anatomical apex. In most instances, this was achieved without binding to the walls of the root canal. The endosonic file also was placed at approximately the same length and used with a light push-pull rasping action around the circumference of the root canals. A stop-watch was used for timing the filing procedures.

In every experiment a group of positive controls without endodontic instrumentation was set up. These positive controls provided measurements of the maximum recoverable bacteria. Results of the test teeth were expressed as percentages of positive controls.

Table. Numbers of teeth used for the experiments in the study.

Comparison of conventional filing and ultrasonication using plain endosonic probe

	Conventional filing	*Ultrasonication*	*Controls (no treatment)*	*Total*
Number of single-rooted teeth	9	9	9	27
Number of multiple-rooted teeth	9	9	9	27
Total	18	18	18	54

Effects of increasing ultrasonication time

Time (min)	*1*	*2*	*3*	*4*	*5*	*Controls (no treatment)*	*Total*
Number of single-rooted teeth	9	9	9	9	9	9	54

Combination of conventional and endosonic filing

	Conventional filing	*Endosonic filing*	*Conventional &endosonic filing*	*Controls (no treatment)*	*Total*
Number of single-rooted teeth	9	9	9	9	27
Number of multiple-rooted teeth	9	9	9	9	27
Total	18	18	18	18	54

Comparison of Ultrasonication and Conventional Filing

The first sets of experiments were designed to determine the efficacy of ultrasonication using a plain endosonic probe compared with mechanical filing. In these experiments in the ultrasonication groups, the endosonic probe was inserted for 3 min in each root canal as recommended in previous literature (Cunningham et al. 1982a; Ahmad and Ford 1989).

Effect of Increasing Ultrasonication Time

To determine the optimum time required for endosonic removal of bacteria single-rooted teeth were used. Two separate sets of experiments were performed, one using the #15 plain endosonic probe and the other using the #15

endosonic file. In each set of experiments, test groups of nine teeth each were treated for 1–5 min at 1 min intervals, respectively.

Combination of Conventional and Endosonic Filing

Both single-rooted and multiple-rooted teeth were used to determine the efficacy of a combination of conventional and endosonic filing compared with conventional filing alone or endosonic filing alone. This time of treatment in these experiments was standardized at 3 min.

All experiments were performed three times and a mean ± SD obtained in each case.

Decalcification of Teeth

At the end of the experiments, the teeth were decalcified in 2 ml of 32% hydrochloric acid at 37°C. Decalcification of the teeth released all 3H-thymidine-labelled bacteria into the hydrochloric acid. When complete decalcification has been achieved in 2 days, 0.2 ml of hydrochloric acid solution from each decalcified tooth was placed in a vial containing 2 ml of scintillation fluid (Optiphase, LKB, Sweden) and the radioactivity counted in an automated Beckman scintillation counter (LKB, Sweden). The amount of residual bacteria in the teeth was indicated by the number of disintegrations per min (DPM). Since all the test teeth as well as positive controls were decalcified in equal volumes of hydrochloric acid, and equal volumes were removed from each tooth for radioactive counting, the results may be compared directly.

The results were expressed as percentages of the total number of bacteria inoculated:

$$\% \text{ residual bacteria} = \frac{\text{residual DPM} \times 100}{\text{original DPM}}$$

Statistical Analysis

The Student's *t*-test was used for statistical analysis of data.

Results

Comparison of Ultrasonication and Conventional Filing

The results as shown in Fig 1 indicated that in the case of single-rooted teeth, ultrasonication showed a significant decrease in the per cent residual bacteria compared to conventional filing (34.0 ± 8.8% vs 58.8 ± 7.0%, $P < 0.05$). In contrast, in multiple-rooted teeth, no statistically significant difference could be observed in the per cent residual bacteria of mechanically filed and ultrasonicated teeth (43.4 ± 10.6% vs 56.7 ± 6.7%, $P > 0.1$), although there was a trend of less residual bacteria in the ultrasonicated group of teeth.

Effect of Increasing Ultrasonication Time

The results as shown in Fig 2 revealed that the endosonic file consistently removed greater amounts of bacteria at all time intervals compared with the endosonic probe. After 1 min of treatment, the per cent residual bacteria using the plain probe was 64.7 ± 4.5% compared with 38.7 ± 5.1% using the endosonic file. This difference was statistically significant ($P < 0.01$). After 2 min of treatment, per cent residual bacteria using the plain probe was 39.9 ± 1.8% while that using the endosonic file was only 25.0 ± 1.9%, the difference being statistically significant ($P < 0.01$). After 3 min, the per cent residual bacteria using the plain probe was 38.8 ± 3.9% compared with only 20.7 ± 1.5% using the endosonic file ($P < 0.01$). However, after 4 and 5 min the difference in per cent residual bacteria between the groups treated with the plain probe and endosonic file became insignificant ($P > 0.1$). Of note is the extremely low percentage of residual bacteria (7.9 ± 2.2%) in the group of teeth treated with the endosonic file for 5 min.

Combination of Conventional and Endosonic Filing

As shown in Fig 3, in single rooted teeth, conventional filing decreased the per cent residual bacteria to 34.8 ± 1.5 whereas endosonic filing reduced it to 19.9 ± 6.8 ($P < 0.05$). By contrast, a combination of conventional and endosonic filing decreased the per cent residual bacteria further to a low of 4.8 ± 1.0 ($P > 0.01$).

In the case of multiple-rooted teeth, similar trends were observed. Conventional filing yielded a per cent

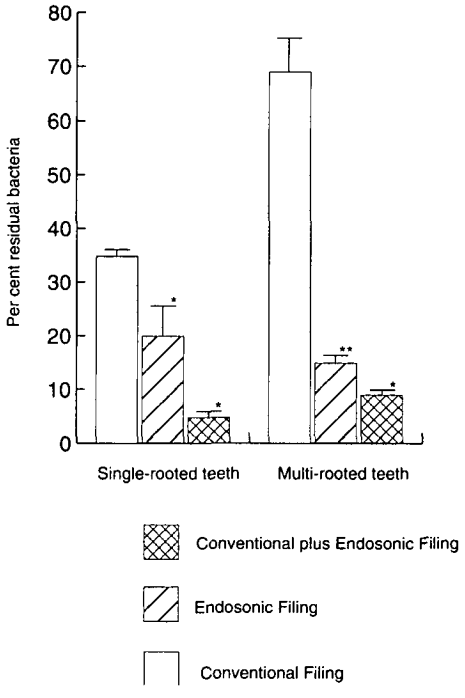

Fig 3. Per cent residual bacteria after conventional filing and ultrasonication using a plain endosonic probe. * $P < 0.05$.

residual bacteria of 68.9 ± 7.4 whereas endosonic filing reduced it to 14.9 ± 1.3 ($P < 0.001$). However a combination of conventional and endosonic filing decreased the per cent residual bacteria to only 9.0 ± 1.1.

Discussion

The debridement effects of endosonics are most probably achieved through the synergistic effects of the biophysical aspects of ultrasound as well as the use of an irrigant (Martin and Cunningham 1985; Walmsley 1987). The two main biophysical effects of ultrasound thought to be of importance in endosonics are termed "cavitation" and "acoustic microstreaming" (Walmsley 1987). During cavitation, bubbles are generated in the liquid which implode with tremendous force creating a pressure-vacuum effect that cleans the root canal walls as well as having a cidal effect on microorganisms (Thacker 1973; Martin and Cunningham 1985). Acoustic microstreaming describes the hydrodynamic shear stresses generated in the ultrasonic field which aid in the removal of debris and smear layers from the walls of the root canal (Ahmad et al. 1987).

Although the efficacy of endosonics has been previously demonstrated in permanent teeth (Martin and Cunningham 1985; Nehammer and Stock 1985; Stamos et al. 1985; Ahmad et al. 1987; Krell et al. 1988), to the author's knowledge, no previous studies are available on primary teeth.

The present study has thus shown that the use of ultrasound also greatly enhances the efficacy of cleaning of the root canals of primary teeth. Ultrasonication for 3 min with a plain endosonic probe was able to remove more than 60% of bacteria in root canals compared to only approximately 40% using conventional hand filing. When the probe was substituted with an endosonic file, the cleaning effect was increased further to about 80% bacteria removed at 3 min of treatment, and more than 90% at 5 min of treatment. However, the most practical and effective technique appears to be a combination of conventional filing followed by 3-min endosonic filing which, in this study, removed more than 95% of bacteria in the root canals.

The results of this study are thus comparable to an in vitro study by Cunningham et al (1982b), which reported an 86% reduction of *Bacillus subtilis* spores after ultrasonic filing of the root canals in contrast to only 62% reduction using hand filing. In addition, our results are also similar to those from the study by Martin (1976) which reported more than 95% growth reduction of common root canal bacteria after ultrasonic cleaning for 5 min.

Furthermore, my results have shown clearly that a combination of ultrasonic and conventional cleaning is extremely efficacious in the removal of bacteria from root canals. This combination technique has not been systematically investigated by previous workers.

Comparison of my results with the two previous in vivo studies in dogs (Barnett et al. 1985; DeNunzio et al. 1989) is difficult, since different experimental methods were involved. Although these studies did not show differences in the antimicrobial effectiveness of ultrasonication over conventional hand filing, technical and experimental design problems may have masked any possible differences. The investigation by Barnett et al. (1985) examined presence/absence of bacterial growth only, and no attempt was made to evaluate the degree of contamination. The study by DeNunzio et al. (1989) involved crushing and other severe physical treatment of the teeth which may have affected the recovery of potentially viable bacteria (Cunningham et al. 1982b).

Our novel method of assessing the efficacy of root canal instrumentation using radiolabelled bacteria has several advantages. First, the organism (*Streptococcus sanguis*) has an ability to adhere to root canal walls which may simulate in vivo endodontic infection (MacFarlane and Samaranayake 1989) compared to the bacterial spores employed by Cunningham et al. (1982b). Second, the amount of bacterial contamination is assessed directly and does not require attempts to regrow the bacteria after the experiments (Barnett et al. 1985; DeNunzio et al. 1989), which is a critical step encumbered with many possible experimental errors.

Although the reliability and validity of this novel method have not been fully established, it is likely the technical advantages may contribute significantly to its success.

In conclusion, this study has shown that ultrasonication is a useful adjunct for endodontic cleaning of primary teeth. Due to the high prevalence of accessory canals (Ringlestein and Seow 1989) and intricate root canal systems of primary teeth (Hibbard and Ireland 1957; Baker et al. 1975) it is reasonable to suggest that ultrasound is more effective than conventional hand filing in the debridement of these canals which are inaccessible to mechanical cleaning. However, due to the close proximity of the permanent tooth germs, it is vital that over-instrumentation past the apex does not occur, since it is possible that ultrasound may have detrimental effects on developing teeth. Further laboratory and clinical trials may be required to determine any possible adverse effects on the succedaneous teeth. However, if applied correctly, endosonics is likely to have a useful role in clinical pediatric endodontics.

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