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Bacterial reduction by extensive versus conservative root canal instrumentation *in vitro*

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Abstract

Objective. This study aimed to test the hypothesis that aggressive dentin removal through greater-tapered instrumentation reduces the intracanal bacteria more effectively than conservative dimension instrumentation. ***Material and methods.*** Twenty extracted human lower premolar teeth were used. After extirpation of the pulps, the teeth were autoclaved and immersed in a broth inoculated with *Enterococcus faecalis* and incubated for 7 days to allow infection of the dentinal tubules. The teeth were divided into 2 experimental groups, each comprising 10 teeth. The teeth were instrumented either with ProTaper or with Hero Shaper nickel-titanium rotary instrumentation techniques. It was calculated that ProTaper theoretically has the potential to remove at least twice the dentin volume compared with Hero Shaper. The apical preparation was standardized to file size 30. Saline solution was used for irrigation. Bacteriological samples were taken before and after instrumentation and plated onto tryptic soy agar, and the reduction in numbers was calculated. ***Results.*** Both instrumentation techniques significantly reduced the number of bacteria in the root canal ($p < 0.05$). Reduction in absolute bacterial numbers was up to 98%. There was no statistically significant difference between the two techniques. ***Conclusions.*** Preparation with an instrumentation technique removing substantial amounts of dentin did not reduce the intracanal bacteria more effectively than a more conservative instrumentation technique.

Key Words: *Bacterial penetration, debridement, endodontics, root canal preparation*

Introduction

Bacteria and their products play an essential role in the pathogenesis of pulpal and periradicular disease [1,2]. Therefore, endodontic treatment should be directed towards prevention of bacterial infection in the root canal or eradication of bacteria in the case of infected root canals. The importance of bacterial elimination from the root canal has been documented clinically: teeth with no detectable micro-organism at the time of root-filling heal in higher proportions than those with a positive culture [3].

Eradication of micro-organisms during endodontic treatment is achieved mainly by instrumentation and antimicrobial irrigation and dressing. While all these procedures are important in obtaining microbe-free canals, instrumentation is of particular importance in the sense that less work remains for the latter procedures if mechanical debridement is carried out efficiently. Irrigation and dressing with antibacterial agents further reduce the number of, and may eliminate, intracanal bacteria [4–6].

Bacteria in the root canal can penetrate into the dentinal tubules at any level (coronal, mid, apical). Increasing the size of the apical preparation reduces the bacterial count and yields cleaner canals, which may support the treatment objectives [7]. However, bacterial penetration is deeper coronally and at mid-root levels than in the apical part [8,9]. Then, variations in taper among different techniques should theoretically lead to variations in their ability to remove bacteria infecting the root canal and the dentinal tubules; root canals instrumented with greater taper files may contain fewer bacteria than those instrumented with smaller taper files.

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This study aimed to test the hypothesis that more bacteria may be left in infected root canals after preparation with a conservative instrumentation technique compared to one that removes a greater volume of dentin.

Material and methods

Preparation and infection of the specimens

Twenty extracted intact human lower premolar teeth with comparable lengths and straight roots were used. Conventional access cavities were prepared and the root canals were instrumented 1 mm short of the radiographic apex to size 20 using K-files. The root canals were irrigated with 17% EDTA and with 5.25% NaOCl, each for 4 min, in order to remove the smear layer. The teeth were then washed thoroughly with distilled water. The tip and the external surface of the roots were sealed with epoxy resin. The teeth were sterilized by means of autoclaving at 121°C for 20 min in distilled water and then transferred into sterile vials each containing 50 ml of Tryptic Soy Broth (Merck, Darmstadt, Germany). Each vial contained 10 teeth. The teeth were incubated overnight at 37°C and checked for turbidity. A 2% inoculum from an overnight culture of *Enterococcus faecalis* (ATCC 29212) was added to the vials and incubated at 37°C, changing the broth every second day, for 7 days. The external surfaces of the teeth were blotted dry using sterile gauze and the teeth were mounted vertically in plaster blocks to facilitate instrumentation and handling.

Instrumentation techniques

The teeth were distributed into 2 groups of 10 each. The lengths of the teeth were comparable among the groups. A total of 10 ml 0.9% saline solution was used for irrigation of each tooth.

Group 1: The root canals were instrumented using the ProTaper rotary instruments (Dentsply, Ballaigues, Switzerland) with a TCM Endo II (Nouvag AG, Goldach, Switzerland) electric handpiece adjusted to 300 rpm. The preparation was in a crown-down manner. The instrumentation sequence was: (1) S1 file (shaping file no. 1; taper 0.02–0.11; size 18.5) was used to one-third of the working length; (2) SX file (auxiliary shaping file; taper 0.035–0.19; size 19) was used to one-half of the working length; (3) S1 file was used to one-half to two-thirds of the working length; (4) S2 file (shaping file no. 2; taper 0.04–0.11; size 20) was used to two-thirds of the working length; (5) F1 file (finishing file no. 1; taper 0.07–0.055; size 20) was used to the full working length; (6) F2 file (finishing file no. 2; taper 0.08–0.055; size 25) was used to the full working length; (7) F3 file (finishing file no. 3; taper 0.09–0.055; size 30) was used to the full working length.

Group 2: The root canals were instrumented using the Hero Shaper rotary instruments (MicroMega, Besancon, France) with the TCM Endo II electric handpiece adjusted to 300 rpm. The preparation was in a crown-down manner. The instrumentation sequence was: (1) File size 30, taper 0.06 was used to one-third of the working length; (2) File size 25, taper 0.06 was used to two-thirds of the working length; (3) File size 20, taper 0.04 was used to the full working length; (4) File size 25, taper 0.04 was used to the full working length; (5) File size 30, taper 0.04 was used to the full working length.

Bacterial sampling

The root canals were sampled for bacterial counting before and after instrumentation. Prior to sampling, the root canal was filled with sterile saline solution using a sterile disposable injector. Then a sterile no. 15 file was inserted into the root canal and the canal walls were slightly touched with an in-out motion circumferentially, moving the file once around the periphery of the canal. Sterile paper points were placed in the root canal and allowed to saturate. Three paper points were used for each root canal. The paper points were transferred to a vial containing 1.5 ml of sterile saline solution and glass beads, and vortexed. Following serial dilution, droplets of 20 μl were cultured on tryptic soy agar (TSA) plates at 37°C for 24 h. Colony-forming units (CFU) were counted and the absolute number of bacteria was calculated. The reduction in absolute bacterial numbers was expressed as percentage values.

Volume calculations

The outline of each instrumentation technique was drawn according to the size and taper of the instruments and the instrumentation sequence described, and also considering that the working length was 21 mm. The diameters were taken from the manufacturer's specifications. The theoretical volume removed with each technique was calculated using a standard software program [10] and according to the ensuing profile (Figure 1). The calculated volume values were of the apical 14 mm of the whole working length. The minimum preoperative pulpal space was assumed to be the volume corresponding to a size 20 (taper 0.02) file, and the material removed by each technique was calculated by subtracting the volume of the pulpal space from the total instrumentation volume. Under these conditions, the preoperative pulpal space volume was calculated to be 1.34 mm^3 as a minimum value. The total instrumentation volumes for ProTaper and Hero

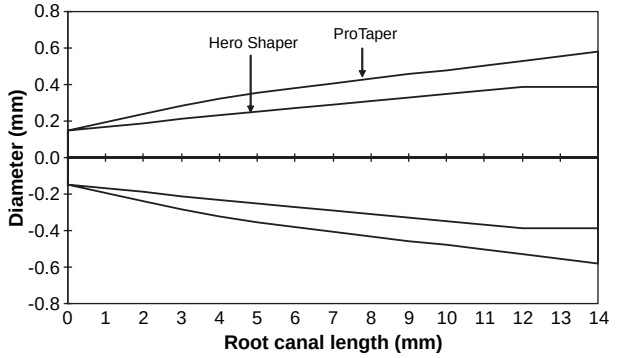

Figure 1. Theoretical preparation profiles achieved by the instrumentation techniques employed in this study.

Shaper were 7.40 and 3.88 mm^3, respectively. Accordingly, the removed material volumes were 6.06 and 2.54 mm^3, respectively.

Statistical analysis

Data obtained from samples taken before and after instrumentation were analyzed statistically for differences within each group using the Wilcoxon test and between the groups using the Mann-Whitney U-test. The statistical significance was set at $p < 0.05$.

Results

The preoperative and postoperative absolute bacterial numbers and percentages of reduction for the absolute values are given in Table I. Both techniques significantly reduced the number of bacteria in the root canal ($p < 0.05$; for ProTaper $p = 0.005$ and for Hero Shaper $p = 0.005$). The reduction was up to 98%. A total elimination of the bacteria was not achieved with any of the techniques. Difference between changes in bacterial numbers achieved with two instrumentation techniques was statistically insignificant ($p = 0.631$).

Discussion

This study was not designed to determine the relative merit of different instrumentation systems. Rather, it was the purpose to see if fewer bacteria would be recovered from infected root canals instrumented more extensively than from canals subjected to a more conservative instrumentation.

Table I. Pre-operative and post-operative means and percent reduction means and standard deviations (SD) for absolute bacterial numbers (n = 10)

	Pre-op CFU values Mean (SD)	Post-op CFU values Mean (SD)	% Reduction in CFU values Mean (SD)
ProTaper	$5.62\ (6.99) \times 10^6$	$5.02\ (8.79) \times 10^4$	97.4 (3.6)
Hero Shaper	$2.16\ (1.34) \times 10^7$	$1.51\ (1.05) \times 10^5$	98.3 (2.6)

However, the main finding in this study was that similar amounts of bacteria could be recovered from teeth in both categories. While instrumentation significantly reduced the number of the bacteria, there was invariably more than 10^4 bacteria left in the root canal postoperatively.

In the present study, the apical preparation was standardized in both techniques to a size 30. The two techniques for rotary instrumentation were selected among current systems, which by design remove very different amounts of dentin. A non-bactericidal solution was used as irrigant to limit the bacterial reduction effect to that of the mechanical preparation. However, it has to be taken into consideration that irrigation *per se*, although non-bactericidal, may account for part of the bacterial reduction, due to the mechanical washing effect [11], but the extent of this effect was not investigated in this study. The study design sought to make the effect of the saline irrigation similar for both groups.

Enterococcus faecalis was chosen as the marker strain, because it is a concern for persistent endodontic infections and periradicular inflammation [12] and it can readily infect the dentinal tubules [13].

The root canals were enlarged to size 20 before being artificially infected. The preoperative pulpal space volume was then considered the volume of the size 20 file (1.34 mm^3 along the 14 mm cutting portion) as a minimum value. Regarding the data on molar root canal volumes [14], it is likely that the actual volume of the pulpal space of a mandibular premolar may be greater than 1.34 mm^3. If the preoperative pulpal space volume is greater than 1.34 mm^3, the difference between the removed material volume by the tested instrumentation techniques becomes more pronounced (e.g. if the preoperative pulpal space volume was 3.00 mm^3 rather than 1.34 mm^3, there would be a 5-fold difference between the volume removed by the two techniques, instead of about 2-fold, as presented). In any case, one of the techniques (ProTaper) is more aggressive than the other (Hero Shaper). While the calculations performed in this study provide basic knowledge of the material removal capacity of an instrumentation technique, computed tomography measurements of changes caused by different instrumentation techniques have confirmed that significantly more dentin was removed with ProTaper on mesial root canals of molars compared with Hero Shaper [15].

It was expected that the more aggressive removal of dentin (ProTaper) would eliminate more bacteria and lead to lower bacterial counts in the final sample. However, this was not the case in this study. Siqueira et al. [16] compared conventional taper files (0.02 mm/mm) with greater taper files (0.06), and found no significant difference in bacterial reduction. In the mentioned study, further apical instrumentation with the conventional files to a larger size

was significantly more effective in eliminating bacteria from the root canal than greater taper files that stopped at a smaller apical size, supporting the concept that the size of the apical preparation is important in intracanal bacterial reduction [7,17].

One reason that no significant difference was found between the two instrumentation techniques may be related to the pattern of bacterial infection of the dentinal tubules. This pattern may affect the results of studies on *in vitro* infected teeth. Studies artificially infecting the root canal and the dentinal tubules, in general, employ relatively short durations of incubation: 1 day to 1 week [4,5,16,18]. In this case, it is possible that the bacteria penetrate sufficiently through the dentinal tubules to be recovered even beyond the largest size file, but fail to form dense populations in the more luminal parts of the dentinal tubules. Then instrumentation techniques would not reflect any difference in their bacterial reduction abilities, in spite of taper differences. Prolonged root infection periods (e.g. more than 3 weeks), however, may change the results of the experiment, i.e. favoring the greater taper file over the smaller taper file, as denser bacterial populations (possibly rarefying gradually towards the cementum direction) form at the luminal side of the root dentin after longer periods of incubation [13]. Then the more aggressive instrumentation may be more efficacious. Another reason may be related to the anatomy of the root canal. Mandibular premolars, in horizontal section, have an oval-shaped root canal which is broad in the buccolingual direction until the middle-third of the root canal [19]. Both rotary instrumentation techniques tested in this study were used with an in-out motion and with no lateral pressure applied on the root canal walls. While attempts were made to include teeth with a round and regular pulp canal, it is possible that surfaces harboring bacteria in ovoid extensions remained untouched by the instruments, which were later sampled through the circumferential filing motion with the size 15 hand-file, thereby yielding recovery of bacteria of similar quantity in both groups. The extent of the touched/untouched surfaces, however, remained unknown in this study.

In conclusion, preparation with an instrumentation technique designed to remove substantial amounts of dentin did not reduce the intracanal bacteria more effectively than a more conservative instrumentation technique.

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