JOURNAL OF ENDODONTICS
Printed in U.S.A.
VOL. 25, NO. 5, MAY 1999

Mechanical Reduction of the Bacterial Population in the Root Canal by Three Instrumentation Techniques

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The in vitro reduction of the bacterial population in the root canal by the mechanical action of instrumentation and irrigation was evaluated. Root canals inoculated with a *Enterococcus faecalis* suspension were instrumented using hand Nitiflex files, Greater Taper (GT) files, and Profile 0.06 taper *Series 29* rotary instruments. Irrigation was performed using sterile saline solution. Root canals were sampled before and after instrumentation. In the group of the Nitiflex files, samples were also taken after each file size. After serial dilution, samples were plated onto *Mitis-Salivarius* agar, and the colony forming units grown were counted. All techniques and instruments tested were able to reduce significantly the number of bacterial cells in the root canal. Instrumentation to a Nitiflex #30 was significantly more effective than GT files. There were no significant differences when comparing the effects of the Profile instrument #5 with either the GT files or the Nitiflex #30. Enlargement to a Nitiflex #40 was significantly more effective in eliminating bacteria when compared with the other techniques and instruments tested ($p < 0.05$). The results of this study showed that the instrumentation and irrigation can mechanically remove more than 90% of bacterial cells from the root canal.

Because pulpal infection plays a role in the development of periradicular lesions (1), endodontic treatment must be directed toward the elimination of bacteria, their products, and substrate from the root canal system. It has been demonstrated that eradication of endodontic infection enhances the success rate of the endodontic therapy (2).

During endodontic treatment, bacterial reduction or elimination may be achieved by both chemomechanical preparation and intracanal dressings. Although it seems unreasonable to place particular emphasis on any endodontic procedure, chemomechanical preparation may be considered an essential step in root canal disinfection (3). The removal of irritants from the root canal is conducted by means of the mechanical action of instruments and the flow and backflow of the irrigant solution (4). In addition, antibacterial irrigants may significantly help to eliminate bacterial cells from the root canal system (5).

Previous studies, in which no antibacterial irrigants were used, have reported that the mechanical action of instrumentation and irrigation was effective in reducing the number of bacterial cells in the root canal (3, 6). However, total elimination of bacteria was not observed in most of the cases. Ingle and Zeldow (7) observed that, immediately after instrumentation, using sterile water as irrigant, 80% of the initially infected root canals yielded positive cultures. At the beginning of the second appointment, 48 h later, this number increased to 95.4%. Byström and Sundqvist (3), using physiological saline solution during instrumentation, found that bacteria persisted in about half of the cases despite treatment on five successive occasions. Teeth where the infection persisted were those with a high number of bacteria in the initial sample.

Several brands and designs of files are manufactured from a nickel-titanium (NiTi) alloy, which has a very low modulus of elasticity, superior flexibility in bending, and great resistance to torsional fracture (8). NiTi instruments may be as aggressive as or better than stainless-steel files in removing dentin (9). In addition, they are more resistant to wear than their stainless-steel counterparts (9).

Recently, NiTi files with increased tapers and different designs have been developed. The Greater Taper (GT) files (Tulsa Dental Products, Tulsa, OK) are four hand instruments with a triangular cross-section, each possessing 0.06, 0.08, 0.1, and 0.12 mm/mm tapers. All GT files have an ISO tip size of 20. Their flutes are machined in reverse direction when compared with conventional files. As these files' tapers become greater, the length of fluted cutting surfaces become progressively shorter (10).

Rotary NiTi instruments have become available in the last few years. The Profile rotary instruments (Tulsa Dental Products, Tulsa, OK) have a 0.04 or 0.06 mm/mm taper, which is double or triple the standard 0.02 mm/mm taper in conventional instruments. In addition, the tip diameter of these instruments increases 29% per size file, unlike the ISO standard 0.05 or 1.0 mm. The resulting tip diameters in millimeters are 0.129, 0.167, 0.216, 0.279, 0.360, 0.465, 0.600, 0.775, and 1.0 (11). These instruments have a

U-cross sectional design with radial land areas, which is reported to cut equally over 360° with a planing action and to be self-centering (11). In addition, Profile instruments show a bullet nosed tip with rounded transitional angle.

To date, there is limited information about the effectiveness of current instrumentation techniques and instruments to mechanically reduce the bacterial population inside the root canal. In an in vivo study, Dalton et al. (12) found that 0.04 taper NiTi rotary files and stainless-steel hand K-file step-back instrumentation are equally effective techniques for reducing intracanal bacteria. However, neither technique could predictably render canals free of bacteria.

The purpose of this study was to compare the intracanal bacterial reduction provided by instrumentation using hand NiTi K-type files, GT files, and Profile 0.06 taper *Series 29* rotary instruments.

MATERIALS AND METHODS

Thirty-five extracted human lower bicuspids with a single root canal, checked by radiographs, were selected for this experiment. Conventional access preparations were made and the root canals were instrumented 1 mm beyond the apical foramen with K-type files up to size 20, under irrigation with tap water. Working length was established at the apical foramen. After root canal preparation, the enlarged apical foramen was sealed by means of epoxy resin to prevent bacterial leakage. To make both handling and identification easier, the teeth were then mounted vertically in plaster blocks and sterilized overnight by ethylene oxide gas.

Sterilized plaster blocks containing the teeth were opened in a laminar air flow cabinet. A suspension was prepared by adding 1 ml of a pure culture of *Enterococcus faecalis* (ATCC 29212), grown in brain heart infusion broth (Difco, Detroit, MI) for 24 h, to fresh brain heart infusion broth. Each root canal was completely filled with the *E. faecalis* suspension using sterile 1 ml tuberculin syringes. Sterile K-type files #15 were used to carry the bacterial suspension to the working length. The blocks were then placed inside sterile plastic bags and incubated at 37°C for 24 h.

The root canals were then divided into three groups accordingly to the instrumentation technique used as follows.

Group 1—10 root canals were handly instrumented using Nitiflex files (Maillefer, Ballaigues, Switzerland) with alternated rotary motions (ARM), as described by Siqueira (13). A #25 Nitiflex file was inserted into the root canal to a point where it bound slightly and then turned clockwise, with no more than one quarter rotation. It was then turned counterclockwise with light apical pressure. Counterclockwise rotation was also no more than one quarter turn. These motions were repeated continuously until the file reached working length. Alternated rotary motion was maintained in this position for few seconds. The file was withdrawn 1 to 2 mm, still oscillating, then replaced to the working length. This continuous oscillation associated with the up-and-down motion was repeated until the file was able to slide easily to the working length. Each sequentially larger file was worked in a similar fashion. Apical preparation was completed by enlargement through a #40 Nitiflex file.

Group 2—10 root canals were enlarged to their full lengths using GT files with 0.10 and 0.12 tapers as recommended by the manufacturer (10). A GT file of 0.10 taper was introduced in the root canal and then rotated counterclockwise one quarter turn to engage dentin. Afterward, the file was turned clockwise with firm apical pressure until the file failed to engage dentin during rotation. The file was turned one quarter turn counterclockwise to load the flutes and then removed from the canal. After cleaning the flutes with sterile moist gauzes, the root canal was irrigated and the file reinserted in the canal. The steps described were repeated until the GT file of 0.12 taper reached the working length.

Group 3—10 root canals were instrumented using the Profile 0.06 Taper *Series 29* rotary instruments in a crown-down manner as recommended by the manufacturer (11). Instruments were used in a Profile electric handpiece (Tulsa Dental Products, Tulsa, OK) adjusted to 250 rpm. A size 7 Profile (tip diameter of 0.465 mm) was used to prepare the coronal third of each canal in a gentle in and out motion. Once this file had reached the desirable length, it was removed and the root canal irrigated. A size 6 Profile (tip diameter of 0.360 mm) was used in the middle third. Preparation was then completed by using a size 5 Profile (tip diameter of 0.279 mm) at the working length.

Root canals were sampled before and after instrumentation. In group 1, samples were also taken after each file size, just after irrigation. Canals were filled with sterile 0.85% saline solution and each sample was taken by using three paper points. After initial sampling, all root canals were irrigated with 1 ml of 0.85% saline solution. In group 1, canals were irrigated with 1.5 ml of saline after each file size. Although irrigation was also done frequently in groups 2 and 3, standardization was difficult. However, each root canal was always irrigated with a total volume of 7 ml of 0.85% sterile saline solution. Irrigant was delivered in the canals by means of a 3 ml plastic syringe with a 23-gauge needle. All procedures were performed by one operator (J.F.S.J.). Each set of instruments was used to prepare no more than three canals.

Paper points used to sample the canals were transferred to tubes containing 1 ml of 0.85% saline solution and vortexed for 1 min. After 10-fold serial dilutions in saline, aliquots of 0.1 ml were plated onto *Mitis-Salivarius* agar plates and incubated at 37°C for 48 h. The colony forming units grown were counted and a log transformation calculated. Five sterilized and noncontaminated root canals were sampled as a control.

Data obtained from samples taken prior, during, and after instrumentation were analyzed statistically for differences inside groups using the paired t test and between groups by means of the nonpaired t test, with similar variances based on the F test. The significance level was established at 5% ($p < 0.05$).

After preparation, the teeth were steam-sterilized, removed from plaster blocks, and each root was transversally sectioned at three levels: cervical, middle, and apical. Sections were further examined using a stereomicroscope (Zeiss, Oberkochen, Germany) at a ×10 magnification.

RESULTS

The mean number of bacterial cells in the initial samples from the root canals prepared by the ARM instrumentation was 2.7×10^6. After instrumentation with files #30, #35, and #40, the mean values of the number of bacterial cells decreased to 5×10^4, 1.3×10^4, and 1.1×10^4, respectively. The mean reduction of bacteria was of 98.17%, 99.5%, and 99.57% for canals prepared to Nitiflex file sizes 30, 35, and 40, respectively. All of these three file sizes were significantly effective in reducing the bacterial population in the root canal. The differences between each size file in eliminating bacteria from canals were also significant at the 5% level. The most significant intracanal bacterial reduction was obtained after instrumentation to a file size 40.

TABLE 1. Mean values of the quantity of bacterial cells (in log numbers) in the root canal before and after instrumentation using the different files and techniques

Instruments	Initial (Mean ± SD)	Final (Mean ± SD)	Reduction (Mean (%))
Nitiflex—hand (size 30)	5.86 ± 0.78	4.18 ± 0.73	98.17
Nitiflex—hand (size 35)	5.86 ± 0.78	3.77 ± 0.59	99.5
Nitiflex—hand (size 40)	5.86 ± 0.78	3.59 ± 0.70	99.57
GT files—hand (0.12 taper)	5.12 ± 0.81	3.99 ± 0.73	94.17
Profile 0.06—rotary (#5)	6.27 ± 0.58	4.75 ± 0.66	97.26

Profile rotary instruments and GT files provided a decrease of 97.26% and 94.17% in the number of viable bacteria in the root canal, respectively. For the Profile group, the means of the bacterial cell numbers at the initial and the final samples were 4.6×10^6 and 1.2×10^5, respectively. Initial samples from the root canals prepared by the GT files contained a mean of 5.8×10^5 bacterial cells, whereas the mean number of bacteria in the final samples was 3.4×10^4.

By comparing the samples taken before and after instrumentation, it was possible to observe that all techniques and instruments tested were able to reduce significantly the number of bacterial cells in the root canal ($p < 0.05$).

When comparison was done between groups, the ARM instrumentation to a Nitiflex #30 resulted in significantly less quantifiable growth than instrumentation using the GT files. Statistical analysis failed to show a significant difference between the Profile and the GT files instrumentation. No significant difference was detected when comparing the bacterial reduction provided by the Profile rotary instruments and ARM instrumentation to a Nitiflex #30. However, instrumentation to a Nitiflex #40 was significantly more effective in eliminating bacteria from the root canal when compared with Profile instrumentation to a file #5 ($p < 0.05$). Data are summarized in the Table 1.

Statistical analysis revealed that the inoculum size was the same for the three techniques used herein. Samples taken from the control group yielded negative growth, confirming that the specimens were effectively sterilized before use.

Canals instrumented by the three techniques were round and centered at most apical sections (Fig. 1). The root canals were also round at some sections from the middle and coronal thirds. However, at other sections from these two more coronal thirds, root canals were either oval or irregular in shape. It seemed that some walls were not prepared by instruments, despite the technique performed (Fig. 1).

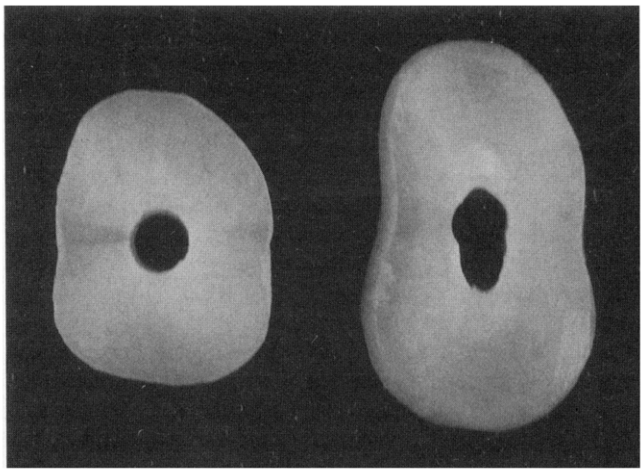

FIG 1. Cross-sections of instrumented root canals. (*Left*) Canal is round at this apical section. (*Right*) Irregular shape of the root canal at the middle third. Some walls were apparently untouched by instruments.

DISCUSSION

E. faecalis, a facultatively anaerobic Gram-positive coccus, is a normal commensal adapted to the ecologically complex environments of the oral cavity, gastrointestinal, and vaginal tracts (14). This bacterial species was chosen for use in this study because it is often involved in persistent endodontic infections (15) and is one of the most resistant species found in the oral cavity, having the ability to survive under unusual environmental stresses (14). *Mitis-Salivarius* agar was used because it just allows the growth of streptococci and some enterococci, including *E. faecalis*. Thus, the risk of false results due to the growth of potential bacterial contaminants, which might have occurred during handling, was reduced.

The 0.85% saline solution has no antibacterial effects on *E. faecalis*, because this bacterial species can growth in the presence of 6.5% NaCl solution (16). Because no antibacterial irrigant was used, elimination of bacteria was just dependent on the mechanical action of both instruments and irrigation. The results of this study showed that instrumentation and irrigation can mechanically remove >90% of the bacterial cells from the root canal.

There were no significant difference between the Profile rotary instrumentation to #5 (equivalent to a file size 28) and the ARM instrumentation to a Nitiflex #30, although these instruments have different tapers. However, canal preparation to a Nitiflex #30 taper 0.02 was significantly more effective than GT file 0.12 taper (diameter tip equivalent a file size 20) in reducing the intracanal bacteria. This finding was unexpected. One millimeter short of the tip, Nitiflex #30 and GT 0.12 have the same diameter (i.e. 0.32 mm). From this point toward the beginning of the flute cutting surfaces, the GT file 0.12 is progressively larger than the Nitiflex #30. Theoretically, the GT file 0.12 should cut more dentin. Moreover, lesser volume of irrigant was used when working to a Nitiflex #30 than to a GT file 0.12. Differences occurred probably due to anatomical variations or different file motions or both.

Instrumentation to a Nitiflex #40 removed significantly more bacteria than preparation to a Profile instrument #5. At 3 mm from the tip, these two files have the same diameter: 0.46 mm. From this point to the instrument tip, Nitiflex #40 is larger than Profile 0.06 taper #5. The better results obtained with instrumentation to a Nitiflex #40 can be explained by the difference in the instrument diameters, although the influence of anatomical variations must be considered.

After instrumentation by the ARM technique to a file size 30, a significant number of bacterial cells was removed from the root canals. Nevertheless, it was evident that the quantity of bacteria in the root canal was significantly reduced after each sequential increase in file size. This finding was consistent with that of Orstavik et al. (6), which showed that, the larger the root canal preparation, the higher the efficacy in reducing the infection level of the root canal. In clinical practice, the extent of instrumentation will depend on the root dimension and the presence of curvatures.

Hand and rotary NiTi instruments can predictably enlarge curved root canals, while maintaining the original path, to sizes not routinely attainable with stainless-steel files (17). Large preparations can incorporate more anatomical irregularities and allow the removal of a substantial number of bacterial cells from the root canal. In addition, instrumentation to larger file sizes can also result in better irrigant exchange in the apical third (18).

Although a considerable bacterial reduction was achieved by the three techniques tested, bacteria were never thoroughly eliminated from the root canals regardless of the instrumentation technique and file sizes used. Whereas minor anatomical irregularities may be incorporated in the preparation, areas such as fins and ramifications, which possibly were not detect by radiographs, might have harbored bacteria. These areas are commonly unaffected by instruments and irrigants during root canal preparation (4). Bacteria located inside dentinal tubules may also not be eliminated by instrumentation. In addition, some root canals used in this investigation were oval in cross-section, particularly in their coronal two-thirds. Instrumentation was performed using modified watch-winding (Nitiflex and GT files) and reaming (Profile) motions. In the coronal two-thirds of oval canals, these motions overprepared the canal on some walls and hardly prepared other walls. Further modifications in these instrumentation techniques might prevent this last undesirable situation.

Remaining pathogens may survive in sufficient numbers in the root canal to jeopardize the outcome of root canal treatment (2). Therefore, the need to use antibacterial irrigants and medicaments to maximize bacterial elimination from the root canal becomes evident. Stewart (19) and Auerbach (20), in clinical investigations, reported negative cultures in >70% of initially infected root canals after chemomechanical preparation using antibacterial irrigants. Siqueira et al. (5), in a laboratory study, found that irrigation with an antibacterial irrigant was significantly more effective than saline solution in rendering canals free of bacteria. Likewise, intracanal medications used between appointments may successfully help to eliminate surviving bacteria not eliminated during chemomechanical preparation (2, 3).

The use of an antibacterial irrigant was omitted in the present study to assess separately the mechanical effects of instrumentation and irrigation. Our results indicated that these mechanical effects cause a significant decrease in bacterial cell numbers in the root canal. The most dramatic bacterial reduction was obtained after larger preparation. Because mechanical means are insufficient to completely eradicate root canal infection, the use of adjunct chemical substances possessing antibacterial properties becomes necessary.

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