Comparative evaluation of apical extrusion of intracanal bacteria using ProTaper Next, Mtwo, and ProTaper rotary systems: An in vitro study

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Abstract

Aim: The aim of this study was to evaluate the number of intracanal bacteria extruded apically after instrumentation with three different nickel–titanium rotary instruments.

Materials and Methodology: Forty freshly extracted mandibular premolars were selected, access cavities were prepared, and the teeth were mounted in the bacterial collection apparatus. Root canals were contaminated with a suspension of Enterococcus faecalis and incubated for 24 h at 37°C. The contaminated teeth were divided into four groups of 10 teeth each according to the rotary system used for instrumentation: Group 1: ProTaper universal files, Group 2: MTwo files, Group 3: ProTaper Next files, and Group 4: Control group (no instrumentation). Bacteria extruded after preparations were collected into vials. The number of colony-forming units (CFUs) was determined for each sample.

Statistical Analysis: The data obtained were analyzed using the one-way analysis of variance followed by post hoc Tukey’s test with a P = 0.05 as the level for statistical significance.

Results: The results suggested a statistically significant difference in the number of CFUs between four experimental groups (P < 0.001).

Conclusion: Least amount of bacterial extrusion was seen in ProTaper Next Group while more bacterial extrusion was seen in MTwo Group.

Keywords: Apical bacterial extrusion; Enterococcus faecalis; MTwo files; ProTaper files; ProTaper Next files

INTRODUCTION

The successful endodontic treatment must be directed toward the elimination of bacteria, their products from the root canal system, and this objective can be achieved by thorough chemo-mechanical preparation. Despite strict length control, almost all instruments and preparation techniques used for the root canal treatment are associated with debris extrusion, which contains dentin chips, pulp tissue, microorganisms and/or irrigants, into the periradicular tissue. This extrusion may potentially result in postoperative flare-ups.¹ Bacteria extruded mainly include Gram-positive, Gram-negative bacteria, and obligate anaerobes. Enterococcus faecalis (E. faecalis) has been identified as a species most commonly recovered from the posttreatment diseases.²

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In recent times, various rotary systems with varying instrument designs have been introduced and thus differences exist in terms of apical extrusion of debris. Previous studies reported that ProTaper Universal (DENTSPLY Maillefer) (PTU) rotary files extruded greater amounts of debris that has been attributed to their aggressive cutting ability.

Recently, the ProTaper Next system (DENTSPLY Maillefer) with M-wire nickel–titanium (Ni-Ti) configuration has been introduced. M-wire technology helps to improve file flexibility and resist cyclic fatigue while retaining cutting efficiency of the instrument. Moreover, also, this rotary system ensures only two-point contact with the canal wall at a time, assuring efficient cleaning of the canal.

MTwo endodontic instruments (VDW, Munich, Germany) are newer generation rotary files with an italicized “S” transverse section and two cutting edges with a noncutting tip are designed with minimum radial contact that permits for continuous upward shifting of dentin chips.

The aim of this in vitro study was to compare the apical extrusion of *E. faecalis* using three rotary Ni-Ti instruments (ProTaper Next, MTtwo, and ProTaper).

**MATERIALS AND METHODOLOGY**

Forty freshly extracted human, single-rooted mandibular premolar teeth with mature apices and root curvatures between 0° and 10° were selected. All teeth were analyzed radio graphically in bucco-lingual and proximal directions to ensure for the presence of single and noncalcified canals. The teeth were cleaned of debris and soft-tissue remnants and endodontic access cavities were prepared with endo access bur No: 2 (DENTSPLY Maillefer) using a super torque high-speed hand piece and the pulp remnants were extirpated with a fine-barbed broach. A model system described by Myers and Montgomery 1991 et al. was used to evaluate bacterial extrusion [Figure 1].

The teeth were inserted under pressure into perforated rubber stoppers of 10 ml glass vials. Two coats of nail varnish were applied to the external surface of all roots to prevent bacterial microleakage through the lateral canals. The rubber stopper with the tooth was then fitted into the opening of the vial. The specimens were then divided into four groups of ten specimens each (*n* = 10). The root canals in three experimental groups were instrumented with three different rotary endodontic systems while in the fourth group which acted as a control, no instrumentation was attempted.

A suspension of pure culture of *E. faecalis* (ATCC-29212) was prepared by adding 1 mL pure culture of *E. faecalis* to freshly prepared brain–heart infusion broth and incubated for 24 h. Then, McFarland standard no. 0.5 was used to evaluate the broth to ensure that the number of bacteria was 1.5 × 10^8 colony-forming units (CFUs)/ml. Each root canal was filled with the *E. faecalis* suspension using sterile pipettes in a Class II vertical laminar airflow cabinet to prevent any airborne contamination and a size 10 K-file was used to carry the bacteria down the length of the canals. The contaminated root canals were placed in an incubator at 37°C for 24 h for bacterial multiplication.

Before the experiment, sterilized glass vials were filled with 0.9% NaCl (saline) solution. The tooth-rubber stopper-needle unit was then fixed into the mouth of the vials. The specimens were then divided into four groups of ten specimens each (*n* = 10). The root canals in three experimental groups were instrumented with three different rotary endodontic systems while in the fourth group which acted as a control, no instrumentation was attempted.

A sterile, 10 K-file was placed 1 mm beyond the foramen to remove the nail varnish that covered the apical foramen., with the same 10-k file, 1 mm short of the file penetration was considered as WL. In this way, standard size of foramen and apical patency was achieved. The WL determined manually was again confirmed with an electronic apex locator (DENTSPLY Mallifer Propex Pixi) after fitting the tooth with rubber stopper into the vial. The entire model system was then sterilized in ethylene oxide gas sterilizer (3M, USA) for a 12-h cycle.

**Figure 1:** The experimental model system
the apical to coronal direction during irrigation. The flow of irrigant was standardized to a constant speed of 1 mL/4 sec controlled by a chronometer.

**Group I – ProTaper universal**
ProTaper rotary files were used in a crown-down manner according to manufacturer’s instructions using a gentle in-and-out motion. An SX file was used at half of the WL, S1, and S2 files were carried to 2/3 of the WL, and F1, F2, F3 sequentially to full WL.

**Group II – MTwo**
All MTwo instruments were used to the full length of the canal (single-length technique) at a rotational speed of 280 rpm according to the manufacturer’s instructions using small, stroking brushing movements. The instrumentation sequence was as follows: Size 10/.04 taper, size 15/.05 taper, size 20/.06 taper, size 25/.06 taper, and size 30/.05 taper.

**Group III-ProTaper next**
All ProTaper Next files were used in a crown-down manner according to the manufacturer’s instructions with a gentle in-and-out motion at 300 rpm and 2 Ncm torque. The instrumentation sequences were X1 (17/04), X2 (25/06), and X3 (30/07).

**Group IV (control)**
All the samples in the control group were kept under laminar airflow cabinet for a time period of 1 min, without attempting instrumentation and were taken out.

Single operator under aseptic conditions carried the canal preparation, whereas bacterial extrusion was assessed by another investigator who was blinded regarding the experimental groups. Prior to and at the end of canal preparation, 0.01 mL of solution was collected from all the forty experimental vials to count the bacteria. Using a bacterial inoculation loop, the bacterial suspension was placed on brain–heart agar plates. All the agar plates were placed in an incubator at 37°C for 24 h. After 24 h, colonies of bacteria were counted using the classical bacterial counting technique (Collins et al. 2004), and they were counted as number of CFUs.

**RESULTS**
The soft ware used was statistical package for the social sciences soft ware version 24.0IBM Corp. Armonk, NY, USA was used for the statistical analysis. Mean and standard deviation were estimated from the sample for each study group. The mean values were compared by the one-way analysis of variance appropriately followed by post hoc Tukey test. Post hoc Tukey test was employed to identify the significant groups. In the present study, the level of significance was set at $P = 0.05$.

Table 1 shows the mean counts, standard deviation, and post hoc Tukey test of extruded bacteria ($E. faecalis$) between different experimental groups. Table 2 and Graph 1 show mean comparison between the groups.

Mean CFU was compared in all the groups, and there was a statistically significant difference among the groups ($P < 0.001$). The mean amount of extruded bacteria ranged between 0 CFU/ml and $13.56 \times 10^3$ CFU/ml. Post hoc test suggested that Group 4 (Control) had significantly lower mean CFU than all the groups.

The highest amount of bacterial extrusion was seen in Group 2, and the least amount of bacterial extrusion was observed with Group 3.

**DISCUSSION**
The primary goal of the root canal treatment is to eliminate microorganisms, thereby reducing the periradicular inflammation. Hülsmann and Hahn stated that in any endodontic treatment, an instrument used in an apical direction or an instrument acting as a plunger may result in apical extrusion of material such as dentin chips, pulp tissue, microorganism, and irrigation solution. Most NiTi rotary instruments work in the coronoapical direction.
In this study, during the root canal irrigation, the needle was gently inserted up to apical 3 mm of WL because it ensures adequate irrigant flow through the root canal, thereby creating less apical pressure and maintaining minimal extrusion of the irrigant.\cite{13}

In the present study, ProTaper Next extruded significantly least amount of bacteria compared to MTwo and ProTaper rotary file systems. Koçak et al. in their study found that bacterial extrusion was comparatively less when instrumented with ProTaper Next Ni-Ti instruments.\cite{14} The current study used rotary instruments with different cross-sectional design: ProTaper Next—rectangular cross section, ProTaper-convex triangular cross section, and MTwo-S-shaped cross section. ProTaper Next instruments have M-wire technology with off-centred cross section and generates snake-like swaggering movement along the active part of the file, thus reducing the screw effect, the unwanted taper lock,\cite{15} thereby decreasing the file-root dentin contact and provides more cross-sectional space for enhanced cutting, loading and allowing the debris to travel in apico-coronal canal direction when compared to the file systems with center of mass and axis of rotation in line with the center of axis of the file.

The common design feature of the ProTaper Next and PTU systems is the presence of progressive and regressive percentage tapers on a single file. However, the PTU F2 instrument has a 0.08 taper at the apical 3 mm, whereas the ProTaper Next X2 instrument has a 0.06 taper at the apical 3 mm. The large apical taper may result in more aggressive preparation of the root canals, which could have led to a larger quantity of debris being extruded apically.\cite{16}

Another reason for this finding may be the number of instruments from each system used to prepare a canal. Specifically, the number of instruments used was three files for the ProTaper Next group, five files for the MTwo group, and six files for ProTaper group. In addition, a reduction in the number of instruments decreases the number of walls played during instrumentation, one of the reasons why systems with smaller numbers of files result in less extrusion of debris.\cite{17}

In the present study, MTwo Rotary Ni-Ti instruments extruded a greater amount of bacteria in comparison with ProTaper and ProTaper Next. The results of this study were in correlation with the study done by Garlapati et al.\cite{18} This may be attributed to the standardized length preparation technique (single-length technique) and also to the s-shaped cross section with double-cutting edge geometry of MTwo instruments. Because of these design characteristics, they remove an adequate amount of dentin in a short period of time, thereby unable to displace the debris coronally, leading to significantly greater amount of apical extrusion.

Graph 1: Comparison of mean values among all the groups

with push–pull rotation filing movements during the canal preparation.\cite{6}

The present research used single-canaled mandibular premolar teeth; this is to minimize nonstandardized preparations that are more likely to occur in curved canals and to ensure that bacterial extrusion is due to instrumentation and not due to the tooth morphology.

Myers and Montgomery\cite{3} showed that debris extrusion can be minimized when WL was taken 1 mm short of the canal length. Beeson et al.\cite{7} reported that significantly less debris extrusion when instrumentation done 1 mm short of apex compared to the instrumentation performed till apical foramen. The present study used electronic apex locator (DENTSPLY Mallet Propex Pixi) to make sure that WL of the canals was maintained 1 mm short of the apex, so that variables which may affect results could be minimized.

\textit{E. faecalis} was used as the bacteriological marker as it is implicated in persistent root canal infections and is identified as the species most commonly recovered from the root canals of teeth with posttreatment disease.\cite{8} The apical diameter of master apical instruments in all the groups was standardized at ISO size 30 to avoid any variations in the amount of extruded bacteria due to the size of apical enlargement.\cite{9} Therefore, any bacteria extruded apically could be attributed to the design of the rotary file and instrumentation technique used in that particular group.

Irrigation is an important phase in cleansing the canal. In the present study, saline was used as irrigant as it has no antibacterial effect, and the irrigation was done passively to ensure that bacterial extrusion is only due to the mechanical action of the rotary instruments being tested. Irrigant was delivered with 27 G side-vented at a constant rate of 1 ml/4 s.\cite{10} Abou-Rass and Piccinino\cite{11} recommended the use of small diameter (27 G or 30 G) needles for irrigation because they provide sufficient volume of irrigant for efficient flushing of debris by fitting at depths just short of the physiological terminus. Various studies have shown that apical extrusion of irrigant is less when irrigation done with side-vented needles.\cite{12} In this study, during the root canal irrigation, the needle was gently inserted up to apical 3 mm of WL because it ensures adequate irrigant flow through the root canal, thereby creating less apical pressure and maintaining minimal extrusion of the irrigant.\cite{13}
of bacteria. Early coronal flaring with the crown down technique used with ProTaper and ProTaper next rotary files, helped in removing majority of microorganisms and reduced the possibility of debris packing, thereby allowing deeper penetration of irrigating solutions and clearing debris from the apical area,\textsuperscript{[19]} reducing the potential of extruding material from the root apex.

The present research was an \textit{in vitro} study, and its results should not be extrapolated to clinical situations, because different results may be achieved \textit{in vivo} as the periapical tissues may serve as a natural barrier, inhibiting bacterial and debris extrusion and also their pressures might differ at the apex with normal or pathological tissues.

**CONCLUSION**

ProTaper Next rotary instruments extruded less number of bacteria where instrumentation was performed by the crown-down technique. MTwo rotary instruments extruded more number of bacteria where instrumentation was performed by the single-length technique.

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**Conflicts of interest**

There are no conflicts of interest.

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