



International Journal of Applied Dental Sciences

ISSN Print: 2394-7489
ISSN Online: 2394-7497
IJADS 2018; 4(1): 225-227
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www.oraljournal.com
Received: 08-11-2017
Accepted: 09-12-2017

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To evaluate the effectiveness in root canal disinfection of three rotary file systems: An *in-vitro* study

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Abstract

Aim: The aim of the study was to evaluate the effectiveness in root canal disinfection in canals, infected with *Enterococcus faecalis* by using Pro Taper, Mtwo and Neoendo Flex rotary file systems.

Methods and Material: The sample size consisted of 45 distobuccal roots of maxillary molars. The canals were sterilized and bio mechanically prepared to ISO #20 and later infected with an inoculation of a culture of *E. faecalis*. After the incubation period samples were collected from these canals and counted for CFUs. According to the type of file systems used, the samples were divided into three groups (n=15). Three roots were left uncontaminated and uninstrumented which served as the control to demonstrate the sterility of the canal after sterilization. After instrumentation, the bacterial samples were again collected from the roots and evaluated for CFU count.

Statistical analysis: The data obtained from the three groups was statistically evaluated by using one way ANOVA test.

Results: All of three groups demonstrated an efficient mean reduction in CFU. Mtwo system produced a 76.68% decrease in CFUs, Pro Taper system 70.56% and the Neoendo Flex system produced a decrease of 68.83% in *E. faecalis* count. Mtwo showed higher percentage reduction than Pro Taper and Neoendo Flex. The difference between Mtwo, Pro Taper and Neoendo Flex was statistically significant ($P < 0.001$).

Conclusion: All the three systems included in the study showed a significant reduction in the number of bacteria in mechanical disinfection of root canals after instrumentation.

Keywords: Disinfection, enterococcus faecalis, colony forming unit

Introduction

In the past few years, endodontics has seen latest advances and applications of technological resources with the aim of improving the prognosis of treatment, increasingly contributing to the preservation of teeth and the orofacial structures. The main aim of endodontics is to reduce the root canal bacterial count during endodontic treatment which may be accomplished by a combination of mechanical instrumentation techniques, irrigation protocols and intracanal antimicrobial medications^[1].

Although all these procedures are important to obtain bacteria-free canals of which instrumentation is the most important one^[2].

One of the new advancement that stand out is rotary instrumentation made of nickel-titanium alloys, featuring the ability to afford a uniform preparation and that too in less time, consequently reducing work fatigue of the clinician and stress imposed on the patient. The main objective of the root canal treatment is to render the root canal system free of microorganisms, necrotic debris and, to obturate the prepared root canal with permanent root filling. Among the various procedures involved in the control of endodontic infection, irrigation also plays an important role in the elimination of microorganisms from the root canal. Irrigants are used during the endodontic treatment to flush out loose debris, to lubricate the dentinal walls, to dissolve organic matter in the canal, and to be antimicrobial^[3].

It has been demonstrated that when no irrigant is used during instrumentation, approximately 70% more debris seemed to remain in the root canals when compared with irrigated canals^[4]. Among the variety of rotary systems developed, the Pro Taper system has progressively tapered files and is comparatively faster in instrumentation resulting in preparations that are more tapered especially in the cervical and middle thirds^[5, 6].

Another system in the market is Mtwo files. It differs from Pro Taper system because of its fixed conicity. When compared with other rotary file systems, Mtwo preparations followed the original canal curvature more closely and demonstrated better cutting efficiency than others [7]. Unlike other Ni Ti systems, Mtwo follows 'single length technique' i. e. all instruments are taken to full working length from the beginning.

Bacteria can become a source for persistent infection as it can recolonize in the root canal space amongst these bacteria, *Enterococcus faecalis* is the most frequently detected species in root filled teeth with persistent lesions [8].

Enterococcus faecalis is gram positive cocci, facultative anaerobes which can withstand wide range of temperatures (i. e. 10 to 45 °C) and can even survive a temperature of 60 °C for 30 min [9].

In our study, we evaluated the effectiveness in disinfection of the preparations carried out by using Pro Taper, Mtwo, and K3 rotary file systems in canals infected with *Enterococcus faecalis*.

Materials and Methods

A total of 45 permanent maxillary human molars extracted for periodontal reasons were used in our study. The distobuccal root were selected to perform the study. They were sectioned at the level of cemento-enamel junction. The canals were instrumented 1 mm short of working length, beginning with ISO size 8 K file (Dentsply Maillefer, Ballaigues, Switzerland). upto ISO size 20 K file (Dentsply Maillefer, Ballaigues, Switzerland). During this procedure, only sterile distilled water was used for irrigation. The apical foramen of the prepared canals was sealed with cyanoacrylate. Later the roots were mounted on plaster and were then placed in 45-ml Falcon plastic tubes to be sterilized in an autoclave to ensure no residual microorganisms are present in the canal. After sterilization, the canals were contaminated by an inoculation with a culture of *Enterococcus faecalis* (ATCC 29212). Sterile ISO size 15 K file (Dentsply Maillefer, Ballaigues, Switzerland) was used to transfer the bacterial suspension throughout the canals.

The specimens were then placed in their respective tubes and incubated at 37 °C for 72 hours in an incubator. After 24 hours of incubation, the canals were filled with Tryptone Soya Broth (S. Merck) to determine the bacterial survival. After completion of incubation period, all the Roots were removed from the tubes. The canals were now filled with sterile peptonated water. Then, the microbiological samples were

collected from each canal by using three sterilized ISO size 20 absorbent points (Dentsply Maillefer, Ballaigues, Switzerland) for 10 seconds each. These paper points were stored in Eppendorf tubes containing 1ml of peptonated water for serial dilutions. For quantitative bacterial assessment, each dilution was seeded on plates containing m-Enterococcus agar medium (S. Merck), which were incubated at 37 °C for 48 hours. After this incubation period, the colony forming units (CFU) were counted.

The teeth were segregated into 3 different groups (n=15), according to the rotary system used for instrumentation: Mtwo group represented the Mtwo system. Pro Taper group represented the Pro Taper system and the Neoendo flex group represents the Neo Endo flex system. Three teeth were not instrumented and were used as the control group until the final experiment for the purpose of demonstrating the sterility of canals.

Each sterilized rotary kit was used for the preparation of not more than four canals. The canals were irrigated by using sterile distilled water that was renewed with every change of instrument. Final irrigation at the end of preparation was done with 5 ml of the same water. The cervical third was straightened with Gates-Glidden 1, 2 and 3 drills. Instrumentation with ProTaper (Dentsply-Maillefer, Ballaigues, CA, USA), Mtwo (VDW; Munich, Germany) and Neoendo Flex (Neoendo, india) system was carried out according to the manufacturer's instructions. The canals were instrumented to size 35/0.04 taper with all three systems. After instrumentation, the canals were again filled with peptonated water, and new samples were collected with sterile ISO size 20 absorbent paper points for later plating and CFU counting. The data obtained from the samples were assessed before and after instrumentation and was statistically evaluated through One way ANOVA test at a 5% level of significance.

Results

When mean percentage reduction was compared for all three groups, Mtwo showed higher percentage reduction than Pro Taper and Neo Endoflex. The difference between Mtwo, Protaper and Neo Endoflex was statistically significant ($P < 0.001$). All of three demonstrated an efficient mean reduction in CFU, in which the Mtwo system produced a 76.68% decrease in CFUs, Pro Taper system 70.56%, the and Neo Endo flex system produced a decrease of 68.83%. In *E. faecalis* count.

Group	N	Before instrumentation	After instrumentation	Percentage reduction	P value
Mtwo	15	94.22x10 ³ ±12.45 x10 ³	17.54x10 ³ ±9.76 x10 ³	76.68±2.69	0.01*
ProTaper	15	91.78x10 ³ ±9.87 x10 ³	21.22x10 ³ ±8.45 x10 ³	70.56±1.42	
Neo Endoflex	15	92.65x10 ³ ±11.98 x10 ³	23.82x10 ³ ±7.59 x10 ³	68.83±4.39	

ANOVA

Discussion

In the present study distobuccal canal of maxillary molars were included as they usually have a single root canal which is generally circular. These teeth have a comparatively high episodes of root canal infections [10].

The strain of *E. faecalis* was used for inoculation as this bacterium is associated with persistent endodontic infections and is also found to be resistant to root canal treatments [11].

These microorganisms are also resistance to Na OCl and high pH of calcium hydroxide. Even within the obturated canals, *E. faecalis* can persist for 1-12 months inside the tubules [12].

Enterococci are also involved in infections of root canal infection and are often isolated from obturated root canals showing chronic periapical infections.

These features confirm the role of *E. faecalis* in the chronic endodontic failures and may be attributed to its resistance to intracanal medicaments [13].

The apical preparation size will affect the success of endodontic therapy as smaller prepared apical diameter will render more root canal surface untouched which can have an impact on the ability to disinfect the root canal [14].

In the present study, all the canals were instrumented to ISO

35/0.04. Significant reduction in the bacterial count were seen here in canals prepared by using rotary instruments such as Pro Taper, Mtwo, and Neo Endo flex rotary systems, as found in other studies involving rotary systems, demonstrating outstanding performance of the instruments evaluated [15-18].

A significant finding of the present study was that no canal was totally free of microorganisms, which validates the existing literature and demonstrate the importance of chemo-mechanical root canal treatment protocol being followed [19, 20].

This study concludes that all three systems, Pro Taper, Mtwo, and Neoendo Flex, significantly reduce the amount of bacteria in the mechanical disinfection of the root canal system, demonstrating that they are suitable for the biomechanical preparation of the root canal system. Maximum percentage reduction in CFU count was seen with Mtwo file system which was statistically significant when compared with the protaper rotary endodontic file system. Percentage CFU reduction with Pro Taper endodontic file system was statistically insignificant with Neoendo Flex rotary endodontic file system.

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