

Comparative Evaluation of Three Rotary File Systems regarding Root Canal Disinfection: An In-Vitro Study

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ABSTRACT

Aim: The purpose of this study was to comparatively evaluate the effectiveness in disinfection of the preparations carried out by using ProTaper, Mtwo, and K3 rotary file systems in canals infected with *Enterococcus faecalis*. **Methods and Material:** The sample size consisted of 41 distobuccal roots of maxillary molars. The canals were sterilized after being enlarged to ISO #20 file and later contaminated with an inoculation of a culture of *E. faecalis*. After the incubation period samples were collected from these canals and seeded on plates for counting of CFUs. According to the type of file system used for instrumentation, the samples were divided into three groups (n=13). Two 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 seeded on plates for CFU count. **Statistical analysis:** The data obtained was statistically evaluated by using one way ANOVA test. **Results:** ProTaper system produced 80.82% reduction in CFUs, Mtwo produced 83.11%, and K3 produced a decrease of 80.14% in CFUs count of *E. faecalis*. The difference between Mtwo and K3 was statistically significant ($p < 0.021$), whereas ProTaper group did not differ significantly either from Mtwo or K3 group. **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 demonstrating that they are suitable for this purpose.

KEYWORDS: Endodontics, Disinfection, *Enterococcus Faecalis*, Colony Forming Units (CFUs)

INTRODUCTION

The main objective of any root canal treatment is to render the root canal system free of microorganisms, necrotic debris, and canal irregularities and, to fill the prepared root canal with permanent root filling.¹ Dental fraternity all over the world is constantly looking for better means to clean and shape the root canal system in less time while maintaining its original shape. One step to achieve this goal was the introduction of rotary NiTi root canal file system. During the last decade, various rotary NiTi file systems were introduced into the market. Various studies have confirmed the efficacy of nickel-titanium rotary instruments regarding the production of a well-tapered root canal form rendering them suitable for obturation, with minimal transportation of the original canal.^{2,3}

Among the variety of rotary systems developed, the ProTaper system catches the eye as this system has progressively tapered files in the same instrument and is comparatively faster in instrumentation,⁴ resulting in preparations that are more tapered especially in the cervical and middle thirds.^{5,6} Another prominent system is Mtwo, which is in the market since 2003. It differs from ProTaper 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 NiTi systems, Mtwo follows 'single length technique' i.e. all instruments are taken to full working length from the beginning.^{8,9} One more rotary file system is K3, which has radial land relief in combination with slightly positive rake angle, a flattened non cutting tip, an asymmetrical constant tapered design along with variable helical flute and core diameter. The K3 instruments are made of 55-nitinol, and have a rounded transitional angle. Twenty K3 instruments are available in sizes 15-60 having two different tapers (0.04 and 0.06) and are been recommended to be used following crown-down technique.¹⁰

Whenever suitable opportunity is there, bacteria can recolonize the root canal space, and may become a source for persistent infection. In clinically infected canals, bacteria are generally found within dentinal tubules. Amongst these bacteria, *Enterococcus faecalis* is of interest because it is the most frequently detected species in root filled teeth with persistent lesions.¹¹

Enterococcus faecalis is gram positive cocci which can represent itself as single, in pairs, or as short chains. Enterococci can grow in an environment with or without oxygen, hence are called facultative anaerobes. Vast quantities of enterococci [10^5 - 10^8 colony-forming units

How to cite this article:

Rai K, Goel M, Sachdeva GS, Verma S, Mandhotra P. Comparative Evaluation of Three Rotary File Systems regarding Root Canal Disinfection: An In-Vitro Study. *Int J Oral Health Med Res* 2016;3(1):54-57.

(cfu) per gram of feces] are present in the human intestinal lumen and are usually found to be harmless to their hosts. Enterococci are also found in human female genital tracts and oral cavity but are comparatively lesser in numbers. They can catabolize various energy sources like carbohydrates, glycerol, lactate, malate, citrate, arginine and many keto acids. Enterococci have the ability to survive adverse conditions like high alkaline pH and increased salt concentrations. They can withstand ethanol, bile salts, azide, heavy metals, detergents, and even desiccation. Enterococci can grow in wide range of temperatures (i.e. 10 to 45°C) and can even survive a temperature of 60°C for 30 min.¹²

MATERIALS AND METHODS

For the present study, 41 extracted permanent maxillary human molars were used. The distobuccal root of the selected molars was sectioned at the level of cementoenamel junction. The respective canals were instrumented 1 mm short of apical foramen, beginning with ISO size 8 K file (21mm, Dentsply-Maillefer Switzerland) upto ISO size 20 K file (21mm, Dentsply-Maillefer Switzerland). During this procedure, only sterile distilled water was used for irrigation. The apical foramen of the prepared canals was sealed with cyanoacrylate (Super Glue, Degushi). Later the roots were mounted on plaster (Dentex). The mounted roots were placed in 45-ml Falcon plastic tubes (Polylab) to be sterilized in an autoclave. After sterilization, the canals were contaminated by an inoculation with a culture of *Enterococcus faecalis* (ATCC 29212). Sterile ISO size 15 K file (21mm, Dentsply Maillefer 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. All the Roots were removed from the tubes after the completion of incubation period, and the canals were 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) 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=13), according to the rotary system used for instrumentation. ProTaper group represents the ProTaper system; Mtwo group represents the Mtwo system and the K3 group represents the K3 system. Two 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 K3 (SybronEndo, West Collins, CA, USA) system was carried out according to the manufacturer's instructions. The canals were instrumented to size 35/0.04 taper with all three systems. Due to limited size available in ProTaper system, enlargement to ISO size 35 was performed with the profile instrument 35/0.04 (Dentsply-Maillefer, Ballaigues, Switzerland). 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 K3 and ProTaper. The difference between Mtwo and K3 was statistically significant (P <0.021), however, ProTaper group did not differ significantly either with Mtwo or K3 (Table 1). All of three demonstrated an efficient mean reduction in CFU, in which the Mtwo system produced a 83.11% decrease in CFUs, ProTaper system 80.82%, the and K3 system produced a decrease of 80.14%. With regard to the positive control group, there was no detectable bacterial growth, thereby demonstrating the sterile condition of the root canal system before contamination with *E. faecalis*.

| Grp | N | Before instrumentation (CFU/ml) (Mean±SD) | After instrumentation (CFU/ml) (Mean±SD) | Percent reduction (Mean±SD) | P-value |
|-----------|----|---|---|-----------------------------|---------|
| Pro Taper | 13 | 91.61x10 ³ ±14.4x10 ³ | 17.46x10 ³ ±3.04x10 ³ | 80.80±24.74 | 0.020* |
| Mtwo | 13 | 94.15x10 ³ ±92x10 ³ | 15.84x10 ³ ±2.5x10 ³ | 83.11±26.38 | |
| K3 | 13 | 92.92x10 ³ ±10.1x10 ³ | 18.61x10 ³ ±4.31x10 ³ | 80.14±29.26 | |

Table 1- *Enterococcus faecalis* CFUs count before and after instrumentation

DISCUSSION

Distobuccal canal of maxillary molars is used in this study as they are the part of tooth group with comparatively high episodes of root canal treatments. Their selection also standardized the samples as distobuccal root usually have a single root canal which is generally circular. These teeth do have some difficulties during endodontic treatment regarding access preparation and root canal curvature.¹³

In the present study, *E. faecalis* is used as this bacterium is associated with persistent endodontic infections and is

also found to be resistant to root canal treatment protocol¹⁴ including resistance to NaOCl and high pH of calcium hydroxide. As a result of collagen's attraction, this bacterium penetrates the interior of dentinal tubules and can develop favourable conditions for survival. Even within the obturated canals, *E. faecalis* can persist for 1-12 months inside the tubules.¹⁵ Enterococci are involved in a array of infections in human beings infecting the urinary tract, biliary tract, abdomen, and endocardium. The role of enterococci in root canal infections is well known, however because the majority of initial colonizers are gram negative species, so enterococci contribute very less to initial flora. Enterococci are often isolated from obturated root canals showing chronic periapical pathology. All these evidences confirm the role of *E. faecalis* in the chronic endodontic failures and it may be attributed to its resistance to intracanal medicaments, its ability to endure prolonged starvation periods and its tendency to survive as single organism independent of the support of other bacteria.¹⁶

There is a mention in the literature regarding the apical preparation size and its relation with 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.¹⁷ In the present study, all the canals were instrumented to ISO 35/0.04. ProTaper, Mtwo, and K3 rotary systems have different characteristics with respect to their conicity, but all have shown excellent results regarding preparation and amount of time required.

Significant reduction in the bacterial count were seen here in canals prepared by using rotary instruments, as found in other studies involving rotary systems,¹⁸⁻²¹ demonstrating outstanding performance of the instruments evaluated. 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^{22,23} being followed.

CONCLUSION

This study concludes that all three systems, ProTaper, Mtwo, and K3, 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 K3 rotary endodontic file system. Percentage CFU reduction with ProTaper endodontic file system was statistically insignificant with Mtwo or K3 rotary endodontic file system.

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Source of Support: Nil
Conflict of Interest: Nil