Original Article

Apical Extrusion of Intracanal Bacteria with Single File and Multifile Rotary Instrumentation Systems

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Abstract

Aims and Objectives: Instrumentation techniques may cause extrusion of microorganisms and their products into the periapical region resulting inflammation and treatment failure. The aim of this *ex vivo* study was comparing the apical bacterial extrusion in canals prepared with single file versus multiple file rotary systems.

Materials and Methods: Ninety-two human single-rooted mandibular first premolars were used. Endodontic access cavities were prepared, and root canals were contaminated with an *Enterococcus faecalis* (*E. faecalis*) suspension. The samples were incubated at 37°C for 30 days; the contaminated teeth were divided into four groups of 20 specimens each (1: Reciproc, 2: Mtwo, 3: Neoniti A1, 4: Safesider). Six teeth were not infected and each were prepared with one of the above instruments were considered as negative and six teeth which had been previously infected, were used as positive control groups. Extruded bacteria from the apical foramen during instrumentation were collected into vials containing 0.9% NaCl. The microbial samples were taken from the vials and incubated in brain heart agar medium for 24 h. The resulting bacterial titer, in colony-forming units per mL, was determined. The data entered into SPSS 18 software and were analyzed by Kruskal–Wallis and Mann–Whitney U-tests at 0.05 significance level.

Results: Mtwo multifile system showed significantly less bacterial extrusion than Safesider (P = 0.015) and Neoniti A1 (P = 0.042) but did not show significant difference with Reciproc system (P = 0.25).

Conclusions: All instrumentation systems extruded bacteria beyond the apical foramen. However, this study showed that Mtwo multifile rotary system extruded fewer bacteria.

Keywords: *Apical extrusion, bacteria, engine-driven techniques, rotatory instruments*

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Introduction

Complete debridement of the root canal system using endodontic files and irrigating solution is essential to improve the success rate of endodontic treatment. Meanwhile, during root canal preparation, instrumentation techniques may cause extrusion of the irrigating solution, dentin chips, necrotic tissues, pulp tissue remnants, microorganisms, and their products into the periapical region resulting inflammation and treatment failure.[1,2]

Extrusion of bacteria and their products into the periradicular region can cause an acute inflammatory response and pain or flare-up after instrumentation.[2,3]

The severity of this response is dependent on the number of extruded bacteria that is a quantitative factor or pathogenicity (virulence) that is a qualitative factor[4] of these two factors, what that can be controlled by the dentist is the quantitative factor. On the other hand,

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when virulent type of pathogenic bacteria push out to the periradicular region during instrumentation even a small amount of infected debris have the potential to cause acute inflammation and pain or flare-up.

Hence, it seems logical that with reducing the amount of bacteria and their byproducts, inflammatory reactions be minimized during and after endodontic treatment.[5]

Studies have shown that all instrumentation systems extruded bacteria beyond the apical foramen[5,6] even if the preparation is shorter than apical constriction.[7-9]

Extruded bacteria from the root canal into the periapical region are Gram-positive, Gram-negative, and obligatory anaerobic bacteria[10] *Enterococcus faecalis* is one of this bacteria that was found in associated with posttreatment apical periodontitis.[10,11] and it is likely that extruded into the periapical region during instrumentation and be reside there.

Advancement in the cutting ability, surface treatment, cross-section, rake angle, tip design, and metallurgical properties of rotary files, leading to the production and supply of instruments with the ability to cut differently in various movements. Of these, researches about the single file and multifile rotary systems with different cross-sections and flute numbers in full rotation and reciprocation movements seem necessary. It is shown that instrumentation with reciprocal motion, accelerate mechanical enlargement of the root canal system.[12]

However, little information is available about the apical extrusion of bacteria in reciprocating systems compared to full-sequence rotary instrumentation; so the aim of this *ex vivo* study was to compare the apical bacterial extrusion of the canals prepared with single file versus multiple file systems in reciprocating movements and full rotation preparation.

Materials and Methods

This experimental study was conducted using simple random sampling. This study was reviewed and approved by the Ethics Committee of Zahedan University of Medical Sciences under approval letter No: 7240. A total number of 92 extracted single-root mandibular first premolars with closed apex were selected. The teeth had approximately 21 mm length, and the initial size of the apical foramen was as the same size as ≠15 k file. To ensure the single canal, digital periapical radiographs were taken buccolingually and mesiodistally. All root curvatures were in the range of 0–10 mm which was determined by Schneider method.[13]

Calcified canals and the teeth with large apical foramen were excluded from the study. Root surfaces were cleaned from debris and tissue remnants, and the samples were stored until use in normal saline at +4°C.

Access cavity was prepared with a diamond fissure bur under water and air spray with high-speed handpiece, apical patency was controlled and working length was determined with a # 15 K file one mm short of the apex.

1. Test apparatus

Kustarci *et al*.[14] method, was used for the study so that a vial with rubber stops were used for each tooth. A hole was created with a heat instrument at the center of the vial, and the tooth was inserted inside it under pressure to cementoenamel junction level. It was then fixed with cyanoacrylate glue. Two layers of nail varnish applied on the outer surface of the roots to prevent bacterial leakage of lateral canals. The apical portion was suspended in vials so the vial act as a collecting container for extruded materials from the apical foramen. The rubber stop was vented with a ≠25 needle insertion to equalize the air pressure inside and outside the vial. The whole system was sterilized by ethylene oxide gas for 12 h at 74°C

Contamination with *Enterococcus faecalis*

A pure culture of *E. faecalis* (ATCC 29212) was used to infect the root canal.

The bacteria were grown by adding 1 ml of a pure culture of *E. faecalis* (BHI-Difco, Detroit, MI, USA) in brain heart infusion broth for 24 h. Then, 0.5 McFarlane of cultured solution was used to ensure that the number of bacteria was reached to 1.5×10^8 CFU/mL.

First, a #15 K file was driven 1 mm beyond the apex to be resolved obstruction in the apical foramen. Then, each canal was filled with a solution of *E. faecalis* by a sterile micropipette and using a ≠10 K file the solution was transferred toward the apical foramen.

The vials were filled with 0.9% NaCl solution, and then, the rubber stop of the vial containing the needle was mounted. Infected roots were stored for 30 days at 37°C.

BHI of infectious canals was renewed every day till biofilm formation.

Root canal preparation

After 30 days, infected roots were randomly divided into four groups of 20 each:

Group 1: Reciproc (VDW, Munich, Germany), Group 2: Mtwo (VDW, Munich, Germany), Group 3: Neoniti A1 (Neoniti A1, France), and Group 4: Safesider, (Endo Express Safesiders Essential Dental systems, south Hackens ask, NJ). Six mandibular first premolars were not infected and each were prepared with one of the above instruments and were considered as negative

control group. To check whether the biofilm was still viable after 30 days six teeth which had been previously infected, were used as positive controls.

Instrumentation was done with VDW Silver electric motors (VDW, Munich, Germany) by one operator according to the manufacturer's instructions, below laminar hood, and under aseptic conditions to prevent airborne bacterial contamination.

Overall, 10 ml of normal saline was used for irrigation of each canal, with disposable syringes and gauge ≠27 needle. The needle was inserted into the canal until resistance was felt and irrigation was done without binding. In all groups, stainless steel ≠15 k file was used as glide path to ensure openness of the canal.

Reciproc group

Reciproc files (VDW) ≠25/0.08 entered the canal and used with slow in-and-out pecking motion without completely removing the instrument from the canal, according to the manufacturer's instructions.

The range of motion of in-and-out was not more than 3–4 mm. After three motion or when the additional force was needed to penetrate deeper, or when the penetration resistance was felt, instrument was removed from the canal and the flutes were cleaned with moistened gauze, canal patency was checked with ≠10 K file (Dentsply Maillefer) and root canal preparation continued until the instrument reached the working length.

Mtow group

Mtow files were used with standard technique (single-length technique) with small stroke brushing motion. Canal patency and glide path were controlled at the working length using ≠10 K file. Files sequences were including ≠10/0.04, ≠15/0.05, ≠20/0.06, and ≠25/0.06 to the working length.

Neoniti A1 group

The characteristics of these newly generated file are homogeneous rectangular cross-section and multiple tapers during each instrument. Full series if these files consisted of C1 and A1 with three tip size of ≠20, ≠25, and ≠40. The applied file in this study was ≠25/0.08 with 350 rpm and torque 1.5N/cm to the working length.

Safesider group

After working length determination, instruments of this system done orderly with a reciprocating motion along the working length. First ≠20 Safesider reamer was used along the working length, then ≠2 Peeso Reamer was used for flaring and creating straight line access, then the sequence of ≠25, ≠30, and ≠35 stainless steel Safesider reamer were used to the apex.

No. 40 Safesider stainless steel reamer instrumented 1 mm short of the apex, No. 2 Gates Glidden was used to further straighten and deepen the flare of the canal then the apex instrumented with No. 30/.04 and No. 25/.08 NiTi Safesider reamer.[15]

At the end of the canal preparations, 0.01 ml of NaCL solution harvested for titration of bacteria and cultured in brain heart agar and then incubated at 37°C for 24 h. Bacterial colonies were counted and were recorded in data collecting form. Data entered into SPSS 18 (SPSS Inc., Chicago, IL, USA) software and analyzed with Kruskal–Wallis and Mann–Whitney U-tests at 0.05% significance level.

Results

No growth was observed in the negative control group. All positive controls demonstrated bacterial growth after the experimental time interval. No significant differences were found in the number of colony-forming units (CFU) between Reciproc and other systems and between Safesider and Neoniti A1 ($P > 0.5$). On the other hand, Mtow group was associated with significantly less CFU than Safesider ($P = 0.015$) and Neoniti A1 ($P = 0.42$, while did not show significant difference with Reciproc system ($P > 0.05$). The mean and standard deviation of CFU are shown in Table 1, and pair comparison of instruments is shown in Table 2.

Discussion

The findings of this study indicated that all instrumentation systems caused periapical bacterial extrusion, while

Table 1: The average number of colony forming units and standard deviation of extruded bacteria in the study groups

Groups	*n*	CFU mean	SD	95% CI		Lowest	Highest
				Down	Up		
Reciproc	20	21.20	28.522	0.7965	41.603	0.00	72.00
Mtwo	20	5.20	8.791	1.089	11.488	0	28.00
Neoniti A1	20	30.70	36.086	4.885	56.514	0	91.00
Safesider	20	33.10	29.591	11.931	54.268	0	81.00

Tests=Kruskal-Wallis Mann-Whitney U-test. CFU=Colony forming units, SD=Standard deviation, CI=Confidence interval

Table 2: Pair comparison of instruments

Instruments	*P Value*
Reciproc and safe sider	0.315
Reciproc and Neoniti A1	0.393
Recipro and Mtwo	0.247
Safesider and Neoniti A1	0.680
Safesider and Mtwo	0.015*
Neoniti A1 and Mtwo	0.042*

*The differences are significant

Mtwo systems produced significantly less bacterial extrusion compared to the Safesider and Neoniti A1 files but Reciproc file. This finding is consistent with the Bruklein and shafer. They reported that Full-sequence rotary instrumentation was associated with less debris extrusion compared with the reciprocating single-file systems[16].

In contrast De-Deus et al noted that conventional multi rotary system extruded significantly more debris than reciprocating groups[5]. This difference can be attributed to the file type, number of used files during instrumentation, cutting efficacy, cutting edge and cross section.

It has shown that reciprocating motion may be produced more debris extrusion than the full rotatory systems. The reason could be that preparation with reciprocal files is significantly faster than other file which in turn may provide more debris extrusion.[15]

This difference may be related to the number of used files, so that the more number of files used, regardless of the file type, the more bacteria or debris exited coronally.

On the other hand, in canal preparation with Mtow instruments, at first, the smaller and less tapered instruments were used that it would increase the possibility of debris egress from the coronal portion of the canal. So that the file number used in the apical region in Neoniti A1 was ≠25/0/08, while the last Mtwo file was ≠25/0.06, perhaps increasing the diameter of the instrument without coronal flaring, has increased the apical extrusion of bacteria.

The results of the present study showed that Mtwo files create significantly less bacterial extrusion than Safesider, which this difference can be attributed to three factors:

1. Reciprocation systems have larger cutting angle and smaller releasing angle and flute can only push debris into the apical area
2. In Safesider systems, although the aspect of file with flat-sided design collect more debris probably some of debris remains in the canal wall and despite irrigation, extruded to the periapical area by brushing motion of the file or with the next file entry.
3. In the Safesider system, the number of applied instruments is approximately 10, that is more than the number of Mtwo system, which could possibly be the reason for debris extrusion. Although this comment is in sharp contrast with the results of the Camps and Pertot study.[17] They proposed that Safe sider instruments for possessing of flat-sided design, have a smaller cross-section and more space is created between the instruments and the canal walls. Hence, this extra space allows more debris collection that can easily be removed from the coronal portion of the canal.[17]

This study showed that Mtow files create significantly less bacterial extrusion than Neoniti A1. Neoniti A1 is a file that has not screwing effect and can be used easily and safely even in curved canals and reaches to the apical part, because of the rounded tip of the file, maintains the apical morphology and ultimately raises the success rate of root canal filling.[18] According to preliminary results, this file can be used as single file technique with continuous rotation after orifice opener.[19]

In the present study, Neoniti A1 was used as a single-instrument without the use of Neoniti C1 (orifice opener) at the apex from the beginning. It is likely that the lack of coronal flaring caused more debris pushed beyond the apex.

Robinson *et al*. compared the remaining debris in the mesiobuccal canal walls of the mandibular first molars which prepared with two single-file reciprocal and multifile full rotational systems. Their results showed that in case of presence of isthmus and protrusion, use of multifile rotary systems create significantly cleaner with less remaining debris than reciprocating systems.[20]

In this study, single-rooted premolar teeth were used which usually lacks isthmus and have more rounded cross-section. Therefore, debris and bacteria in the canal should either be removed from the coronal region or part of it be extruded to the periapical region. The results of our study showed that Mtow multifile rotary system extruded fewer bacteria than Safesider, Neoniti A1, and Reciproc; however, the difference with Reciproc system was not statistically significant.

It should be noted that unlike the *ex vivo* environment, the presence of periapical tissues in clinical conditions, created back pressure, which prevents the ingress of debris and bacteria[21] and modeling of these conditions (use of the floral foam) also have their own problems.[22] The establishment of apical patency and use of normal saline in this study, instead of sodium hypochlorite and other irrigants, make the difference with clinical conditions, so extending this results to the clinical conditions should be done with caution.

Conclusion

All instrumentation systems extruded bacteria beyond the apical foramen. However, this study showed that Mtwo multifile rotary system extruded fewer bacteria.

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research of dental school, Zahedan University of medical sciences.

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Conflicts of interest

There are no conflicts of interest.

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