



# Apical extrusion of intracanal biofilm after root canal preparation using Revo-S, Twisted File Adaptive, One Shape New Generation, ProTaper Next, and K3XF instrumentation systems

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**Objective:** To evaluate the amount of bacteria extruded apically during instrumentation using different nickel titanium (NiTi) rotary instruments.

**Methods:** Eighty extracted single-rooted human mandibular premolar teeth were inoculated with *E. faecalis* to obtain biofilm formation and the re-inoculation procedure was performed on the first, fourth, seventh and tenth days. The infected root canals were prepared using Revo-S (RS), Twisted File Adaptive (TFA), One Shape New Generation (OSNG), ProTaper Next (PTN), and K3XF instrumentation systems. The amount of apially extruded bacteria was incubated in brain heart infusion agar and it was measured in colony forming units per milliliter. Data were analyzed using the one-way analysis of variance (ANOVA) and Tukey post-hoc tests.

**Results:** The RS group caused more bacterial extrusion compared to other groups ( $p<0.05$ ). Although the OSNG and K3XF caused the less amount of bacteria extrusion than TFA and PTN groups ( $p<0.05$ ), there was no statistically significant difference between OSNG and K3XF ( $p>0.05$ ). TFA extruded more bacteria compared to the PTN group ( $p<0.05$ ).

**Conclusion:** All NiTi instrumentation systems were associated with apical extrusion of biofilm. OSNG and K3XF appear to be safer instruments in terms of biofilm extrusion compared to other instruments. The apically extruded bacteria may vary according to the instrument metallurgy, kinematics and design.

**Keywords:** Apical extrusion; biofilm; rotary files.

Root canal preparation is one of main stages of endodontic treatment. Moreover, undesirable results may occur during this important stage. One of the crucial issues which might occur during preparation is the extrusion of

bacteria, irrigants, necrotic tissues, and filling materials into periapical tissues, leading to periapical inflammation and postoperative flare-ups.<sup>[1]</sup> Specifically, more severe tissue reactions are observed when instrumentation is not

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limited within the root canal system.<sup>[2]</sup> Furthermore, the microbial colonies extruded from the root canal system to the periradicular tissues are one of the most significant causes of inter-appointment flare-ups.<sup>[1]</sup> Therefore, it is necessary to minimize the amount of apically extruded material to prevent postoperative reactions.

Microorganisms and debris remaining within the root canal system play a significant role in the outcome of endodontic treatment. Microbiological research has recently focused on resistant biofilms to elucidate the real causes of failed endodontic treatments. A commonly used definition of a biofilm is a “microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other”. In a biofilm, cell densities are significantly higher than in a planktonic culture.<sup>[3]</sup> Biofilm is the community of microorganisms that may be isolated from infected root canals. Biofilms have special characteristics such as the difficulty of elimination from root canals. As they are commonly existing structures in root canals contributing to the failure of endodontic treatment, we used biofilms similar to other recent studies.<sup>[4,5]</sup>

Various researchers emphasized the importance of extrusion of infected debris that may be incurred during the instrumentation with different preparation techniques and instruments, even when preparation is maintained short of the apical foramen.<sup>[6–10]</sup> The amount of debris extrusion may differ according to the preparation techniques and the design of the file systems.<sup>[10]</sup>

Revo-S (RS; Micro-Mega, Besancon, France) system was developed with a distinctive asymmetric cross-section intended to decrease the stress on the instrument.<sup>[11]</sup> Recently, new generations of nickel titanium (NiTi) rotary instruments with higher flexibility and greater cutting efficiency have been introduced. The Twisted File Adaptive (TFA; Sybron Endo, Amersfoort, The Netherlands) represents one of the most advanced endodontic NiTi rotary files in the market. It has 3 unique design features: the R-phase heat treatment, twisting of the metal, and special surface conditioning. These features specifically contribute to the prevention of extrusion beyond the apical foramen<sup>[12,13]</sup> and provide greater flexibility.<sup>[14]</sup>

Another recently introduced root canal single file preparation system is One Shape New Generation (OSGN; Micro Méga). The OSNG file has an asymmetric cross-sectional geometry and longer pitch that ensure an optimal and improved cutting action in three zones of the root canal.<sup>[13]</sup> This design of OSNG file may increase the available volume for upward debris elimination and downward movement may offer effective apical progression.<sup>[14]</sup>

K3XF (SybronEndo) is the next generation of K3 (SybronEndo) instrumentation system. The manufacturer claims that K3XF provides clinicians with the basic features of the original K3 plus an extraordinary new level of flexibility and resistance to cyclic fatigue with the proprietary R-phase technology. These features of K3XF file were found to be effective in decreasing the extrusion of bacteria.<sup>[15]</sup>

The ProTaper Next instruments (PTN; Dentsply Maillefer, Ballaigues, Switzerland) are made from M-wire that is claimed to improve file flexibility and resistance to cyclic fatigue whilst retaining cutting efficiency.<sup>[16,17]</sup> These new generation instruments incorporate a snake-like swagging movement as the file advances into the root canal. This design of file may help to result in less debris extrusion from the apical constriction. A survey of the literature shows that few studies have evaluated the efficacy of these NiTi systems on extrusion of intracanal biofilms.

In light of this information, the purpose of current study was to investigate the extrusion of biofilms during instrumentation of root canals using RS, TFA, K3XF, PTN, and OSNG nickel titanium (NiTi) rotary systems.

## Materials and methods

### Tooth selection and preparation

The study was approved by the Local Ethics Committee on Human Research of Cumhuriyet University (2014-10-21). Eighty extracted human single-rooted mandibular premolar teeth were used for this study. Criteria for tooth selection included a single root canal, no visible crown and root caries, fractures, or cracks, no signs of internal or external resorption or calcification, a completely formed apex, and a curvature  $<5^\circ$ . The teeth were cleaned of debris and soft tissue remnants and were stored in physiological saline solution at  $+4^\circ\text{C}$  until usage.

Endodontic access cavities were prepared using diamond (Endo Access Bur; Dentsply Maillefer) with a high-speed hand piece under water cooling. The pulp chambers were accessed and the canal patency was then established with a size 10 K-file (Dentsply Maillefer). Canals that were patent to greater than International Standards Organization (ISO) size 15 were discarded<sup>[18]</sup> and eighty teeth were finally selected. To ensure standardization and obtain a reference point, both cusp edges of each tooth were flattened and all teeth was standardized to 19 mm. A size 10 K-file (Dentsply Maillefer) was introduced into the canal until the file tip was observed at the apical foramen. The working length (WL) was determined by subtracting 1 mm from this measurement.

### Test apparatus

A previously described method was used<sup>[19,20]</sup> for the experimental evaluation. The vials with rubber stoppers were adjusted for use by using a heated instrument to create a hole through the center. A hole was created on each stopper and a 25-G needle was inserted alongside the stopper to equalize the air pressure inside and outside the tubes. Then, each stopper with the tooth and the needle was attached to its Eppendorf tube, and the tubes were fitted into vials. The entire apparatus was handled only by the outer vial. In no case was the inner Eppendorf tube touched with fingers. All vials were covered with aluminum leaf to prevent the operator from viewing debris extrusion during the instrumentation phase. All teeth were coded and then randomly assigned to 5 groups of 16 specimens each.

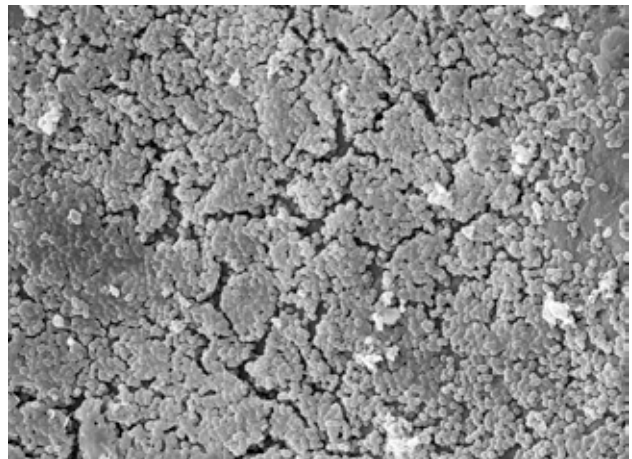
The entire model system was sterilized in ethylene oxide gas for a 12-h cycle using the anprolene and 74°C gas sterilizer (Andersen Products Inc., Haw River, NC, USA).

### Contamination with *E. faecalis* Biofilm

*E. faecalis* (ATCC 29212) strains were cultured on blood agar (Brain-heart infusion agar, Acumedia Manufactures, Inc., Lansing, Michigan, USA) and were incubated at 37°C for 24 h. Before each experiment, 0.5 McFarland turbidity was set with a kristalspec™ device. Then sub-culturing was performed on Trypticase soy broth (Detroit, Michigan, USA) and the strains were incubated aerobically at 37°C for 24 h. The turbidity of *E. faecalis* culture was adjusted to No. 0.5 Mc Farland Standard. The value of 10 µl of bacterial suspension (final concentration of about  $1.5 \times 10^8$ ) were transferred to the mechanically expanded lumen of the root canal using a sterile micropipette except 10 canals to be used as negative controls and then kept at 37°C for 24 h. The access cavities were then sealed with Cavit™ temporary filling material (3M ESPE, Dental products, USA). All samples were stored at 37°C for 10 days in a humid atmosphere and the re inoculation procedure was repeated every 72h with fresh culture at first, fourth, seventh and tenth days. The obtained biofilm is shown in Figure 1.

### Experimental groups and instrumentation procedures

One operator, using aseptic techniques performed the canal preparation and sampling procedures on each specimen under a Class I laminar airflow cabinet to prevent airborne bacterial contamination. Engine-driven instruments were used according to the manufacturer's instructions. The instrumentation sequences used were as follows:



**Fig. 1.** Stereomicroscopic image of developed *E. faecalis* biofilm in a root canal.

### Revo-S (RS) Group

The RS files (Micro-Mega, Besancon, France) were used with a torque-controlled electric motor (VDW Silver; VDW GmbH, Munich, Germany) at a rotational speed of 300 rpm and 0.6 Ncm torque. SC1 (size 25, .06) was used to enlarge the coronal two-thirds of the root canal. Then, SC2 (size 25, .04 taper), SU (size 25, .06 taper), AS30 (size 30, .06 taper), and AS35 (size 35, .06 taper) were used at the WL.

### Twisted File Adaptive (TFA) Group

TFA (SybronEndo, Orange, CA, USA) instruments were used with the TFA program of their motor in a sequence of ML1 (size 25, .08) and ML2 (size 35, .06). ML1 and ML2 instruments were taken carefully into the canal until the WL was achieved, using a Sybron Elements motor (SybronEndo, Orange, CA, USA) selected in the adaptive motion. Each instrument in TFA technique was moved in apical direction using an in-and-out motion of about 3 mm in amplitude with a light apical pressure.

### One Shape New Generation (OSNG) Group

A 'new generation' OSNG file (Micro-Mega, Besancon, France) (size 25, .06) was used with in-and-out movements without pressure at a rotational speed of 400 rpm and 2 Ncm torque at the WL, using a torque-controlled electric motor (VDW Silver; VDW). Then, OSNG Apical 1 (size 30, .06 taper) and 2 (size 37, .06 taper) were used at a rotational speed of 400 rpm and 1 Ncm torque at the WL.

### K3XF Group

K3XF instruments were used with a gentle in-and-out

motion at a rotational speed of 400 rpm using a Sybron Elements motor (SybronEndo, Orange, CA, USA). K3XF orifice opener file (size 25, .10) was used one half of the WL. Then, the other K3XF files (size 25, .04, size 25, .06, size 30, .06 taper, and size 35, .06 taper) were used at the WL.

### ProTaper Next (PTN) Group

PTN instruments were used with a gentle in and out brushing motion at a speed of 300rpm with light apical pressure. For optimum usage, torque control device was at 2 N cm<sup>-1</sup>. All instruments were used to full working length. The instrumentation sequence was used as follows: X1 (size 17, 0.4 taper), X2 (size 26, 0.6 taper), X3 (size 30, 0.7 taper), and X4 (size 40, 0.6 taper) instruments at the WL.

All systems were used in a sequence of the file recommended by the manufacturer for each specific system used. The total amount of distilled water solution used for each instrumentation system was 10 ml; by using a syringe and a 29-gauge double-side port NaviTip irrigation needle (Ultradent, South Jordan, UT, USA). The irrigation needle was placed as deep as possible into the canal without resistance until 1 mm short of the WL. The flutes of the instrument were cleaned after each removal from the root canal of the instrument. Canal patency was checked using a size 10 K- file. All root canal preparations were completed by a single operator.

### Evaluation of apically extruded bacterial

Evaluation was performed by a second examiner who was blinded to group assignment. Paper points were placed in the root canals before instrumentation to control the

evaluation of biofilm formation. The extruded bacteria were detected in the vial. Then, the CFUs incubated in brain heart infusion agar were calculated.

### Statistical analysis

The variation data for the irrigation solutions were analyzed using SPSS statistical software (SPSS 22.0, SPSS Inc., Chicago, USA). The data were subjected to statistical analysis among the five different groups using one-way ANOVA. Tukey's post-hoc test was applied when significant differences appeared, in order to examine pairwise differences at a significance level of 0.05.

### Results

The mean and standard deviations of apically bacteria extruded in each group is shown in Table 1. The results of the one-way ANOVA test indicated that RS group extruded significantly more bacteria than all other groups ( $p < 0.05$ ). TFA group caused statistically more bacteria extrusion significant differences when compared with OSNG, K3XF, and PTN ( $p < 0.05$ ). Although PTN produced significantly less bacteria extrusion than RS and TFA, it caused more bacteria than OSNG and K3XF ( $p < 0.05$ ). There was no statistically significant difference between OSNG and KXF groups ( $p > 0.05$ ).

### Discussion

Various root canal instruments and irrigation systems have been produced and assessed in terms of their debris and microorganisms extrusion. Because the apical extrusion of debris has a deleterious effect on the prognosis of root canal treatment. Seltzer, et al.<sup>[2]</sup> indicated that a quiescent chronic inflammatory periapical lesion may give a violent

**Table 1.** Mean (Standard Deviation) together with their statistical comparisons, minimum and maximum values were obtained from all groups

Groups	Mean (SD) (CFU mL <sup>-1</sup> )	Minimum (CFU mL <sup>-1</sup> )	Maximum (CFU mL <sup>-1</sup> )
Group 1 Revo-S	27.80 (4.98)	20.00	36.00
Group 2 Twisted File	23.80 (3.24)	18.00	30.00
Group 3 One-Shape New Generation	16.35 (2.94) <sup>a</sup>	13.00	26.00
Group 4 K3xf	17.15 (3.41) <sup>a</sup>	10.00	23.00
Group 5 Protaper Next	20.45 (2.93)	16.00	27.00

By the one way ANOVA,  $F=35.385$ ;  $p=0.000$  ( $p < 0.05$ ).

Groups with the same letter (a) was not significantly different at  $p > 0.05$  by Tukey's Test.



inflammatory reaction after the initiation of root canal treatment. When these factors are taken into account, extrusion of intracanal material plays a significant role in the overall success of endodontic treatment.

In a biofilm, cell densities are significantly higher than in planktonic culture.<sup>[3]</sup> Therefore, most biofilm cells have higher levels of secondary metabolites, waste products and secreted factors when compared with planktonic bacteria. Moreover, biofilms have lower nutrient and oxygen limitation.<sup>[21]</sup> Additionally, biofilms are known to express genes different from planktonic cells. They are much more metabolically active than planktonic cells in the stationary phase. Biofilms are also difficult to remove from root canals and serve as important factors in failed endodontic treatments. For these reasons, we used biofilms to assess apical extrusion similar to previous studies.<sup>[4,5]</sup>

Endodontic engine-driven NiTi instrumentation systems have recently been redesigned in an attempt to perform more efficient root canal treatments. One of these systems, K3XF, has been investigated in terms of debris extrusion. Garlapati, et al.<sup>[15]</sup> evaluated the number of apically extruded bacteria during root canal preparation using 4 rotary instrumentation techniques. Although less bacterial extrusion was found in the K3 group, the maximum amount of extruded bacteria was determined with Mtwo. Furthermore, H K S, et al.<sup>[22]</sup> evaluated the apical extrusion of intracanal debris during root canal instrumentation using hand instruments, ProTaper, K3, and LightSpeed LSX. They found no statistically significant difference among the tested NiTi instruments. In another study, Nagaveni, et al.<sup>[23]</sup> evaluated the weight of debris and volume of irrigant extruded apically after endodontic instrumentation using ProTaper Universal, HeroShaper, RaCe, and K3. All of the tested instrumentation systems caused measurable apical extrusion of debris and irrigants. HeroShaper, K3 and RaCe systems produced less extruded debris and irrigant than the ProTaper system. Madhusudhana, et al.<sup>[24]</sup> assessed apical debris and irrigant extrusion using ProTaper, K3, Mtwo systems. All instrumentation techniques produced significant amount of extruded debris and irrigant. Moreover, no statistically significant differences were found between the tested NiTi systems. K3XF caused small amounts of bacteria to be extruded and no significant difference was noted among the K3XF and OSNG systems. This result may be associated with configurational differences between K3XF and OSNG like blades, flutes, helical angle and pitch shapes that provided a variable design along the cutting part. Although in the present study, there were a few variations such as the number of inoculated bacteria,<sup>[15]</sup> the type of instrumentation systems used,<sup>[22,23]</sup> and irrigation solu-

tion,<sup>[24]</sup> the results showed similarity to the above-mentioned studies.<sup>[15,22-24]</sup>

Recently, TFA has been examined in few studies in terms of extrusion. Capar, et al.<sup>[13]</sup> compared the amount of apically extruded debris with ProTaper Universal, PTN, TFA and HyFlex. The TFA and PTN systems extruded significantly less debris than the ProTaper Universal and HyFlex systems. The current study examined and revealed biofilm extrusion during TFA instrumentation. Although, only the TFA systems was used with adaptive motion, a significant amount of biofilm extrusion was observed in this group. The adaptive motion incorporates both reciprocating and rotational motion. Some studies indicated that; rotational motion was associated with less debris extrusion compared to reciprocating motion<sup>[25,26]</sup> The greater amount of extrusion observed with TFA may be explained with the reciprocating component when compared with rotational motion systems such as K3XF, OSNG and PTN in present study.

Yeter, et al.<sup>[27]</sup> compared the weight of apically extruded debris using K-files, and the RS system. Both hand and rotary instrumentation resulted in extrusion of debris beyond the apical foramen. No significant difference was observed between K-files and RS system. RS system has not been evaluated in terms of bacterial extrusion, so far. In the present study, RS file caused more bacterial extrusion compared to the other groups. This may be due to asymmetric triangular cross section with flutes, helical angle and pitch triple helix of RS. In the present study, OSNG caused less amount of bacterial extrusion than all other instrumentation systems. This result may be attributed to the variable, asymmetric and off-centered cross section resulting in an asymmetrical rotary motion. Inherited design provides the following advantages such as; reduced screw-in-effect, reduced torsion, less resistance and stress along the length, minimal cyclic fatigue along the file, easy curvature negotiation, better apical control, and increased debris elimination. Moreover, Mittal, et al.<sup>[28]</sup> compared the amount of apically extruded bacteria with manual, ProTaper Universal and One Shape systems. They concluded that multi-file ProTaper Universal system extruded significantly more bacteria than single-file One Shape system. Similarly, according to the results of present study, single-file system OSNG caused less bacterial extrusion compared to multi-file K3XF, PTN and RS systems, although there was no statistically significant difference between OSNG and K3XF. Another NiTi file that was evaluated by few researchers is PTN. Kirchhoff, et al.<sup>[29]</sup> evaluated the amount of apically extruded debris in flat-oval root canal systems using different instrumentation systems. They observed no significant difference amongst the PTN, WO, and TFA.

Capar, et al.<sup>[13]</sup> compared the amount of apically extruded debris with new endodontic rotary nickel-titanium instruments. PTN instrumentation systems were associated with less debris extrusion compared with other systems. Üstün, et al.<sup>[30]</sup> evaluated the apical extrusion of debris associated with several root canal preparation systems. The Wave One system extruded less debris compared to the TFA and PTN systems. In the present study, a small amount of bacterial extrusion was noted with the PTN system. The offset rectangular mass and eccentric axis of rotation with swaggering motion of PTN may account for such a result. As a result, although PTN extruded less bacteria than RS and TFA, PTN produced more biofilm when compared with OSNG and K3XF.

### Conclusions

All NiTi instrumentation systems were associated with apical extrusion of bacteria. OSNG and K3XF can be preferable as a safer instrument in terms of bacteria extrusion compared to tested other instruments.

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The authors deny any conflicts of interest related to this study.

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