

Apical extrusion of intracanal biofilm using ProTaper Gold, WaveOne Gold, Twisted File Adaptive, OneShape New Generation and K3XF

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ABSTRACT

Objective: To evaluate the bacterial extrusion during instrumentation with different nickel titanium (NiTi) engine-driven instruments.

Methods: Ninety extracted single-canal human mandibular incisor teeth were inoculated with *Enterococcus faecalis* to obtain biofilm formation and were randomly divided to 6 groups ($n=15$). One group served as the control and was not instrumented; the other groups were prepared with ProTaper Gold (PTG; Dentsply Maillefer, Ballaigues, Switzerland), WaveOne Gold (WOG; Dentsply Maillefer), Twisted File Adaptive (TFA; SybronEndo, Orange, CA, USA), One Shape New Generation (OSNG; MicroMega, Besancon, France), and K3XF (SybronEndo) instruments. Bacteria extruded beyond the apical foramen were quantified in colony-forming units per milliliter. The number of colony-forming units in the remaining biofilm was determined for each sample. Data were analyzed using the one-way analysis of variance (ANOVA) and Tukey *post-hoc* tests.

Results: All NiTi instruments resulted in different quantities of bacterial extrusion. The TFA group caused most bacterial extrusion ($p<0.05$). The PTG and WOG groups caused less bacterial extrusion than the OSNG and K3XF groups ($p<0.05$), but there was no statistically significant difference between the PTG and WOG groups ($p>0.05$).

Conclusion: PTG and WOG are preferable system in terms of successful endodontic treatments. The amount of bacterial extrusion is associated with the metallurgy and design of the instrument used.

Keywords: Apical extrusions, Gold rotary systems, Biofilm

INTRODUCTION

Success of endodontic treatment basically depends on various important factors such as diagnosis, access preparation, cleaning and shaping, control of microbial populations. Therefore, the microbiological research has gained crucial role to achieve the elimination of resistance microorganisms such as biofilms during endodontic treatments (1). Biofilm layer has higher cell densities compared with planktonic culture (1). Biofilm is the community of microorganisms that may be isolated in infected root canals. Moreover, biofilms can show strong antimicrobial tolerance through four mechanisms; the physical barrier properties of the extracellular polysaccharide matrix (2), slow growth of bacterial cells residing within a biofilm (3), deep locations of cells that stay alive under decreased oxygen tension (4) and highly resistant phenotypic states (5). These important features indicated that; the elimination of biofilms from root canal are quite difficult so they can simulate the clinical conditions in vivo. Due to these superior properties, we preferred biofilm formation of *E. faecalis* bacteria as used in recent studies (1,2).

The ProTaper Gold (PTG, Dentsply Maillefer, Ballaigues, Switzerland) system is a new type instrumentation system obtains a triangular cross section and a variable progressive taper. This progressively tapered design that serves to improve cutting efficiency and safety provides more durability for ProTaper Gold system. Furthermore, its convex triangular cross-section enhances cutting action while decreasing rotational friction between the blade of the file and dentin. PTG was manufactured with proprietary metallurgy as gold wire. This new production method allows greater flexibility and resistance to cyclic fatigue (3,4). WaveOne Gold (WOG, Dentsply Maillefer) is another new

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generation of reciprocating files. The manufacturers of WOG claim that their technology boosts cyclical fatigue resistance and reduces the screwing effect. Manufacturers also put forth that, the design of WOG has also been optimized to increase cutting efficiency. Moreover, various superior properties of WOG as the gold thermal metallurgy, an optimized cross section, tip diameter and taper provide a greater flexibility and resistance to cyclic fatigue. Additionally, the WOG is offered in four sizes to cover a wider range of canal morphologies.

The other recently introduced root canal single file preparation system is One Shape New Generation (OSNG; Micro Méga). The OSNG instruments have an asymmetric cross-sectional geometry and longer pitch that ensure an improved cutting properties (5). This design of OSNG file may increase the amount of extruded debris and downward movement may offer a effective apical progression (5).

K3XF (SybronEndo) is next generation of K3 (SybronEndo) instrument system. Producers assert that K3XF is manufactured with R-phase technology ensure clinicians superior flexibility and resistance to cyclic fatigue. These features of K3XF file was found effective to decrease the extrusion of bacteria (6).

The present study evaluated and compared the extrusion of bacteria occurred during instrumentation of root canal using ProTaper Gold, WaveOne Gold, Twisted File Adaptive, One Shape New Generation, and K3XF nickel titanium (NiTi) rotary instruments.

MATERIALS AND METHODS

Tooth Selection and Preparation

Informed consent was obtained from the patients before the study and the study was approved by the Local Ethics Committee on Human Research of Cumhuriyet University (2015-10-21).

In the present study, one hundred and thirty single-rooted mandibular permanent incisor teeth were freshly extracted for orthodontic or periodontal reasons. Criteria for tooth selection included no visible root caries, fractures, or cracks, no signs of internal or external resorption or calcification, a completely formed apex, and a curvature $<5^\circ$, in accordance with Schneider's criteria (7). Periapical radiographs (Schick Tech. Inc., Long Island City, NY, USA) were taken in the buccolingual and mesiodistal directions to select only teeth with single root single canal and oval shaped root canals – long/short cross section diameter ratio of ≥ 2.5 , at 5 mm from the apex (8). Then, ninety teeth which were selected according to these criteria was included in the study and cleaned of debris and soft tissue remnants and were stored in physiological saline solution at +4 C until required. The access cavities of ninety mandibular incisor teeth were prepared and the pulp chambers were accessed. Then a size 10 K-file was used to establish the canal patency (Dentsply Maillefer). The root canals working



Figure 1. The imaging of test apparatus using during root canal instrumentation

length and canal width was standardized as 15 and 1.5 mm respectively. The samples that greater than size 15 in terms of International Standards Organization (ISO) were discarded (9) and ninety teeth were finally selected.

Test Apparatus

Firstly, a hole was opened through the center of the rubber stopper of a glass vial. One tooth was inserted into this center and fixed at the cemento-enamel junction. The equalization of the air pressure inside and outside the tubes was provided with a 24-gauge needle that was placed alongside the rubber stopper. Sterilized glass vials were entirely filled with 10 mL of 0.9% saline solution (NaCl). Then, each stopper together with a tooth and needle was placed to its Eppendorf tube, and the tubes were fitted into vials (10). The test apparatus was shown in figure 1. The standardization of foramen and patency was achieved with using a sterilized size 10 K-file that was placed 1 mm beyond the foramen to create a hole. 1 mm of the instrument was extruded one time during this application. All samples randomly assigned to 6 groups of 15 specimens each.

Contamination with *E. faecalis* Biofilm

The cultured *E. faecalis* (ATCC 29212) strains were subcultured (Detroit, Michigan, USA) and incubated aerobically. Using a sterile micropipette, 10 μ l of bacterial suspension (final concentration of about 1.5×10^8) were transferred to the root ca-

nal, then stored at 37 °C for 24 h. The re-inoculation process was reapplied with fresh culture on the first, third, fifth, seventh, ninth and eleventh days.

Experimental Groups and Instrumentation Procedures

Airborne bacterial contamination was prevented with aseptic techniques in a Class I laminar airflow cabinet. All rotary systems were used with torque-controlled endodontic motors (X-Smart, Dentsply Maillefer). The automatic reverse function mode was used except with the TFA. The instrumentation sequences were used with gentle and minimal pressure as follows:

Control Group

The infected root canals were no instrumentation with any rotary system.

PTG Group

PTG instruments were used for root canal instrumentation procedure. The following sequence was used: SX file (size 18, 0.10 taper), S1 (size 18, 0.10 taper) and S2 (size 20, 0.10 taper) files, F1 (size 20, 0.07 taper) file, and F2 (size 25, 0.08 taper) file (full WL). All the PTG instruments were used at 300 rpm with a torque of 3 Ncm for SX and S1 instruments, 1.5 Ncm for F1 instruments, and 2 Ncm for F2 instruments.

WOG Group

WOG Primary file (size 25, 0.08 taper) was used for root canal instrumentation procedure in a reciprocating working motion at 300 rpm with a proprietary motor (X-Smart, Dentsply Maillefer).

TFA Group

TFA (TFA motor; SybronEndo, Orange, Ca, USA) ML1 (size 25, 0.08 taper) instruments were used. The TFA motor (Sybron Endo) was set to the TFA program, which provides torque control and stability. The ML1 instrument was inserted carefully into the canal until the WL was achieved, using a motor set at the adaptive motion.

OSNG Group

Root canals were instrumented with OSNG (Micro-Mega) instruments (size 25, 0.06 taper). The instruments were worked using in-and-out movements and minimal pressure with 300 rpm 2 Ncm torque at the WL.

K3XF Group

K3XF (Sybron Endo) files (size 25, 0.04 taper and size 25, 0.06 taper) were used at the WL with a gentle in-and-out motion at a rotational speed of 300 rpm using a Sybron Elements motor (Sybron Endo).

All systems were used in a sequence of the file recommended by the manufacturer for each specific system used. The canals were irrigated with 2 mL of NaCl between each file size by using a syringe and a 29-gauge double-side port NaviTip

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

Groups	Mean (SD) (CFU mL-1)	Minimum (CFU mL-1)	Maximum (CFU mL-1)
Group 1 <i>Control</i>	33.40 (4.28) †	28.00	40.00
Group 2 <i>Protaper Gold</i>	12.85 (2.41) ‡	10.00	17.00
Group 3 <i>WaveOne Gold</i>	13.29 (2.58) ‡	11.00	18.00
Group 4 <i>Twisted File Adaptive</i>	23.20 (3.27) §	18.00	30.00
Group 5 <i>One-Shape New Generation</i>	16.40 (3.25) ¶	13.00	22.00
Group 6 <i>K3XF</i>	17.12 (2.67) ¶	14.00	23.00

By the one way ANOVA, $F=123.563$; $P=0.000$ ($p<0.05$).

Values with same superscript letter show no statistically different at $p<0.05$ by Tukey's post-hoc Test.

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 the preparations were completed by a single operator.

Evaluation of Apically Extruded Bacteria

The samples were taken from each canal to detect biofilm formation before instrumentation and to excluded the samples that was under 1.5×10^8 CFU/ml of bacterial count in each root canal. After root canal instrumentation, 0.1 ml of normal saline was taken from the experimental vials for counting of bacteria that extruded from the root canal. Colony-forming unit (CFU) counting of the bacteria were performed using blood agar plates and the results were recorded as the number of CFU/mL.

Statistical Analysis

The amount of apically extruded bacteria was analyzed statistically using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. The level of significance was set at $p<0.05$. All statistical analyses were performed with SPSS version 22.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Mean (Standard Deviation) together with their statistical comparisons, minimum and maximum values obtained from all groups are shown in Table 1. No bacterial extrusion was showed in control group ($p<0.05$). The results of the one-way ANOVA test indicated that the TFA instrumentation system indicated significantly higher number of bacterial extrusion compared with other experimental groups ($p<0.05$). Although

OSNG and K3XF instrumentation systems demonstrated no statistically significant difference ($p>0.05$), both groups resulted in a greater amount of bacterial extrusion than the WOG and PTG instrumentation systems ($p<0.05$). There was no significant difference between the PTG and WOG instrumentation systems ($p>0.05$).

DISCUSSION

Various instrumentation systems have been produced in order to reduce the extruded debris and microorganism during endodontic treatment. Because apical extrusion of microorganisms especially intra canal biofilm have high levels of secondary metabolites, waste products, and secreted factors, therefore biofilm has a harmful efficacy on the prognosis of endodontic treatments (11). In present research, single-canal mandibular incisor teeth were preferred to evaluate biofilm extrusion. This simple canal system was selected to minimize complications, non-standardized preparations, and irrigation variance that are more likely to occur in curved canals.

2 mL of 0.9% saline solution was used after every change of instrument to reduce the antibacterial effect of the irrigant and to ensure elimination of the biofilm layer due only to the mechanical action of the instruments. The saline solution was applied with passive injection to prevent debris being forced out of the apical foramen by anything other than the preparation system being tested.

Endodontic NiTi instrumentation systems have recently been redesigned to increase endodontic treatment success. One of the important reasons of post-operative pain is the extrusion of debris that obtain virulent bacteria into the periradicular tissues. If the infected debris is extruded into periapical region during root canal instrumentation, it may cause or increase the various of periradicular inflammation (12,13). Resultly, it is important to be examined the instruments in terms of bacterial extrusion during root canal preparation to prevent post-operative complications. Therefore, the thermomechanical treatment and kinematics features of instrumentations systems must be examined in terms of apical extrusion occurring during preparation. No previous study compared the effect of the PTG, WOG, TFA and OSNG instrumentation systems on bacterial extrusion.

According to the results obtained from this study, TFA group caused a greater amount of bacterial extrusion through the apical foramen than other experimental groups. Burklein et al. (14) examined the effect of several rotary instruments (Reciproc, OneShape, Mtwo and F360) in terms of debris extrusion and also reported that the Reciproc caused greater debris extrusion than other tested systems. Ghogre et al. (15) investigated the amount of intracanal bacteria extruded apically using rotary systems. They indicated larger amounts of apically extruded bacteria after instrumentation using TFA than K3XF. Türker et al. (16) evaluated the bacterial extrusion related with

various NiTi systems. Resultly, TFA instruments caused a higher amount of bacterial extrusion beyond the foramen. The aforementioned studies (14-16) demonstrated similar results as present study. Increasing cutting ability of rotary instruments is usually associated with increasing cleaning efficacy, however it may also enhance debris transportation towards the apex when used in combination with a reciprocal movement (17). Reciprocating motion, may act as a piston, extruding more debris and irrigant than OneShape instrumentation technique. While the file with continuous rotation act like a screw conveyor improving transportation of dentin chips and debris coronally (17).

Several researchers evaluated the effect of K3 instrument on bacterial extrusion during preparation after using different rotary instrumentation techniques (6,18,19). As a result of these researches, K3 may produce measurable different amounts of apical extrusion of bacteria (6,18,19). The amount of bacterial extrusion after K3XF instrumentation system was also investigated in present study. K3XF caused less bacterial extrusion than TFA, this result may related to its unique design feature of variable pitch which helps to prevent the screwing-in effect of instrument. It has increasing variable helical flute angle from tip to handle which helps to dislodge the dentin chips from working area and carried coronally to the orifice. This result shows similarity compared with the upper mentioned studies (6,18,19). This may be also explained with the different cross-sectional geometry that carried coronally the dentinal debris.

The present study also evaluated and compared the efficacy of OSNG and other systems on the apical extrusion of intracanal bacteria. At the same time, no research investigated the effect of One Shape New Generation on apical extrusion. Regarding this issue, Mittal et al. (20) assessed the bacterial extrusion by using ProTaper and One Shape rotary systems. The less amount of bacterial extrusion was detected in One Shape single-file system. Nayak et al. (21) measured the amount of debris extruded apically during instrumentation with using various rotary systems that were manufactured with different kinematics. The One Shape NiTi instrument caused less debris than Reciproc file. As similar to the above-mentioned study results (20,21), although OSNG instrumentation system caused lesser amount of bacterial extrusion than TFA using with adaptive movement, it created more extrusion than Gold systems. The space between the file and the dentinal walls can increase according to different cross-sectional geometry, carried coronally more dentinal debris. Therefore, primarily it is necessary to evaluate these differences of results in terms of file design. For instance, the OSNG opener file removes more dentin in the coronal portion than K3XF, the increased width of the coronal part of the root canal may facilitate transport of bacterial colonies toward the coronal area, thereby decreasing the amount of apically extruded bacteria. This result may be associated with the blade, flutes, helical angle, and pitch shapes of K3XF. Another possibility is that the variable pitch of K3XF increases the transport of debris

to the coronal area. The longer pitch design of the OSNG file may increase the volume of upward bacterial elimination. This result may be due to the variable and asymmetric cross section of the system. The design may provide the following advantages: reduced screw-in effect, reduced torsion, less resistance and stress along the length, easy curvature negotiation, better apical control, and increased debris elimination. Although there were a few differences such as the amount of inoculated bacteria (6), the type of instrumentation systems (18,19), and irrigation solution used during instrumentation procedures (17) compared with other similar studies, the results here are similar to other studies (6,18,19).

The WOG and PTG groups extruded the least amount of bacteria. This superior result may be explained with the assessments of metallurgy, design features and kinematics of these systems. It has been shown that heat-treated alloys have less stiffness (22) and a lower ultimate tensile strength than conventional superelastic wires (23). Both of Gold systems are produced with using different alloys and a new proprietary thermal process named Gold wire in which the ground NiTi files are heat-treated and slowly cooled to obtain super-elastic NiTi files. It could be attributed to the 2-stage transformation behaviour and the high temperatures from which PTG and WOG is produced; as this material has greater flexibility (24) with an elastic modulus lower than that of the austenitic phase (25,26). Consequently, it could be supposed that the martensitic NiTi wire may ensure a lower amount of apical extrusion at a similar torque than austenitic NiTi alloy (26). These metallurgical superior properties that provide less stiffness and reduced restoring force to the instruments (3,4) may explain the least amount of apical extrusion after instrumentation performed by Gold systems.

The design of Gold systems also play a crucial role on apically bacterial extrusion. First of all, the roundly tapered, and semi-active features of WOG reduce the mass of the center of the tip and contribute to less debris extrusion compared with all other groups. The PTG system has a different geometry; smaller dimensions, an off-centered mass, and a regressive taper. The centering ability of PTG instruments may ensure that a greater percentage of dentin thickness is retained in the root canal and may facilitate greater bacteria elimination (27). The convex triangular cross-section and progressive taper enhance the cutting efficacy of PTG, while decreasing rotational friction between the file blade and dentin (28). PTG had a significantly lower torsional resistance. The non-cutting tip design allows each instrument to safely follow the secured portion of the canal, while the small flat area on the tip enhances its ability to find its way through soft tissue and debris (29). Thus, the low transportation and canal wall contact values are other superiorities of WOG and PTG instrumentation systems. These crucial differences of WOG and PTG systems may explain the least amount of bacterial extrusion. On the other hand, although there was no statistically significant

difference between Gold systems, WOG produced less apical extrusion of bacteria than PTG. This can be explained by differences in terms of kinematic and number of file in systems. It has indicated that single-file reciprocating instruments with Gold wire metallurgy caused less bacterial extrusion (30) and debris than conventional multifile rotary systems. The results of the present study may be explained with this observation. Resultly, an increase in the number of files of NiTi instruments may also associate with an increased extruded bacteria. Under the conditions in this study, the Gold systems produced less bacterial extrusion compared with K3XF and OneShape New Generation systems during root canal instrumentation.

CONCLUSION

All instrumentation systems caused apically extruded bacteria during root canal preparation in vitro. WOG and PTG instruments were associated with less bacterial extrusion compared with the OSNG, TFA, and K3XF instruments. Under the light of this study, it can be concluded that; Gold systems may be preferred as safer to minimize the apically extruded bacteria during endodontic treatments.

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