Evaluation of Bacterial Reduction after Root Canal Shaping Using ProTaper Gold and WaveOne Gold Rotary Systems

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Abstract

Objective The present study evaluated bacterial reduction promoted by the WaveOne system (Dentsply Maillefer, Ballaigues, Switzerland) and ProTaper Gold system (PTG; Dentsply Maillefer) in human extracted central incisors.

Materials and Methods Sixty-two maxillary central incisors that were infected with *Enterococcus faecalis* (ATCC 51299) were sterilized with ethylene oxide for 21 days, and then root canal initial bacterial sample was collected with paper points and plated on M-Enterococcus agar. The specimens were randomly divided into two groups according to instrumentation: WaveOne Gold group (n = 30) and PTG group (n = 30). Each group was further subdivided into subgroup A (n = 15) where no activation of the irrigant was performed, and subgroup B (n = 15) where passive ultrasonic activation (PUI) was applied. The other two specimens without contamination were control asepsis. After instrumentation, samples were collected with the use of paper points. The bacterial reduction was calculated using colony-forming unit and quantitative real-time polymerase chain reaction. Data were collected and statistically analyzed.

Results All techniques significantly reduced the number of bacteria in the root canal ($p < 0.05$), in which PTG showed superior bacterial reduction than WaveOne Gold ($p > 0.05$). The aseptic control group did not show any bacterial growth. PUI showed a significant bacterial reduction with the WaveOne Gold group.

Conclusion It can be concluded that the single-file system, WaveOne Gold with the aid of passive ultrasonic irrigation, significantly reduce the bacterial number in the root canal similar to the multifile system, PTG.

Keywords
- bacterial reduction
- *Enterococcus faecalis*
- WaveOne Gold
- ProTaper Gold

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Introduction

One of the major objectives of root canal treatment is the reduction of bacteria flora in the root canal system. Chemomechanical instrumentation achieves a major part of this task.[1,2] Therefore, the development of chemomechanical strategies that maximize root canal disinfection before obturation is of prime importance. Many rotary nickel-titanium systems have emerged to facilitate the cleaning and shaping procedure; however, anatomic complexities might represent physical constraints that pose a severe challenge to adequate root canal disinfection. The introduction of single-file systems in comparison to multiple file systems presents another challenge among endodontists to select the system that can provide a superior bacterial reduction in the root canal.

The WaveOne Gold system (Dentsply Maillefer, Ballaigues, Switzerland), with its reciprocating motion, was shown to have a favorable performance in root canal preparation[3] and bacterial reduction.[4] However, the WaveOne system has also been shown to produce a higher amount of smear layer in the canal.[5] ProTaper Gold (Dentsply Tulsa Dental Specialties, Tulsa, Oklahoma, United States) was recently introduced as a multiple file system that is manufactured with advanced metallurgy through heat treatment technology. ProTaper Gold has two-stage specific transformation behavior and high austenite finish temperatures, which is around 55°C.[6] It consists of three shaping files (SX, S1, and S2) and five finishing files (F1, F2, F3, F4, and F5). It has triangular cross-sections and progressive tapers as ProTaper Universal (Dentsply Tulsa Dental Specialties).[7] In addition, it uses the same rotary motion and settings. However, it was found that it provides less transportation,[8] higher flexibility, and higher cyclic fatigue resistance.[6,9]

Therefore, due to the increasing interest in single-file systems, the aim of the present study was to compare the ability of a single (WaveOne) and a multiple file (ProTaper Gold) systems to reduce the bacterial flora in the root canal system. The null hypothesis tested in this study was that there is no difference in the bacterial reduction between the rotary file systems under investigation.

Materials and Methods

Sample Selection and Classification

The proposed study was approved by the institutional review board (IRB # 16–00287). Maxillary central incisors (n = 62) were collected from 30 to 50 years old patients attending the outpatient clinic of MetroHealth Medical Center Oral Health Department, Ohio, United States. The teeth were sectioned at the cementoenamel junction using a fine steel disc (NTI diamond disc, Axis Dental, United States) mounted on a high-speed handpiece with water coolant. All samples were standardized to 15 mm and instrumented to a working length (WL) of 14 mm up to 15 K-file (Dentsply Maillefer) under irrigation with sterile saline. The root canals were filled with 17% ethylenediaminetetraacetic acid for 2 minutes then washed with 5 mL sterile saline. The apex was covered with composite filling material (3M, Saint Paul, Minnesota, United States), and the root surface was covered using a bonding agent (3M). All samples were fixed onto polystyrene microtiter plates using self-acrylic resin and were sterilized using a steam autoclave (MELAG, Medizintechnik Geneststrase, Berlin, Germany) at 134°C for 15 minutes. Samples were divided into two groups: the WaveOne Gold group (n = 30) and the ProTaper Gold group (n = 30). Each group was further subdivided into subgroup A (n = 15), where no activation of the irrigant was performed, and subgroup B (n = 15) where passive ultrasonic activation (PUI) was applied. Two samples served as the negative controls to assess the sterility of teeth.

Bacterial Culture and Inoculation of Specimens for Biofilm Formation

Enterococcus faecalis (ATCC 51299) was cultured overnight in thioglycollate broth (Remel, Lenexa, Kansas, United States) under the aerobic condition at 37°C. A 0.5 McFarland Standard Solution (MSS; ~1 × 10^8 colony-forming unit [CFU]/mL) was made from this fresh overnight culture. A subsequent 1:10 dilution of 0.5 MSS with thioglycollate broth was prepared to inoculate the teeth specimens by immersing them in 3 mL of the solution and incubated at the aerobic condition at 37°C for 4 weeks. The culture medium was replaced with a fresh thioglycollate broth every other day.[10,11] Negative control samples (n = 2) were incubated in phosphate-buffered saline (PBS; pH 7.4; Life Technologies, Grand Island, New York, United States).

After incubation, positive control samples (n = 4) were randomly selected from each subgroup for scanning electron microscopy (SEM) evaluation (magnification 5000×) for biofilm formation (►**Fig. 1**) and for *Enterococcus* genus-specific quantitative real-time polymerase chain reaction (qPCR).

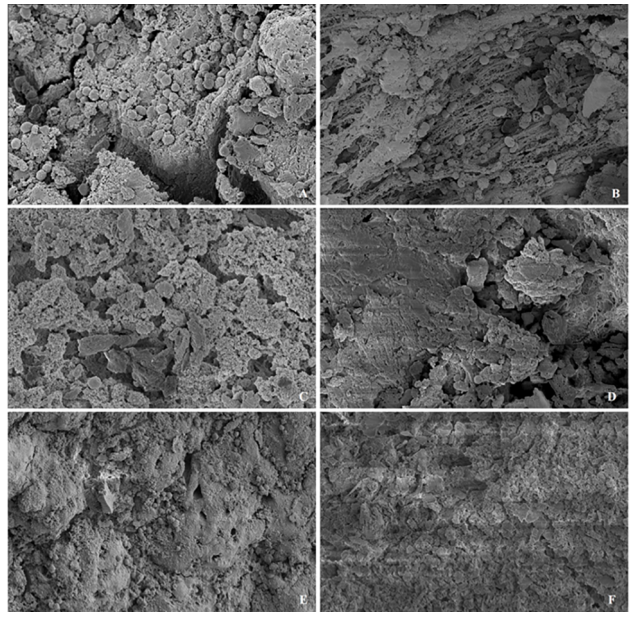

Fig. 1 (**A**, **B**) Untreated positive controls (5000×); (**C**) sample from ProTaper Gold group with passive ultrasonic activation (PUI) (8000×) showing no microorganisms; (**D**) sample from ProTaper Gold group without PUI (5000×) showing few microorganisms; (**E**) sample from WaveOne Gold without PUI (2500×) showing few microorganisms; (**F**) sample from WaveOne Gold with PUI (2500×) showing few microorganisms.

Root Canal Instrumentation

The WL was determined by passing a 10 K-type file through the apical foramen and withdrawing it 0.5 mm. Samples of the ProTaper Gold group were instrumented following the directions of the manufacturer with the following sequence: S1, S2, F1, F2, and F3. Each canal preparation was performed with a new set of instruments mounted on endodontic 6:1 reduction handpiece (Dentsply Maillefer) powered by an electric motor (Dentsply Maillefer) at 300 revolutions per minute and 2.5 Ncm. Samples of the WaveOne Gold group were instrumented following the directions of the manufacturer in a pecking motion until reaching the full WL. The WaveOne Gold files were mounted on a 6:1 reduction handpiece connected to a reciprocating motor (Dentsply Maillefer).

In subgroups, irrigation was accomplished with a 10-mL syringe and a 30-gauge needle (NaviTip). In subgroup B, irrigation was performed with an ultrasonic device (Piezon Master 400) and a stainless steel K-type file size 15 (Endosonore; Dentsply Maillefer), with its power set at the 1⁄4 of the scale. In all subgroups, a total volume of 20 mL NaOCl (1%) was delivered per canal. The flow rate in subgroup A was approximately 5 mL/min. In subgroup B, the delivery rate during the PUI with a continuous flush of the irrigant was 10 mL/min. The insertion depth of the needle and the ultrasonic file was 1 mm short of WL in all subgroups.

Microbiological Assessment

All samples were split mesio-distal into two parts. Two samples from each subgroup were selected for SEM magnification of 10,000× and 20,000×. SEM examination of positive controls showed excellent biofilm formation by *E. faecalis* (ATCC 51299) in the root canal walls after 4 weeks of incubation, and the test organism could clearly be identified based on cocci morphology in clusters (►**Figs. 1A** and **B**).

The remaining samples were sonicated for CFUs and qPCR.[12] Sonication (Branson 8800, model CPX8800H, Branson Ultrasonics, Danbury, Connecticut, United States) was performed for each sample for 5 minutes in 1 mL of PBS. A 10-µL aliquot of the sonicated fluid was plated in triplicates with blood agar (Remel) and incubated overnight at 37°C; then, the CFUs per mL were counted. The remaining sonicated fluid was stored at –70°C for qPCR assay to determine the bacterial load. Total nucleic acid was extracted from 200 µL of sonication fluid from each teeth specimen using the NucliSENS easyMAG automated extraction system (bioMerieux Inc., Durham, North Carolina, United States). The *Enterococcus* genus-specific primers targeting *tuf* gene (Ent1: 5′-TAC TGA CAA ACC ATT CT GAT G-3′ and Ent2: 5′-AAC TTC GTC ACC AAC GCG AAC-3′) were used at 1 µM concentration.[3] The qPCR was performed with a LightCycler 1.5 instruments using LightCycler FastStart DNA Master SYBR Green 1 Hot start reaction mix (Roche Molecular, Indianapolis, Indiana, United States) per manufacturer's instructions. The following thermal cycling protocol was used: 95°C for 10 minutes (preincubation), 45 cycles of 95°C for 10 seconds (denaturation), 55°C for 5 seconds (annealing), and 72°C for 25 seconds (extension), 1 cycle of melt curve with 95°C for 0 second (denaturation), 65°C for 15 seconds (annealing), and 95°C for 0 second (ramp rate = 0.1°C/s for melting), and 1 cycle of 40°C for 30 seconds (cooling). The quantification of bacterial copies was determined from the standard curve generated by testing a known number of bacterial cells using the Roche Molecular quantification procedure.

Statistical Analysis

Statistical analysis was performed using statistical analysis software SPSS (Statistical Packages for the Social Sciences 20.0; IBM, Armonk, New York, United States). One-way analysis of variance followed by Tukey's post hoc test was performed. Significance level was set at 0.05.

Results

Scanning Electron Microscopy

Among representative treated teeth specimens, two showed no viable organisms at all (one treated with ProTaper Gold without prior ultrasonic treatment, ►**Fig. 1C**, **a**nd another treated with WaveOne Gold with prior ultrasonic treatment, ►**Fig. 1F**). In general, less organism burden was observed in experimental teeth specimens compared with untreated controls (►**Figs. 1D** and **E**).

Colony Formed Units

Data collected from the CFU evaluation method is presented in ►**Table 1**. The ProTaper Gold group showed statistically superior bacterial reduction than WaveOne Gold with or without the use of PUI ($p < 0.001$). Compared with the control group, both groups under investigation showed a statistically significant reduction in CFU with or without the use of PUI ($p < 0.001$).

Table 1 Bacterial count (Log10 CFU/mL) for ProTaper Gold and WaveOne Gold with or without ultrasonic

	ProTaper Gold group	WaveOne Gold group	Positive control	*p*-Value
With no PUI	95 ± 72^{Aa}	976 ± 821^{Ba}	$5{,}010 \pm 1{,}345^{Ca}$	< 0.001
Using PUI	103 ± 39^{Aa}	436 ± 469^{Ba}	$4{,}353 \pm 1{,}290^{Ca}$	< 0.001
p-Value	0.7609	0.087	0.27	

Abbreviations: CFU, colony-forming unit; PUI, passive ultrasonic activation.
Note: Different capital letters represent statistically significant differences ($p < 0.05$) in the same row, whereas different small letters represent significant differences in the same column ($p < 0.05$).

Table 2 Quantitative PCR (PCR E+6) for ProTaper Gold and WaveOne Gold with or without ultrasonic

	ProTaper Gold group	WaveOne Gold group	Positive control	*p*-Value
With no PUI	3.06 ± 2.37^{Aa}	7.18 ± 4.33^{Ba}	50.1 ± 17.4^{Ca}	< 0.001
Using PUI	3.45 ± 2.38^{Aa}	5.44 ± 3.31^{Aa}	44.34 ± 5.8^{Ba}	< 0.001
p-Value	0.713	0.326	0.334	

Abbreviations: PCR, polymerase chain reaction; PUI, passive ultrasonic activation.
Note: Different capital letters represent statistically significant differences ($p < 0.05$) in the same row, whereas different small letters represent significant differences in the same column ($p < 0.05$).

Quantitative Real-Time PCR

Data collected from the qPCR evaluation method is presented in ►**Table 2**. The ProTaper Gold group showed statistically superior bacterial reduction than WaveOne Gold without the use of PUI ($p < 0.001$). However, there was no significant difference between both groups upon using PUI. Compared with the control group, both groups under investigation showed a statistically significant reduction in CFU with or without the use of PUI ($p < 0.001$).

Discussion

Evaluation of endodontic instrumentation includes morphometric analysis, microscopic observation of remaining debris,[13] and bacteriological assessment.[14,15] The bacterial reduction was selected for this study because of the utmost importance of canal disinfection in the treatment and prevention of apical periodontitis.

A. faecalis, facultative anaerobic Gram-positive cocci, was selected for this study because it is always involved in resistant endodontic infections, having the ability to survive under extreme environmental conditions,[16,17] which may contribute to bacterial resistance to intracanal antimicrobial procedures.[18] The specimens were infected with *E. faecalis* for 4 weeks to ensure deeper bacterial penetration into dentinal tubules,[19,20] which makes it harder to eliminate them from the infected root canal.[21]

Single-file systems have become recently available for root canal instrumentation, but evidence as to their cleaning and disinfecting abilities is still questionable.[3,22]

The present quantitative results showed that chemomechanical preparation promoted a highly significant reduction in intracanal bacterial count irrespective of the system used for preparation.[14,15,23,24]

Real-time PCR was used as a quantification method in this study, because of its high sensitivity and the ability to detect and quantitate not only cultivable bacteria but also culture-difficult species, and noncultivable.[24]

The use of single-file instrumentation is thought to compromise the disinfection ability. This is because of the claimed simplification of the preparation process hence a decrease in the amount of antibacterial irrigant being used, combined with a shorter time in the canal.[25] This was confirmed in this study, in which significant reduction was observed in favor of the multifile system, ProTaper Gold without the use of activation method. However, the use of PUI as an activation method resulted in a significant reduction in the case of the single-file WaveOne Gold, and hence there was no significant difference between the single and multifile system in that case. These findings were consistent with the conventional CFU counting method. The minor conflict was noticed between both quantification methods in case of absence of activation method as there was no difference in bacterial reduction between the single and multifile systems in CFU count while there was a significant difference in the PCR quantification method. This conflict might be attributed to the fact that dead bacteria that remained in the canal with their deoxyribonucleic acid made it detectable in PCR but not cultivable using the CFU counting method.

Under the conditions of this study, the two instrumentation systems used, single and multifile techniques, significantly reduced the bacterial counts. However, multifile techniques showed better antibacterial results compared with a single-file technique without the use of any activation methods. The use of PUI dramatically improved the antibacterial performance of single files. The use of activation methods is recommended with single-file techniques.

Conflict of Interest

None declared.

References

1 Siqueira JF Jr, Lima KC, Magalhães FAC, Lopes HP, de Uzeda M. Mechanical reduction of the bacterial population in the root canal by three instrumentation techniques. J Endod 1999;25(5):332–335
2 Nakamura VC, Cai S, Candeiro GTM, Ferrari PH, Caldeira CL, Gavini G. Ex vivo evaluation of the effects of several root canal preparation techniques and irrigation regimens on a mixed microbial infection. Int Endod J 2013;46(3):217–224
3 Bürklein S, Hinschitza K, Dammaschke T, Schäfer E. Shaping ability and cleaning effectiveness of two single-file systems in severely curved root canals of extracted teeth: Reciproc and WaveOne versus Mtwo and ProTaper. Int Endod J 2012;45(5):449–461
4 Machado MEL, Nabeshima CK, Leonardo MFP, Reis FAS, Britto MLB, Cai S. Influence of reciprocating single-file and rotary instrumentation on bacterial reduction on infected root canals. Int Endod J 2013;46(11):1083–1087
5 Dietrich MA, Kirkpatrick TC, Yaccino JM. In vitro canal and isthmus debris removal of the self-adjusting file, K3, and WaveOne files in the mesial root of human mandibular molars. J Endod 2012;38(8):1140–1144
6 Uygun AD, Kol E, Topcu MKC, Seckin F, Ersoy I, Tanriver M. Variations in cyclic fatigue resistance among ProTaper Gold, ProTaper Next and ProTaper Universal instruments at different levels. Int Endod J 2016;49(5):494–499

7 Bayram HM, Bayram E, Ocak M, Uygun AD, Celik HH. Effect of ProTaper Gold, self-adjusting file, and XP-endo shaper instruments on dentinal microcrack formation: a micro-computed tomographic study. J Endod 2017;43(7):1166–1169
8 Gagliardi J, Versiani MA, de Sousa-Neto MD, Plazas-Garzon A, Basrani B. Evaluation of the shaping characteristics of ProTaper Gold, ProTaper NEXT, and ProTaper Universal in curved canals. J Endod 2015;41(10):1718–1724
9 Plotino G, Grande NM, Mercadé Bellido M, Testarelli L, Gambarini G. Influence of temperature on cyclic fatigue resistance of ProTaper Gold and ProTaper universal rotary files. J Endod 2017;43(2):200–202
10 Liu Y, Guo L, Li Y, Guo X, Wang B, Wu L. In vitro comparison of antimicrobial effectiveness of QMix and other final irrigants in human root canals. Sci Rep 2015;5:17823
11 Baca P, Mendoza-Llamas ML, Arias-Moliz MT, González-Rodríguez MP, Ferrer-Luque CM. Residual effectiveness of final irrigation regimens on Enteroccus faecalis-infected root canals. J Endod 2011;37(8):1121–1123
12 Ke D, Picard FJ, Martineau F, et al. Development of a PCR assay for rapid detection of enterococci. J Clin Microbiol 1999;37(11):3497–3503
13 Schäfer E, Erler M, Dammaschke T. Comparative study on the shaping ability and cleaning efficiency of rotary Mtwo instruments. Part 1. Shaping ability in simulated curved canals. Int Endod J 2006;39(3):196–202
14 Shuping GB, Ørstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod 2000;26(12):751–755
15 Siqueira JF Jr, Alves FR, Almeida BM, de Oliveira JC, Rôças IN. Ability of chemomechanical preparation with either rotary instruments or self-adjusting file to disinfect oval-shaped root canals. J Endod 2010;36(11):1860–1865
16 Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32(2):93–98
17 Portenier I, Waltimo TM, Haapasalo M. Enterococcus faecalis-the root canal survivor and "star" in post-treatment disease. Endod Topics 2003;6(1):135–159
18 Vivacqua-Gomes N, Gurgel-Filho ED, Gomes BPFA, Ferraz CC, Zaia AA, Souza-Filho FJ. Recovery of Enterococcus faecalis after single- or multiple-visit root canal treatments carried out in infected teeth ex vivo. Int Endod J 2005;38(10):697–704
19 Weiger R, de Lucena J, Decker HE, Löst C. Vitality status of microorganisms in infected human root dentine. Int Endod J 2002;35(2):166–171
20 Haapasalo M, Ørstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res 1987;66(8):1375–1379
21 Chivatxaranukul P, Dashper SG, Messer HH. Dentinal tubule invasion and adherence by Enterococcus faecalis. Int Endod J 2008;41(10):873–882
22 de Carvalho FMA, Gonçalves LC de O, Marques AA, Alves V, Bueno CE da S, De Martin AS. Cleaning effectiveness of a reciprocating single-file and a conventional rotary instrumentation system. Open Dent J 2016;10(1):704–713
23 Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 1997;30(5):297–306
24 Rôças IN, Siqueira JF Jr. In vivo antimicrobial effects of endodontic treatment procedures as assessed by molecular microbiologic techniques. J Endod 2011;37(3):304–310
25 Alves FR, Rôças IN, Almeida BM, Neves MAS, Zoffoli J, Siqueira JF Jr. Quantitative molecular and culture analyses of bacterial elimination in oval-shaped root canals by a single-file instrumentation technique. Int Endod J 2012;45(9):871–877