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ORIGINAL ARTICLE

Comparison of Apical Extrusion of Bacteria After Glide Path Preparation Between Manual K File, One G Rotary, and WaveOne Gold Glider Reciprocation Preparations

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ABSTRACT

Objective: To compare the amount of apically extruded bacteria between hand-filed preparations, rotary and reciprocation glide path preparations in curved canals of extracted teeth infected with *Enterococcus faecalis*.

Methods: Forty mandibular first molar teeth were decoronated, fitted into rubber stoppers and fixed onto glass vials. The mesiobuccal canals from mandibular first molar teeth were infected with *Enterococcus faecalis*, then randomly assigned to one of five groups for glide path preparation: manual stainless-steel file (K-files), rotary file (One G), reciprocating file (WaveOne Gold Glider) and two control groups. After glide path preparation, 0.01 mL of saline was taken from the experimental vials. The solution was plated on tryptic soy agar and colonies of bacteria were counted as colony-forming units. The results were analysed statistically using Kruskal-Wallis and post hoc Mann-Whitney U tests.

Results: The manual K-file group was associated with significantly more bacteria extrusion compared to the rotary and reciprocating groups ($P<0.05$). However, no significant difference occurred between rotary and reciprocation instruments.

Conclusion: All instrumentation techniques resulted in a measurable amount of apical extrusion of bacteria. Manual K-files extruded the highest quantity of bacteria compared to One G rotary file and WaveOne Gold Glider reciprocation file during glide path preparation.

Keywords: Bacterial extrusion, debris extrusion, glide path, root canal preparation

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HIGHLIGHTS

- All instrumentation techniques resulted in a measurable amount of apical extrusion of bacteria.
- Manual K-files extruded the highest quantity of bacteria compared to One G rotary file and WaveOne Gold Glider reciprocation file during glide path preparation.

INTRODUCTION

During root canal preparation of endodontically infected teeth, debris containing bacteria is always extruded beyond the apical foramen (1, 2). The extruded bacteria may be associated with periapical infection and flare-ups (3, 4), post-endodontic pain (4) and could eventually compromise the long-term outcome of root canal treatment (5). The intensity of the host inflammatory response is mainly based on the quantitative factor, the microbial counts, and the qualitative factor, the virulence of bacterial species (4). When virulent clonal types of bacterial species are extruded apically, even a small quantity of infected debris can cause or exacerbate periapical tissue inflammation (3, 4). The clinician does not have control over the qualitative factor because it is related to the composition of the root canal bacterial communities. However, the clinician can control the quantitative factor (number of bacteria) by selecting appropriate instruments and techniques to minimise apical extrusion (2). The number of bacteria extruded apically depends on the number, design, and kinematics (rotary vs reciprocation) of instruments and instrumentation techniques (1, 2, 6-8).

A glide path is a smooth tunnel from the root canal orifice to the physiological terminus of the root canal system (9). A glide path helps prevent taper lock, reduces instrument fracture, canal transpor-

tation, and maintains the canal centring ability (10, 11). Glide paths can be created by hand files or engine driven instruments (rotary/ reciprocating). It has been reported that creating glide paths using manual techniques are difficult and time-consuming, especially in curved canals (12), whilst nickel-titanium rotary instruments have been associated with safe, fast and predictable glide path preparations (11, 13). Creating a glide path before root canal preparation reduces the quantity of debris extruded apically in curved root canals (14). Interestingly, Alves et al. (7) concluded that the apical extrusion of bacteria was more frequent compared to the extrusion of tissue debris after root canal preparation. They found no relationship between the volume of debris and the number of extruded bacteria (7). Consequently, it can be argued that the investigation of the effect of glide path systems on the apical extrusion of bacteria is more important than the extrusion of debris.

One G (MicroMega, Besançon, France) is a single rotary instrument with an asymmetrical cross-section and has an apical size of 0.14 mm and a constant taper of 3%. In addition, the file has three cutting edges, allowing better debris elimination (15). The WaveOne Gold Glider (Dentsply Sirona, Ballaigues, Switzerland) is a single reciprocating glide path instrument manufactured using a gold heat treatment technology. It has a tip diameter of 0.15 mm, a progressive variable taper of 2-6%, a parallelogram horizontal cross-section with two cutting edges (16).

No studies have been conducted to compare the apical extrusion of bacteria when using reciprocating, rotary or manual glide path instruments in curved canals of extracted molar teeth infected with *Enterococcus faecalis*. Hence, the objective of the current study was to compare the apical extruded bacteria between manual (stainless steel hands file), rotary (One G) and reciprocation (WaveOne Gold Glider) glide path file in curved canals in a laboratory setting.

MATERIALS AND METHODS

Specimen selection

The study design was approved by the institutional review board on research and ethics of the International Medical University, Malaysia (BDS I-01/2019 (17)). Forty mandibular first molars extracted for various clinical reasons were used. To avoid the introduction of confounding variables, only teeth that satisfied the following criteria were included:

i) teeth with moderately to severely (10° to 30°) curved mesial roots, with curvature defined by Schneider method (17),

ii) mesial roots with two separate mesial canals and two separate apical foramina,

iii) canals negotiable until the apical foramen, and

iv) canals with an initial apical size equivalent to a size 10 K-file.

Specimen preparation

Under continuous saline irrigation, a diamond disc was used to decoronate the teeth 3 mm above the level of the cementoenamel junction. A reservoir for infection of the root canal was created by partially maintaining the crown structure. Only the mesiobuccal root canals were used in the current study. The mesiobuccal canal was located and the working length was determined with a size 10 K-file (MicroMega) by extruding the file beyond the apical foramen then subtracting 1 mm from the length.

Test apparatus (Fig. 1)

Glass vials (10 mL) with rubber stoppers were autoclaved and punched with a hole in the centre. The teeth were also autoclaved. Each tooth was fixed with Parafilm (Sigma-Aldrich Co, St. Louis, MO, USA), and two coats of nail varnish were used on the tooth's external surface to ensure the set-up was leak-proof. All the experiments were performed in an aseptic condition under a BSL II laminar flow hood (Bioair Safemate, Italy). The apical one-third of the root was suspended within the glass vial. During glide path preparation, any material extruded apically were collected in the glass vial with saline (18).

Contaminating the specimens with *E. faecalis*

E. faecalis (ATCC 29212) was cultured in brain heart infusion agar (BHIA) (Oxoid, Hampshire, UK). A single colony from the agar plate was inoculated in 10 mL of BHI broth and grown overnight, this suspension was adjusted to 0.5 McFarland standard, which approximately has $1.5X10^8$ colony-forming units (CFU)/mL, this suspension was used for inoculation for bacterial biofilm formation in each root canal, the medium was replenished after every 2 days for 30 days. The entire setup was incubated at 37°C.

Glide path preparation

Forty teeth were randomly assigned to five different groups as follows:

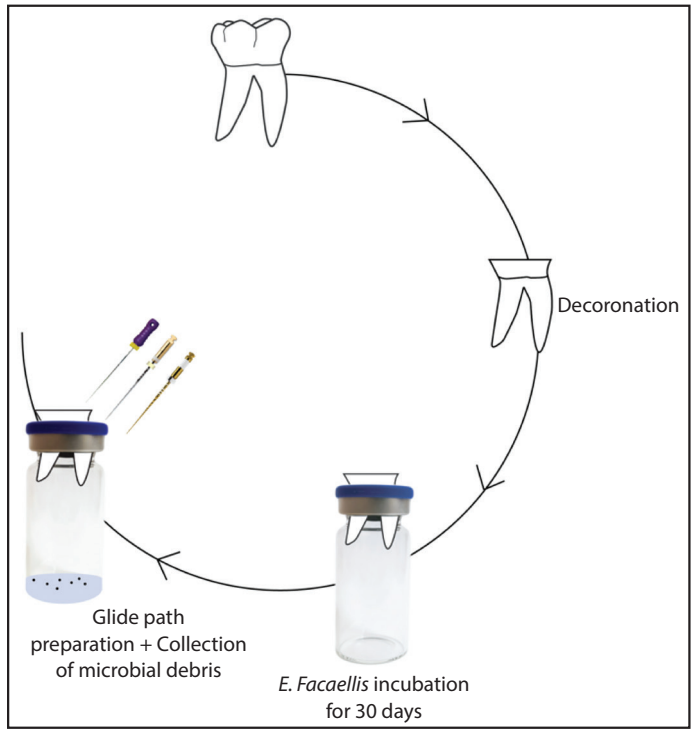

Figure 1. Schematic diagram showing the methodology of this study

Group 1(n=10): Sizes 10, 15 and 20 K-files were introduced sequentially into the root canal to the working length using the balanced force motion (18, 19).

Group 2 (n=10): The One G rotary glide path instrument was operated with an endodontic motor in continuous rotation at 300 rpm and torque of 1.2 Ncm, according to the manufacturer's instructions.

Group 3 (n=10): The WaveOne Gold Glider reciprocating glide path instrument was used in the "WAVEONE ALL" mode of the VDW Reciproc Gold endodontic motor according to manufacturer instructions.

Group 4 (n=5) (Negative Control): Canals were not infected, but glide path preparation was respectively performed with one of the three systems for each tooth sample. The glide path for 2 specimens was created with One G, another 2 specimens with WaveOne Gold Glider and 1 specimen with the K-files.

Group 5 (n=5) (Positive Control): Canals were infected but no glide path was created.

Each instrument was used to prepare only three root canals. A total volume of 6 mL 0.9% NaCl solution were used for each root canal as an irrigating solution. The NaCl solution was delivered into the root canal using a syringe with a 30-gauge stainless steel irrigation needle (Ultradent, Utah, USA) placed passively up to 3 mm from the apical foramen without binding. Apical patency of the root canal was assessed using a size 10 K-file after each instrument. To avoid operator variability, all glide path preparations were performed by a single operator.

Cultivable bacterial counts

Using aseptic techniques, preparation of the glide paths and sampling of the debris were carried out by a single operator within a laminar flow cabinet to prevent microbial contamination. The apically extruded debris was collected in the vials. After the canal preparation, 0.01 mL of NaCl was taken from the experimental vials and serially diluted (1/10), and 100 microlitres of the samples were plated on BHIA. The plates were incubated at 37°C for 18 – 24 hours. The colonies of bacteria were then counted and recorded as colony forming units (CFU).

Statistical analysis

Statistical analysis was performed using SPSS Version 21.0 software (IBM Corp, Armonk, NY). The data were statistically analyzed using Kruskal-Wallis analysis and post hoc Mann-Whitney U test. Statistical significance was set at 95%.

RESULTS

The mean and standard deviation of CFU for the three experimental and two control groups are shown in Table 1. All instruments used to create glide path were associated with an apical extrusion of bacteria. A significant difference occurred in the CFU among the three groups. The manual glide path with K-files had the largest number of extruded bacteria compared to rotary and reciprocating techniques ($P<0.05$). No significant difference was observed in the CFUs between rotary and reciprocating glide path techniques. No growth was found in the negative control group. The least growth was found in the positive control group.

TABLE 1. The mean and standard deviation (SD) of extruded bacteria in colony-forming units (CFU)

Groups	Mean±SD (CFU mL)
K-file	$(3.14\pm1.73)\times10^{7a}$
One G	$(1.92\pm0.39)\times10^{6b}$
WaveOne Gold Glider	$(1.84\pm0.99)\times10^{6b}$
Control (Negative)	0.00^{c}
Control (Positive)	$(3.20\pm0.68)\times10^{4d}$

Different superscript letters between groups indicate significant differences ($P<0.05$)

DISCUSSION

Glide path preparation is highly recommended before using rotary instruments in narrow and curved root canals to reduce the risk of instrument separation by torsion (9, 10, 13, 14, 19). Even though the quantity of extruded bacteria during glide path preparation is lesser than during root canal shaping, the bacterial content extruded is more likely to irritate the periapical tissues (2, 4, 20). The intensity of the inflammatory response of the tissues is dependent on the virulence of the bacteria (4, 20). During instrumentation, bacterial extrusion occurs more commonly compared to debris extrusion and no association was found between the volume of extruded debris and bacterial count extruded (7). Therefore, in the present study, the quantity of extruded bacteria was studied rather than the extrusion of debris following different instruments to establish a glide path.

According to the current study results, all glide path preparation techniques resulted in apical extrusion of bacteria. The K-files showed a significantly higher amount of bacterial extrusion compared to rotary and reciprocating files. This result is consistent with the study by Ferraz et al. (21), who reported that engine-driven instruments extruded lesser debris compared to hand files. The probable reason could be due to the rotatory motion of engine-driven instruments that directs the debris towards the orifice than the apex (21). The balanced force technique for manual glide path preparation was used in the current study as it produces a lesser amount of extrusion compared to other techniques. A push-and-pull filing motion would act as a piston pushing debris through the apex, but a balanced force technique would direct the debris towards the coronal orifice (21). Due to stiffness and push-pull motion with minimal rotation, the glide path preparation with K-files resulted in more debris extrusion compared to rotary instruments while creating a glide path (19). This may also correlate with postoperative pain, as shown by Pasqualini et al. (22), where postoperative pain was lesser and resulted in faster resolution of symptoms with rotary instrumentation as compared to manual glide path instruments.

Apart from the kinematics, the size, cross-sectional design and taper of the instruments used in the current study could also influence the results (14, 19, 23). One study showed that a larger taper might result in higher debris extrusion because of the

greater preparation into the dentinal walls (14). The off-centre design with a 3% taper of One G might help debris removal towards the coronal direction (19).

To the knowledge of the authors, to date, no studies have investigated the apical extrusion of bacteria between reciprocating and rotary single glide path instruments in curved root canals. The results of our study showed that no statistical difference was observed in the amount of bacteria extruded between rotary and reciprocation instruments. A systematic review by Ahn et al. (24) showed conflicting results between the studies that compared the rotary and reciprocation instruments in apical extrusion of debris during canal shaping. The other reason for this difference could be due to the difference in methodology and curvature of the root canals (2). Further studies are needed to support the results of our study.

In our study, we selected *E. faecalis* as a bacteriologic marker because it is easy to grow, colonise and can form biofilms inside the root canals under laboratory conditions. In addition, its association to persistent endodontic infections/root canal failure has been established (25, 26). Other bacteria associated with endodontic infections might need symbiotic support from other bacteria (27). In few studies, *E. faecalis* was grown for 24 hours (3), whereas in the current study, a 30-day *E. faecalis* biofilm growth model was used. The reason is that the mature biofilm (30 days) is a complex structure, which is very difficult to eradicate (28, 29). Likewise, in this study, mesiobuccal canals with apical foramen equivalent to size 10 K-file were selected, and a size 10 K-file was used before using glide-path files. This is important to provide consistency and helps to compare the systems fairly (20). Previous studies showed that extrusion of debris would be more when instrumentation was performed to the apical foramen compared to 1 mm short preparation (30, 31). This is the reason for selecting a working length 1 mm short of the apical foramen in the current study. The working length was adjusted to 13 mm to standardise the depth of shaping and the penetration of irrigants. This attempt was made to exclude a potential effect of varying working length on the apical extrusion of the bacteria (18). A 30-gauge irrigating needle was used in all specimens to maintain uniformity. Our study used 0.9 % NaCl (saline) as an irrigating solution as it has no antimicrobial property. This ensured that bacterial elimination and extrusion was due to the mechanical action of the instruments. Antimicrobial solutions like sodium hypochlorite were not used because the extrusion of the bacteria will not be detected as it eradicates the bacteria (18, 29), and the extrusion of sodium hypochlorite to the vial would deter the bacterial growth (8).

This study has limitations in translating the results to clinical practice due to the following reasons: saline was used as an irrigant when generally antimicrobial agents will be used during cleaning and shaping. In addition, the condition of the pulp or periapical tissues acts as natural barriers that could limit the apical extrusion. The study also lacked simulated periodontal tissues, which generally provides natural back pressure (15, 32) and absence of back pressure, affecting the quantity of debris extrusion (15). It has been suggested that floral foam could be used to simulate the periodontal ligament, however, it might absorb the debris and irrigation solution which affects the experiment results (15, 20, 30). For the above reason, no attempt was made experimentally to mimic the periapical tissues in the current study.

CONCLUSION

All instrumentation techniques resulted in a measurable amount of apical extrusion of bacteria. However, manual K-files extruded the highest quantity of bacteria compared to One G rotary file and WaveOne Gold Glider reciprocation file during glide path preparation.

Disclosures

Conflict of interest: The authors deny any conflict of interest.

Ethics Committee Approval: The study design was approved by the institutional review board on research and ethics of the International Medical University (IMU), Malaysia (BDS I-01/2019 (17)).

Peer-review: Externally peer-reviewed.

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