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Original Research

Quantitative Analysis of Biofilm Removal Following Instrumentation with TRUShape and Vortex Blue File Systems: Microbiological Study

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Abstract

Mesial roots and isthmuses of mandibular molars are difficult areas to obtain adequate disinfection of root canal walls, and consequently microorganisms can survive treatment. The present study compared, through real-time polymerase chain reaction (qPCR), the effectiveness of TRUShape (TS) (Dentsply Tulsa Dental Specialties, Tulsa, OK) and Vortex Blue (VB) (Dentsply Tulsa Dental Specialties, Tulsa, OK) in removing *Enterococcus faecalis* (*E. faecalis*) from the mesial canals and isthmuses of mandibular molars. Fifty extracted human lower molars were inoculated with *E. faecalis* OG1RF for 14 days, and then an initial bacterial sample was collected with paper points from mesiobuccal and mesiolingual canals and isthmuses. The specimens were randomly divided into four groups (n = 10 teeth; 20 canals each), according to instrumentation system: TS 25/0.06, TS 30/0.06, VB 25/0.06 and VB 30/0.06. The remaining 10 teeth were divided between positive control, inoculated teeth without instrumentation or irrigation, and negative controls, teeth without inoculation. After instrumentation, the final sample was taken using paper points and DNA was isolated. Primers specific for *E. faecalis* were used for qPCR. The bacterial reduction between pre- and post-instrumentation was calculated. One-way analysis of variance (ANOVA) with Bonferroni's multiple-comparisons tests were for statistical analysis with significance of ($p < 0.05$). All file systems were able to reduce the load of *E. faecalis* from the prepared root canals, however, TS size 30 removed significantly more bacteria than size 25. Interestingly, regardless of the size, TS files removed significantly more *E. faecalis* biofilm ($p < 0.05$) than did VB files (63.7% vs 50.8% for size 25, and 69.5% vs 56% for size 30). In conclusion, when combined with irrigation, TS file system is more effective than VB in reducing *E. faecalis* biofilms from mesiobuccal and mesiolingual canals and the isthmuses of mandibular molars.

Keywords: biofilms; dental pulp cavity; *enterococcus faecalis*; root canal treatment; trushape; vortex blue

1. Introduction

Successful root canal treatment requires the elimination of pulp tissues, microbial biofilm, and bacterial byproducts from the root canal system [1]. Mechanical debridement is effective in bacterial reduction; however, due to the complexity of the canal system, such as isthmuses, ramifications, and lateral canals, 35–50% of the canal space remains uninstrumented and thereby associated with microbial remnants [2–4]. Mesial roots of mandibular molars commonly contain isthmuses in the apical 3–5 mm, with 22% having complete isthmuses and 37% having partial isthmuses [5,6]. Bacteria or infected tissue left in these spaces can be a cause of persistent periapical lesion and root canal failure [2,7]. The Gram-positive bacterium *E. faecalis* is a natural inhabitant of the gastroenterological tract of humans and is commonly associated with hospital acquired illnesses as well as endodontic infections [8]. Moreover, *E. faecalis* can survive root canal treatment due to its ability to invade dentinal tubules and to withstand harsh environments such as nutrient deprivation, and tolerance to disinfectants and high pH [9]. In addition, it develops resistance to antibiotics, so it has become a major threat in hospital settings [10,11].

The chemomechanical preparation of the root canal system is the most important phase for bacterial elimination in endodontic treatment [12]. Currently, there are several file systems with different properties in relation to the type of alloy, cutting efficiency, and shaping ability. TS system (Dentsply Tulsa Dental Specialties, Tulsa, OK) consists in an S-shape design with four different tips (20, 25, 30, 40) and taper 0.06 in the apical 2 mm [13]. This unique design allows greater adaptation to the canals various shapes. According to the manufacturer, this system creates an envelope of motion that can predictably better disrupt biofilms allowing for bacterial reduction. Another instrumentation system is the VB rotary files (Dentsply Tulsa

Dental Specialties, Tulsa, OK). This system contains a distinctive color with a visible titanium oxide layer, as a result of new processing of NiTi wire [14]. The manufacturers claim that VB has a 42% higher peak torque strength increase over M-Wire NiTi. VB system works in a rotational, non-reciprocating movement. Both systems tested are made of NiTi.To date, there are no *ex vivo* studies in the literature comparing with through quantitative analysis, the ability of mechanical instrumentation with TS and VB in bacteria removal. Thus, the purpose of our study is to compare the effectiveness, by real-time PCR, of TS and VB in removing *E. faecalis* from the mesial canals and isthmuses of mandibular molars.

2. Materials and Methods

2.1 Teeth Selection and Specimen Preparation

Sample size calculation was based a commonly used effect size of (=1) for endodontic file systems in extracted teeth with power of 80% [15]. Our experimental unit selected for our experiment was a tooth. The One-way ANOVA statistical test (G*Power 3.1 for Macintosh; Heinrich Heine, Universität Dusseldorf, Dusseldorf, Germany), with effect size = 1, $\alpha = 0.05$ and $\beta = 0.95$ suggests a minimum total sample ideal size of 24 teeth.

Fifty human permanent mandibular molar teeth were collected with patients' consent from adult patients requiring extractions under a protocol approved by the University of Florida Institutional Review Board, Gainesville, Florida (201500591). Teeth were stored at 6% NaOCl until use. All selected teeth had a mesial root curvature of 30° to 40° and closed apices. Specimens were examined under the microscope and digital imaging to confirm the curvature using the Bramante technique [16]. The crown of each tooth was removed at the level of the cementoenamel junction (CEJ) to maintain a standardized length of 12 mm on each mesial root and to facilitate better visualization of the presence of isthmuses communicating both canals. Apical patency was confirmed in each canal using #10 stainless steel K-type file (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA). Mesiobuccal (MB) and mesiolingual (ML) canal lengths were recorded to confirm a consistent working length of 12 mm. Teeth were autoclaved and mounted in tissue culture plates with polyvinylsiloxane, so the apices were blocked to simulate the occlusion of the tissue, PDL, and not allow free flow of irritants out the apex (Fig. 1).

2.2 Bacterial Inoculation

Gates Glidden drills size # 1 and # 2 (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) were used 4 mm from the CEJ, to provide enough flare for biofilm inoculation. 1x10e7 E. faecalis OG1RF were inoculated into the sterilized canals of each tooth using a sterile syringe and a 30-gauge irrigation needle (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA), except for the negative controls that received Brain Heart Infusion broth (BHI) media (Millipore Sigma, St. Louis, MO, USA) only. After inoculation, teeth were kept upright in 18-well plates containing BHI and incubated at 37 °C in a 5% CO_2 for 14 days follow our lab protocol [17].

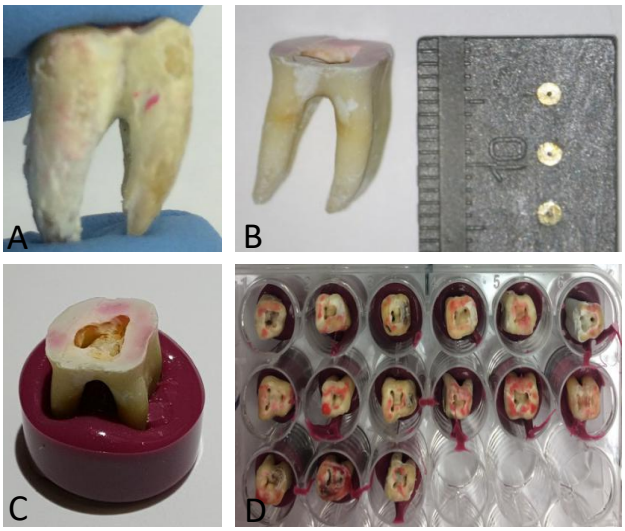

Fig. 1. Visual demonstration for the teeth samples. (A) Extracted tooth after occlusal reduction. (B) Demonstration of measurement of the tooth length and canal curvature. (C) The prepared sample embedded in polyvinylsiloxane. (D) 18-well plate used for samples incubation.

2.3 Root Canal Instrumentation

Mesial canals were instrumented with two different file systems (TS and VB). The irrigation technique for all samples was done using a 30 gauge Maxi-probe needle with an up and down motion of the needle stopping 3–4 mm short of the working length. The irrigation protocol was sodium hypochlorite 8.15%, and final irrigation was performed with 5 mL of Qmix® (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) using EndoActivator (Dentsply Sirona, York, PA, USA). Teeth were divided into 4 experimental groups (10 teeth; 20 canals each) and 2 control groups (5 teeth, 10 canals each) as follows: Group I: TS size 25/0.06; Group II: TS size 30/0.06; Group III: VB size 25/0.06; Group IV: VB size 30/0.06; Negative control: Teeth without inoculation; Positive control: Inoculated teeth without instrumentation or irrigation following our previous protocol. Each system was used to instrument the lingual and buccal canals of the mesial root of the corresponding tooth in the assigned group. According to recent study, Niti files like TS and VB experience and increased cyclic fatigue at 37 °C, when compared to 20 °C [18]. Thus, instrumentation was carried at room temperature of 25 °C. Procedure was performed by a calibrated single endodontic resident.

2.4 Bacterial Sample Collection

Prior to instrumentation, a sterile fine paper point was placed in each canal (MB and ML) and isthmuses for 10 seconds, for bacterial sample collection and quantification. The three paper points were then transferred into sterile Eppendorf tubes containing 1 mL of sterile sodium-magnesium buffer (pH 7.4) after which bacterial DNA was extracted and analyzed by qPCR to determine the initial bacterial load. Following instrumentation and irrigation, a post-treatment sample was also collected in a similar method for further processing. The time of paper point placement and method of sample collection was constant throughout all groups to prevent any variation. All samples were collected in triplicate and the average bacterial load was calculated.

2.5 Real-Time Polymerase Chain Reaction (qRT-PCR)

Total DNA was harvested with a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA), according to manufacturer instructions. Then, the presence of *E. faecalis* was quantified by qRT-PCR using primers specific for *E. faecalis* [19] in a 50 μL reaction containing, 25 μL of SYBR Green Super Mix (BioRad, Hercules, CA, USA), 23 μL of DNA and 0.2 μM of each primer. Then, DNA was amplified in the CFX-96 Real Time machine (BioRad, Hercules, CA, USA) using the following settings: 95 °C for 4 min, then 45 cycles of 95 °C for 1 min, 55 °C for 45 sec. Melting curve analysis was performed after amplification to confirm the specificity of the amplified reaction. Data were normalized by 16S rRNA. Expression values of *E. faecalis* were determined pre- and post-instrumentation, and data were expressed as a percentage reduction in the expression of *E. faecalis* post-instrumentation compared with pre-instrumentation. All steps were performed in a laminar flow hood under sterile environment.

2.6 Statistical Analysis

Statistical analyses were performed with GraphPad Software (GraphPad Software Inc., La Jolla, CA, USA), where Kolmogorov Smirnov test was used to test for normal distribution and to account for multiple comparisons, a one-way analysis of variance (ANOVA) with Bonferroni's multiple-comparisons test was used to determine significance ($p < 0.05$ was considered significant). All error bars represent standard deviation (SD). The null hypothesis in this study is that there is no significant difference in *E. faecalis* when either of the file systems was used.

3. Results

All inoculated teeth allowed for growth and amplification of *E. faecalis*. There was no significant difference in the bacterial load between each sample prior to instrumentation. Absence of detectable DNA in the negative control teeth following qRT-PCR insured complete elimination of *E. faecalis* in the teeth canal systems prior to inoculation. The bacterial loads from teeth treated with VB or TS as determined by qRT-PCR were compared to those from the positive control teeth, which were not subjected to instrumentation or irrigation. Treatment with each of the file systems resulted in a significant decrease in bacterial load, though the reduction was greater for those teeth treated with TS (Fig. 2). Expectedly, for both file systems, a larger size file resulted in enhanced reduction of bacterial biofilm. For example, VB 25/0.06 reduced *E. faecalis* by 50.8% $\pm$ 3.21 compared to 56.0% $\pm$ 3.71 reduction observed by 30/0.06 ($p = 0.0138$). Similarly, TS with size 25/0.06 reduced bacterial load by 63.7% $\pm$ 2.53 while size 30/0.06 resulted in 69.5% $\pm$ 2.32 ($p = 0.0074$) (Fig. 2).

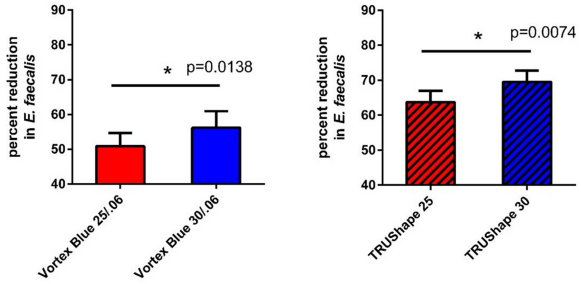

Fig. 2. Percentage of *E. faecalis* reduction after instrumentation by either VB or TS file systems. Error bars represent standard deviation. The number 25/0.06 and 30/0.06 represent the file size and taper respectively ($p < 0.05$).

Furthermore, the two file systems were compared. For the same file size and taper, TS showed significantly enhanced reduction in *E. faecalis* as compared to the VB counterpart (63.7% $\pm$ 2.53 vs 50.8% $\pm$ 3.21 for size 25, and 69.5% $\pm$ 2.32 vs 56.0% $\pm$ 3.71 for size 30) ($p < 0.0001$) (Fig. 3). Overall, TS size 30/0.06 removed about 70% of the inoculated *E. faecalis,* thereby the most effective file size and system tested in this study (Fig. 3).

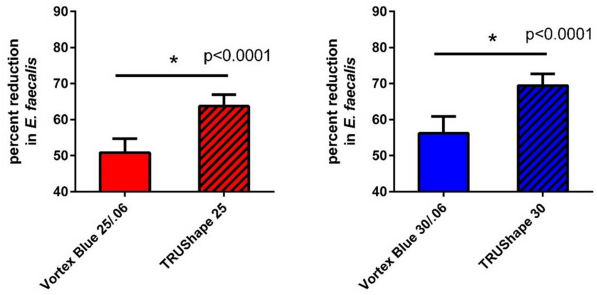

Fig. 3. Percentage of *E. faecalis* reduction after instrumentation by either 25/0.06 and 30/0.06 sizes of VB or TS file systems. Error bars represent standard deviation ($p < 0.0001$).

4. Discussion

In this study, we found that TS file system has removed more *E. faecalis* from the canal system of the mesial root of the mandibular molar compared to VB file system. *E. faecalis* was chosen as a model microorganism because of its high prevalence in endodontic infections, and because several previous studies have used the microbial reduction of *E. faecalis* as a way to analyze different instrumentation techniques and irrigation protocols [4,12,20–22]. *E. faecalis* plays a major role in endodontic infections due to its ability to form resistant biofilms, invade the dentinal tubules, compete with other microorganisms and low nutrient requirements [8,9,23]. Moreover, *E. faecalis* has a remarkable ability to withstand chemomechanical procedures of root canal cleaning and shaping for both treated and untreated teeth [20,21]. It can also survive intracanal calcium hydroxide-based medications because of the presence of a proton pump that allows pH homeostasis [23]. Although the maximum microbial reduction was approximately 70%, which differs from some studies that found a reduction of 100% [22,24,25], this difference can be explained by difference on methodologies. The studies that achieved complete eradication used culture methods for bacteria detection [22,24,25], while our study used qPCR, which is a more sensitive and strategic method to detect and quantify specific targeted bacterial genes [9].

The present *ex vivo* study was performed to compare the ability of TS and VB file systems to remove *E. faecalis* from mesial roots and isthmuses of mandibular molars. This type of tooth was selected due to its high complexity, which makes it a common challenge for adequate endodontic cleaning, shaping, and disinfection. Studies have demonstrated that many areas of the root canal remain unistrumented after chemomechanical preparation, regardless of the instrumentation technique used, and these sites can be a safe harbor for bacteria and their toxins [4,26]. These untouched areas often retain pulp tissue remnants and bacteria [26] that can perpetuate the endodontic disease, thus compromising the success of the treatment. In addition to the anatomical complexity of the mesial canals, the isthmuses may be present in 54–64% of the cases [27,28]. Isthmus configuration may not be affected by mechanical instrumentation, and disinfection depends mainly on the action of the irrigation solutions. It has been reported that apical diameter of the mesial root canals of mandibular first molar were in the range of 0.24–0.33 mm, so files with apical sizes of 0.25 and 0.30 are the commonly used finishing file sizes [29]. Sodium hypochlorite is the most commonly used root canal irrigant during chemomechanical preparation, and can enter small areas that the instrument cannot reach, thereby improving disinfection [30,31].

The use of a file with a design that adapts to the anatomical irregularities of the root canal in conjunction with irrigating solutions is an effective method for eliminating, or at least reducing, the microbial load, thus contributing to treatment sucess. The irrigation protocol used in the present study was sodium hypochlorite, due to its potent antimicrobial activity and capacity of dissolution of organic tissues [11,32]. Qmix® associated with sonic irrigation (EndoActivator) was chosen as the final irrigation solution due to its effective antimicrobial action against *E. faecalis* [33,34].

The present study demonstrated that TS produced greater microbial reduction in comparison with VB, independent of the tip size (25 or 30). This finding is in agreement with another study, also using species of *E. faecalis*, which found that TS was able to remove more bacteria from the root canal when compared to Twisted files [35]. In a recent cone beam computerized tomography (CBCT) study comparing the two systems, it was found that TS can reach a greater area of the canal surface [36]. A viable explanation for this reduction is possibly related the S-shape design of the file, which creates an envelope of motion allowing the file to adapt to the anatomical irregularities of the canal system at smaller apical sizes. TS allows for lower apical transportation and greater dentin conservation than VB [13], and does not induce the formation of new dentin microcracks [37]. Therefore, the TS file design supports the concept of minimally invasive endodontics, which aims to preserve tooth structure and to avoid possible fracture that would result in poor long-term prognosis.

Our study confirmed that the files has succeeded in reducing the bacterial load in the canal system. We have shown for the first time that TS file resulted in a greater reduction than did VB in both file sizes of 25 and 30. While the present study was limited to a single bacterial species, we expect that polymicrobial biofilm to be more resistant to canal instrumentation. To expand on this *ex vivo* study, future studies with samples collected from patients' teeth post instrumentation with either of these two file systems could provide stronger evidence on performance of these two files. We hope that our finding would help clinicians make the proper decision in their treatment approach. Further studies on other teeth and root systems such as the mesiobuccal root of maxillary first molar highly recommended.

5. Conclusions

Within the limitations of this *ex vivo* study, it can be concluded that TS file system associated with sodium hypochlorite and Qmix® irrigation is more effective than VB in reducing *E. faecalis* biofilms from mesiobuccal and mesiolingual canals and the isthmuses of mandibular molars. Possibly due to its shaping design, TS allows preparation of canals to smaller sizes, preserving dentin while achieving optimal disinfection.

Author Contributions

Conceptualization—RP and SW; methodology—SW, JA and JL; software—MM; validation—MM, SW and

RP; formal analysis—SB; investigation—SB, AP, MM and JL; resources—RP; data curation—MM, SW; writing - original draft preparation—MM and SB; writing - review and editing—JK, AP and MM; visualization—SB; supervision—RP and SW; project administration—RP. All authors have read and agreed to the published version of the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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