Comparative Analysis of Protaper and Waveone Systems to Reduce *Enterococcus Faecalis* from Root Canal System in Primary Molars – An *in Vitro* Study

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Objective: *To assess, in vitro, the ability of the ProTaper™ and WaveOne™ systems to reduce Enterococcus faecalis contamination in primary molars.* ***Study design:*** *Sixty roots of primary molars were contaminated with E. faecalis. Roots were randomly allocated to one of four groups (n=20): ProTaper™, WaveOne™, control A, or control B. The files used were S1 and S2/F1 and F2 (ProTaper™ system) and 25.08 (WaveOne™ system). In control group A, the root canal was left uninstrumented, whereas in control group B, the root canal was irrigated with NaCl 0.9%. E. faecalis was sampled from the root canal system before and after instrumentation and the Wilcoxon test and Mann–Whitney U were used.* ***Results:*** *There were no differences in E. faecalis counts between pre-instrumentation counts in the ProTaper™ and WaveOne™ (p>0.05). The ProTaper™ system led to an 89.36% reduction in E. faecalis burden, versus 78.10% with the WaveOne™ system (p>0.05). Instrumentation time was shorter with WaveOne™ (p<0.0001).* ***Conclusions:*** *The ProTaper™ and WaveOne™ systems were equally effective in reducing Enterococcus faecalis in primary molars. The WaveOne™ system was associated with shorter instrumentation time.*

Key words: *endodontics, primary dentition, root canal preparation, Enterococcus faecalis.*

INTRODUCTION

Chemical disinfection and mechanical preparation of root canals in primary teeth is mostly performed with manual instruments[1,2]. However, the advent of nickel–titanium (NiTi) rotary instruments offered a new alternative for root canal instrumentation . Advances in rotary instrumentation have streamlined endodontic techniques and reduced operative times[3,4].

The ProTaper™ system is a NiTi rotary system based on a multiple-taper design. Each file is designed specifically for one region of the root canal system. Endodontic systems have recently been launched that seek to streamline the instrumentation process by enabling single-file endodontic treatment. In these systems, the file performs a reciprocating motion, which requires special motors. Such single-file systems include Reciproc™ and WaveOne™[5,6]. The single-file WaveOne™ system was associated with reduced modification of the canal curvature as compared with the ProTaper™ system[7].

In pediatric dentistry, use of single-file instrument systems for root canal preparation can be an alternative for optimization of endodontic treatment. Use of these instruments may shorten instrumentation time and, consequently, chair time[2,6,8,9]. The root canal anatomy of primary molars varies considerably. This could be explained by secondary dentin formation and physiological root resorption can reconfigure the root canal system, which may reach up to six canals. Pulp and/or periodontal inflammation can cause pathologic changes in this programmed physiological root resorption and also complicate the root-root canal morphology. In addition to the apical foramen and large accessory canals (lateral

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and furcation canals), dentinal tubule exposure due to physiological root resorption may also cause structural alteration and increase the permeability of the root surface to microbial toxins. Consequently, the inter-radicular bone lesion in primary molars can be found anywhere along the root or in the furcation area[10]. Microbiological profile of symptomatic teeth with primary endodontic infections and the species found in higher counts (10^{-5}) in exposed pulp space cases were *Eubacterium saburreum*, *Fusobacterium nucleatum* subsp. *vincentii*, *Tannerella forsythia*, *Enterococcus faecalis*, *Neisseria mucosa*, *Campylobacter gracilis*, and *Prevotella nigrescens*, whereas in unexposed pulp space cases, the most prevalent bacteria were *F. nucleatum* subsp. *vincentii*, *N. mucosa*, *E. faecalis*, *E. saburreum*, *C. gracilis*, and *Porphyromonas gingivalis*. Counts of *F. nucleatum* subsp. *vincentii*, *Campylobacter showae*, *Capnocytophaga sputigena*, *Treponema socranskii*, *Porphyromonas endodontalis*, *Eikenella corrodens*, and *Capnocytophaga ochracea* were significantly higher in unexposed pulp space cases[11].

There is a paucity of studies on the use of the WaveOne™ system in deciduous teeth. Therefore, the purpose of this investigation was to assess the ability of the WaveOne™ and ProTaper™ systems to reduce *Enterococcus faecalis* contamination in primary teeth, as well as compare the instrumentation time of these systems.

MATERIALS AND METHOD

This study was approved by Ethics Committee (CE/UCS-150/2012) and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

The sample comprised 60 roots of 27 deciduous molars extracted at the Pediatric Dentistry clinic of our institution and donated (by patients or their legal guardians) for research by means of a Tooth Donation Form.

Inclusion criteria

- At least two-thirds of the root present;
- No pathological internal or external root resorption;
- No internal or external furcation perforation;
- Moderate root angulation (root curvature radius 10–20 mm, angle of the curvature 25º–39º)[12].

Clinical procedure

Access to the root canal system was obtained with high-speed (Dabi-Atlante Ltda., São Paulo, Brazil) round diamond burs (KG Sorensen, São Paulo, Brazil) under constant cooling. Coronally divergent axial walls were created with a round-end tapered diamond bur (3082, KG Sorensen, São Paulo, Brazil). Working length was determined by inserting a manual #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the canal and advancing until the active tip was visible at the apical foramen. The length was marked, the instrument withdrawn and the working length established as 1 mm short of the canal length.

To facilitate bacterial contamination of the root canal system, all canals were initially instrumented with a manual #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) down to working length[4]. Teeth were then sterilized by the moist heat method in an autoclave, at 121ºC for 15 minutes. Two teeth were selected at random and submerged in Brain Heart Infusion (BHI) (Difco, Michigan, USA) broth for 24 hours to serve as a control for effectiveness of sterilization. As no microbial growth was observed, the sterilization method was deemed reliable. Root canals were contaminated with *Enterococcus faecalis* ATCC 19433 of human origin standard strain (LabCenter, São Paulo, Brazil), standardized to 0.5 McFarland turbidity. Briefly, each root canal was irrigated with 1 mL of *E. faecalis* standard strain using a 5-mL syringe (BD Plastipak™, Paraná, Brazil) with needle (BD PrecisionGlide™, Paraná, Brazil).

Contaminated specimens were transferred to sterile plates (Costar, Nova York, USA) containing standard strain and sterile BHI broth (Difco, Michigan, USA). Samples were inoculated in anaerobic jars (Oxoid Ltd., Hampshire, England) for 5 days at 37°C in 85% nitrogen (N_2), 10% carbon dioxide (CO_2), and 5% hydrogen (H_2), obtained using the Anaerogen™ anaerobic atmosphere generation system (Oxoid Ltd., Hampshire, England).

Canals were randomly allocated to one of the four treatment groups. Two control groups were established: in control group A, the root canal was left uninstrumented, whereas in control group B, the root canals were irrigated with NaCl 0.9% (Arboreto, Minas Gerais, Brazil) solution[9,13]. Depending on group allocation, canals were instrumented with either the ProTaper™ (Dentsply Maillefer, Ballaigues, Switzerland) or the WaveOne™ (Dentsply Maillefer, Ballaigues, Switzerland) instrument systems. Treatment sequences were as follows: for the ProTaper™ system (Dentsply Maillefer, Ballaigues, Switzerland, batch 0064570), S1, S2, F1, and F2 files, with a 300 rpm handpiece (S1 and S2, 3 N torque and brushing motion; F1 and F2, 2 N torque and back-and-forth motions)[2,9], powered by an X-Smart™ endodontic motor (Dentsply Maillefer, Ballaigues, Switzerland) (Figure 1). The instruments for cervical and medium preparation were divided into Shaping 1 (S1), Shaping X (SX), and Shaping 2 (S2), and those used for final shaping and apical preparation were divided into Finishing 1 (F1), Finishing 2 (F2), and Finishing 3 (F3) (Figure 2). For the WaveOne™ system (Dentsply Maillefer, Ballaigues, Switzerland, batch 99506250), a 25.08 file powered by an X-Smart plus™ (Dentsply Maillefer, Ballaigues, Switzerland) endodontic motor was used (Figure 3). The WaveOne™ system is composed of three files: small with 0.06 taper, primary and large with 0.08 taper and 21, 25, and 31 mm length (Figura 4). Instrumentation was performed using in-and-out motions[4]. In each group, instruments were changed when the files reached full working length. At each instrument change, the root canal system was irrigated with 1 mL of sodium hypochlorite 1.0 % (ASFER, São Paulo, Brazil). The total volume of sodium hypochlorite (ASFER, São Paulo, Brazil) used for irrigation was 4 mL with the ProTaper™ system and 1 mL with the WaveOne™ system. Irrigation was carried out using a plastic syringe and 27-gauge side-vented Monoject™ stainless steel needle (Ultradent Products, South Jordan, USA). The needle was placed into the canal without binding and kept within 3 mm of the working length throughout the process. *E. faecalis* was collected from the canal before and after instrumentation using sterile paper points (Dentsply Maillefer, Ballaigues, Switzerland) of appropriate diameter for bacterial collection. Paper points (Dentsply Maillefer, Ballaigues, Switzerland) were introduced into the canal, where they remained for 30 seconds, and immediately placed in individual test tubes containing 4.5 mL of BHI (Difco, Michigan, USA) broth. All teeth were instrumented by the same practitioner[1,4].

Figure 1. X-Smart™ rotary device used for ProTaper™ files.

Figure 2. ProTaper™ files used in this study: A-S1 (18/02), B-S2 (20/04), C-F1 (20/07), and D-F2 (25/08).

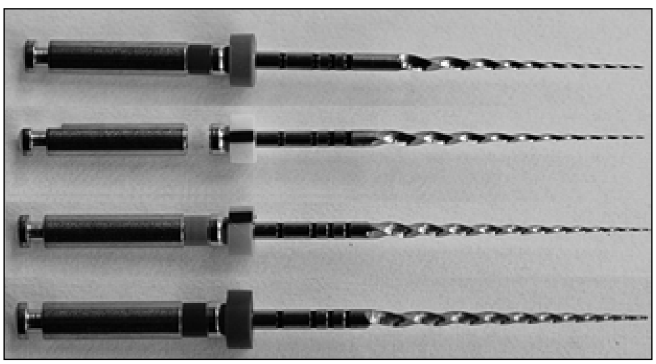
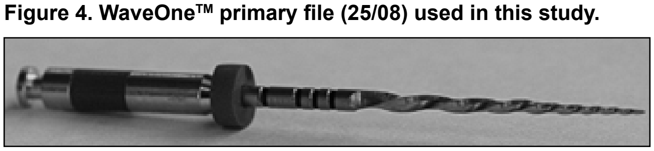


Figure 3. X-Smart plus™ device used for WaveOne™ files.

Figure 4. WaveOne™ primary file (25/08) used in this study.



Statistical analysis

Results were analyzed in the Biostat 4.0 software environment. Descriptive analyses were carried out, and the Wilcoxon test and Mann–Whitney *U* were used to compare *E. faecalis* counts. The Mann–Whitney *U* was used to compare instrumentation times and percentage of *E. faecalis* reduction between the two systems.

RESULTS

Fourteen second maxillary molars, three first maxillary molars, one first mandibular molar, and nine second mandibular molars were used in this study (Table 1). There were no differences in *E. faecalis* counts among the control, ProTaper™, and WaveOne™ groups before instrumentation ($p>0.05$) (Table 2). The results showed that random allocation of the roots into four different groups was effective and did not interfere with *E. faecalis* counts before instrumentation.

There was a significant reduction in *E. faecalis* contamination after instrumentation with the ProTaper™ and WaveOne™ systems ($p<0.0001$). There were no significant differences in *E. faecalis* contamination before instrumentation with the ProTaper™ and WaveOne™ systems ($p=0.3793$), indicating that microbiological standardization was effective, as there were no differences among the groups in baseline *E. faecalis* counts. There were no significant differences in the microbial reduction achieved after instrumentation with the ProTaper™ and WaveOne™ systems ($p=0.1636$); therefore, the use of four files (ProTaper™ system) promoted the same radicular root disinfection as the use of only one file (WaveOne™ system) (Table 2).

The percentage reduction of *E. faecalis* showed no significant differences between the ProTaper™ and WaveOne™ systems ($p<0.05$), while operative times were shorter with the WaveOne™ system as compared with the ProTaper™ system ($p<0.0001$) (Table 3).

DISCUSSION

Pulp necrosis of primary teeth may lead to periapical disease and consequently affect the permanent tooth bud[14]. The objective of endodontic treatment of primary teeth with pulp necrosis is to eradicate endodontic infection and prevent early tooth loss, so as to preserve the health of the permanent tooth bud[15,16].

The endodontic microbiota are polymicrobial and dynamic[2,14,16,17] , and the efficacy of endodontic treatment depends on its ability to reduce microbial burden[1,9,14,15]. During the progression of endodontic infection, a variety of interactions and commensalism processes take place among bacteria within the root canal system, leading to a reduction in the number of facultative anaerobes and an increase in strict anaerobes. This phenomenon is due to oxygen consumption and to the establishment of a low redox potential[18]. However, facultative anaerobes—despite lower numbers as disease progresses—may remain latent for a long time and can survive wide variations in environmental conditions. Notable microorganisms of this class include *Enterococcus faecalis, Staphylococcus*, and *Actinomyces*[19]. *E. faecalis* is biofilm-forming; produces the so-called aggregation substance, surface adhesins, and lipoteichoic acid; produces extracellular superoxide; expresses gelatinase and hyaluronidase; and is capable of recolonizing treated canals[14,20,21]. It is a Gram-positive facultative anaerobe and is highly prevalent in the setting of endodontic retreatment[2,14,21]. *E. faecalis* can survive

Table 1. Differences between types of teeth regarding preparation with both systems

Number of teeth used	Teeth	Number of canals used	Groups
1	First mandibular molar	1-D 1-MB	Control ProTaper™
9	Second mandibular molar	1-D 3-ML 4-MB 3-D 2-ML 3-MB 2-D 4-ML 2-MB	Control Control Control WaveOne™ WaveOne™ WaveOne™ ProTaper™ ProTaper™ ProTaper™
3	First maxillary molar	1-P 2-MB 1-DB 1-MB 2-DB	Control Control WaveOne™ WaveOne™ ProTaper™
14	Second maxillary molar	3-P 3-DB 2-MB 3-P 3-DB 4-MB 2-P 3-DB 4-MB	Control Control Control WaveOne™ WaveOne™ WaveOne™ ProTaper™ ProTaper™ ProTaper™

D: distal; MB: mesiobuccal; ML: mesiolingual; P: palatal; DB: distobuccal

Table 2 ***E. faecalis*** **counts in each sample group (CFU/mL).**

	CT B 0.43±0.79	PT_{pre} 0.17±0.22	PT_{post} 0.01±0.01	WO_{pre} 0.41±0.62	WO_{post} 0.16±0.39
CT A 0.31±0.29	0.2853	0.0859	<0.0001	0.5885	<0.0001
CT B 0.43±0.79	———	0.4989	<0.0001	0.6554	<0.0001
PT_{pre} 0.17±0.22	———	———	<0.0001	0.3793	<0.0001
PT_{post} 0.01±0.01	———	———	———	<0.0001	0.1636
WO_{pre} 0.41±0.62	———	———	———	———	<0.0001

Expressed as arithmetic means, standard deviations (±), and P-values. Wilcoxon test and Mann–Whitney *U* test.

CT A: control group A; CT B: control group B; PT_{pre}: contamination before instrumentation with the ProTaper™ system; PT_{post}: contamination after instrumentation with the ProTaper™ system; WO_{pre}: contamination before instrumentation with the WaveOne system; WO_{post}: contamination after instrumentation with the WaveOne™ system.

Table 3 Percentage reduction of ***Enterococcus faecalis*** **and operative time (seconds) before and after instrumentation with the ProTaper or WaveOne systems.**

	ProTaper™	WaveOne™	p-value*
PR	89.36 (±17.32)^{a}	78.10 (±23.98)^{a}	0.0742
OT	29.06 (±14.97)^{a}	11.73 (±3.92)^{b}	<0.0001

PR: percentage reduction; OT: operative time. Expressed as arithmetic means and standard deviations (±). *Mann–Whitney *U*. Different letters (horizontal lines) denote statistically significant differences.

for prolong periods under adverse nutritional conditions[22]. The survival of *E. faecalis* is associated with its ability to penetrate the dentin tubules and bind to collagen molecules by producing adhesin enzymes[23]. In view of the microbiological features of *Enterococcus faecalis,* such as virulence, ability to recolonize root canals, survival in nutritionally deprived settings, and high prevalence in cases of endodontic retreatment, we chose to contaminate the root canals of the deciduous molars used in this study with a standard strain of *E. faecalis*[2,14].

NiTi instruments are a useful alternative in pediatric dentistry due to reduced shaping time as compared with manual techniques[2,8,25]. In the present study, both the ProTaper™ and WaveOne™ systems produced significant reductions in *Enterococcus faecalis* contamination, with no significant difference between the two. Instrumentation with the ProTaper™ system comprised a sequence of four files (S1, S2, F1, and F2)[2,9] and irrigation with 1 mL of sodium hypochlorite 1% at each instrument change, for a total of 4 mL of irrigant. Conversely, instrumentation with the WaveOne™ system comprised a single file (25.08) and irrigation with 1 mL of the same solution. There were no statistically significant between-group differences. Regarding helical angles, the greater the angle, the greater the ability of the instrument to remove debris from the root canal system. ProTaper™ and WaveOne™ files have variable-pitched flutes: the helical angle increases progressively along the shank, from the tip of the instrument toward the base. Therefore, all files used in this study have a flute profile favorable for debris removal during instrumentation. The ProTaper™ system uses a continuous rotation motion, whereas the WaveOne™ system uses a back-and-forth reciprocating motion[4,7,26]; that is, with each counterclockwise motion, the file penetrates and cuts dentin, removing debris, whereas with each clockwise motion, the file exits the root canal system before it can "lock" or become jammed in the canal. Furthermore, the WaveOne™ 25.08 file has a larger pitch (smaller number of flutes) and lower mass the fewer flutes on an instrument, the greater its flexibility, increasing cutting efficiency and improving clearance of debris from within the canal. However, the rotary instrumentation was associated with less debris extrusion compared with the use of reciprocating single-file systems[5]. In this study, instrumentation time was found to be shorter with the WaveOne™ system than with the ProTaper™ system. This may be explained by the single-file nature of the WaveOne™ system, whereas four files were used with the ProTaper™ system. Therefore, the WaveOne™ system is indicated for endodontic preparation of primary teeth due to its ability to reduce microbial contamination significantly in a short operative time. However, as this is an *in vitro* study, further clinical research is required to evaluate the behavior of the ProTaper™ and WaveOne™ systems in the endodontic treatment of primary teeth.

CONCLUSIONS

Both the ProTaper™ and WaveOne™ systems, used in conjunction with chemical disinfection with 1.0% sodium hypochlorite, were able to reduce *Enterococcus faecalis* counts in primary molars.

Use of single-file instrumentation systems produced an *Enterococcus faecalis* reduction equivalent to using a sequence of four files in the root canals of primary molars.

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