Original Research

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An ex vivo antimicrobial evaluation after the preparation with XP-Endo Shaper and Trunatomy systems

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Abstract

AIM. The aim of this study was to evaluate bacterial reduction in root canals with the XP-Endo Shaper (XP) and Trunatomy (TN) systems.

MATERIALS AND METHODS**.** Twenty-eight permanent human type I, oval-shaped Vertucci premolars and straight root canals were contaminated with Enterococcus faecalis for 30 days at 37°C. Samples were collected prior to instrumentation. The teeth were divided into two groups (n = 14) and processed with the tested groups at 37°C: XP group – (30/0.04) and TN group – Small (20/0.04) and Prime (26/0.04). Biological samples before and after instrumentation were collected using a sterile paper cone inserted into the canal for one minute. Bacteria were counted using colony forming units (CFU/mL) and results were subjected to Kruskal-Wallis test at 5 level of significance.

RESULTS. Both the XP and TN systems significantly reduced bacterial counts ($p < 0.0001$), but did not eliminate bacteria in the root canals.

CONCLUSIONS. Both the Trunatomy and XP-Endo Shaper systems were similar in terms of antimicrobial efficacy, but neither system was able to eliminate bacteria from the root canals.

Keywords: endodontics, Enterococcus faecalis, root canal treatment

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Экспериментальная ex vivo оценка антимикробной эффективности после препарирования с использованием систем XP-Endo Shaper и TruNatomy

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Резюме

ЦЕЛЬ. Целью данного исследования было оценить степень снижения бактериальной обсемененности в корневых каналах после обработки с использованием систем XP-Endo Shaper (XP) и TruNatomy (TN).
МАТЕРИАЛЫ И МЕТОДЫ. Двадцать восемь постоянных человеческих премоляров типа I по Вертуччи с овальной формой и прямыми корневыми каналами были заражены *Enterococcus faecalis* в течение 30 дней при температуре 37°C. Образцы собирались до начала инструментальной обработки. Зубы были разделены на две группы (n = 14) и обработаны при 37°C: группа XP – инструмент (30/0.04); группа TN – инструменты Small (20/0.04) и Prime (26/0.04). Биологические образцы до и после обработки собирались стерильным бумажным штифтом, введенным в канал на одну минуту. Подсчет бактерий производился по числу колониеобразующих единиц (КОЕ/мл), результаты анализировались с использованием критерия Крускала–Уоллиса при уровне значимости 5%.
РЕЗУЛЬТАТЫ. Обе системы – XP и TN – достоверно снижали количество бактерий в корневых каналах ($p < 0.0001$), однако не обеспечивали полного их удаления.
ВЫВОДЫ. Системы TruNatomy и XP-Endo Shaper продемонстрировали сопоставимую антимикробную эффективность, однако ни одна из них не обеспечила полной элиминации бактерий из корневых каналов.

Ключевые слова: эндодонтия, Enterococcus faecalis, лечение корневых каналов

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INTRODUCTION

One of the main aims of endodontic treatment is to prevent or promote the healing of apical periodontitis by cleaning, shaping, disinfecting and filling the root canal system [1]. The microorganisms can organize themselves into biofilms, which increases their resistance to endodontic procedures and makes root canal disinfection a challenge [2].

One microorganism of interest in endodontics is Enterococcus faecalis, which has been extensively studied as it is particularly associated with persistent endodontic infections, which are a common cause of treatment failure due to their resistance in intracanal procedures [3]. This bacterium has an inherent resistance to various treatments, including irrigation solutions, intracanal medications [4], antibiotics and pH changes [5]. Its ability to adhere, multiply, invade, resist host defenses and compete with other bacteria increases its virulence [6].

Mechanical preparation is the most critical phase of endodontic treatment, and great development has been made in the development of instruments [7]. Studies show that even with different techniques, complete removal of organic debris [8] and bacteria [9] is difficult to achieve. Automated systems based on a nickel-titanium alloy that work with continuous rotary motion have become widely accepted in recent decades [9]. These files, with innovative designs, manufacture, alloy composition and heat treatment [10], have redefined root canal instrumentation.

Some of these systems are XP-Endo Shaper (FKG Dentaire, La Chaux de Fonds, Switzerland) and TruNatomy (Dentsply Sirona, Ballaigues, Switzerland). These files have an innovative design and use rotary motion to enhance the root can preparations [7], considered effective for complex anatomy that often harbor resistant microorganisms such as *Enterococcus faecalis* [5; 6].

The XP-Endo Shaper (FKG) with its serpentine design and NiTi MaxWire alloy adapts to the irregularities of the canal and minimizes stress on the dentin wall. It transitions from a straight, martensitic shape to a spoon shape at body temperature and uses its elasticity and shape memory effect to expand and contract during rotation to improve cleaning and disinfection. This dynamic ability improves root canal treatments by reaching hard-to-reach areas and effectively agitating the irrigant [7; 11]. The instrument is 30/.01 (size/taper), but can have a final canal preparation of 30/.04 [12].

Another new system is the Trunatomy (Dentsply Sirona), which ensures superior peri-cervical dentin preservation thanks to a special instrument geometry and heat treatment [13]. The Trunatomy instruments are characterized by flexibility, regressive tapering and a decentralized parallelogram cross-section and faithfully reproduce the anatomy of the canal [14]. This system comprises five instruments for cervical preparation (Orifice Modifier 20.08), the glide path (Glider 17.02v) and three sizes for canal shaping: Small (20.04v), Prime (26.04v) and Medium (36.03v) [11].

Endo Shaper (FKG Dentaire) and the Trunatomy (Dentsply Sirona) system. The null hypothesis tested was that there would be no difference between the groups in terms of bacterial reduction [15; 16].

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki. This study approved by the local ethics committee because it involved biological material (Opinion: 5.351.873, 14th April, 2022). The manuscript was written according to the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines [17] (Fig. 1).

The number of 14 samples per group was based on previous works [18; 19]. The sample was calculated using the t-test, with a minimum difference between the means of the treatments of 0.74, a standard deviation of 0.81–0.78, a number of treatments of 2, a test power of 0.80 and an alpha of 0.05.

Sample selection and standardization

The criteria included the selection of permanent human mandibular premolars with fully developed, singular roots, a straight single canal curvature < 10° according to Schneider [20] and a Vertucci type I and an oval shape (in which the buccolingual diameter is twice as large as the mesiodistal diameter in the first two thirds of the canal). Teeth with fractures, calcifications, dilacerations or previous endodontic treatment were excluded. Radiographs were taken in both vestibulo-lingual and mesiodistal orientations to confirm compliance with the inclusion criteria.

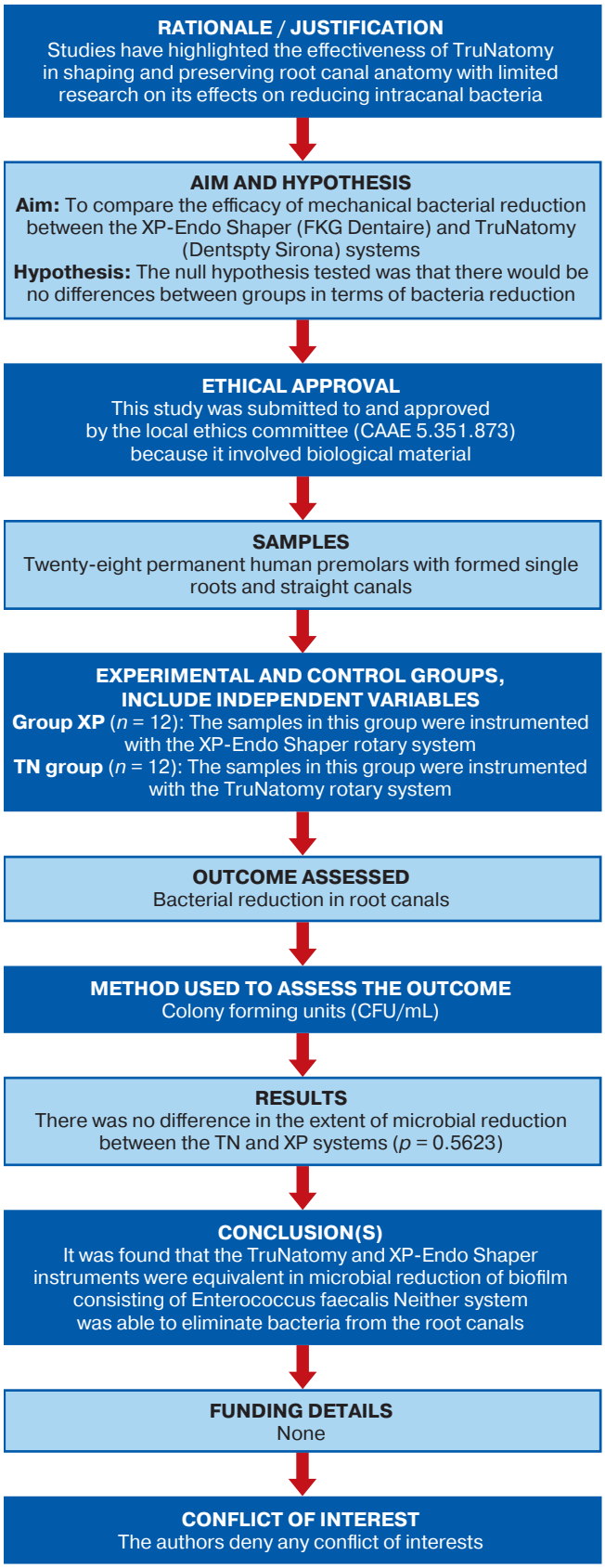

Fig. 1. Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines

Рис. 1. Рекомендуемые элементы отчета для лабораторных исследований в эндодонтии (PRILE) – рекомендации 2021 г.

The teeth were ultrasonically cleaned to remove any residual periodontal ligament or calculus. They were stored in 0.1% thymol until the start of the study. In preparation for the experiments, the roots were thoroughly rinsed under running water for one hour to remove all thymol residues. They were then dried with a stream of air and gauze. The samples were then flattened with a diamond disk attached to a ruler and measured with a conventional ruler, ensuring standardization to a length of 15 mm.

The working length (WL) was determined with a K#10 file (K-File, Dentsply Maillefer, Ballaigues, Switzerland) up to the apical foramen, whereby the WL was determined by subtracting 1 mm. The canal was then enlarged with a K#20 file (K-File, Dentsply Maillefer, Ballaigues, Switzerland) to standardize the original canal diameter and make room for bacterial contamination.

Micro-CT Scanning

All teeth were scanned in a micro-CT device (Sky-Scan 1273; Brucker micro-CT, Kontich, Belgium) at 70 kV, 114 mA, 12 µm pixel size, 360° around the vertical axis, with 0.5 rotation step and 2 average frame and with a 1.0 mm thick aluminum filter. After scanning, the N. Recon v.1.6.9.16 software (Bruker micro-CT) was used for the image's reconstruction (ring artifact correction of 5, a beam hardening correction of 50% and a smoothing of 8). The 3-dimensional volume and surface area were measured using CTAn v1.14.4.1 software (Bruker micro-CT).

The CTAn v1.14.4.1 software (Bruker micro-CT) was also used to measure the major diameter of the buccolingual root canal three millimeters from the apex. Root canals that had a buccolingual distance that was at least three times the mesiodistal distance were considered oval [21]. This procedure allowed the samples to be matched and randomly divided in two experimental groups according to Enterococcus faecalis contamination.

Enclosure of the roots in silicone

To prevent the irrigation solution from leaking through the apical foramen during root preparation, the root apices were first covered with utility wax. These roots were then placed in plastic cubes with 4 cm and a diameter of 3 cm, filled with silicone and finally covered with a thin layer of cyanoacrylate around the root to ensure stability. The teeth were then sterilized into an autoclave at 121°C for 15 minutes and subsequently contaminated with *E. faecalis.*

Contamination of the sample

The target microorganism for the infiltration test was E. faecalis (ATCC-29212), which was cultured and stored in BHI liquid medium (Brain Infusion Hearth – BHI – Difco – Detroit, Michigan, USA) with 20% glycerol. To prepare the inoculum, 100 µL of the E. faecalis broth was transferred to 2 mL of BHI broth and kept in the oven at 37°C for a maximum of 24 hours. After this time, the broth became turbid, which was compared with the Mac Farland 10 scale (1.0x10 CFU/mL). 37 g of BHI was dissolved in 1 liter of demineralized water and distributed into smaller containers. After the BHI was prepared, it was autoclaved for 40 minutes at 121 °C, pH 7.4, 0.2 at 25c with typical (g/liter).

Twenty microliters (µL) of the suspension at the final concentration was added to the root canals using a pipette. A sterile, absorbent cotton was moistened, and placed in four wells of each cell culture plate. The lid of the plate was closed and sealed with adhesive tape and the whole was incubated in an oven at 37°C with 5% CO_2 for 30 days, when the used absorbent cotton was changed in plates wells [4]. During the contamination period, the bacteria viability was checked every three days and BHI was added daily to keep the strains viable to confirm that the contamination was effective. The container was then opened, and the contaminated teeth were used to start the experiment.

Root canal Instrumentation

Once the biofilm had matured, the individual samples were removed prior to instrumentation and randomly allocated into experimental groups (n = 12):

– Group TruNatomy (TN);

– Group XP – Endo Shaper (XP).

Samples were collected in a laminar flow chamber using a sterile cone of absorbent paper No. 20 (Endopoints, Manacapuru, Amazonas, Brazil) immersed in the canal for 1 minute. The samples were then transferred to an Eppendorf tube containing 5 mL of sterile 0.85% saline solution and shaken for 30 seconds (Vortex CP 600 Plus, Phoenix, Araraquara, Brazil). Serial dilutions (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) of this suspension were prepared and transferred to test tubes. Aliquots of 0.1 mL of the suspension and each dilution were seeded in Petri dishes containing BHI agar. The plates were incubated in an oven with 5% CO_2 at 37°C for 24 hours. The number of CFU/mL per culture plate was then counted before the root canals preparations.

After the antimicrobial samples prior to instrumentation, 5 mL of sterile 0.9% saline solution was used as irrigant, and the canals were instrumented. Root canal preparation was performed at 37°C in a heating cabinet (800-Heater; PlasLabs, Lansing, MI) built into the laminar flow. Instrumentation of the root canal was performed as follows:

Group XP (n = 12): Samples in this group were instrumented with the XP-Endo Shaper rotary system. While the canal was filled with 0.9% saline, a K#15 file (K-File, Dentsply Maillefer, Ballaigues, Switzerland) was inserted third by third up to the WL. The file reached the WL by long, even back and forth movements in the longitudinal direction. The movements were wide and uninterrupted so that the conical shape of the canal was defined. Once the WL was reached, 3 incremental movements were done. The teeth were instrumented with the X-Smart Plus motor (Dentsply Maillefer, Ballaigues, Switzerland) at 800 rpm and 1.5 Ncm. At each instrumented root third, the canal was irrigated with 5 mL of sterile 0.9% saline solution (Farmax, Brazil) using a conventional irrigation syringe (Ultradent, USA) and a 25x4 irrigation needle (Injex, São Paulo, Brazil)

and the glide path was performed with a K#20 hand file. A 30/ 04 gutta-percha cone was used to check the final preparation diameter.

TN group (n = 12): The specimens in this group were instrumented with the TruNatomy rotary system. While the canal was filled with 0.9% sterile saline, a K#15 file was inserted third by third up to the WL. Initially, the Orifice Modifier 20.08 instrument was used, followed by the 17.02v, 20.04v, 26.04v and 36/.03 instruments. The torque used was 1.5 Ncm and the speed was 500 rpm with continuous rotation using the X-Smart Plus motor. Each time the instrument was used, three to four in and out movements were performed until the WL was reached.

All procedures were performed in a cabinet at 37°C with a heater (800-Heater; PlasLabs, Lansing, USA) to simulate body temperature. In both groups, each root canal was irrigated with 25 mL of saline after 3 preparation cycles (1 cycle corresponds to 3 back and forth movements).

Sample collection after instrumentation

After the final irrigation, the samples of each group were taken again using sterile paper cones, which remained in the canal for 1 minute. After 1 minute, the cones were removed and stored in a plastic container. The dilutions were prepared in test tubes according to the same protocol as the previous collection.

Statistical analysis

The results were analyzed using BioEstat 5.3 and the Shapiro-Wilk normality test was used. The sample showed non-normal behavior, then Kruskal-Wallis parametric test (Student-Newman-Keuls) was chosen, considering a significance level of 5%.

RESULTS

The previous count showed no significant difference between the groups before the preparation (0.5546). The results showed a significant intragroup microbial reduction after root canal instrumentation with the TN ($p < 0.0001$) and XP ($p < 0.0001$) systems. Also, no difference was found in an intergroup comparison between the TN and XP systems ($p = 0.5623$) (Table 1).

Table 1. Kruskal-Wallis (Student-Newman-Keuls) statistical test of colony forming units/mL (log10) of the sample groups

Таблица 1. Результаты статистического анализа методом Крускала-Уоллиса (и критерия Стьюдента – Ньюмена – Келса) количества колониеобразующих единиц/мл (log10) в исследуемых группах

	TN		XP	
	PC	CAI	PC	CAI
MN	4.28	3.33	5.46	3.38
MX	6.30	4.98	7.05	5.40
MD (ID)	5.98(0.50)^{A}	3.97(0.55)^{B}	6.00(0.74)^{A}	4.09(1.24)^{B}
MA (SD)	5.79 (0.52)	4.00 (0.45)	6.08 (0.47)	4.29 (0.68)

Note: TN – TruNatomy system; XP – XP-Endo Shaper system; PC – prior collection; CAI – collection after instrumentation; MN – minimum values; MX – maximum values; MD – median; ID – interquartile deviations; MA – arithmetic means; SD – standard deviation; $p < 0.0001$

Примечание: TN – система TruNatomy; XP – система XP-Endo Shaper; PC – забор до инструментальной обработки; CAI – забор после инструментальной обработки; MN – минимальные значения; MX – максимальные значения; MD – медиана, ID –межквартильные размахи; средние MA – арифметические значения; SD – стандартное отклонение; $p < 0.0001$

DISCUSSION

The primary goal of endodontic treatment involves thorough cleaning, disinfection and proper root canal obturation [22]. However, the complicated anatomy often harbors microorganisms in the bifurcations, isthmi and dentinal tubules, making them resistant to conventional mechanical and chemical treatments. This resistance could contribute to the failure of endodontic therapy [23].

In the present study, the instrumentation capacity of 2 rotary systems for the removal of E. faecalis was compared. Despite the different designs, cross-sections and heat treatments, there was no difference in the ability to disinfect root canals with the XP-Endo Shaper and the TruNatomy system. Both systems promoted partial bacterial reduction. The null hypothesis was therefore accepted.

Oval mandibular premolars were selected for this study because cleaning and shaping these teeth is challenging [12]. This is because the canal usually has a round cross-section during rotary instrumentation and the polar areas in oval canals remain unprepared.

In this study, the final preparation size was 30/0.04 for XP-Shaper and 36/0.03 for Trunatomy. Despite the differences in final apical diameter, studies have shown that both systems have similar capabilities in reducing microbial presence in root canals. This consistency of results suggests that factors such as instrument flexibility, cutting efficiency and ability to move irrigants may play a more critical role than preparation size alone [11]. The design and material composition of the instruments facilitate thorough cleaning of the canal so that even complicated canal anatomies can be reached effectively. These results emphasize the sophistication of modern endodontic instruments, where design complements size to achieve the desired results in bacterial reduction [11].

Enterococcus faecalis was focused on in this study because it appears to be the most common microorganism in persistent endodontic infections [24]. This bacterium tolerates hostile alkaline pH values and resists prolonged nutrient deprivation with reduced metabolic activity in treated root canals [25]. In addition, mature E. faecalis biofilms in root canal dentin exhibit greater resistance to disinfectant solutions than young biofilms [26].

In this study, a culture-based microbiological test based on an ex vivo approach was chosen. Despite newer, more advanced methods for evaluating cell cul-

tures and root canal disinfection, this method is widely supported in the literature [11; 27]. Collection via sterile paper tips, followed by serial dilution and seeding in culture media, facilitated the enumeration of colonies in Petri dishes [28]. Although these systems have similarities in potential bacterial reduction in root canals, it is important to emphasize the method chosen for this study, the bacterial counting [27; 28], which is crucial for the evaluation of instruments and substances. which is crucial for the evaluation of instruments and substances. However, it must be acknowledged that the use of paper points may underestimate the bacterial count in the canal system, as bacteria located in areas further away from the main canal lumen, such as dentinal tubules, isthmuses and bifurcations, may not be detected [1].

Other studies investigating bacterial reduction are in agreement with these results [2; 29]. It has been shown that the reciprocating, manual and rotary technique in combination with 2.5% NaOCl is equally effective in reducing the number of microorganisms in the oval root canals [2]. The efficacy of Trunatomy and Rotate in the disinfection of root canals [29]. Although without significant differences, the preparation with XP-Endo Shaper resulted in a significant reduction in bacteria compared to TruNatomy instruments [14] or compared to compared to Reciproc [30], which contradicts the present study.

The results of the study emphasize the continuing need for research and innovation in endodontics and highlight the importance of developing new instruments, techniques and strategies for thorough root canal disinfection, especially in cases with resistant microorganisms. Although the study provides valuable insights, the limitations of the study caution against immediate application of the results in clinical practice. It suggests that further research, including clinical trials, is needed to validate and build on the observations.

CONCLUSION

It was found that the Trunatomy and XP-Endo Shaper instruments were equivalent in microbial reduction of biofilm consisting *of Enterococcus faecalis*. Neither system was able to remove bacteria from the root canals. Therefore, the integration of effective irrigation protocols and solutions is crucial to improve the antibacterial efficacy of these systems. Continued research and development are essential to refine these technologies and improve the clinical outcomes of endodontic therapy.

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