

Comparative evaluation of antibacterial efficacy of Two shape, TruNatomy files against enterococcus faecalis: An invitro study.

Abstract:

Aim: The present study aim to assess the antibacterial efficiency of Two shape(TS) and Tru Natomy files(TRN) files against Enterococcus faecalis.

Materials and Methodology: Forty human mandibular premolars with single roots were collected and decoronated to standardize the lengths. Thespecimens were then mounted in acrylic resin and E. faecalis suspension was used to contaminate the specimens and they were incubated for three weeks. All the specimens were divided randomly into four groups. Instrumentation was done using Twoshape files (TS): Group I, TruNatomy files (TRN): Group II & Manual Crown Down technique (CD) using Gatesglidden drills and Hand files: GroupIII with sterile distilled water asirrigant. Group IV was served as control group. Microbial samples were collected using paper points without instrumentation of the samples and after instrumentationfromintra canal environment, which were diluted and late rplate dontryptic soybroth (TSB).

Results: Significant difference was seen amongst the experimental groups ($P < 0.05$), before and after instrumentation. TS and TRN resulted in more bacterial reduction than CD technique ($P < 0.05$) whereas TSand TRN were almost similar in their efficacy ($P > 0.05$).

Conclusion: TS and TRN promoted higher bacterial reduction than CD technique, with TS demonstrating the highest antibacterial efficacy.

Keywords: Twoshape, Tru Natomy, Enterococcusfaecalis

Introduction:

The purpose of endodontic treatment is to effectively reduce the bacterial load and facilitates complete cleaning, shaping and sealing of root canal system. The removal of microorganisms plays an important role in root canal treatment, failing of which will lead to pulpal and periapical diseases.[1] Among the microorganisms that harbor the root canal, Enterococcus faecalis is the one which penetrate deep into dentinal tubules (1100µm), henceforth very difficult to remove. This is the reason why it plays a major role in endodontic failure. [2]

Previously hand files were used to disinfect the root canal system. Mechanical preparation results only in 50-60% cleaning and shaping while the rest is done by the use of irrigating solutions.[3] With the advent of rotary endodontics

and Ni-Ti instruments, the root canal treatment becomes cost efficient and time saving. Over the years Ni-Ti instruments not only evolved from rotary to reciprocating motion, but also advanced in terms of flexibility, cutting ability.4But the drawback is that Ni-Ti instruments during biomechanical preparation may lead to formation of smear layer. This led to usage of demineralizing agents like Ethylene diamine tetraacetic acid (EDTA), Phytic acid etc. Thus judicious use of hand files, rotary files with demineralizing agents followed by

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copious irrigation will lead to complete disinfection of root canal system.⁵ Till now several studies were conducted to know the antibacterial efficacy of different rotary and reciprocating files against *E. faecalis*. They documented that there is a possibility that greater taper files could reduce more bacteria relatively to smaller taper files.^[6]

Two shape (TS) Micromega, Tulsa Dental Specialties, Johnson, WA, USA and TruNatomy (TRN) DENTSPLY Sirona Maillefer, Ballaigues, Switzerland and are two novel files systems introduced recently in endodontics. [7, 8] Till now their antibacterial efficacy is not yet documented. So the purpose of this study was to evaluate their efficacy against *Enterococcus faecalis*.

Materials & Methodology:

Preparation of samples:

Forty human teeth with single roots were collected and stored in 10% formalin until use. The specimens were decoronated by diamond disc and root lengths were standardized to fifteen millimetres. The working length was determined by a #15 K-file (Dentsply, Maillefer, Ballaigues, Switzerland) on a millimetre short of apex. Irrigation was performed with 5ml of distilled water. Cyanoacrylate resin was used to seal the apical for a men. Nail varnish was used to cover the root surface and the specimens were fixed individually in acrylic resin blocks. Autoclaving of specimens was done at 121°C and 15 psi pressure for 15 min.

Contamination of samples:

A suspension of 10 µl of *Enterococcus faecalis* (ATCC 29212) in sterile tryptic soy broth (TSB; Difco, Le Pont-de-Claix, RA, France) was prepared and standardized to #4 McFarland scale. Then, the samples were divided randomly into four groups of ten teeth each. Forty root canals were contaminated with the *E. faecalis* suspension by an insulin syringe. The plates were shaken (Fisher Vortex Genie 2; Fisher scientific, Bohemia, NY, USA) during 5 min to remove air pockets and to promote better bacterial penetration into dentinal tubules. The specimens were incubated at 37 °C for 21 days. The root canal contents were replaced with fresh TSB every 48 h. After the incubation period, the root canals were filled with distilled water. Initial samples (S1) were collected using three sterilized size 15 paper points (Dentsply Maillefer), which

were inserted into the root canals up to working length for 1 minute each. The points were stored in tubes containing 500 µL of peptone water followed by agitation in vortex for 1 minute, then, 6-fold serial dilutions were prepared. Different dilutions were plated in triplicate on m-Enterococcus agar culture medium (Difco). The plates were incubated at 37 °C for 48 h, and the bacterial count was measured (in CFU mL⁻¹).

Instrumentation of specimens:

In Group I, Two shape (TS) files were used. Canals were prepared initially using One G (Micro-Mega), 2Shape TS1 (25/.04) and TS2 (25/.06) files at 300 rpm and 1.2 N/cm torque values using VDW Gold (VDW, Munich, Germany) endodontic motor.

In Group II, instrumentation with TruNatomy (TRN) system was initiated with orifice enlarger and prime size file was used (26/.04). The speed and torque were adjusted to 500 rpm and 1.5 N/cm, respectively using VDW Gold (VDW, Munich, Germany) endodontic motor.

In Group III, CD technique was used where coronal shaping was done using Gates Glidden drills (#1, 2, and 3) and apical shaping was done with K-files till #25.

Group IV (Control group) specimens were left un-instrumented.

Irrigation during instrumentation was performed with a total of 10 mL of distilled water by using a syringe and a 29-gauge NaviTip (Ultradent Products, South Jordan, USA), which was taken up to 2 mm short of the working length through an in-and-out motion associated to aspiration (Neovoni, NRS Indústria, Barueri, SP, Brazil) for better flow. The irrigation was repeated with each time after withdrawing.

Data Collection after Instrumentation:

To determine the bacterial count (in CFU mL⁻¹) immediately after instrumentation (S2), the root canal was filled with distilled water, and scraping using a size 25 Hedström file (Dentsply, Maillefer) was performed. The file was sectioned below the handle and dropped into a tube containing 500 µL of peptone water. Three sterilized size 25 paper points

(Dentsply, Maillefer) were inserted into the root canal for 1 min each and were stored in the same tube as the file. The root canals were filled with distilled water and were closed by using cotton.

Statistical Analysis:

The mean and standard deviation values for the bacterial counts before and after instrumentation were calculated. In I, II & III groups. A Student's t-test for paired samples was used to compare the bacterial count before and after instrumentation. An ANOVA followed by a Turkey's Test was used to compare the antibacterial efficacy of each file system. A p-value <0.05 will be considered as statistically significant.

Results:

The mean and standard deviation values of bacterial count for I, II & III groups before and after instrumentation are shown in Table 1. The reduction in bacterial load in each of the instrumented groups is shown in Table 2. The results showed that there was statistically significant difference in between the two groups.

Group	N	Bacterial count before instrumentation	Bacterial count after instrumentation
Group I (Two shape)	10	7.39(±0.01)A	5.59(±0.02)B
Group II (TruNatomy)	10	7.41(±0.02)A	5.29(±0.01)B
Group III (Manual Crown down)	10	7.37(±0.01)A	6.18(±0.01)B

Table 1. Mean and standard deviation values for the bacterial count (in cfu/ml) before and after the instrumentation.

*Different upper case letters in the same row represent statistical difference between groups.

Group	N	Percentage (%) reduction in bacterial load
Group I (Two shape)	10	98.3A
Group II (TruNatomy)	10	97.7A
Group III (Manual Crown down)	10	92.2B

Table 2. Mean and standard deviation of bacterial load

reduction values (expressed as percentage) after instrumentation with respective file systems.

The different uppercase letters in the column represent statistically significant difference between the groups.

Discussion:

The route of entry for microorganisms into dentinal tubules is multinodal. Caries, cracks in dentin, leakage around cavosurface margins may cause the microorganisms to penetrate deep into dentin and cause infections of coronal and radicular tooth structure.[9,10] Henceforth root canal treatment aims to minimize intracanal bacteria and helps in achieving healing of periradicular tissues.[11] The minimum the bacterial load the better the treatment outcome.[12] Additive use of endodontic instruments with irrigants aids in complete disinfection of tooth structure before three dimensional obturation to create a hermetic seal.[13]

The present invitro study focused on the bacterial reduction efficacy of two different rotary systems used in the root canals. Two shape (TS) is made up of T wire that is heat treated and has cross section which is triangular in shape and asymmetrical all over. It has Simplified intuitive sequence with 2 shaping instruments in continuous clockwise rotation. 2 shape consists of 4 files - 2 shaping files {TS1 (25/.04), TS2(25/.06)} and 2 finishing files {F35 (36/.06), and F40 (40/.04)}. Advantages of 2 shape file are user friendly and exceptional negotiation of curvatures due to its flexibility.[7, 14]

Other file used in the study is TruNatomy (TRN) file, which have parallelogram cross section with off centered design. Sequence of instruments in TRN are an orifice modifier TRN OM (20/.08 taper) and a glide-path file TRN Glider (17/.02), three shaping instruments in three different sizes: Small (20/.04), Prime (26/.04) and Medium (36/.03). There is difference in wire design compared to other Ni-Ti rotary systems (0.8mm & 1.2mm respectively). Advantages of these files are ease of placement into canals due to regressive tapers, slim design, instrument geometry and shorter handle (9.5mm), conserves tooth structure.[8, 15, 16]

The microbial count was determined using plate culture method in this study, because of its ease of use and effectiveness. [17-20] Apart from that it also has resemblance to quantitative Polymerase Chain Reaction method.[21] The antibacterial efficacy was evaluated against *Enterococcus faecalis* in the present study as it is the most common microorganism that can be isolated from failed root canal treatment cases. The reason for this is its drug resistance which gives the ability to survive even in unfavourable

conditions and its depth of penetration into tubules of dentin. [22- 24] Further it is also well documented that streptococcus and enterococcus are the bacteria that initially invade the dentinal tubules.[9]

Both file systems TS and TRN promoted a higher bacterial reduction when compared to the manual crown-down technique using G.G drills and hand files. The asymmetrical triangular cross-section and progressive movement in three waves attributed to about 98.3% bacterial reduction in TS group. TRN accounted for 97.7% bacterial reduction, due to its flexibility obtained from heat treatment and accessibility obtained from shorter handle. Group I (TS) and II (TRN) showed statistically significant reduction in bacterial load when compared to group III (CD). Although the bacterial reduction was slightly higher in group II (TS) compared to group III (TRN), the results were not statistically significant. The results that were obtained in group IV (CD) were similar to study done by Machado et al in year 2017. [25]

Bacterial reduction was accomplished only with mechanical preparation without using any antibacterial irrigants.[26-28] In the present study also only distilled water was used for irrigation, during instrumentation. Even demineralizing agents like EDTA were not used to avoid their antibacterial effect. Still it was evident that bacterial reduction was significant with use of these novel file systems. But the role of antibacterial agents like sodium hypochlorite and chlorhexidine as irrigants in reducing the root canal microbes was well documented in the literature.[29-31] Furthermore, in debriding the root canal, activated irrigation may play an important role.[32,33] Hence, different irrigation protocols using various chemical substances should be considered for further studies.

Conclusion:

With use of rotary endodontics, more promising results can be obtained in eliminating bacteria from infected root canals particularly while doing retreatment. However one cannot ignore the conjunctive results that are obtained with the use of irrigants.

References:

1. Gorduysus M, Nagas E, Torun OY, Gorduysus OA. Comparison of three rotary systems and hand instrumentation technique for the elimination of *Enterococcus faecalis* from the root canal. *Australian Endodontic Journal* 2011; 37(3): 128-133.
2. Vatkar NA, Hegde V, Sathe .Vitality of *Enterococcus faecalis* inside dentinal tubules after ve root canal disinfection methods. *Journal of conservative dentistry* 2016; 19(5): 445.
3. Baker NA, Eleazer PD, Averbach RE, Seltzer S. Scanning electron microscopic study of the efficacy of various irrigating solutions. *Journal of Endodontics*, 1975;1:127-35.
4. Adina-Bianca Pi□, Ioana-AmaliaBorcean, Ionela-AndreeaVărgatu, Andreea Mai, Maryam Shyblak, Samir Mokdad,et al. Evaluation of the time and efficiency of trunatomy, vdw.rotate, protaper gold and reciproc blue in shaping root canals - an in vitro study. *Romanian Journal of Oral Rehabilitation* 2020; 12(3).
5. Peters OA, Laib A, Gohring TN, BarbakowF. changes in root canal geometry after preparation assessed by high-resolution computed tomography. *Journal of Endodontics* 2001;27:1-6
6. Vossoghi M, Vossoghi M, Shahriari S, Faramarzi F, Mashouf R Y, Farhadian M. Efficacy of a Novel Rotary System in Reduction of Intracanal Bacteria: An in Vitro Study. *Iranian endodontic journal* 2016; 11(3): 219-21.
7. Staffoli S, etal. Comparison of shaping ability of ProTaper Next and 2Shape nickel—titanium files in simulated severe curved canals. *GiornaleItaliano di Endodonzia* 2018.
8. DentsplySirona. TruNatomy Brochure. Available at <https://www.dentsplysirona.com/en/explore/endodontics/trunatomy.html>

9. Love RM, Jenkinson HF. Invasion of dentinal tubules by oral bacteria. *Critical reviews in oral biology & medicine* 2002; 13(2): 171-183.
10. Brittan JL, Sprague SV, Macdonald EL, Love RM, Jenkinson HF, West NX. In vivo model for microbial invasion of tooth root dentinal tubules. *Journal of Applied Oral Science* 2016; 24(2): 126-135.
11. Ferrer Luque CM, Bejarano I, Ruiz Linares M, Baca P. Reduction in *Enterococcus faecalis* counts—a comparison between rotary and reciprocating systems. *International endodontic journal* 2014; 47(4): 380-386.
12. Siqueira Jr JF, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. *Journal of endodontics* 2008; 34(11): 1291-1301.
13. Siqueira Jr JF, Alves FR, Almeida BM, De Oliveira JCM, Rôças IN. Ability of chemomechanical preparation with either rotary instruments or self-adjusting file to disinfect oval-shaped root canals. *Journal of Endodontics* 2010; 36(11):1860-65.
14. AhmetDemirhanUygun, EyüpCandaşGündoğdu, HakanArslan, İbrahim Ersoy. Efficacy of XP-endo finisher and TRUShape 3D conforming file compared to conventional and ultrasonic irrigation in removing calcium hydroxide. *Aust Endod Journal* 2017;43(2):89-93.
15. Elnaghy AM, Elsaka SE, Mandorah AO. In vitro comparison of cyclic fatigue resistance of TruNatomy in single and double curvature canals compared with different nickel-titanium rotary instruments. *BMC Oral Health* 2020; 20:38.
16. Van der Vyver PJ, Vorster M, Peters OA. Minimally invasive endodontics using a new single-file rotary system. *Int Dent – African ed.* 2019; 9(4):6–20.
17. Siqueira Jr JF, Alves FR, Rôças IN, Almeida BM, Neves MAS, Zoffoli J. Quantitative molecular and culture analyses of bacterial elimination in oval shaped root canals by a single file instrumentation technique. *International endodontic journal* 2012; 45(9): 871-877.
18. Machado MEDL, Nabeshima C, Leonardo MFP, Reis FAS, Britto M.L.B, Cai S. Influence of reciprocating single file and rotary instrumentation on bacterial reduction on infected root canals. *International endodontic journal* 2013; 46(11):1083-1087.
19. Nabeshima CK, Caballero-Flores H, Cai S, Aranguren J, Britto MLB, De Lima Machado M E. Bacterial removal promoted by 2 single file systems: Wave One and One Shape. *Journal of endodontics* 2014; 40(12): 1995-98.
20. Tewari RK, Ali S, Mishra SK, Kumar A, Andrabi SMUN, Zoya A, et al. Mechanical reduction of the intracanal *Enterococcus faecalis* population by Hyflex CM, K3 XF, ProTaper Next and two manual instrument systems: an in vitro comparative study. *Journal of investigative and clinical dentistry* 2016; 7(2): 168-173.
21. Alves FR, Rôças IN, Almeida BM, Neves MAS, Zoffoli J, Siqueira Jr J F. Quantitative molecular and culture analyses of bacterial elimination in oval shaped root canals by a single file instrumentation technique. *International Endodontic Journal* 2012; 45(9): 871-7
22. Abbaszadegan A, Gholami A, Ghahramani Y, Ghareghan R, Ghareghan M, Kazemi A et al. Antimicrobial and cytotoxic activity of Cuminumcuminum as an intracanal medicament compared to chlorhexidine gel. *Iranian Endodontic Journal* 2016; 11(1):44-46.
23. Giardino L, Estrela C, Generali L, Mohammadi Z, Asgary S. The in vitro effect of irrigants with low surface tension on *Enterococcus faecalis*. *Iranian Endodontic Journal* 2015; 10(3): 174-6.
24. Tabrizzadeh M, Rasti M, Ayatollahi F, Mossadegh MH, Zandi H, Dehghan F, et al. Antimicrobial activity of calcium hydroxide and betamethasone on *Enterococcus faecalis*; an in vitro assessment. *Iranian Endodontic Journal* 2015; 10(3): 184-7
25. Machado MEDL, Nabeshima CK, Caballero-Flores H, Elmadjian-Filho M, Duarte MAH, Odinola-Zapata R, et al. Instrument design may influence bacterial reduction during root canal preparation. *Brazilian Dental Journal* 2017; 28(5): 587-91.
26. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *European Journal of Oral Sciences* 1981; 89(4):321-28.
27. Dalton BC, Ørstavik D, Phillips C, Pettiette M, Trope M. Bacterial reduction with nickel-titanium rotary instrumentation. *Journal of Endodontics* 1998; 24(11): 763-67.
28. Siqueira Jr JF, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. *International Endodontic Journal* 1999; 32(5): 361-69.

29. Dametto FR, Ferraz CCR, De Almeida Gomes BPF, Zaia AA, Teixeira FB, De Souza-Filho F J. In vitro assessment of the immediate and prolonged antimicrobial action of chlorhexidine gel as an endodontic irrigant against *Enterococcus faecalis*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005; 99(6): 768-72.
30. Dornelles-Morgental R., Guerreiro-Tanomaru JM, De Faria-Júnior NB, Hungaro-Duarte M A, Kuga MC, Tanomaru-Filho M. Antibacterial efficacy of endodontic irrigating solutions and their combinations in root canals contaminated with *Enterococcus faecalis*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011; 112(3): 396-400.
31. Nakamura VC, Cai S, Candeiro GTDM, 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. *International Endodontic Journal* 2013; 46(3): 217-24.
32. Blank-Gonçalves LM, Nabeshima CK, Martins GHR, De Lima Machado M.E. Qualitative analysis of the removal of the smear layer in the apical third of curved roots: conventional irrigation versus activation systems. *Journal of Endodontics* 2011; 37(9): 1268-71.
33. Ordinola Zapata R, Bramante CM, Aprecio RM, Handysides R, Jaramillo DE. Bio film removal by 6% sodium hypochlorite activated by different irrigation techniques. *International Endodontic Journal* 2014; 47(7): 659-66.