Dental Research Journal

Original Article

Comparative evaluation of *Enterococcus faecalis* counts in different tapers of rotary system and irrigation fluids: An *ex vivo* study

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Received: November 2017
Accepted: January 2018

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ABSTRACT

Background: Bacteria and their by-products are etiological factors for the failure of endodontic treatment. Reduction of root canal bacterial contamination is one of the chief aims of root canal therapy. The aim of this study was to compare the effects of different rotary file tapers and two irrigation fluids on *Enterococcus faecalis* counts.

Materials and Methods: In this *ex vivo* study Root canals of 72 human upper lateral incisors were enlarged to ISO #20 K-file. Then, the samples were sterilized and inoculated with *E. faecalis* for 72 h, divided into six experimental groups and prepared with #30 Flexmaster files with 0.02, 0.04, and 0.06 tapers and two different irrigation solutions such as normal saline and sodium hypochlorite. The control group (n = 10) was subdivided into two groups with or without bacterial inoculation and no mechanical instrumentation. Cleaning efficacy was evaluated in terms of the reduction of colony forming units (CFUs). *T*-test, ANOVA, Duncan, and Tukey tests were applied to the groups. A significant level of α = 0.05 was set for comparison between the groups.

Results: The canals instrumented with 0.06 taper exhibited greater significant reduction in CFUs compared to canals instrumented with 0.04 and 0.02 taper ($P < 0.05$); 0.04 taper also resulted in greater significant reduction in CFUs than 0.02 taper ($P < 0.05$). In addition, no significant differences were observed in *E. faecalis* counts between the two irrigation fluids ($P > 0.05$).

Conclusion: Under the conditions of this study, root canal preparation with greater taper resulted in canal cleanliness and better debridement.

Key Words: Bacterial load, dental instruments, *Enterococcus faecalis*, root canal preparation

INTRODUCTION

Bacteria and their by-products have a major role in pulpal and periapical pathogenesis.[1-5] It seems reasonable that elimination or reduction of pathogens as a prime objective in the successful treatment of apical periodontitis leads to successful treatment of this condition. In fact, when previously infected canals are rendered negative in bacterial sampling, an improved prognosis has been achieved.[6,7]

Among bacteria, *Enterococcus faecalis* is the most consistently reported organism from former

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How to cite this article: Navabi AA, Khademi AA, Khabiri M, Zarean P, Zarean P. Comparative evaluation of *Enterococcus faecalis* counts in different tapers of rotary system and irrigation fluids: An *ex vivo* study. Dent Res J 2018;15:173-9.

cases. This organism is resistant to most intracanal medicaments and can survive up to a pH value of 11.5.[8] *E. Faecalis* can also survive prolonged starvation and grow as a monoinfection in treated canals in the absence of synergistic support from other bacteria.[9] Moreover, endodontic infections with *E. faecalis* are usually difficult to eliminate, even with intracanal calcium hydroxide dressing.[10]

Reduction in bacterial counts is accomplished by a triad of mechanical instrumentation such as cleaning and shaping with various irrigating solutions, and disinfection with intracanal medicaments.[4,11-13] When endodontic instruments were manufactured by the ISO, it was believed that the only way to reach the irrigation fluids to the critical apical 3-mm of the root canal, was apical preparation as wide as possible to reduce the microbial population and increase cleanliness.[14-17] Currently, after the introduction of nickel-titanium rotary systems, it is suggested that increasing the root canal taper should be implemented with apical preparation as narrow as possible.

Buchanan refers to advantages of "variable taper" as tapering with easy and simple use, enhanced and predictable cleaning and obturation outcomes even in inexperienced hands, adequate coronal enlargement, full deep shape, and apical resistance form in a simple instrument sequence.[18] In a recent study, de Gregorio *et al.* assessed only the effect of apical size and taper on irrigation solution volume delivered without analyzing the effect of irrigation solution on cleanliness and suggest that apical preparation of 40# taper 0.06 significantly increase the volume of irrigant at the working length.[19]

In a study rather similar to ours, Arvaniti and Khabbaz, Mohammadzadeh Akhlaghi *et al.*, Cohenca *et al.*, and Moshari *et al.* investigated the effects of taper, size and irrigation on root canal cleanliness. They stated greater size and taper with positive pressure irrigation could reduce the count of root canal bacteria. Our detailed methodology, however, is different from theirs.[2,20-22]

The aim of the present study, therefore, was to investigate the effects of different irrigation fluids and taper of the rotary system on root canal cleanliness in terms of decreases in colony forming units (CFUs) in the 3-mm apical area of the powdered root. The null hypothesis stated that the increase in taper does not affect *E. faecalis* counts.

MATERIALS AND METHODS

In this *ex vivo* study atotal of 82 human upper lateral incisors with one root canal, extracted due to orthodontic or periodontal reasons, were collected. Before preparation, all the teeth were radiographed in buccolingual and mesiodistal directions to exclude teeth with any aberrant canal morphology and to confirm a single canal. Teeth with severe root curvature, cracks or fractured roots, calcified root canals, immature apices, and decayed and filled teeth were excluded from the study. Ethics approval was granted by the Ethics Committee of Islamic Azad University, Isfahan (Khorasgan) branch (UREC 23810201902002).

The teeth were cleaned and debrided gently by the use of periodontal curette right after extraction. Then, they were immersed in 5.25% sodium hypochlorite (NaOCl) solution (Shamin chemical Co. Tehran, Iran) for 30 min for surface disinfection and stored in 0.9% sterile normal saline solution (Daroopakhsh, Tehran, Iran). In the first place, the teeth were cut from cementoenamel junction perpendicular to their long axis with a diamond disk (Horico H557F220) 12 mm from the root tip [Figure 1]. Patency of the root canals and presence of one root canal were ensured by using #10 and #15 K-files (Densply/Maillefer, Ballaigues, Switzerland). To reduce confounding variables, all the samples were primarily instrumented up to #20 K-file (Densply/Maillefer, Ballaigues, Switzerland) under copious irrigation with distilled water up to 11 mm working length. Then, roots were soaked in 17% ethylenediaminetetraacetic acid (Vericom, Korea) for 10 min, followed by

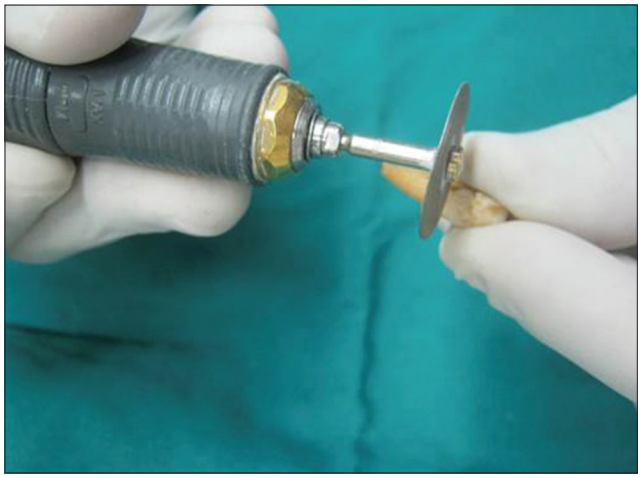

Figure 1: Standard cutting from cementoenamel junction.

5.25% NaOCl for 10 min; finally, the samples were rinsed with sterile water. After preparation, the roots were randomly divided into six experimental groups (n = 12) and two control groups (n = 5).

All the samples were placed in brain-heart infusion (BHI) broth and autoclaved for 20 min 121°C and 15 psi. All the samples were autoclaved again in the same way as the first time for better accuracy. To ensure sterilization, all the samples were incubated separately in a micro-tube containing BHI for 24 h under aerobic and aseptic conditions at 37°C. If turbidity was observed in the micro-tube, it meant incomplete sterilization and the process of sterilization was repeated.

In this study, to create standard and controlled infection in all the cases, we used *E. faecalis* as a resistant bacterial species. This Gram-positive anaerobic bacterial species were obtained from the Department of Microbiology, Faculty of Medicine, Tehran University of Medical Sciences with an ID code of ATCC-29212. Furthermore, we used bile esculin agar since it allows the growth of enterococci and Streptococci, including *E. faecalis*.

A suspension of bacteria was prepared by adding 1 mL of a pure culture of *E. faecalis* (ATCC2912) grown in BHI broth; 0.05 mL of suspension was injected by volume sampler into each canal of the experimental group and positive control group [Figure 2]. The access cavity was sealed with intermediate restorative material Cavit (Premier Dental Products Co., Philadelphia, PA, USA). The roots were incubated at 37°C for 72 h separately.

The samples were kept sterile and divided into six groups (n = 12) after the incubation period. Preparation steps were performed as follows:

In all the groups, recapitulation with #15 K-file (Densply/Maillefer, Ballaigues, Switzerland) between different instruments was carried out, and after each file, 2 mL of irrigation solution was flushed. Canal preparation was carried out by single-length technique with Flexmaster rotary instruments.

- Group I – Preparation was carried out with this sequence: Flexmaster 0.02 taper, #30; Flexmaster 0.04 taper, #30; Flexmaster 0.06 taper, #30, with 2 mL of 2.5% NaOCl irrigation after each file
- Group II – Preparation was carried out with this sequence: Flexmaster 0.02 taper, #30; Flexmaster 0.04 taper, #30, with 2 mL of 2.5% NaOCl irrigation after each file

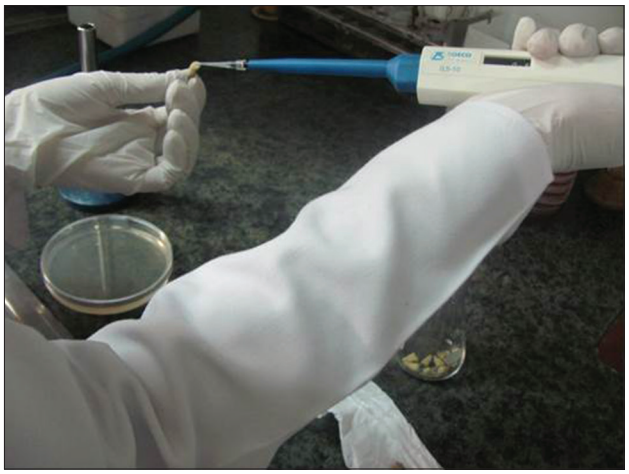

Figure 2: Contamination of samples.

- Group III – Preparation was carried out with this sequence: Flexmaster 0.02 taper, #30, with 2 mL of 2.5% NaOCl irrigation after each file
- Group IV – Preparation was carried out with this sequence: Flexmaster 0.02 taper, #30; Flexmaster 0.04 taper, #30; Flexmaster 0.06 taper, #30, with 2 mL of normal saline irrigation after each file
- Group V – Preparation was carried out with this sequence: Flexmaster 0.02 taper, #30; Flexmaster 0.04 taper, #30, with 2 mL of normal saline irrigation after each file
- Group VI – Preparation was carried out with this sequence: Flexmaster 0.02 taper, #30, with 2 mL of normal saline irrigation after each file
- Group VIIa – No instrumentation was performed after bacterial inoculation
- Group VIIb – Neither bacterial inoculation nor mechanical instrumentation was performed.

After canal preparation and final rinsing with 10 mL of distilled water, 3-mm apical area of the root was powdered with a carbide bur driven by a Micromotor (NSK, Japan), low speed of 500 rpm by rinse of sterile distilled water and wait for drying. Then, transferred powders into tubes containing sterile BHI in the same condition for all the groups. After 10-fold serial dilutions in saline solution, aliquots of 0.1 mL were plated onto nutrient agar plates and incubated at 37°C for 48 h [Figure 3]. The CFUs were counted manually using a pen and click-counter after 24 h of growth.

RESULTS

The comparison between the groups, according to two-way ANOVA with regard to the reaction, showed

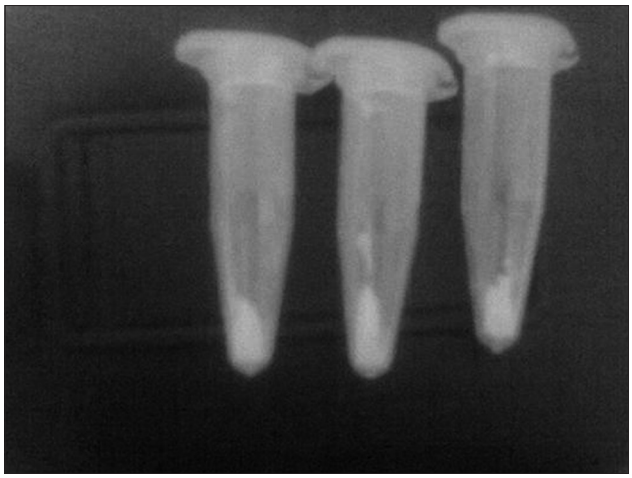

Figure 3: Microbiologic evaluation.

no interplay between 2.5% NaOCl and normal saline groups. However, one-way ANOVA showed significant differences between the groups.

Duncan test was used to assimilate *E. faecalis* counts in prepared teeth with 0.02, 0.04, 0.06 taper and 2.5% NaOCl as an irrigation solution. The test showed significant differences, suggesting that high tapering reduced bacteria more than low tapering. Different tapers in normal saline groups yielded the same results as the NaOCl groups.

E. Faecalis counts in prepared teeth with 0.02 taper and NaOCl and normal saline as irrigation solutions were assessed using *t*-test which showed no significant differences ($P = 0.27$); 0.04 and 0.06 taper with different irrigation solutions also showed no significant differences with $P = 0.42$ and $P = 0.33$, respectively.

Canals instrumented with 0.06 taper exhibited greater reduction in CFUs compared to canals instrumented with 0.04 and 0.02 tapers ($P < 0.001$). Instrumentation with 0.04 taper also yielded a greater reduction in CFUs compared to canals instrumented with 0.02 taper ($P < 0.001$). Thus, canals instrumented with greater taper were cleaner, with no significant difference between the two irrigation solutions. CFU counts in each specimen in different groups are presented in Table 1.

Table 1: Colony-forming unit counts in each of the specimens in different groups

Irrigation fluid	0.02 taper		0.04 taper		0.06 taper	
	SD	Mean	SD	Mean	SD	Mean
NaOCl 2.5%	22.49	113.66	13.49	97.83	10.87	77.83
Normal saline	20.68	123.58	22.70	104.08	17.56	89.41

SD: Standard deviation

DISCUSSION

The aim of the present study was to evaluate the effect of different irrigation solutions and taper of rotary systems on *E. faecalis* counts.

E. faecalis is a resistant bacterial species that believed to be the cause of endodontic treatment failure.

Based on the results of the present study, 2.5% NaOCl and normal saline did not exhibit significant differences. Selection of 2.5% NaOCl solution was based on the fact that lower or higher NaOCl concentrations have shown no significant differences in their antibacterial effects.[23]

This is consistent with Shabahang and Torabinejad who suggested the ineffectiveness of NaOCl to consistently disinfect root canals. They demonstrated that 50% of the root canals remained contaminated with *E. faecalis* despite irrigation with 1.3% or 5.25% NaOCl.[24] Another study used 1.25% NaOCl and found that about 38.1% of root canals remained contaminated with bacteria.[25] also Sjögren *et al*. in their clinical study stated that after the use of 0.5% NaOCl in debridement process, 40% of root canals remained infected.[26] Siqueira *et al*. studied on human extracted teeth were infected with *E. faecalis* and supposed after NaOCl 4% irrigation, 30%–40% of the root canals still contained viable bacteria.[27]

Gonçalves *et al*. and Rôças and Siqueira. reported reductions in bacterial counts with both NaOCl and chlorhexidine irrigants, with no significant differences between them.[28,29]

A substantial reduction in the bacterial counts was observed after chemomechanical preparation using either irrigant. This finding is consistent with many other studies,[11,30,31] confirming the essential role of chemomechanical procedures in eliminating intraradicular bacteria.[29]

Although there are some studies in contrary to our results,[32] it may be attributed to different methodologies.

However, antibacterial effects of NaOCl are recognized, the exact mechanism of microbial killing is not well clarified.[26] Therefore, we concluded that 2.5% NaOCl can reduce bacterial contamination, with no significant effect on *E. faecalis*. This can shows that elimination of *E. faecalis* might be more attributed to the mechanical action of instruments.

Another finding of the present study on different tapers is that root canal taper affected its cleanliness. This result can be compared with some previous studies, carried out by considering both size and taper. Mohammadzadeh Akhlaghi *et al*., Albrecht *et al*., Usman *et al*. and Lumley suggested that the greater size and taper could reduce the bacterial load.[2,33-35] Our results about greater taper are consistent with these studies.

To achieve the main aim of this study, we evaluated studies that prepared root canals with the same size but with different tapers. Consistent with our study results, Lee *et al*. suggested that an increase in taper leads to better debridement.[36] Singla *et al*. reported a significant decrease in bacterial counts with progressively larger tapers.[37] Lumley also reported that canals shaped with hand files of greater taper were significantly cleaner.[35] These findings are consistent with those of Dalton *et al*., Byström and Sundqvist.[38-40]

The results of Siqueira *et al*. were in contrast to the results of the present study. They suggested that canal preparation with different tapering had no effect on further reduction in bacterial counts in root canals.[41] The use of mandibular premolars in their research might be one of the reasons for the absence of significant differences in the results of their study. The cross-section of mandibular premolars is oval and wider in buccolingual direction. Despite the two instrumentation techniques that were performed in their study, the oval walls might remain unfilled. To solve this problem, anterior teeth with one root canal and circular cross-section anatomy were used in our study.

Arvaniti and Khabbaz also suggested that the cleanest part of the canal is in the middle third, with a statistically significant difference from the apical third. In their study, debris removal was almost complete with all the tapers, whereas the smear layer was not removed because of inadequate irrigation fluids.[20] According to this study, we designed our study to analyze the 3-mm apical area of the root canals.

None of the previous studies considered the penetration of bacteria into dentinal tubules. Hence, we were encouraged to plan a study with a different and accurate methodology. The study was therefore carried out by powdering 3-mm root end with a carbide bur, instead of paper points, piezo drills, or optical microscope observation.[37,38,42-44]

It is noteworthy that the strength of teeth with root canal treatment directly depends on the remaining intact tooth structure. Endodontic treatment processes result in the loss of tooth structure and weak root canal walls.[45,46]

CONCLUSION

The results of the present study showed that high tapering is more effective in root canal cleanliness, reducing *E. faecalis* counts to almost zero level. However, other clinical effects of larger instrumentation, including compromised restorability, fracture susceptibility, and canal path alterations should also be considered when using any instrumentation technique. Thus, root canals should be flared as needed and prepared in a conical shape with a gentle taper. Further studies are suggested to evaluate the effects of different tapers on VRF strength of the root.

Financial support and sponsorship
Nil.

Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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