Reverse Rotary Instrumentation in the Apical Third of the Root Canal System: An Scanning Electron Microscope Analysis

Abstract

Aims: The aim of the present study was to evaluate the efficacy of reverse rotary instrumentation in disinfection of the root canal at the apical third and qualitative confirmatory analysis using the scanning electron microscope (SEM). Subjects and Methods: Sixty single-rooted mandibular premolars were instrumented up to Protaper rotary file size F2 and contaminated with a known species of Enterococcus faecalis (ATCC 29212). The samples were then divided into three groups; Group 1: Experimental group—irrigation by agitation of 1% NaOCl with reverse rotary instrumentation; Group 2: Negative control—no irrigation; and Group 3 positive control—irrigation with 1% NaOCl using a 30-gauge needle. The colony forming units of all the groups were checked. SEM analysis of the samples was focused on the apical third to confirm the absence of E. faecalis biofilms. The data obtained were statistically analyzed by the Fisher’s exact test and Pearson’s Chi-square test.

Results: Group I and III showed significant reduction in the growth of E. faecalis (P ≤ 0.001). SEM confirmed dense bacterial colonies in the Group II consistent with biofilm formation and reduction in bacterial colonies in Group I and II. Conclusion: Agitation with reverse rotary instrumentation in the apical third of the root canal along with 1% sodium hypochlorite proved effective in disinfection of the apical third of the root canal, which was further confirmed by scanning electron microscopic analysis. Hence, it can be used as an adjunct during rotary instrumentation in efficient cleansing of the root canal system in the apical third of the root canal system.

Keywords: Apical third, disinfection, Enterococcus faecalis, reverse rotary instrumentation, sodium hypochlorite

Introduction

Endodontic success necessitates the removal of vital and necrotic pulpal tissue, infected dentin, and dentinal debris to eliminate most of the microorganisms from the root canal system (European Society of Endodontology 1994 and American Association of Endodontists 1998). Despite the advent of newer instruments and techniques, the design and the physical limitations of the endodontic instruments can cause inadequate disinfection of the root canal system. For the long-term preservation of endodontically treated teeth, the eradication of persisting bacteria in the distant areas of the tubular root canal system poses a major challenge. Enterococcus faecalis is known to proficiently invade dentinal tubules, further survive chemomechanical instrumentation and intracanal medication also; adapt to altered nutrient supply and continue to remain viable within the dentinal tubules. Nonetheless, elimination of endodontic infection follows a different pathway due to the anatomical challenges of the root canal. Host measures that are sufficient to eliminate the infective microorganisms in other sites are unable to completely eliminate endodontic infections and a sequence of procedures in the control of endodontic infections are required: instrumentation and irrigation, intracanal medicaments used between appointments, permanent root filling, and coronal restoration. Mechanical instrumentation not only removes microbes from the main root canal spaces but also enhances irrigation, placement of medication, and the root filling. Irrigation supports mechanical instrumentation, by reducing friction and removing dead and living microbes from the root canal and its complexities such as fins, isthmi, and cul-de-sacs, where instrumentation is potentially impossible.

Microorganisms either remaining in the root canal space after treatment or recolonizing the filled canal system are established to be the main cause of

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endodontic failure. Effective irrigants act as bactericidal agents, tissue solvents, lubricants, and remove organic and inorganic debris. Irrigation is an essential part of root canal debridement because it allows for cleaning beyond what might be achieved by root canal instrumentation alone. Byström et al. established that mechanical instrumentation of the root canal using saline irrigation alone frequently leaves cultivable bacteria in the canal system. Thus, disinfectants such as sodium hypochlorite are essential. The apical third of the root canal is the most difficult area to clean due to the complex anatomy of this region such as deltas, lateral canals, isthmuses, and ramifications. Shaping procedures can be completed more easily, quickly, and predictably using nickel-titanium (NiTi) rotary instruments, yet effective cleansing of the root canal system, especially in the apical one-third, has not yet been demonstrated.

More importantly, these irrigants must be brought into direct contact with the entire canal wall surfaces for effective action, particularly for the apical portions of small root canals. Throughout the history of endodontics, endeavors have continuously been made to develop more effective irrigant delivery and agitation systems for root canal irrigation.

According to Gutmann, a rotary NiTi instrument that fits loosely in the canal can be used in reverse to place the material apically; the file is placed only to the middle third, and the rotary action will carry the material apically. This hypothesis can be utilized to deliver irrigant solutions into the apical third of the root canal, where effective cleansing has not been demonstrated.

The aim of the present study was to evaluate the efficacy of reverse rotary instrumentation using 1% sodium hypochlorite in debridement of the root canal at the apical third.

Subjects and Methods

Sixty, freshly extracted single-rooted human incisors with fully developed apices were collected and stored in 0.9% physiologic saline for ≤4 weeks. Teeth were disinfected and handled according to the recommendations and guidelines laid down by the Occupational Safety and Health Administration and Centers for Disease Control and Prevention.

Preparation of bacteria

A pure bacterial culture of the Gram-positive cocci Escherichia coli (ATCC 29212) was grown and harvested by placing in the Mueller-Hinton nutrient broth and incubated for 24 h at 37°C under aerobic conditions. The turbidity of the Mueller-Hinton broth containing E. coli was adjusted to McFarland 0.5 standard, which corresponds to $1.5 \times 10^6$ colonies. Then, the 20 µL of the bacterial culture was transferred into canal lumen of the mechanically enlarged root canals using a sterile micropipette and incubated for 48 h at 37°C.

Specimen preparation

The crowns of sixty freshly extracted single-rooted human incisors were sectioned 2 mm above the cementoenamel junction using a high-speed diamond bur (SS White® Burs, USA, ISO 9001) with water coolant. Two coats of nail varnish were applied to seal the apex. Working length was determined with the help of the digital radiography (Intra SkanDigi, Skanray Technologies, Mysore, India). The roots were prepared by serial preparation to #30 K-file for easier inoculation of bacteria. Saline was used as an irrigant between each step of the preparation for removal of debris. The preparation was stopped at the apical constriction. Rotary NiTi files were used to instrument the canals with crown-down technique to a size F2 at 300 rpm. The apical portion was tapered with 0.02 tapered NiTi hand files to an ISO #35 to ensure the removal of debris at the apical third.

All the specimens were randomly divided into three groups of 20 specimens each and subjected to irrigation:

- **Group 1 (Experimental group):** Canals were inoculated with E. faecalis, 1% sodium hypochlorite was deposited using a 27-gauge needle and reverse rotary motion was performed with Protaper Sx series placing the instrument at the middle-third. A 27-gauge needle was used as it required just the deposition of the irrigant into the canal.
- **Group 2 (Positive control):** Canals were inoculated with E. faecalis and were irrigated with 1% sodium hypochlorite using a 30-gauge needle. A 30-gauge needle allows for better penetration of the irrigant into the apical third of the canal due to the thinner diameter of the needle. Hence, it was used for irrigation in this group.
- **Group 3 (Negative control):** Canals were not contaminated with E. faecalis. This was done to evaluate that there should be no contamination of the canals by other microorganisms.

To standardize the irrigant delivery into the root canal system, all the teeth were uniformly irrigated with 2 ml of 1% sodium hypochlorite.

For bacterial sampling

After irrigation, paper points were used to collect the samples from the teeth and were placed in brain-heart infusion broth in microtubes and incubated for 24 h. Samples from microtubes showing turbidity were streaked with nichrome wire loops on agar plates and incubated at 37°C for 48 h, for the confirmation of E. faecalis colony growth. Colony-forming units (CFUs) were counted and the purity of the cultures was confirmed by Gram staining and colony morphology.

For scanning electron microscope analysis

The samples were then prepared for scanning electron microscope (SEM) analysis. Teeth were...
longitudinally grooved and split. Samples were rinsed in phosphate-buffered saline, soaked in glutaraldehyde for 1 h, and postfixed in 1% OsO₄ (osmium tetrachloride) for 30 min. Samples were then dehydrated, mounted on SEM discs, and spatter coated with palladium gold. The analysis of the samples focused on the apical third, and photographs were taken within 2 mm of the working length.

Statistical analysis of the data was done using Pearson’s Chi-squared test and Fisher’s exact test.

The samples were subjected under ×1000 and ×3000 magnification for scanning electron microscopic analysis.

Results

Antimicrobial analysis

Significant results were seen experimental group –10³ CFU/ml and the positive control group –10⁵ CFU/ml as compared to the negative control group –10⁸ CFU/ml.

Significant results were seen experimental group, 76.9% of experimental cases showed 10³ CFU/ml, 71.4% of positive control cases showed 10⁵ CFU/ml as compared to the 87.5% negative control cases which showed 10⁸ CFU/ml [Figure 1].

Scanning electron microscopic analysis

Significant reduction in the number of colonies as compared to the negative and positive controls were seen in the experimental group [Figure 2]. At ×1000 magnification, a clear area of dentinal tubules was seen in the experimental group. At ×3000 magnification, significant reduction in the cell aggregation of bacteria covering the dentinal tubules was seen. Reduction in the smear layer was also seen. In the positive control group, at ×1000 magnification, cell aggregates of bacteria were seen covering the dentinal tubules, and at ×3000 magnification, bacterial biofilms were seen covering the dentinal tubules [Figure 3]. It also showed a reduction in the bacterial colonies and smear layer when compared to the negative group which showed the presence of bacteria over the root canal surface at ×1000 magnification and dense bacterial biofilms over the root canal surface at ×3000 [Figure 4].

Discussion

Microorganisms in the root canals have long been recognized as the primary etiologic factors in the development of pulp and periapical lesions.[14] Elimination of the microorganisms is one of the important objectives for successful root canal treatment. It is necessary to chemically debride teeth with complex internal anatomy or other irregularities that might be missed by instrumentation of the root canals.[15] Hence, the use of irrigants during root canal treatment is of prime importance.

In the present study, 1% NaOCl was used as an irrigant as it is a broad-spectrum antimicrobial agent. It’s powerful oxidative activity not only dissolves the pulpal and dentinal tissue but also flushes them out of the canals.[15] This is in agreement with studies done by A. U. Eldeniz et al. 2007 and Rupali Karale et al. 2011.[16,17] Even though being one of the most widely used irrigants in endodontic therapy its toxicity to periapical tissues remains a principal concern.[18] NaOCl is best known for its strong antibacterial activity. It kills bacteria very rapidly even at low concentrations. Hypochlorous acid disrupts oxidative phosphorylation and other membrane-associated activities as well as DNA synthesis.[19]

Severe irritations have been reported when concentrated solutions were inadvertently forced into the periapical
tissues during irrigation or leaked through the rubber dam. Furthermore, a 5.25% solution significantly decreases the elastic modulus and flexural strength of human dentin compared to physiologic saline, while a 0.5% solution does not. This is most likely because of the proteolytic action of concentrated hypochlorite on the collagen matrix of dentin. The reduction of intracanal microbiota, on the other hand, is not any greater when 5% sodium hypochlorite is used as an irrigant as compared to 0.5%. From in vitro observations, it would appear that a 1% NaOCl solution should suffice to dissolve the entire pulp tissue in the course of an endodontic treatment session. Hence, based on the currently available evidence, there is no rationale for using hypochlorite solutions at concentrations over 1% wt/vol.\textsuperscript{[21,22]}

\textit{E. faecalis} (ATCC 29212) was selected as the test organism because it is commonly associated with root canal failure cases and persistent apical periodontitis. It is also resistant to interappointment calcium hydroxide dressing.\textsuperscript{[23]} They also have the ability to reside in the canals without the support of other microorganisms and under specific conditions have the ability to infect the whole length of tubules within few days.\textsuperscript{[4]}

Gomes et al. tested \textit{in vitro} the effect of various concentrations of NaOCl against \textit{E. faecalis}. \textit{E. faecalis} was killed within 30 s by the 5.25% solution, while 10 and 30 min was required for killing all bacteria by 2.5 and 0.5% solutions, respectively.\textsuperscript{[23]} The higher resistance of \textit{E. faecalis} to hypochlorite as compared with the yeast \textit{Candida albicans} was suggested also by Radcliffe et al.\textsuperscript{[20]}

However, the study is in contrast to the results reported by Haapasalo et al. who demonstrated rapid killing of \textit{E. faecalis} strains in logarithmic and stationary growth phase by even 0.001% NaOCl.\textsuperscript{[7]}

Different techniques and devices have been proposed to improve the flow and distribution of irrigating solutions within the root canal system. Even with the use of rotary instrumentation, the NiTi instruments currently available only act on the central body of the canal, leaving canal fins, isthmi, and cul-de-sacs untouched after completion of the preparation.\textsuperscript{[24-26]} These areas might harbor tissue debris, microbes, and their by-products, which might prevent close adaptation of the obturation material and result in persistent periapical inflammation.\textsuperscript{[27]} Therefore, irrigation is an essential part of root canal debridement because it allows for cleaning beyond what might be achieved by root canal instrumentation alone.\textsuperscript{[28]} Throughout the history of endodontics, endeavors have continuously been made to develop more effective irrigant delivery and agitation systems for root canal irrigation. Previous studies have stated that the most important factor is the delivery system and not irrigating solution \textit{per se}.\textsuperscript{[24]}

Irrigation is most feasible in the instrumented areas because the irrigation needle can follow the smooth path created by the instruments. Hence, in the present study, apical enlargement was done until Protaper size F2 for easy penetration of irrigant into the canals. Visibility in microcomputed tomography scans indicates that the debris also contains a considerable proportion of inorganic material.\textsuperscript{[23]} Although at present it is not known how the debris can best be removed, it is likely that physical agitation and the use of demineralizing agents are needed in addition to hypochlorite. In the present study, the orifice opener, Sx was used to agitate and deliver the irrigant in reverse rotary motion into the apical third of the root canals. This method of delivery was based on Gutmann’s hypothesis that a rotary NiTi instrument that fits loosely in the canal can be used in reverse to place the material apically; the file is placed only to the middle third, and the rotary action will carry the material apically, which was validated in a study done by Simcock et al. for evaluating the efficacy of delivery of intracanal medicament.\textsuperscript{[29]} In the present study, the same hypothesis was adapted for delivery of irrigant into the apical third of the root canal system, wherein there was a significant reduction in the growth of the CFUs in the apical third of the root canal system using this technique. The scanning electron microscopic analysis confirmed the reduction in the \textit{E. faecalis} biofilms compared to the negative and positive controls.

Conventional irrigation with syringes has been advocated as an efficient method of irrigant delivery before the advent of passive ultrasonic activation.\textsuperscript{[26]} The technique involves dispensing of an irrigant into a canal through needles/cannulas of variable gauges, either passively or with agitation. The latter is achieved by moving the needle up and down the canal space. Some of these needles are designed to dispense an irrigant through their most distal ends, whereas others are designed to deliver an irrigant laterally through closed-ended, side-vented channels.\textsuperscript{[18]} It is crucial that the needle/cannula should remain loose inside the canal during irrigation. This allows the irrigant to reflux and causes more debris to be displaced coronally, while avoiding the inadvertent expression of the irrigant into periapical tissues. One of the advantages of syringe irrigation is that it allows comparatively easy control of the depth of needle penetration within the canal and the volume of irrigant that is flushed through the canal.\textsuperscript{[26]} In the present study, conventional needle irrigation was performed with a 30-gauge needle. This group was considered to be
the positive control. Significant reduction in the CFUs was observed in comparison to the negative control where no irrigation was performed but not as significant as the experimental group.

SEM analysis was performed to confirm the effectiveness of irrigation at the apical third of the root canal based on the presence or absence of bacterial biofilms. Significant reduction in the number of colonies as compared to the negative and positive controls was seen in the experimental group. Furthermore, smear layer removal and a clear layer of dentin was seen. This confirmed the effectiveness of the agitation by reverse rotary instrumentation in the apical third of the root canal system. The positive control group also showed a reduction in the bacterial colonies and smear layer when compared to the negative group which showed dense bacterial colonies but not as much as the experimental group.

**Conclusion**

Delivery of an irrigant in the apical third of the root canal system poses to be a challenge in successful endodontic treatment. In the present study, agitation of 1% sodium hypochlorite with reverse rotary instrumentation demonstrated efficacious disinfection of the apical third of the root canal system, which was further confirmed by scanning electron microscopic analysis. Thus, this technique can be effectively used as an adjunct during cleaning and shaping for thorough disinfection of the apical third of the root canal system.

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**Conflicts of interest**

There are no conflicts of interest.

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