EGYPTIAN DENTAL JOURNAL

VOL. 68, 1967:1978, APRIL, 2022

PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360

CONSERVATIVE DENTISTRY AND ENDODONTICS

Submit Date : 2022-02-08 • Accept Date : 2022-03-16 • Available online: 2022-03-30 • DOI : 10.21608/edj.2022.120697.1974

CUMULATIVE IMPACTS OF ENDODONTIC PROCEDURES ON ENTEROCOCCUS FAECALIS: AN IN VITRO STUDY

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ABSTRACT

Objectives: Assessment of the cumulative impacts of endodontic procedures using novel BioAKT Endo root canal irrigant on Enterococcus faecalis in comparison with sodium hypochlorite irrigant.

Materials and Methods: Eighty single rooted premolar teeth were collected for this study. The crowns were sectioned and the length of the roots was standardized to 15 mm. The roots were then initially cleaned, shaped, and inoculated with E. faecalis. The excess broth was removed by sterile paper points and then sample 1 (S1) was taken. The roots were divided into eight groups, groups 1-4 were instrumented and irrigated with BioAKT Endo root canal irrigant then sample 2 (S2) was taken. Groups 5-8 were instrumented and irrigated with 5.25% NaOCl. Standard laboratory methods were followed to determine the number of CFU for the tested organism. Data were collected and analyzed. A specimen from each group was randomly selected and investigated under scanning electron microscope (SEM) to evaluate the bacterial contamination on the root canal walls initially and after the endodontic procedures.

Results: Marked bacterial reduction was detected in all groups after instrumentation and irrigation with BioAKT Endo or 5.25%NaOCl, however NaOCl groups were superior to BioAKT Endo groups. The maximum bacterial reduction was achieved in group 5 followed by 7, 6, 8,1,3,2, and 4 in descending order. Scanning electron microscopic evaluation confirmed the final microbial results.

Conclusions: BioAKT Endo is considered a novel irrigant material for root canals with a marked and promising anti-bacterial activity. Further in-vivo and in-vitro studies are required to investigate this class of endodontic irrigants.

KEYWORDS: BioAKT Endo, Sodium hypochlorite, Ethylenediamine tetraacetic acid, Enterococcus faecalis.

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INTRODUCTION

Pulpal and periapical pathosis as well as the prognosis of endodontic treatment are primarily influenced by bacteria and their by-products. Therefore, the main objective, in contemporary root canal treatment, is complete eradication or even reduction of the bacteria inside the canals to a lower level to preclude periapical pathosis or allow its resolution. During root canal treatment, bacterial eradication is aaccomplished mainly by a mechanical instrumentation, different antiseptic irrigation solutions and intracanal medicaments.[1]

Despite adequate cleaning, shaping and irrigation with disinfectants or antiseptics, achieving a bacteria free root canal space is unfeasible. Therefore, there is a concern about the fate and implications of the residual micro-organisms inside the root canal, since rapid bacterial multiplication to almost the initial numbers may occur in empty canals within 2-4 days.–However, it has commonly been assumed that the application of an interappointment medicament such as calcium hydroxide can reduce the residual bacteria or prevent their multiplication. Potent eradication of infection before obturating the root canal system improves the prognosis of endodontic treatment.[2,3]

Persistent apical lesions after endodontic treatment have revealed different microbial flora than that detected in untreated necrotic canals. In necrotic root canals, studies have recorded typically polymicrobial flora with approximately equal proportion of both gram-positive and gram-negative bacteria and with dominance of anaerobic bacteria.[4] Whereas the microbial flora of retreated canals are described as mono-infection of predominant gram-positive microorganisms, and proportions of facultative and obligate anaerobes are approximately equal.[5-7]

Enterococcus faecalis (E. faecalis), a gram-positive facultative anaerobe, is considered as one of the predominant bacteria involved in cases of failed endodontic therapy and in asymptomatic cases than in symptomatic ones.[8] Its resistance to several irrigation solutions and medications can be attributed to its capability of attaching to the dentin, invasion of the dentinal tubules as well as formation of biofilm organized communities, resulting in long-term survival inside the root canals.[9]

Ideal endodontic irrigating solutions must have wide spectrum antimicrobial effect, capability for smear layer removal, organic tissue solvent and nontoxic to surrounding tissue. Sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) are the main irrigating solutions most worldwide utilized in root canal treatment. NaOCl has the ability to dissolve vital and necrotic organic non-mineralized tissue, thus described as a bactericidal agent. However, NaOCl it does not affect the mineralized ingredients of the smear layer formed after root canal biomechanical preparation.[10,11]

Consequently, complete removal of the smear layer requires a metal chelator as an adjuvant irrigating solution. Although EDTA is the chelating agent of choice, it has toxic effect on the periradicular tissue and can not eradicate bacteria which negatively affects the prognosis. Moreover, EDTA is used in a relatively high concentration (15-17%) in dentistry, which renders it as a pollutant. As a result, it is mandatory to find substitute chelating agents for root canal treatment.[12,13]

Silver and its compounds have been known for their antibacterial properties and recently used as a disinfectant in medical field. A novel irrigating solution based on a patented blend of electrolytically generated silver ions (0.003%) in citric acid (4.846%) (BioAKT, New Tech Solutions s.r.l., Brescia, Italy), has been tested as an innovative biomaterial for root canal cleaning and disinfecting. This patented aqueous disinfectant is an antimicrobial agent based on a stabilized silver ion complex. By a special electrochemical process with silver and citric acid, a silver ion is weakly bonded to a citrate ion developing the molecular complex AgC6H7O7

that is rapidly efficacious against a broad spectrum of bacteria, viruses, and fungi due to its bioavailability. [14]

Before a chemical agent is launched to the market, biocompatibility must be carefully evaluated. Recently, another silver citrate solution (BioAKT Endo, New Tech Solutions s.r.l., Brescia, Italy) has been launched to create a new two-in-one root canal irrigating solution in clinical applications. Interestingly, this new irrigating solution was reported as non-toxic and biocompatible, however, its antimicrobial activity, biocompatibility, and composition are rarely reported in literature. [14,15]

The objective of this research is to assess the cumulative impact of endodontic procedures using BioAKT Endo root canal irrigant on Enterococcus faecalis in comparison with sodium hypochlorite irrigant.

MATERIALS AND METHODS

Teeth Selection

Eighty caries-free freshly extracted human mature single rooted premolar teeth were collected and selected for this study and following the protocol reviewed and approved by the Ethical Committee, Faculty of Dentistry, Mansoura University. They were extracted in the dental clinic of Oral Surgery Department, Faculty of Dentistry, Mansoura University for orthodontic purposes. All patients signed an approval consent form. The storage protocols followed the international and institutional infection control guidelines. All of the external debris were removed with an ultrasonic scaler, and the teeth were stored in distilled water.

Teeth preparation

All teeth were decoronated at the level of cementoenamel junction using diamond instrument. The length of the roots was standardized to 15 mm. Remnants of pulp tissue, and necrotic debris were removed with suitable size nerve broach. All the roots were initially prepared by K files up to size #30 as an initial master apical file (MAF) to the full length (15mm) and 2 ml 5.25% NaOCl was used for irrigation after each file size. The apical foramina were sealed by epoxy resin and three layers of nail polish to prevent leakage of microbial suspension. The specimens were placed in sterilization pouches and sterilized in the autoclave at 121°C for 15minutes at a pressure of 15 psi. The sterilized pouches were opened in a laminar flow chamber and a sample root was used to test the efficacy of sterilization; a sterile H-file was used to scrap the root canal walls and the cut dentine chips were cultured on blood agar and nutrient agar plates. The plates were placed in the incubator for 24 hours at 37 °C. After the incubation period, the agar plates were examined visually and were found to be free of any microbial growth which proved the efficacy of sterilization.

Inoculation with Enterococcus faecalis

Under complete aseptic conditions in laminar flow chamber the microbial count of E. faecalis was adjusted to 1.5 x 108 cell forming units (CFUs)/ml in nutrient broth using McFarland scale, then 10 µl of the mixed inoculum of this culture was injected in each root using a micropipette. The root canal orifices were subsequently covered with sterile tin foil to avoid seepage of the microbial suspension, then placed in sterile Eppendorf tubes and held vertically in perforated tray (Rack of Widal) and incubated at 37°C for 7 days to give chance for the microorganism to establish the biofilm and to penetrate dentinal tubules. The excess broth was removed from the root canal with sterile paper points at the end of the incubation period, and the first microbial sample (S1) was taken.

Microbial sampling technique

All microbial samples were collected by injecting 10 µl of transport media (Stewart Transport Media) in the canal with micropipette and gentle scraping

the root canal wall with a new size of H file to contaminate the transport media inside the canals. Then, a sterile paper cone was placed in the canal for 1 minutes, each paper cone was placed in test tube containing 1ml of sterile saline solution, and vortexed for 30 seconds. After 10 folds dilutions; aliquots of 100 µl were plated on blood agar plates and at once spreaded widely with fine wire loop. The plates were incubated at 37°C for 24 hours. After incubation, the number of CFU was calculated from the average colony count per plate.

The microbiologic sample S1 was taken from all specimens at the end of the incubation period after inoculation following the steps mentioned previously. Afterward, the specimens were divided according to type of irrigants into eight groups: groups 1-4; BioAKT Endo was used for irrigation. Groups 5-8; sodium hypochlorite (NaOCl) 5.25% was used for irrigation. Description of all the microbiological samples collected after endodontic steps are listed in Table 1.

TABLE (1): Description of microbiological samples

Sample	Description of microbiological samples.
S1	Sample after inoculation.
S2	Sample after instrumentation and irrigation.
S3	Sample after removal of smear layer.
S4	Sample after medication with calcium hydroxide for seven days.
S5	Sample after 7 days from empty canals sealed with sterile cotton pellet and temporary filling.

Root canal preparation completion

The root canals were prepared at the predetermined working length under complete aseptic condition in a laminar flow chamber to avoid contamination. All of the root canals were prepared by ProTaper NEXT rotary files (Dentsply Maillefer, Ballaigues, Switzerland) in a brushing motion with rotational speed of 300 rpm and torque 2.0-5.2 (using X-Smart Plus electric motor), till X4 file (40/6) and irrigation with the assigned irrigant. Irrigation with 2ml of 0.5% BioAKT Endo was used after each file size for groups 1-4 (40 roots). And 5.25% sodium hypochlorite for groups 5-8 (40 roots)

A-BioAKT Endo groups (1-4):

Group 1

Ten specimens were instrumented up to size X4 file (40/6) as mentioned above (Root canal preparation completion) using 0.5% BioAKT Endo as an irrigant, canals were dried with sterile paper points, and steps for S2 collection was followed. The smear layer formed on the canal walls was removed using 2 ml of EDTA 17% for one minute and washed with 2ml of sterile saline before sample 3 (S3) collection. Calcium hydroxide paste Metapaste (Meta Biomed Co, Ltd, Chungbuk, Korea) was placed inside the canal with hand lentulo spiral until the canal was completely filled and confirmed radiographiclly. The canal orifices were sealed with temporary filling, and incubated at 37˚C for 7 days. Calcium hydroxide was removed at the end of the incubation period by scraping with H file, and for deactivation; 2 ml of 5% citric acid was used followed by 2 ml of sterile saline for washing, and then sample 4 (S4) was taken. The empty canals were sealed with sterile cotton pellet and temporary filling and incubated in the same conditions for 7 days after which sample 5 (S5) was taken.

Group 2

Ten specimens were prepared as in group 1 until S3. Then the orifices were sealed with sterile cotton pellet and temporary restoration and incubated at 37°C for 7 days after which S5 was taken.

Group 3

Ten specimens were prepared as in group 1 until S2, then Calcium hydroxide paste was applied inside the canals, and the orifices were sealed with

temporary filling, and incubated at 37°C for 7 days. Calcium hydroxide was removed at the end of the incubation period as described previously, and then S4 was taken. The orifices were sealed with sterile cotton pellet, temporary filling and incubated at 37°C for 7 days after which S5 was taken.

Group 4

Ten specimens were prepared as in group 1 until S2, and the orifices were sealed with sterile cotton pellet, temporary filling and incubated at 37°C for 7 days after which S5 was taken.

B-Sodium hypochlorite groups (5-8):

Group 5:

Ten specimens were finally instrumented as mentioned before (root canal preparation Completion), using 5.25% NaOCl as an irrigant. Then steps for S2 collection were followed. The smear layer formed on the canal walls was removed using 2 ml of EDTA 17% over one minute and washed with 2ml of sterile saline before S3 collection. Calcium hydroxide paste was applied inside the canal with hand lentulo spiral until the canal was completely filled and confirmed radiographiclly. The canal orifice were sealed with temporary filling, and incubated at 37°C for 7 days. Calcium hydroxide was removed at the end of the incubation period by scraping with H file, and for deactivation; 2 ml of 5% citric acid was used followed by 2ml of sterile saline for washing, and then S4 was taken. The empty canals were sealed with sterile cotton pellet and temporary filling and incubated in the same conditions for 7 days after which S5 was taken.

Group 6:

Ten specimens were prepared as in group 5 until S3. Then the orifices were sealed with sterile cotton pellet and temporary filling and incubated at 37°C for 7 days after which S5 was taken.

Group 7:

Ten specimens were prepared as in group 5 until S2, then Calcium hydroxide was applied inside the canals, and the orifices were sealed with temporary filling, and incubated at 37°C for 7 days. Calcium hydroxide was removed at the end of the incubation period as described previously, and then S4 was taken. The orifices were sealed with sterile cotton pellet, temporary filling and incubated at 37°C for 7 days after which S5 was taken.

Group 8:

Ten specimens were prepared as in group 5 until S2, and the orifices were sealed with sterile cotton pellet, temporary filling and incubated at 37°C for 7 days after which S5 was taken.

Preparation for Scanning electron microscopy:

Along the entire length of each root, longitudinal grooves were cut. Then the roots were split into two halves with chisel and hammer. Each half was then gently washed in 0.2 M potassium phosphate buffer (PBS), pH 7.2, at 4°C. The specimens were then fixed in 2% glutaraldehyde at 4°C for 24 hour, and washed with PBS for 15 minute and then fixed for 12 hour at 4°C in 1% (wt /vol) osmium tetroxide. Potassium phosphate buffer was used as a final wash and dehydration was performed with an ascending concentration of acetone series 30%, 60% and 100% for 10 minutes each. The specimens were then dried by using a SAMDRIPVT-3 critical point dryer apparatus (Tousimis Research Crop., Rockville, MD) using liquid CO2 replacement. Each half was mounted and coated with 200 layer of gold palladium, and viewed in JEOL JSM-35Cf scanning electron microscope at 25 KV.. The middle third of the root canals was scanned and representative area of each specimen was photographed. Photographs were evaluated for heavy, moderate and mild presence of E. faecalis.

RESULTS

The data were collected and subjected to statistical analysis using One way ANOVA test.

A-BioAKT Endo groups (1-4):

All specimens (40) showed bacterial growth in S1. After chemomechanical root canal instrumentation and irrigation with BioAKT Endo, the microbial samples (S2) showed constant significant reduction (92.95%) as shown in groups 1-4 (Table 2-5) compared to S1 ($P< 0.05$).

Group 1

The use of EDTA had no significant effect on the bacterial count (S3) as shown (Table 2) where the reduction was slightly increased to 93.44 % ($P> 0.05$).The use of calcium hydroxide for 7 days increased the percentage of bacterial reduction to 95.92% (S4), however there was no significant difference compared to (S3). Significant bacterial regrowth presented as decrease in the percentage of reduction (91.37%) after 7 days, (S5) compared to S4 ($P<0.05$) as shown in Table 2.

Group 2

The use of EDTA had no significant effect on the bacterial count as shown in (S3) where the reduction was 93.69% ($P>0.05$). Significant bacterial regrowth was recorded to 88.96% in S5 compared to 93.69% in S3 ($P< 0.05$) Table 3.

Group 3

Calcium hydroxide increased the bacterial reduction in S4 (95.01%) with no significant difference compared to S2 ($P >0.05$) Table 4. Significant bacterial regrowth after 7 days (S5) was recorded (88.13%) compared to S4 (95.01%) where ($P< 0.05$).

Group 4

Significant bacterial regrowth presented as decrease in the percentage of reduction was recorded (85.36 %) in S5 after 7 days from leaving the canal empty (without the use of EDTA or calcium hydroxide medication) compared to S2 (92.95%) ($P< 0.05$) Table 5.

TABLE (2): The mean bacterial count and reduction percentage of group1.

	Mean Bacterial Count	Reduction Percentage of the Bacterial Count	P -Value
S1	4.5×10^7		
S2	2.8×10^6	92.95%	< 0.05
S3	2.3×10^6	93.44%	> 0.05
S4	1.6×10^6	95.92%	> 0.05
S5	3.2×10^6	91.37%	< 0.05

TABLE (3): The mean bacterial count and reduction percentage of group 2.

	Mean Bacterial Count	Reduction Percentage of the Bacterial Count	P -Value
S1	4.5×10^7		
S2	2.8×10^6	92.95%	< 0.05
S3	2.4×10^6	93.69%	> 0.05
S5	5.0×10^6	88.96%	< 0.05

TABLE (4): The mean bacterial count and reduction percentage of group 3.

	Mean Bacterial Count	Reduction Percentage of the Bacterial Count	P -Value
S1	4.3×10^7		
S2	2.8×10^6	92.95%	< 0.05
S4	2.4×10^6	95.01%	> 0.05
S5	4.8×10^6	88.13%	< 0.05

TABLE (5): The mean bacterial count and reduction percentage of group 4.

	Mean Bacterial Count	Reduction Percentage of the Bacterial Count	P -Value
S1	4.5×10^7		
S2	2.8×10^6	92.95%	< 0.05
S5	6.0×10^6	85.36%	< 0.05

B-Sodium hypochlorite groups (5-8):

All specimens (40) showed bacterial growth in S1. After chemomechanical root canal instrumentation and irrigation with 5.25% sodium hypochlorite; the microbial samples (S2) showed constant significant reduction (96.99%) in groups 5-8 as shown in (Table 6-9) compared to S1 ($P< 0.05$).

Group 5

The use of EDTA had no significant effect on the bacterial count (S3) as shown in Table 6 where the reduction was slightly increased to 97.4 % ($P> 0.05$). The use of calcium hydroxide for 7 days increased the percentage of bacterial reduction to 98.9% (S4), however there was no significant difference compared to S3. Significant bacterial regrowth was presented as decrease in the percentage of reduction (96.31%) after 7 days (S5) compared to S4 (98.9%) ($P<0.05$) Table 6.

Group 6

The use of EDTA had no significant effect on the bacterial count as shown in S3 where the reduction was 97.4 % ($P >0.05$). Significant bacterial regrowth was recorded (95.11%) in S5 compared to 97.4% in S3 ($P< 0.05$) Table 7.

Group 7

Calcium hydroxide increased the bacterial reduction in S4 (97.68%) with no significant difference compared to S2 (96.99%) ($P >0.05$) Table 8. Significant bacteria regrowth was recorded (94.96 %) in S5 after 7 days compared to S4 (97.68%) where ($P< 0.05$).

Group 8

Significant bacterial regrowth presented as decrease in the percentage of reduction was recorded 93.33% in S5 after 7 days from leaving the canal empty (without the use of EDTA or calcium hydroxide medication) compared to S2 (96.99%) ($P< 0.05$) Table 9.

TABLE (6): The mean bacterial count and reduction percentage of group 5.

	Mean Bacterial Count	Reduction Percentage of the Bacterial Count	P -Value
S1	4.5×10^7		
S2	1.2×10^6	96.99%	< 0.05
S3	1.0×10^6	97.4%	> 0.05
S4	3.4×10^5	98.9%	> 0.05
S5	1.5×10^6	96.31%	< 0.05

TABLE (7): The mean bacterial count and reduction percentage of group 6.

	Mean Bacterial Count	Reduction Percentage of the Bacterial Count	P -Value
S1	4.5×10^7		
S2	1.2×10^6	96.99%	< 0.05
S3	1.0×10^6	97.4%	> 0.05
S5	1.9×10^6	95.11%	< 0.05

TABLE (8): The mean bacterial count and reduction percentage of group 7.

	Mean Bacterial Count	Reduction Percentage of the Bacterial Count	P -Value
S1	4.5×10^7		
S2	1.2×10^6	96.99%	< 0.05
S4	8.7×10^6	97.68%	> 0.05
S5	2.0×10^6	94.96%	< 0.05

TABLE (9): The mean bacterial count and reduction percentage of group 8.

	Mean Bacterial Count	Reduction Percentage of the Bacterial Count	P -Value
S1	4.5×10^7		
S2	1.2×10^6	96.99%	< 0.05
S5	3.0×10^6	93.33%	< 0.05

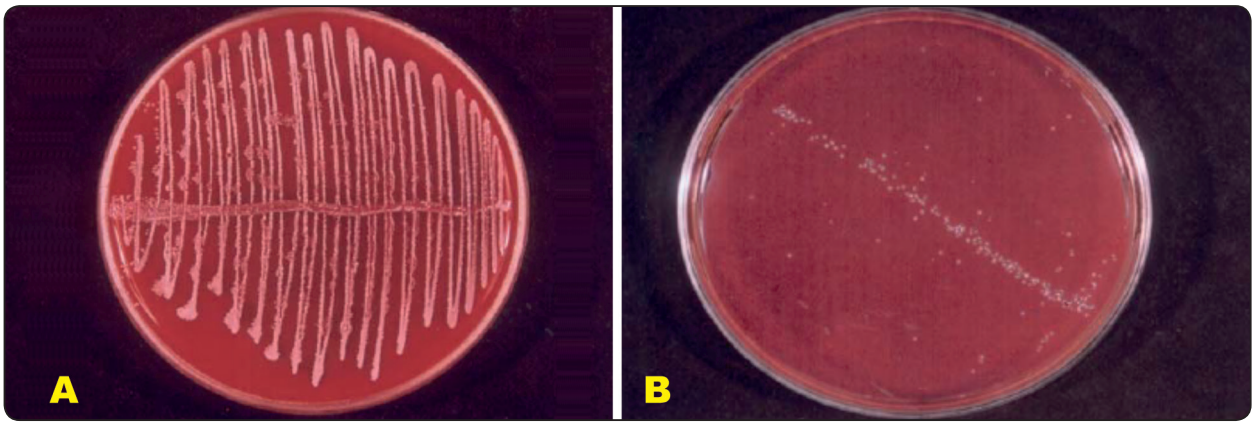

Fig. (1) (A) Photograph showing the growth of E. faecalis colonies after root canal contamination S1. (B) Photograph showing the effect of root canal instrumentation, irrigation with BioAKT Endo, 17% EDTA application and Calcium hydroxide dressing for 7 days on E. faecalis (S4).

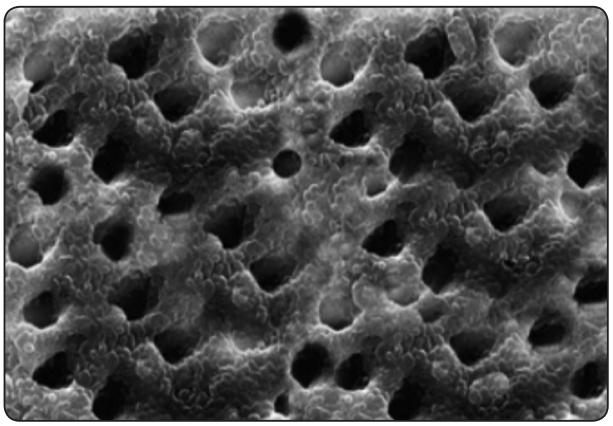

Fig. (2) Photomicrograph of root canal wall (middle) X= 17, 00 after contamination with E. faecalis

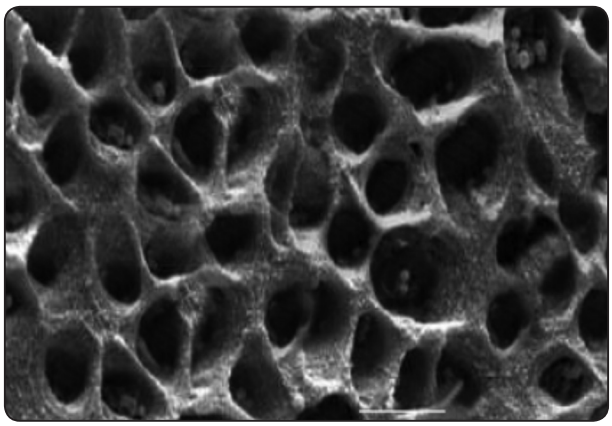

Fig. (3) Photomicrograph showing the root canal wall X= 15,00 after instrumentation, irrigation with 0.5% BioAKT Endo, 17% EDTA application and CH dressing for 7 days in group 1.

Scanning electron microscopic observations

The ultrastructural observations of the root canal infection after initial root canal instrumentation with E. faecalis are reported in the scanning electron micrographs shown in (Figures 2). The findings of this study showed true bacterial reduction achieved by mechanical instrumentation using 0.5% BioAKT Endo, 17% EDTA and calcium hydroxide for one week. Few colonies forming units of E. faecalis are scattered as in group 1 (Figures 3).

DISCUSSION

Enterococcus faecalis has been observed the most predominant bacteria in teeth with endodontic infections and periradicular inflammation. It is one of the most resistant species in oral cavity and hence it is difficult to be eradicated during root canal treatment. The objective of this research was to monitor the cumulative impact of endodontic biomechanical instrumentation, irrigation and intracanal medication to find out the most accepted protocol for E. faecalis eradication from infected failed root canals. The methodology used in this

study was to mimic the in vivo condition as assured by SEM evaluation rather than studies used direct contact methodologies.[16]

Ideal properties of an endodontic irrigant include its capability of smear layer removal, biocompatibility with vital tissues, and broad-spectrum antimicrobial effect. However, currently available irrigants do not satisfy all these properties. Despite its well-recognized drawbacks, NaOCl is considered as the golden standard disinfectant in endodontic treatment because of its broad-spectrum antimicrobial activity and its organic solvent ability. NaOCl lacks the capability of removing inorganic materials, therefore using chelating agents after NaOCl treatment is highly recommended.[17,18] As a chelating agent in endodontic treatment, EDTA is typically used to remove smear layer. However, the adjuvant drawbacks of EDTA and its lacking antimicrobial activity, have aroused the search for substitute agents. Previous researches have demonstrated that the golden standard irrigation protocol is (NaOCl followed by EDTA) may not eradicate neither E. faecalis nor C. albicans biofilms. This was not related to the buffering effect of dentine or the complex anatomy of the root canal but related to the high resistance of biofilm to antimicrobials. Consequently, it is recommended to find out a chelating agent with efficient antimicrobial properties.[19-21]

BioAKT as a novel metabolic substrate based on silver citrate, was tested for endodontic irrigation (solution pH ~ 1.7). BioAKT citric acid-based complex increased cell viability significantly at 0.5% concentration. Recent studies have demonstrated antimicrobial activity of BioAKT on dentin discs from human teeth contaminated with E. faecalis biofilm. Conversely, there is lack of data available about the concentration of the various chemicals constituting BioAKT, the instructions for its clinical use, the safety data sheet, as well as its antibacterial activity. Based on the above fore mentioned data, the present study was concerned with evaluating the antibacterial action of this new class of irrigants on E. faecalis in laboratory settings.[14,15]

In the present study, samples from all groups (1-8) were obtained just after biomechanical root canal preparation to monitor their antimicrobial actions immediately after instrumentation and irrigation (S2), and the residual effect of these solutions after 7 days in groups 4 and 8 (S5). Mechanical effects during instrumentation and irrigation are generated by the flow and back flow of irrigant inside the root canal. The bacterial populations inside root canal are significantly reduced by the mechanical effect of the instruments and the irrigant irrespective of the type of the used irrigant.[22] This has been confirmed in our study through the marked bacterial reduction in S2 of groups 1- 4 and 5-8 were 92.95% (instrumentation and irrigation with BioAKT Endo) and 96.99% (instrumentation and irrigation with 5.25 % NaOCl) respectively.

Although the mechanical effect of irrigation has been demonstrated to minimize intracanal bacteria count, the current results demonstrated that 5.25 % NaOCl was highly effective (96.99%) in the first microbiological sampling obtained just after instrumentation and irrigation in groups 5-8 (S2) than BioAKT Endo in S2 of groups 1-4 (92.95%). However, another study found significant antimicrobial activity of BioAKT Endo.[14,15] The heterogeneity in the results could be related to the differences in methodology and groups. This signify the use of 5.25% NaOCl as antibacterial irrigant, however it did not achieve bacteria free root canal which is in agreement with Byström, and Sundqvist, 1985.

Also, that E. faecalis was able to recolonize in the root canal after 7 days from leaving the canal empty and sealed with temporary filling in groups 4 and 8. The residual effect of 5.25% NaOCl was superior to BioAKT Endo as shown in bacterial reduction percentage 93.33% and 85.36% respectively. Sodium hypochlorite 5.25% concentration was selected because it is the most commonly used concentration for endodontic treatment.[23]

The significance of the smear layer covering the entire root canal wall remains controversial.

Several studies have mentioned that the smear layer might prevent the penetration of intracanal disinfectant and filling material into dentinal tubules, suggesting that chemomechanical cleaning should be supported by removal of smear layer. Therefore, the combination of NaOCl and EDTA is strongly recommended for irrigation of root canal during instrumentation.[24,25] Our results in this research confirm this recommendation through the significant increase in the bacterial reduction in S3 (97.4%) after the use of 5.25% NaOCl and 17% EDTA compared to 93.44% after the use of to BioAKT Endo and 17% EDTA. This combination effectively has removed the smear layer which may enhance medication or root canal sealer penetration inside the dentinal tubules and hence eliminates bacteria remnants in the root canal system and improve the success rates.[25]

Calcium hydroxide has been widely used as an intracanal medication to reduce bacteria counts that can survive after biomechanical instrumentation and is currently acknowledged to be one of the most antimicrobial dressings used during root canal therapy. Calcium hydroxide as an intracanal medicament was evaluated because it is widely used in endodontics, although some controversies exist about its effectiveness against E. faecalis. The exact mechanism of action of calcium hydroxide is still unknown, however, its antimicrobial activity is generally related to the release of hydroxyl ions in an aqueous environment, producing a PH of approximately 12.5. E. faecalis can grow at an alkaline PH (9.6) that normally inhibits other bacteria. The results of this research showed that calcium hydroxide decreased the mean bacterial count in all medicated groups (1, 3, 5 and 7) but did not establish bacteria free canal. This may be due to the critical PH greater than 11 is not reached in dentine after application of calcium hydroxide due to the low permeability and dentine buffering action.[26,27]

Our results are in accordance with other recent studies which have shown the limited ability of calcium hydroxide to render completely bacteria free root canal.[26-28] Inspite of the limited capability of calcium hydroxide to eliminate E. faecalis from root canals;[29] our results showed that calcium hydroxide influences the rate of bacterial regrowth when the canals were left empty for 7 days and then S5 was taken. The bacterial regrowth was markedly less in the medicated groups compared to non medicated groups whether irrigated with sterile saline or NaOCl. The medicated groups (1 and 5) showed bacterial reduction in S5 (91.37% and 96.31%) whereas, non medicated groups (2 and 6) showed marked bacterial regrowth in S5 (88.96% and 95.11%) respectively. These results emphasize the importance of using irrigating solutions and intracanal medications with antimicrobial properties which is recommended by other researchers.

In this study the most effective protocol which achieved maximum percentage of bacterial reduction was presented in group 5 where instrumentation and irrigation with 5.25% NaOCl, followed by removal of smear layer with 17% EDTA and intracanal medication with calcium hydroxide for 7 days with temporary sealing possessed 98.9% bacterial reduction. The effect of residual microorganisms can be eliminated or rendered harmless by entombing them through the full obturation with root canal sealer which has antimicrobial activity and gutta percha points.

CONCLUSIONS

Based on the limitation of the present study; the following conclusions can be reported: -

1. Instrumentation and irrigation with BioAKT Endo or sodium hypochlorite 5.25% had significantly reduced bacterial count, however sodium hypochlorite was superior to BioAKT Endo.
2. Smear layer removal enhances the effect of intracanal medication. Implication of 17% EDTA as a conditioner before sealing protocol is recommended.
3. Calcium hydroxide had antibacterial effect but it did not render bacteria free root canals

4. Leaving root canals empty without intracanal medication enhanced bacterial recolonization.
5. BioAKT Endo is considered as a novel irrigant material for root canals with a marked and promising anti-bacterial activity. Further in-vivo and in-vitro studies are required to investigate this class of endodontic irrigants

RECOMMENDATIONS

From this study we recommend:

1. The use of 5.25% sodium hypochlorite, 17% EDTA and calcium hydroxide endodontic protocol in retreatment cases.
2. Further investigation for the use of this novel root canal irrigant (BioAKT Endo) with different concentrations and their effects against Enterococcus faecalis biofilm and smear layer.
3. Clinical evaluation of the cumulative impacts of instrumentation, irrigation with (BioAKT Endo), intracanal medication, and obturation on E faecalis is needed.

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