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In vitro evaluation of photodynamic disinfection and conventional root canal irrigation protocol

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ABSTRACT

Background. The root canal treatment can lead to great success, but the infection within insufficiently treated root canal led to post-treatment disease. This research set out to evaluate photodynamic disinfection (PDD) efficacy in preventing the growth of microorganisms with conventional root canal irrigations regime in vitro.

Method. Sixty teeth were extracted and gathered with a single root and the crowns of all teeth were trimmed to have 14 mm root teeth. The autoclave was used for sterilizing all teeth, which were fitted in glass tube perpendicular, at a temperature of 121°C for a period of 15 minutes. Afterwards, a sterile broth containing *Enterococcus faecalis* (*E. faecalis*) was added to the teeth. After that, the tubes were kept in an aerobic environment at 37 °C for a period of thirty days, every two days fresh broth was added. To evaluate the effectiveness of various disinfectants teeth were randomly divided into 6 groups according to disinfections protocol, each group contained 10 post-infected teeth. After completing the treatment, using a size #35 Hedstrom file, dentin chip samples were obtained from within the canals, and the samples were cultured on Mueller–Hinton agar and then bacterial colonies were counted in each group types. The experiments were repeated in triplicate and duplicate. The data consisted of the number of colonies forming units (CFUs) both before and after the treatments.

Result. The current study showed a significant difference between pre- and post-treatment results in each group. Irrespective of the photosensitizer used in photodynamic disinfection (toluidine blue O or riboflavin), PDD was significantly better than control group ($P < 0.05$). there was no significant difference between PDD with toluidine blue O or riboflavin ($P > 0.05$). Among the other groups, there was not a significant difference in colony count (PDD, NaOCl; $P > 0.05$).

Conclusion. This study presents evidence that the employment of toluidine blue O and riboflavin in photodynamic disinfection to the regular chemo-mechanical irrigation procedure yielded the most favorable outcomes. These substances effectively reduced the quantity of (*E. faecalis*) in infected root canals. Therefore, they may serve as a valuable addition to root canal disinfection procedures.

Keywords: Photodynamic disinfection, Enterococcus faecalis, Root canal, LED, Biofilms

Introduction

The main goal of endodontic therapy is to eradicate or substantially reduce the amount of bacteria in the root canal [1].

Conventional endodontic treatments are frequently unable to entirely eliminate microorganisms due to the intricate anatomy of root canals. Evidence indicates that residual bacteria can be found in over half of the prepared root canal surfaces just prior to obturation [2].

The main cause of pulpal and periapical illnesses is persistent pathogenic microorganisms within the root canal system [3,4].

E. faecalis strains primarily contribute to the development of pulpal and periradicular disorders [5]. While there is less information available on the various genotypes of this bacterium species, it possesses multiple types of surface and secretory virulence factors [4]. Biofilm creation is a crucial element in the pathogenicity of *E. faecalis* [6], biofilm-related endodontic infections pose a special challenge for *E. faecalis* because biofilm modifications enhance the pathogen's pathogenicity and resistance to antimicrobial agents and immune system components [7]. It is difficult and complex to completely eradicate the bacteria causing persistent root canal infections using traditional treatment methods [8].

Culturing investigations reveal that the frequently isolated microorganisms in persistent periapical lesions (mostly endodontic failures) is (*E. faecalis*), a facultative anaerobic gram-positive cocci [9], 18% of primary endodontic infections and 67% of secondary endodontic infections have been linked to *E. faecalis*. [10].

Furthermore, lasers can be employed to conduct final root canal disinfection in addition to irrigant. In endodontic research, antimicrobial photodynamic disinfection (PDD) has been investigated more frequently over the past decade [11,13].

As opposed to photo thermal, PDD has minimal to no heating and is primarily an indirect effect. A photo-activated material (the dye) is needed for PDD. After being applied, the microorganisms absorb the pigment. Next, the bacteria are subjected to light of a particular wavelength. Intracellular generation of reactive oxygen species (ROS) takes place as light activates the absorbed dye. These extremely reactive chemicals interfere with regular metabolic processes [12,13].

PDD is a process that utilizes non-toxic photosensitizer (PS) chemicals which are activated by a certain wavelength of light [14]. Upon irradiation, the photosensitizer undergoes a reaction with molecular oxygen, resulting in the generation of high ROS. These ROS are responsible for causing damage and ultimately leading to the killing of microorganisms [12]. The photosensitizer attaches to the bacteria cell membranes, which then triggers the production of singlet oxygen upon exposure to radiation. In the end, the bacteria die because the singlet oxygen breaks down their cell wall [13].

PS is a crucial element of PDD that becomes active when exposed to light of a specific wavelength. Toluidine blue O (TBO) is a photosensitizer with a blue tint. It is amphiphilic. TBO has a positive charge and a low molecular weight. It absorbs red light most strongly at wavelengths between 620 and 660 nanometers. Root canal infections consist of a combination of both Gram-negative and Gram-positive bacteria. However, the amphiphilic nature of TBO makes it a suitable option for disinfecting root canals and has antibacterial impact on both types of bacteria. Furthermore, research has demonstrated that the firm attachment of TBO to *E. faecalis* makes it a more potent agent than methylene blue photosensitizer for root canals disinfection [15,16].

Riboflavin (RFV) is a photosensitizer with a yellow tint that shows great potential as an alternative photosensitizing substance. A micronutrient and an intrinsic photosensitizer, it produces ROS upon exposure to blue light [17]. This material has excellent biocompatibility and may be easily activated using available LED lamps. That is specifically designed for the purpose of curing composite materials in dental offices. Due to its favorable and widely recognized toxicological and pharmacokinetic characteristics [18].

This study utilized a light-emitting diode (LED) as a source of light instead of a laser, which is worth emphasizing. Many research investigations examining the antibacterial properties of PDD utilize lasers that are more expensive than LED lamps and are subject to strict power output restrictions to prevent harm to surrounding tissues.

Currently, there is a shortage of studies examining the effectiveness of toluidine blue O and riboflavin, both used with LED light, in reducing *E. faecalis* biofilm on extracted teeth.

The purpose of this research is to compare the effectiveness of different photosensitizers and LED wavelengths when used alone as photodynamic with low energy LED light and in conjunction with traditional disinfectants.

Methods

The study received ethical approval from the research ethics committee of Al-Basrah university's college of dentistry in Al-Basrah, Iraq.

Tooth selection and preparation. This experimental in vitro study was performed on 60 extracted human teeth. The teeth were maxillary and mandibular canines, maxillary incisors and mandibular premolars. These teeth were extracted due to periodontal issues or as part of an orthodontic treatment plan. The teeth exhibited a single canal and a single root, with straight and intact roots of nearly similar length. There was no evidence of apical resorption or having endodontic disinfection. Consequently, teeth that had pulpal calcification, decay in the crown or root, fractures in the root, multiple roots, bent roots, or had undergone previous endodontic disinfection were not included. Prior to the experiment, the teeth had been disinfected and preserved in a saline solution.

Root canal preparation. All the teeth are cleaned with dental scaler and periodontal curette for removal of residual tissue. Using a dental laboratory engine and a sectioning disc under running water, the crowns of the teeth are removed. This process results in a 14 mm-long root. By using gates Glidden drills widen and taper the root canal orifice. By visually inspecting a size 10 manual K-file and placing it 1 mm from the apical foramen to assess the working length. Root canals were shaped with Rotary files (plex-v, Orodeka file, LTD China) from size 15 to 40 and irrigated with 2 mL of 3% sodium hypochlorite (NaOCl) after the use of each instrument. One skilled researcher handled all aspects of root canal preparation. 2 ml of 5% sodium thiosulfate were used to neutralize the remaining intracanal NaOCl [19]. to remove the smear layer and smear plugs, each canal was irrigated with 2 mL of 17% Ethylenediaminetetraacetic acid (EDTA) after that being cleaned with distal water. Next the teeth were autoclaved at 121°C for 15 min at 26 psi.

To make the handling easier we used glass tube vial (K3 EDTA Tube 7 ml 13×75mm) after cleaning it in distal water to fixed the roots by using silicon impression material. Teeth were inserted with the root embedded in the silicon material in the glass tube in which the canal orifice is free of silicon in upright position where the cervical portion facing upwards and left 10 mm free space in

the top of the tube to the broth material so that the broth could cover the tooth's root and then autoclaved again at 121°C for 15 min at 26 psi, then aseptically stored until use.

Biofilm formation and bacterial inoculation. Following sterilization, 100 µL of *E. faecalis* at a concentration of 0.5 McFarland was introduced into the root canals to create biofilm. After that, the specimens were incubated for 30 days at 37ºC in an aerobic environment. To maintain bacterial viability, fresh broth was added every two days.

Distribution of experimental groups:

Group 1: teeth treated only with distal water it represents the control group.

Group 2: teeth treated with riboflavin + 480 nm LED.

Group 3: teeth treated with toluidine blue O+630 nm LED.

Group 4: teeth subjected to treatment of 3% NaOCl and 17% EDTA,

Group 5: teeth subjected to treatment of 3% NaOCl and 17% EDTA and riboflavin + 480 nm LED.

Group 6: teeth subjected to treatment of 3% NaOCl and 17% EDTA and toluidine blue O + 630 nm LED.

Procedure of Disinfection:

Sodium Hypochlorite (NaOCl)

Using a 30-gauge irrigation needle to disinfect specimens with 10 ml of 3% NaOCl at a flow rate of 3–3.5 ml per minute. Using rotating file agitation (extending up to the working length) with 35 No. file (plex-v, Orodeka file, LTD China), After that, irrigate with 2 ml 17% EDTA (Ultradent, South Jordan, USA), which was left in the canal for one minute.

Antimicrobial photodynamic disinfection with toluidine blue O

For the red-light source in groups 3 and 6, a light-emitting diode (LED) device (Fotosan 630, Korea, MDD, CMS Dental Denmark) was employed. This system has a wavelength spectrum of 630 nm and an output power of 2,000_4,000 mW/cm2. TBO (0.1 mg/mL) was utilized as a PS agent. The root canal was filled with a TBO solution using a 30-gauge irrigation needle. The endodontic plastic tip

was attached to the LED device and placed into the root canal without exerting any pressure, and light activation was performed for a duration of 30 seconds.

Antimicrobial photodynamic disinfection with riboflavin

A 0.1% riboflavin solution containing the photoactive material was injected into the root canals, thereafter, employing a blue light activation LED for 30 seconds using light cure device (O-Star, Guilin Woodpecker medical instrument Co.) at 1000–2000 mW/cm^2, 385–515 nm.

NaOCl and PDD

The specimens from Group 5 and Group 6 were initially disinfected using NaOCl and EDTA, and then underwent photodynamic disinfection.

SEM (Scanning Electron Microscopy)

Scanning electron microscope (SEM) was used to confirm the presence of the *E. faecalis* biofilm. From the available samples, five teeth were randomly selected. The root surfaces were prepared with longitudinal grooves using a high-speed diamond bur, while ensuring that the inner part of the root canal was not affected. After that, a stainless-steel chisel was used to split the roots into two sections via these grooves. After being fixed for 24 hours in 2% formalin, the samples were incubated in a graded sequence of ethanol concentrations (50, 70%, 90%, and 100%, twice) for 20 minutes. Afterward, they were allowed to dry overnight at room temperature in the open. Subsequently, the specimens were then sputtered coated with gold alloy and examined by using scanning electron microscope (ThermoFisher Scientific, Oregon, USA) at 1 kV.

Microbial sampling and quantification of colony-forming units

A preliminary sampling. Prior to disinfection of the teeth, a 100 µl sample of the microbial suspension was obtained and transferred to a sterile tube containing 1 milliliter of distilled water. The mixture was then vigorously mixed for 20 seconds and subsequently serially diluted 10 times. Subsequently, 100 µl of each solution was evenly distributed on a Mueller–Hinton agar (Merck Co., Germany) plate and subjected to incubation at 37°C for a duration of 24 hours. The bacterial colonies were quantified based on the colony-forming units per milliliter (CFU/mL).

Final Sampling. To inactivate the remaining NaOCl present in the canal, the teeth in the NaOCl groups (Group 4,5,6) were irrigated for one minute using 2 ml of 5% sodium thiosulphate. The root canal was cleaned with 5 ml of sterile saline and left

for 30 seconds in order to standardize all the groups. After that, the root canals were filed aggressively with an H-file size 35(20). This process made it possible for the biofilm to come down and for the bacteria that remained inside but were inaccessible to paper points to be extracted. Subsequently, paper points were inserted into the root canals up to the working length (WL) for a duration of 1 minute to collect samples for microbiological analysis. After that, the paper point was put into microtubes with 500 µl of the nutrient broth solution and vortexed for 20 seconds to thoroughly mix it. Subsequently, the sample was serially diluted. Subsequently, 100 µl of each solution was evenly distributed onto a Mueller–Hinton agar plate and subjected to incubation at 37°C for a duration of 24 h. The quantity of bacterial colonies was assessed based on the CFU/ml measurement.

Statistical analysis

The data was analyzed using SPSS software version 21. Descriptive statistics and statistical measures, including the t-test and one-way analysis of variance (ANOVA), were employed to examine the data. Nonparametric tests were utilized when the normality hypothesis was not proven. The tests were conducted using a significant threshold of 0.05.

Results

Based on the findings of our investigation, all the groups that received treatment showed a noticeable decrease in the number of *E. faecalis* biofilms in the root canal compared to the control groups (specifically, the group that used water as a control; $p<0.05$). The group that had the lowest average value of colony-forming units per milliliter (CFUs/mL) was the one that used sodium hypochlorite (NaOCl) and toluidine blue O (TBO) as PDD. This was followed by the group that used NaOCl and riboflavin PDD. The distal water group had the greatest mean value. Each group exhibited a noteworthy decrease in the quantity of biofilm generated by *E. faecalis* both before and after the treatment. Furthermore, there was no statistically significant difference ($P>0.05$) between the photodynamic treatment alone and the combination of NaOCl and photodynamic disinfection as show in table 1.

On biofilm forms of *E. faecalis* strains, the effects of TBO PDD and NaOCl solutions, as well as riboflavin PDD and NaOCl solutions, were comparable to those of conventional irrigation solutions with NaOCl.

The CFU/mL mean values (standard deviation) for the groups acquired at the two evaluation times are displayed in Table 1.

Figure 6 is a bar graph that displays the average microbial reduction values.

Table 1 show the mean and Standard Deviation CFUs of bacteria before and after disinfection and significant differences in comparison with control group.

Group	N	Mean before treatment	Standard Deviation before treatment	Mean after treatment	Standard Deviation after treatment	Percentage of the reduction in comparison with the Control	P value
Distal water (control group)	10	65×10^{11}	15×10^{12}	19×10^{5}	29×10^{5}		
Riboflavin	10	14×10^{11}	20×10^{11}	15×10^{4}	26×10^{4}	99.99	0.001
TBO	10	32×10^{11}	50×10^{11}	18×10^{3}	14×10^{3}	99.99	0.001
NaOCl	10	41×10^{11}	71×10^{11}	67×10^{1}	63×10^{1}	99.99	0.001
NaOCl + Riboflavin	10	23×10^{11}	42×10^{11}	49×10^{1}	51×10^{1}	99.99	0.001
NaOCl + TBO	10	74×10^{11}	79×10^{11}	44×10^{1}	53×10^{1}	99.99	0.001

Discussion

E. faecalis is the most commonly found species in endodontic infections and is a significant factor in the development of persistent periradicular lesions following root canal treatment [21]. The use of disinfecting chemicals to disinfect root canals is the primary and essential step in removing germs from the root canal system, dentinal tubules, and the periapical region [22]. The most common approach for reducing bacteria in infected root canals is through mechanical instrumentation. However, completely eliminating germs from the root canal is a challenging endeavor. Multiple studies have indicated that chemomechanical methods are not effective in completely eradicating germs from diseased root canals [23].

Paper points or dentin chips collected from the canal walls can be used to collect samples from within the root canal. Both of these methods are viable options for root canal sampling. While paper point sampling has been commonly utilized in several research because it's simple to use, it alone gathers specimens of intracanal fluid-containing planktonic microorganisms [24). We collected dentin chips in our study because they allow us to sample biofilm-like formations holding to the canal

wall, including microorganisms that have entered deep into the dentinal tubules by using Hedstrom file [2,24].

An essential aim of modern clinical microbiology is to develop innovative approaches that can efficiently reduce the prevalence of biofilm infections in the wall of the affected root canal.

PDD is a form of antimicrobial treatment that has been the subject of various research [25] and the effectiveness of which has been proven in endodontics. One of its advantages is the targeted elimination of bacteria without harming normal tissue or hurting neighboring tissues [26].

Based on the current study, groups 2 and 3, who underwent PDD alone, demonstrated a significant decrease in microbial content compared to the control group. Nevertheless, the total eradication of *E. faecalis* from root canal samples was not achieved. The results agree with previous research, which similarly observed significant reductions in microbial content with the use of PDD. However, this reduction was not enough to completely eliminate *E. faecalis* [27,28].

Riboflavin (RFV) has several benefits as a photosensitizer for the purpose of PDD opportunities on a theoretical level. It is an extremely biocompatible chemical that is safe to employ intraorally [29]. Due to its pale-yellow hue, it does not cause as much discoloration on the tooth's hard tissues compared to TBO. This is particularly beneficial in areas of the teeth that are aesthetically significant. Additionally, it can be stimulated using readily available blue light generating LED light cure device. As a result, there would be little effort required to implement PDD with RFV and blue light in dental procedures. Prior research has demonstrated that blue light producing LED lamps used for composite curing can stimulate RFV, leading to the generation of ROS [17]. Nevertheless, our investigation unequivocally demonstrates that the antibacterial efficacy of PDD with RFV and blue light was inferior to that of PDD utilizing TBO and red light. The limited efficacy of PDD RFV can be attributed to a reduced generation of ROS from RFV in comparison to TBO [17].

As comprehensive root canal disinfectant, PDD cannot be implemented in the absence of chemical irrigation. According to these findings, PDD is believed to possess an extra antibacterial impact during root canal irrigation, particularly against resistant pathogens.

NaOCl is recommended as the main irrigation solution in endodontics because of its broad-spectrum antibacterial effectiveness and its capacity to dissolve organic compounds [30].

Research investigations have demonstrated that PDD has shown greater efficacy compared to NaOCl [31] or as effective as NaOCl,[32], others, however, experienced different outcomes and felt it was less successful [28], these studies were using laser or another photosensitizer. The documented variances can be attributed to variations in methodology, NaOCl concentrations, and the diversity of PDD processes.

In this study, groups 5 and 6 (NaOCl + EDTA + PDD) demonstrated the most effective outcomes in terms of lowering the presence of *E. faecalis* within the root canal area being better than NaOCl and EDTA with no PDD. The discovery has validated the hypothesis of the current study, which posited that photodynamic disinfection would enhance the decontamination of the root canal system, and this in harmony with a study of Vaid et al. the study examined the collective impact of methylene blue dye and diode laser as PDD and a 2.5% (NaOCl) solution on *E. faecalis*, in comparison to the effects of saline and NaOCl irrigation alone, they found that PDD and NaOCl solution was the most efficient method for root canals disinfection that contained mature *E. faecalis* biofilms [33]. Also, Garcez et al [34] have a study on the impact of diod laser PDD in endodontic retreatments while in vivo. The researchers discovered that the use of PDD in addition to traditional endodontic treatment results in a notable decrease in bacterial presence following the use of NaOCl, hydrogen peroxide, and EDTA for irrigation. Furthermore, PDD proves to be effective against bacteria that are resistant to several drugs.

Although PDD is believed to possess an extra antibacterial impact during root canal irrigation, particularly against resistant pathogens. Additional research is required, particularly focusing on roots that have multiple canals, to evaluate the elimination of bacteria from challenging anatomical regions. Additionally, the effectiveness of PDD should be tested on other bacterial species, and additional evaluation should be conducted through clinical trials.

Conclusion

Within the constraints of the study, the combination of root canal irrigation using NaOCl and EDTA, along with photodynamic disinfection using LED light, demonstrated the most effective antimicrobial outcomes.

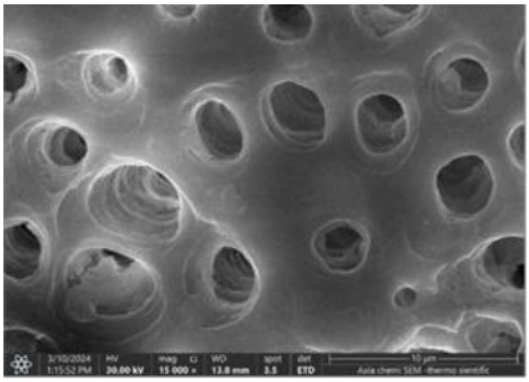
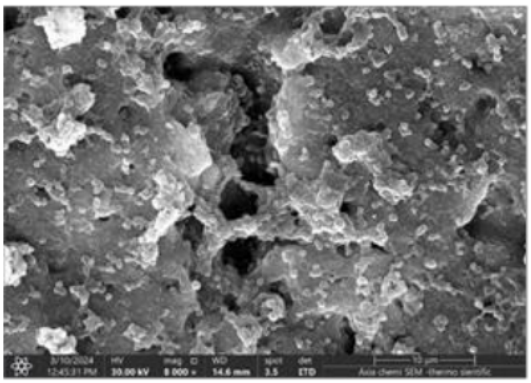


Figure 1: show the clean dentinal tubule in sterile tooth specimen . Magnification 15000x



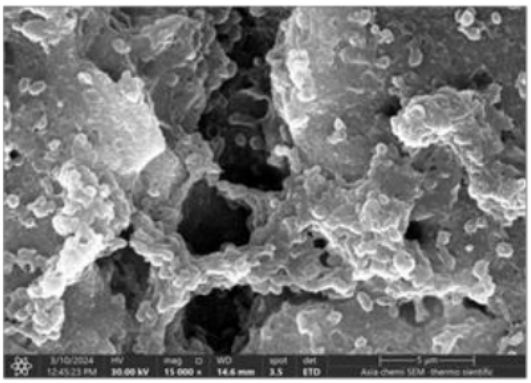
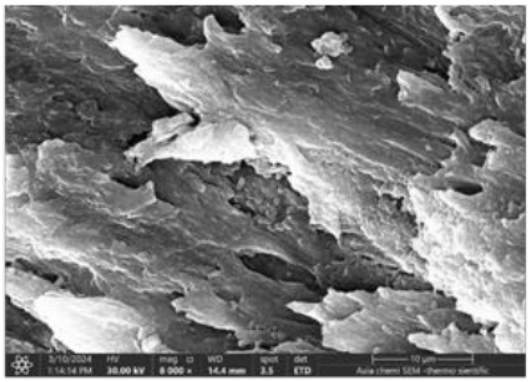


Figure 1: show the biofilm colonization of *E. Faecalis* in the dentinal tubule in the root canal lumen before disinfection treatment. A 8000x Magnification, B 15000x Magnification.



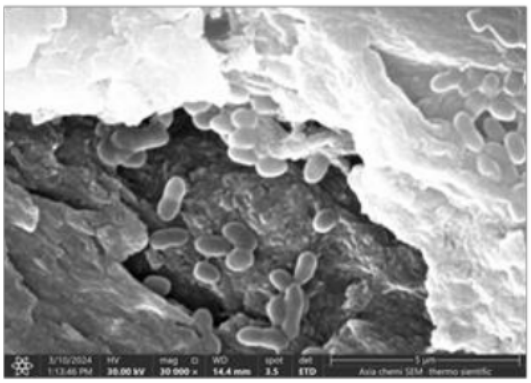


Figure 1: show the biofilm colonization of *E. Faecalis* in the dentinal tubule in the fracture surface. A. 8000 x Magnification, B 30000x Magnification

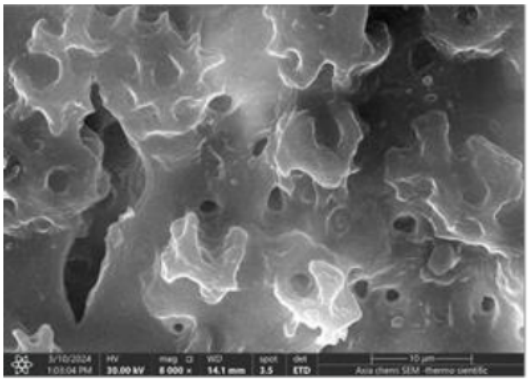


Figure 4 show the root canal lumen after disinfection with PDD. 8000x Magnification.

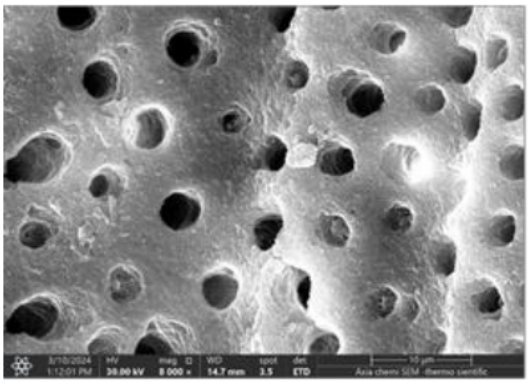


Figure 5 show the root canal lumen after disinfection with NaOCl and PDD. 8000x Magnification

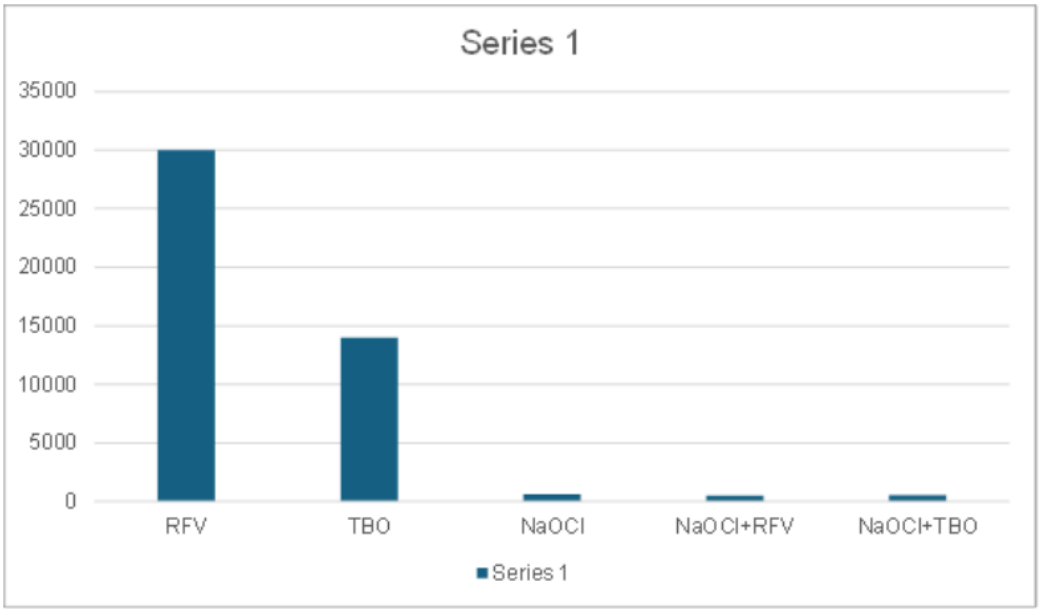


Figure 6: show the reduction of CFUs of bacteria after disinfection.

Disclosure

None

Reference

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