

# Effectiveness of different root canal irrigation protocols in treatment of immature permanent teeth

Krasimir Hristov<sup>1</sup>, Nataliya Gateva<sup>2</sup>

1. Assistant professor, Department of Pediatric Dentistry, Faculty of Dental Medicine, Medical University - Sofia;
2. Associate professor, Department of Pediatric Dentistry, Faculty of Dental Medicine, Medical University - Sofia

## Abstract

**Introduction:** The aim of this study was to determine the efficiency of 6 irrigation protocols in eliminating bacteria in root canals of immature permanent teeth.

**Materials and methods:** Root segments of 10 mm were created from immature permanent teeth. The segments were inoculated with *Enterococcus faecalis* for 2 weeks. Canals were disinfected by 1) passive ultrasonic irrigation; 2) continuous irrigation with sodium hypochlorite and EDTA; 3) XP-Endo finisher, sodium hypochlorite and EDTA; 4) XP-Endo finisher, sodium hypochlorite and citric acid; 5) Gentlefile brush, sodium hypochlorite and EDTA; 6) Gentlefile brush, sodium hypochlorite and citric acid.

Dentinal chips were collected from the canals. Colony forming units were counted, and the effectiveness of each disinfection protocol was reported in terms of log<sub>10</sub> bacterial growth reduction.

**Results:** The number of microorganisms in the ultrasound-activated samples was reduced the least, followed by those prepared according to the American Association of Endodontic Protocol, and segments prepared with Gentlefile and EDTA. The reduction in the number of colony forming units is most

significant in the samples treated with XP-Endo Finisher and EDTA, with XP-Endo Finisher and citric acid, and Gentlefile and citric acid, with no statistically significant difference between the three groups ( $p>0.05$ ).

**Conclusions:** By supplementing the irrigation protocol with minimal mechanical instrumentation, a more efficient reduction of the microflora is achieved in immature permanent teeth.

**Keywords:** immature permanent teeth, irrigation protocol, citric acid

## Introduction

Immature permanent teeth with pulp necrosis are challenging for treatment due to the importance of bacteria and debris elimination without removal of root dentin. Endodontic treatment of such teeth is similar to the treatment of a very wide and oval root canal - a problem considered in modern endodontics (1).

Mechanical instrumentation of oval root canals with hand and rotary files has been shown to leave untreated surfaces (2-6). These spaces may contain residual pulp or bacterial biofilm in infected root canals that may affect adversely the treatment outcome (7).

Most of the microorganisms are in the main canal or in planktonic form. The main canal has many branches - lateral canals, isthmuses, anastomoses, niches, all of which are interconnected (8). Irrigation and disinfectants are effective against microorganisms in a planktonic form, but their effectiveness is reduced against microorganisms in biofilms or invading lateral canals or irregularities. In order for the irrigants to be effective enough in these cases, the biofilm must be removed mechanically (8).

## Aim

The aim of this study is to evaluate and compare the ability of six different irrigation protocols to remove microorganisms from the root canal of immature permanent teeth.

## Material and methods

The study was performed on 70 immature permanent teeth extracted for the purpose of orthodontic treatment from healthy children between the age of 12 to 17. The teeth were included in the study after the parents signed informed consent for their use. The roots were prepared as follows: the coronary part of the tooth was removed with a diamond separator (Superflex, Edenta, Switzerland) and a root segment of 10 mm was created. This was done to minimize variations in the root canal anatomy in order to achieve a real comparability between the results for colony forming units (CFUs). The pulp tissue was removed from the root canal using a barbed broach (VDW, München, Germany).

Samples were apically sealed with light-curing resin composite (Admira, VOCO, Cuxhaven, Germany) and sequential irrigation was carried out with 5 ml 2.5% NaOCl, 5 ml 17% EDTA, 5 ml 2.5% NaOCl to remove the smear layer. The roots were then placed for 48 hours in distilled water. The samples were autoclaved at 121° C for 20 min and stored in sterile phosphate buffer at 4° C until used. Prior to inoculation of the segments with bacterial suspension, the phosphate buffer solution was aspirated under sterile conditions.

The root segments were immersed in brain-heart infusion broth inoculated with *Enterococcus faecalis*. Two segments were immersed in sterile control medium to confirm that no contamination of the samples occurred during the experiment. Samples were incubated for 14 days at 37° C and 5% CO<sub>2</sub>. The culture medium was changed every two days.

When a dense biofilm was confirmed by scanning electron microscopy, the infected 70 root segments were randomly divided into 7 groups of 10 samples in each group. An endodontic needle with a lateral opening (Endo Top Irrigation Needles, Cerekamed, Poland) was used to deliver the irrigation, adjusted to working length of 9 mm. The 7 groups were as follows:

- **First group** - irrigation with phosphate buffer solution to determine the initial number of microorganisms;
- **Second group** - passive ultrasonic irrigation - 5 ml 1.5% hypochlorite for 20s, three cycles; 17% EDTA 1 min. A wireless ultrasonic handpiece (Ultra X, Eighteeth, Changzhou Sifary Technology Co., China) with an activation tip (X Silver, Eighteeth, Changzhou Sifary Technology Co, Chi) is used;
- **Third group** - continuous irrigation with 20 ml of 1.5% hypochlorite for 5 min, followed by 20 ml of 17% EDTA for 5 min, recommended by the American Association of Endodontics;
- **Fourth group** – instrumentation with XP-Endo finisher - 5 ml 1.5% hypochlorite for 60 s, three cycles of 20 s, at 800 rpm and torque of 1 N/cm, 17% EDTA 1 min - three cycles of 20 s;
- **Fifth group** - instrumentation with XP-Endo finisher - 5 ml 1.5% hypochlorite for 60 s, three cycles of 20 s, at 800 rpm and torque of 1 N/cm, 10% citric acid for 1 min - three cycles of 20 s;
- **Sixth group** – instrumentation with Gentlefile brush - 5 ml 1.5% hypochlorite for 60 s, three cycles of 20 s, 17% EDTA 1 min - three cycles of 20 s with the commercially available handpiece.
- **Seventh group** - Gentlefile brush - 5 ml 1.5% hypochlorite for 60 s, three cycles of 20 s, 10% citric acid for 1 min - three cycles of 20 s with the commercially available handpiece.

After irrigation, each canal was rinsed with 5 ml of saline. The root wall was brushed with a No. 3 Peeso reamer and the dentinal chips were transferred to a sterile eppendorf tube with added brain-heart infusion broth. Three sterile paper points were used to absorb residual fluid from the root canal. They were placed in the same tube. The cultures were incubated in blood agar at 37 ° C and 5% CO<sub>2</sub> using the streak culture method. After 24 hours, CFUs were counted, and the mean CFU counts were calculated. The effectiveness of each disinfection protocol was reported in terms of log<sub>10</sub> bacterial growth reduction. The data were analyzed using software SPSS ver. 19. To identify any significant differences between the groups Tukey multiple comparison test was performed.

## Results

The mean values of the colony forming units of the remaining microbial organisms after treatment with the respective irrigation protocols were determined for each group (Table 1, Diagram 1). No microorganisms were isolated from the sterile control groups.

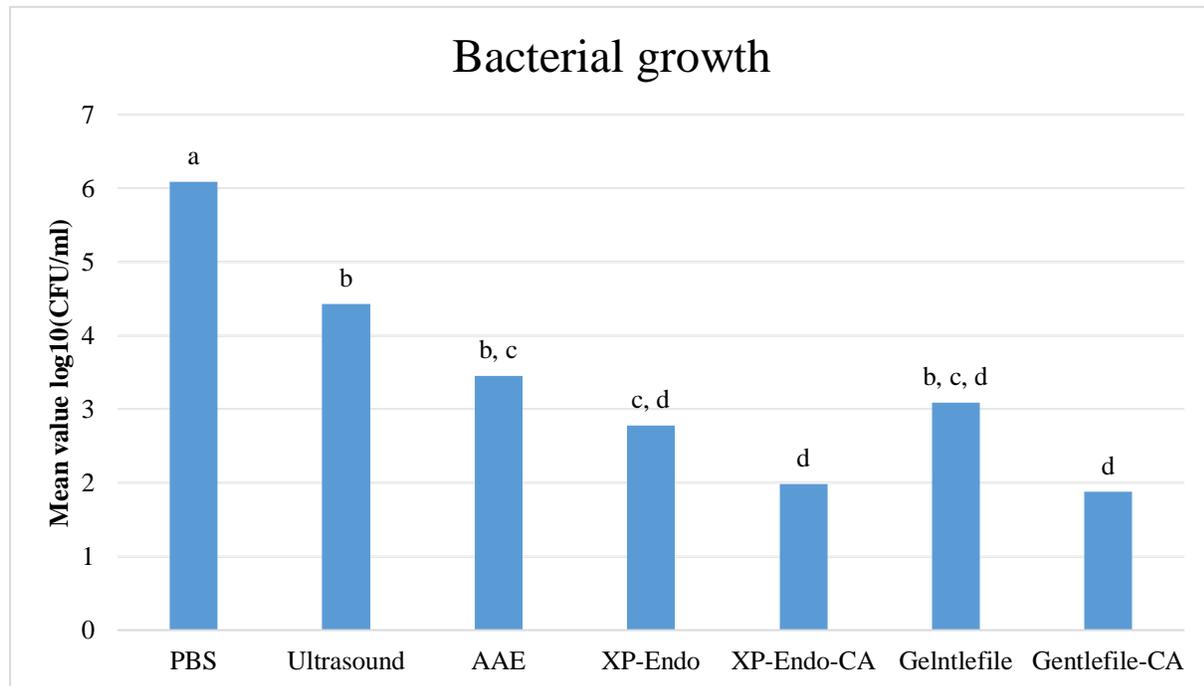
**Table 1. Bacterial growth in each experimental group. SD standard deviation**

|            | PBS     | Ultrasound | AAE     | XP-Endo | XP-Endo-CA | Gentfile | Gentfile-CA |
|------------|---------|------------|---------|---------|------------|----------|-------------|
| Mean value | 6,09247 | 4,43167    | 3,45105 | 2,78061 | 1,98061    | 3,09187  | 1,87604     |
| SD         | 0,32031 | 0,12229    | 0,1548  | 1,50215 | 1,05729    | 1,64652  | 1,01147     |
| Min        | 5,90308 | 4,30102    | 3,30102 | 0,00    | 0,00       | 0,00     | 0,00        |
| Max        | 6,69897 | 4,60205    | 3,69897 | 3,90308 | 2,69897    | 4,17609  | 2,60205     |

In order to evaluate the effectiveness of each of the protocols applied, a comparative evaluation of the mean bacterial residuals between the groups was performed using the Tukey test (Table 2).

**Table 2. Differences between groups according to mean values of residual bacteria (Log10 (CFU/ml), Post Hoc Tukey HSD Analysis).**

| Tukey HSD   |               |                                |          |                         |             |
|-------------|---------------|--------------------------------|----------|-------------------------|-------------|
|             |               | Difference in mean value (A-B) | p        | 95% Confidence interval |             |
| Group A     | Group B       |                                |          | Lower limit             | Upper limit |
| PBS         | Ultrasound    | 1.660799755                    | 0.009185 | 0.274654441             | 3.046945069 |
|             | AAE           | 2.641417752                    | 0.000005 | 1.255272438             | 4.027563066 |
|             | XP-Endo       | 3.311854256                    | 0.000000 | 1.925708942             | 4.69799957  |
|             | XP-Endo-CA    | 4.111854256                    | 0.000000 | 2.725708942             | 5.49799957  |
|             | Gelntlefile   | 3.000593756                    | 0.000000 | 1.614448442             | 4.38673907  |
|             | Gentlefile-CA | 4.216430005                    | 0.000000 | 2.830284691             | 5.602575319 |
| Ultrasound  | AAE           | 0.980617997                    | 0.334705 | -0.40552732             | 2.366763311 |
|             | XP-Endo       | 1.651054501                    | 0.009798 | 0.264909187             | 3.037199815 |
|             | XP-Endo-CA    | 2.451054501                    | 0.000023 | 1.064909187             | 3.837199815 |
|             | Gelntlefile   | 1.339794001                    | 0.064673 | -0.04635131             | 2.725939315 |
|             | Gentlefile-CA | 2.55563025                     | 0.000010 | 1.169484936             | 3.941775564 |
| AAE         | XP-Endo       | 0.670436504                    | 0.759395 | -0.71570881             | 2.056581818 |
|             | XP-Endo-CA    | 1.470436504                    | 0.030616 | 0.08429119              | 2.856581818 |
|             | Gelntlefile   | 0.359176004                    | 0.985223 | -1.02696931             | 1.745321318 |
|             | Gentlefile-CA | 1.575012253                    | 0.016048 | 0.188866939             | 2.961157567 |
| XP-Endo     | XP-Endo-CA    | 0.8                            | 0.580793 | -0.58614531             | 2.186145314 |
|             | Gelntlefile   | -0.3112605                     | 0.993058 | -1.69740581             | 1.074884814 |
|             | Gentlefile-CA | 0.904575749                    | 0.432673 | -0.48156957             | 2.290721063 |
| XP-Endo-CA  | Gelntlefile   | -1.1112605                     | 0.198980 | -2.49740581             | 0.274884814 |
|             | Gentlefile-CA | 0.104575749                    | 0.999987 | -1.28156957             | 1.490721063 |
| Gelntlefile | Gentlefile-CA | 1.215836249                    | 0.122788 | -0.17030906             | 2.601981563 |



**Figure 1. Bacterial growth in each experimental group. The different letters indicate a statistically significant difference between the groups ( $p < 0.05$ ).**

The statistical analysis revealed that the average number of colony forming units in each group was significantly lower than the phosphate buffered segments (group 1,  $p < 0.05$ ). In the other groups, the number of microorganisms in the ultrasound-activated samples (group 2) was reduced the least, followed by those prepared according to the recommendation of the American Association of Endodontic Protocol (group 3) and segments prepared with Gentlefile and irrigated with EDTA (group 6).

The results suggest that the obtained antibacterial effect is due to the disinfectant properties of the irrigants, rather than to their rinsing action. This is confirmed by the statistically significant difference in the mean number of microorganisms in group 1 treated with phosphate buffer solution and the other groups.

The reduction in the number of CFUs is most significant in the samples treated with XP-Endo Finisher with EDTA (group 4), with XP-Endo Finisher and citric acid (group 5) and the samples treated with Gentlefile and citric acid (group 7), with no statistically significant difference between the three groups ( $p > 0.05$ ). The data show that minimal mechanical instrumentation of the root canal with appropriate files significantly improves the removal of bacteria from the dentin surface with a shorter treatment protocol.

## Discussion

Effective removal of bacterial biofilm contributes to the success of regenerative endodontics (9). It is known that root canal infection is always polymicrobial (10). In our study, we used a monomicrobial biofilm model to avoid all possible variations caused by interspecies interactions (11). *E. faecalis* was selected as the test species because these bacteria have the ability to form biofilms, survive under severe conditions and counteract the antimicrobial effect of calcium hydroxide (11). Moreover, *E. faecalis* is often isolated from the root canal in failed endodontic treatments (12, 13).

In all protocols, we used 1.5% NaOCl instead of 2.5% NaOCl, as it is known that high sodium hypochlorite concentration impairs stem cell attachment to the dentin surface (14), may prevent odontoblast differentiation mediated by growth factors since it denatures these factors (15) and it is toxic to the stem cells from apical papilla (16). Decreasing the concentration of the solution leads to a decrease in its toxicity, but also in the antibacterial effect and the ability to dissolve tissues (17). Concentrations of 1.5% NaOCl are recommended for the purpose of regenerative endodontics by AAE (18), and concentrations of 2.5% NaOCl are preferred by the European Society of Endodontics (19). Traditional endodontic disinfectants only limit the infection (16). To use their full capacity, these solutions must be activated. Our study examined the effect of different methods of activating irrigation solutions on immature permanent teeth with pulp necrosis. Only one of the protocols (group 3) does not use tools for activating the solutions, while the others use passive ultrasonic irrigation (group 2), the innovative XP-endo Finisher system (groups 4 and 5) and Gentlefile system (Groups 6 and 7). The number of microorganisms in ultrasound-activated samples was reduced the least, followed by those prepared according to the protocol recommended by the American Association of Endodontists (group 3). In fact, the AAE protocol relies on longer irrigation time and more hypochlorite and EDTA to cope with the infection. Theoretically, the effect of passive ultrasonic irrigation is inversely proportional to the thickness of the microbial biofilm and the width of the root canal (20). Immature permanent teeth have wide oval canals, and this explains the insufficient effect of passive ultrasonic irrigation on their disinfection. Our results confirm this.

There is little data in the specialized literature on the use of citric acid, especially in permanent immature teeth. Exogenous citrate added to the culture medium or released from the biomaterial during its absorption is known to increase alkaline phosphatase gene expression and osteoblastic phenotypic progression both in vitro and in vivo (21).

Promising results have been published on the effect of citric acid on the vitality of fibroblasts and macrophages (22-24) and on its use as an irrigant in the endodontic protocol for permanent teeth (25). In the same time citric acid has weaker proinflammatory action compared to EDTA-based solutions and it plays important natural role in the tricarboxylic acid cycle, thus regulating energy homeostasis and cellular metabolism (26, 27).

In two of the protocols, we used citric acid to find out if it would increase the effectiveness of the applied irrigation protocol. Citric acid has similar to EDTA effect. The use of 10% citric acid in combination with 2.5% NaOCl is known to be a good approach for removing the smear layer from the root canal dentin and opening the dentin tubules. Its efficacy does not differ from that of 17% EDTA (25). In the same time citric acid is well tolerated by the stem cells from apical papilla, i.e. it is not more toxic than EDTA for the apical papilla, which is important for immature permanent teeth with pulp necrosis. Our results indicated that the use of citric acid in the irrigation protocol (groups 5 and 7) significantly improves its antibacterial effectiveness in

immature permanent teeth, compared to other groups in which it is not included in the irrigation protocol (Table 2). There are no published previous research on the effects of citric acid as a part of the disinfection protocol in immature permanent teeth.

Our study demonstrates the superiority of new root canal systems in the disinfection of root canals in immature permanent teeth. The best results are demonstrated with the XP-Endo Finisher protocols with EDTA and citric acid (groups 4 and 5) and Gentlefile with citric acid (group 7). These root canal instruments were chosen because of the manufacturer's claims that they do not change the shape of the root canal and remove a minimal amount of dentin from the root walls (28-30).

The effectiveness of the XP-Endo Finisher system (groups 4 and 5) proven by our study is in accordance with results from other studies. In the study of Azim et al. XP-endo Finisher effectively reduced bacteria in the root canal (98.2%), showing a statistically significant difference when compared to PIPS-erbium: yttrium aluminum garnet laser (89.6%) and EndoActivator (93.3%). The XP Endo finisher also has a high efficiency in killing bacteria in the dentin tubules and at 50 microns depth in the coronary, middle and apical thirds shows the highest percentage of killed bacteria - between 78% and 82%. This may be due to the combination of the mechanical action of the file and the simultaneous activation of irrigation solutions, thereby facilitating the removal of microorganisms from the canal wall (31).

## Conclusion

By supplementing the irrigation protocol with the use of the XP-Endo Finisher and Gentlefile Brush systems, a more efficient reduction of the microflora in the root canal is achieved without unnecessary dentin removal. The data show that this minimal mechanical scraping or brushing of the root canal with appropriate files significantly improves the removal of bacteria from the dentin surface and can be used as an alternative, effective and minimally invasive method of disinfection in regenerative endodontics. Our study demonstrated that the low-toxic citric acid improves the antibacterial action of chemical disinfection. Therefore, citric acid would be a useful supplement to the irrigation protocol, given its weaker proinflammatory action compared to EDTA-based solutions, as well as its important natural role in the tricarboxylic acid cycle, which is important for regulating energy homeostasis and cellular metabolism.

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### Corresponding author:

Krasimir Hristov,  
Department of Pediatric Dentistry,  
Faculty of Dental Medicine,  
Medical University- Sofia,  
1 Georgy Sofiyski Str.  
tel: +359 883 411 743  
email: khristov87@gmail.com