

Effect of Different Obturation Materials on Residual Antimicrobial Activity of 2% Chlorhexidine in Dentin at Different Time Intervals: An Ex Vivo Study

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Abstract

Objectives: The aim of this study was to evaluate the effect of gutta percha/AH26 and Resilon/RealSeal SE on residual antimicrobial activity of chlorhexidine (CHX) in human root dentin and suggest the best filling material when CHX is used as final irrigant.

Materials and Methods: One-hundred and forty-four single-rooted human teeth were selected for this study. Canals were instrumented to the apical size #35. Smear layer was removed using 5.25% NaOCl and 17% EDTA and then 108 teeth were irrigated with 2% CHX and randomly divided into three groups of gutta percha/AH26, Resilon/RealSeal SE and positive controls. Each group was divided into three subgroups for different time intervals (one, three and six weeks). Thirty-six teeth, as negative controls, were irrigated with saline and obturated with gutta percha/AH26 and Resilon/RealSeal SE. Dentin powder was prepared at the afore-mentioned intervals. After exposure to *Enterococcus faecalis* for 24 hours, colony forming units (CFUs) were counted and residual antimicrobial activity was calculated. The data were analyzed using the Kruskal Wallis test and one-way ANOVA. The significance level was set at $P < 0.05$.

Results: The antimicrobial activity of CHX gradually decreased in a time-dependent manner but it maintained over 95% of its antimicrobial activity after six weeks. Moreover, Resilon/RealSeal SE significantly decreased the antimicrobial activity of CHX in comparison with gutta-percha/AH26 ($P < 0.05$).

Conclusion: After a final irrigation with CHX, gutta-percha/AH26 is a better choice for root canal obturation.

Key words: Chlorhexidine; Gutta-Percha; Epoxy Resin AH-26; Resilon Sealer
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INTRODUCTION

Success of endodontic treatment is directly related to efficient removal of pathogenic microorganisms from the root canal system [1].

Several techniques are available to reduce the microbial load of the root canal system such as mechanical root canal instrumentation, use of

irrigating solutions and intracanal medicaments. Although mechanical preparation can reduce the number of bacteria, antimicrobial irrigants have an important role in effective elimination of bacteria [2,3].

Enterococcus faecalis is the most common *Enterococcus* species frequently isolated in patients with periodontitis, gingivitis and failed root canal therapy [4,5]. Some characteristic features of this bacterium such as proton pump made it a resistant bacterium [6]. Studies have demonstrated that calcium hydroxide is not able to completely remove resistant bacteria such as *E. faecalis* [7]. On the other hand, various materials such as serum proteins, hydroxyapatite and collagen can affect antimicrobial efficacy of irrigants [8,9]. Therefore, we should select an irrigant with maximum efficacy for elimination of bacteria. It should also be able to preserve its antimicrobial activity in presence of dentin and filling materials and have substantivity after a long period of time.

Chlorhexidine is a broad-spectrum antibacterial agent effective on Gram-positive and Gram-negative bacteria and fungi. Due to cationic nature of CHX, it has the ability to adhere to negatively charged surfaces of bacteria and increase their permeability [10]. Chlorhexidine has sustained antimicrobial activity via bonding to dentin [11]. According to some recent studies, final irrigation of root canal with CHX can produce the highest level of sustained antimicrobial activity [12,13]. Nevertheless, it is important whether CHX can maintain its antimicrobial activity in presence of filling materials or not.

Gutta-percha is the most commonly used filling material but it cannot provide an appropriate seal against bacteria [14]. Another material, Resilon/RealSeal, was introduced aiming to form a monoblock with the root canal walls. The second generation consists of two parts of Resilon and Epiphany self-etch sealer (Resilon/RealSeal SE). Some studies showed that Resilon "monoblock" system was

associated with lower incidence of apical periodontitis, which may be due to its superior resistance to coronal microleakage [15]. This study was planned to assess the residual antimicrobial activity of CHX some time after root canal obturation. The null hypothesis of this study was that the residual antimicrobial activity of CHX would not be affected by canal filling materials.

MATERIALS AND METHODS

Tooth and dentin powder preparation:

One-hundred and forty-four single-rooted human teeth with no cavities and resorption were selected for this study. The teeth were soaked in 5.25% NaOCl for 30 minutes. Calcified root canals with more than one canal were excluded. Access cavity was prepared and working length was determined. Root canals were instrumented to the apical size #35 using Mtwo rotary files (VDW, Munich, Germany) according to the manufacturer's instructions. All canals were irrigated after using each file by 2 mL of 2.5% NaOCl. To prevent bacterial leakage, apical foramina were sealed with wax. Nail varnish was applied on the surface of roots in two layers. Three mL of 17% EDTA (Meta Biomed Co. Ltd., Mandaluyong, Korea) was used for one minute to remove the smear layer. Then, the canals were irrigated with saline and dried. Finally, 108 teeth (for test groups and positive control) were rinsed with 5 mL of 2% CHX (Consepsis®, Ultradent, South Jordan, UT, USA) for 10 minutes. Thirty-six remaining samples that were not irrigated with CHX were used as negative controls.

The teeth were randomly divided into the following groups:

1- Gutta-percha/AH26 group (n=45):

Root canals were dried with paper points (Ariadent, Tehran, Iran) and obturated with gutta-percha (Ariadent, Tehran, Iran) /AH26 (Dentsply, DeTrey, Germany) using lateral compaction technique. Then, they were restored with Coltisol (AriaDent, Tehran, Iran).

The teeth were randomly divided into three subgroups (n=15). Samples were packed and sterilized by gamma radiation at the dose of 40 k gray (ISO standard, 11137). After sterilization, samples were maintained in an incubator at 37° C and 100% humidity. One, three and six weeks after canal obturation, dentin powder was prepared. For dentin powder preparation, the teeth were longitudinally split in half by a high-speed diamond bur. After removing the root filling material, dentin powder was obtained from 2 mm apical to the cemento-enamel junction using a low-speed round bur. Area for dentin powder preparation had a length of 3 mm and a depth equal to the diameter of a #7 round bur. Dentin powder of each sample was collected in a sterile 1.5mL Eppendorf tube and prepared for microbiological tests.

2- *Resilon/RealSeal SE group (n=45):*

Root canals were dried and obturated with Resilon/RealSeal SE (Pentron Clinical Technologies, Wallingford, CT, USA) using lateral compaction technique. After cutting and packing Resilon, it was light-cured for 40 seconds according to the manufacturer's instructions. Then, the teeth were restored with Coltosol and randomly divided into three for group 1.

3- *Positive control (n=18):*

After canal preparation and final rinsing with CHX, the root canals remained unfilled and restored with Coltosol. Then, they were randomly divided into three subgroups (six teeth for each interval). Sterilization and dentin powder preparation were done as in group 1.

4- *Negative control obturated with Gutta-percha/AH26 (n=18):*

After root canal preparation, canals were irrigated with 5mL of normal saline and obturated with Gutta-percha/AH26. Samples were randomly divided into three subgroups (six teeth for each interval). The next steps were done as in group 1.

5- *Negative control obturated with Resilon/RealSeal SE (n=18):*

The procedure was the same as in group 4 except that instead of Gutta-percha/AH26, Resilon/RealSeal SE was used for root canal obturation.

Microbiological procedure:

Lyophilized *E. faecalis* (ATCC 25922, obtained from Rayen Biotechnology Co. Ltd., Tehran, Iran) was cultured in brain heart infusion (BHI) broth (Merck, Darmstadt, Germany). It was incubated at 37°C for 24 hours. Bacterial cultures were adjusted to a concentration of 10⁸ colony forming units (CFU)/mL by colony counting and optical density was adjusted to 0.4 by spectrophotometry (Biophotometer, Tokyo, Japan). Then, the exact density (CFU/mL) of each suspension was verified on BHI plates. Dentin powder was prepared as previously described. Freshly prepared dentin powder was mixed with 500µL of bacterial suspension. Also, 500 µL of bacterial suspension without dentin powder was used as control. The tubes were kept in aerobic conditions at 37°C for 24 hours. Ten-fold serial dilution was done by transferring 50 µL of each sample into tubes containing 450 µL of BHI broth. One hundred µL of third and fourth dilutions were cultured onto BHI plates. Bacterial growth and concentration were assessed. Blind cultures on BHI plates were done to check possible contamination.

Residual antimicrobial activity was calculated using the formula below:

$$\frac{[\text{Bacterial suspension (CFU/mL)} - \text{each sample (CFU/mL)}]}{\text{Bacterial suspension (CFU/mL)}} \times 100$$
 For comparison between groups at each time point, nonparametric Kruskal-Wallis and Dunn tests were used because of the absence of normal distribution. For comparison between different time intervals, One-way ANOVA and Tukey's HSD test were used for groups with normal distribution and nonparametric Kruskal-Wallis and Dunn test were used for groups that did not have a normal distribution. P<0.05 was considered statistically significant.

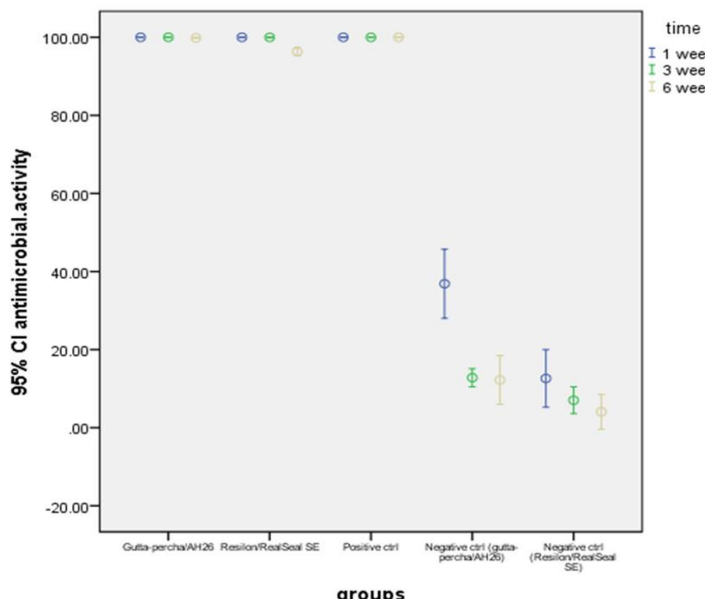


Fig. 1. Comparison of residual antimicrobial activity of chlorhexidine in different groups.

RESULTS

The mean percentages of residual antimicrobial activity of CHX with standard deviations for each group are listed in Table 1.

Statistical analyses showed a significant relationship between the filling material and residual antimicrobial activity of CHX (P<0.05).

The results showed that residual antimicrobial activity of CHX was significantly higher in gutta-percha/AH26 samples than in Resilon/RealSeal SE samples (P=0.02 at one week and P=0.003 at three and six weeks, respectively).

Pairwise comparisons between other groups revealed a significant difference between the antimicrobial activity of CHX in the positive control and Resilon/RealSeal SE samples at three and six weeks (P=0.07 at one week and P<0.001 at three and six weeks); nonetheless, the difference was not significant for gutta-percha/AH26 samples at all time points (P>0.05). Also, there was a significant difference between positive control and negative control samples (both gutta-percha/AH26 and Resilon/RealSeal SE) and negative controls with gutta-percha/AH26 or Resilon/RealSeal SE groups (all Ps<0.001).

Table 1. The mean and standard deviation of percentage of residual antimicrobial activity of chlorhexidine for each group.

| Groups | Time intervals | Residual antibacterial activity of chlorhexidine (%) ± SD | | |
|--------------------------------------|----------------|---|----------------|--------------|
| | | At one week | At three weeks | At six weeks |
| Gutta-percha/AH26 | | 100 ± .00 | 99.99 ± .0006 | 99.85 ± .17 |
| Resilon/RealSeal SE | | 99.99 ± .0001 | 99.98 ± .01 | 96.33 ± 1.89 |
| Positive control | | 100 | 100 | 100 |
| Negative control-gutta-percha/AH26 | | 36.86 ± 8.44 | 12.81 ± 2.21 | 12.23 ± 5.97 |
| Negative control-Resilon/RealSeal SE | | 12.62 ± 7.02 | 7.03 ± 3.25 | 4.07 ± 4.22 |

For comparison of different time points, further analyses revealed a significant difference between one and three weeks, one and six weeks and three and six weeks in both gutta-percha/AH26 and Resilon/RealSeal SE samples ($P=0.001$, $P<0.001$ and $P=0.001$ for gutta-percha/AH26 and $P=0.002$, $P<0.001$ and $P=0.002$ for Resilon/RealSeal SE, respectively).

In gutta-percha/AH26 negative control, the differences between one week and two other time points were statistically significant ($P<0.001$). In Resilon/RealSeal SE negative control, only the difference between one week and six weeks was statistically significant ($P=0.02$). Residual antimicrobial activity in gutta-percha/AH26 negative control was higher than that in Resilon/RealSeal SE negative control at each time point although it was not statistically significant.

In general, antimicrobial activity of CHX decreased in a time-dependent manner; however, its reduction was faster in Resilon/RealSeal SE samples than in gutta-percha/AH26 samples. The highest antimicrobial activity was found in at one week in gutta-percha/AH26 and positive control samples and the lowest was found at six weeks in Resilon/RealSeal SE samples. Comparative graph for different groups is shown in Fig. 1.

DISCUSSION

The current study assessed the residual antimicrobial activity of CHX after canal obturation with gutta-percha/AH26 and Resilon/RealSeal SE. In spite of a gradual time-dependent decrease in antimicrobial activity of CHX, the results indicated that at the end of six weeks after canal obturation, CHX maintained over 95% of its antimicrobial activity regardless of the root filling material. Moreover, it was concluded that Resilon/RealSeal SE significantly decreased the antimicrobial activity of CHX in comparison with gutta-percha/AH26. Accordingly, it is suggested that after final

irrigation with CHX, gutta-percha/AH26 would be a better choice for root canal obturation. Microorganisms are responsible for development of pulpal and periapical diseases and reinfection of root canals. Facultative bacteria, such as *E. faecalis* play a key role in recurrent infections of root canals [1]. This bacterium is known as a resistant bacterium because of its ability to grow at high salt concentrations, tolerate temperature and pH alterations (due to its effective proton pump) and remain viable but not cultivable (VBNC); thus, it can resist common antimicrobial agents [16]. Therefore, an efficient endodontic irrigant with sustained activity may significantly affect the long-term success of endodontic treatment. Chlorhexidine is a broad-spectrum antibacterial agent with good efficacy against Gram-positive and Gram-negative bacteria and fungi. Depending on the concentration of CHX, it can be bacteriostatic or bactericidal. Positively charged CHX can interact with negatively charged surfaces of bacterial cell membrane [10]. At low concentrations, it reversibly damages the bacteria by causing leak out of the low-weight molecular substances. At high concentrations, it causes coagulation of the cytoplasm and results in death of bacteria [7]. Chlorhexidine has an acceptable antibacterial activity comparable to that of sodium hypochlorite. Oncag et al. [17] compared antibacterial activity of 5.25% NaOCl, 2% CHX and 0.2% CHX+0.2% Cetrimide against *E. faecalis*. They concluded that 2% CHX and 0.2% CHX+0.2% Cetrimide were significantly more effective against *E. faecalis* than 5.25% NaOCl. Another study by Vianna et al. [18] showed that the time required for eliminating all tested microorganisms was the same for 1.0% and 2.0% CHX and 5.25% NaOCl. They concluded that antimicrobial action is related to concentration and form of the irrigant as well as microbial susceptibility. According to studies about the effect of CHX on biofilms [19, 20], sodium hypochlorite is absolutely superior to CHX in disrupting the biofilms.

Shen et al. [21] reported that mature and nutrient-limited biofilms are more resistant to CHX than young biofilms. As a whole, it seems that antibacterial effects of CHX and NaOCl, when used in identical concentrations, are similar but sodium hypochlorite is superior to CHX in disrupting bacterial biofilms.

Other characteristic features of CHX are substantivity and anti-collagenolytic activity. Its structure consists of two 4-chlorophenyl rings, two bisguanide groups and a hexamethylene chain [10]. Chlorhexidine is a cationic bisguanide molecule that acts through a cationic-anionic reaction rather than a deposition mechanism [12]. After ionization in water, protonated amine groups of CHX interact with anionic molecules in dentin such as phosphoproteins, hydroxyl and carboxyl groups of collagen and etc. This mechanism explains the substantivity of CHX to oral/dental structures [22]. Khademi et al. [23] investigated the substantivity of 2% CHX five minutes after the application and reported its substantivity up to four weeks. According to Rasimick et al, [13] the half-life of CHX was 14 weeks on dentin. Various potential factors such as dentin, collagen proteins and hydroxyapatite along with filling materials and sealers can affect antimicrobial activity of endodontic irrigants [8,9,24]. Rosenthal et al. [24] evaluated the substantivity of 2% CHX in root canal dentin in presence of gutta-percha/AH26.

They investigated CHX substantivity by both quantification and qualification methods. According to quantification results, approximate concentrations of CHX were halved at three weeks. According to qualification results, CHX retained in root canal dentin in antimicrobially effective amounts and its antibacterial activity was approximately 83% and 40% at six and twelve weeks, respectively. Our results reported higher antibacterial activity (99%) in gutta-percha/AH26 at six weeks. What made Rosenthal et al, and the current study different from previous studies was that antimicrobial

activity of CHX was assessed in root canals filled with sealer and filling materials. This made these studies more clinically relevant.

Epoxy resin sealers, like AH26, have binding ability to dentin [25], acceptable physical properties [26], appropriate antimicrobial activity [27] and good apical seal against micro-leakage [28]. Lee et al. [25] reported that AH26 has the highest adhesion to dentin and gutta-percha in comparison with other tested sealers. AH26 can bind to amino groups of exposed collagen by its open epoxide rings [25].

Moreover, there are amine groups in CHX molecule [12]; accordingly, AH26 might be capable of forming covalent bond with CHX by its epoxide rings. This may explain higher antimicrobial activity of CHX in canals obturated with gutta percha /AH26 in our study. Since, there is no study showing the above-mentioned reaction, further studies in this regard are recommended.

According to the broad-spectrum inhibitory effect of CHX on matrix metalloproteinase, it is suggested that CHX can significantly increase the resin–dentin bond stability [29]. Thus, it can improve the bond strength of resin sealers to root canal dentin. Prado et al. [30] reported that a final flush with 2% CHX significantly reduced the coronal microleakage of teeth filled with gutta-percha/AH Plus or Resilon/Real Seal SE. Furthermore, Sharifian et al. [31] suggested that 2% CHX is a good conditioner for root canal dentin before obturation with Resilon/Epiphany SE.

Resilon/Epiphany SE was introduced aiming to form a monoblock with canal walls [32]. Studies showed that there was superior resistance to coronal microleakage in Resilon/Epiphany SE than in gutta-percha/AH26 [14,15]. On the other hand, acidic sealers (like Epiphany SE) might alter local pH of dentin; besides, pH has an important role in effectiveness of antimicrobial agents and their diffusion rate [13]. For this reason, we can explain lower residual antimicrobial activity of

CHX in canals obturated with Resilon/Epiphany SE in our study. The results of this study are in accordance with those of two recent studies by Bolhari et al, [33,34] that showed lower stability and antimicrobial activity of MTAD in root canal dentin after obturation with Resilon/RealSeal SE.

Consequently, we suggest that gutta percha/AH26 is significantly superior to Resilon/Epiphany SE regarding preservation of antimicrobial activity of CHX. Also, the results of Sharifian et al. [31] were not in contrast to our results because first of all their study results were not statistically significant. Second, they did not assess gutta-percha/AH26 and did not compare it with Resilon/Epiphany SE.

In this study, the samples were sterilized by gamma Irradiation instead of autoclave to eliminate contamination before culturing; because, autoclaving might denature the collagen proteins in the dentin and affect the retention of CHX. Ruhl et al. [35] showed that gamma irradiation at the dose of 12 k gray does not affect the composition and integrity of human saliva proteins. But further studies on the effects of gamma sterilization on dentin components are required. It should be noted that all of these results are related to in vitro studies and more clinical studies are needed to investigate the effect of root canal filling materials on substantivity of endodontic irrigants and success/failure of treatment.

CONCLUSION

Within the limitations of this study, although the antimicrobial activity of CHX had a gradual, time-dependent decrease, CHX maintained over 95% of its antimicrobial activity after six weeks. Moreover, Resilon/RealSeal SE significantly decreased the antimicrobial activity of CHX in comparison with gutta-percha/AH26. Accordingly, it is suggested that after final irrigation with CHX, gutta-percha/AH26 would be a better choice for root canal obturation.

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