ว ทันต จุฬาฯ 2559;53-64
CU Dent J. 2016;39:53-64

บทวิทยาการ
Original Article

The effect of AH plus and Zinc Oxide Eugenol-based root canal sealers on preventing residual bacteria in dentinal tubules from accessing the root canal

Napapas Nidhidvanich, D.D.S., M.Sc.[1]
Panida Thanyasrisung, D.D.S., Ph.D.[2]
Pavena Chivatxaranukul, D.D.S., Grad. Dip. Clin. Dent., Ph.D.[1]

[1]Department of Operative Dentistry, Faculty of Dentistry Chulalongkorn University, Bangkok, Thailand
[2]Department of Microbiology and RU on Oral Microbiology and Immunology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

Abstract

Background/objectives To investigate the ability of two root canal sealers in preventing residual bacteria in dentinal tubules from accessing the main root canal space.

Materials and methods Thirty-five sterilized root samples were randomly assigned to 5 groups: Gutta percha+AH Plus sealer (AH Plus, n=10), Gutta percha+Zinc oxide eugenol sealer (ZOE, n=10), Gutta percha without sealer (GP, n=5), Flowable resin composite coating (composite, n=5), and non-infected samples+GP (aseptic control, n=5). All samples, except the aseptic control, were incubated with *Enterococcus faecalis* for 4 weeks. All canals were chemo-mechanically prepared and obturated according to the assigned groups. After 1 week, the filling materials, except that of the composite group, were removed and replaced with media. To investigate the presence of residual bacteria that access and regrow in the root canal space, the added-media were collected for culture and replaced with fresh media every day, up to 30 days. Data were analysed by Fisher-exact and Kruskal-Wallis test.

Results Number of samples showing residual bacteria accessing and regrowing in the canal space in AH Plus group was lower than that of the GP group ($p < 0.05$), while the number in the ZOE group was similar to that of the GP group ($p = 0.50$). However, both AH Plus and ZOE sealer significantly delayed the regrowth of residual bacteria, compared to the GP group ($p < 0.001$ and < 0.01, respectively).

Conclusion Although root canal sealers could not completely prevent the residual bacteria in the dentinal tubules from accessing root canal space, they possessed the ability to prevent or delay such circumstance to some extent. AH Plus was more effective than ZOE sealer in preventing the residual bacteria from accessing the root canal space.

(CU Dent J. 2016;39:53-00)

Key words: *AH Plus root canal sealer, Bacterial entombment, Enterococcus faecalis, prevention & control, root canal therapy*

Correspondence: Pavena Chivatxaranukul, pavena.c@chula.ac.th

Introduction

Bacterial infection of the root canal system is the main cause of apical periodontitis (Moller et al., 2004). Therefore, the aim of root canal treatment is to eliminate the bacteria present in the root canal. However, complete bacterial elimination is difficult to achieve, even after chemomechanical preparation of root canal has been completed (Fabricius et al., 2006; Nair, 2006; Harrison et al., 2010). Root canal obturation is associated with a reduction in the number of viable bacteria in root dentine and periapical tissue inflammation (Katebzadeh et al., 1999; Wang et al., 2014). In addition to the host immune response, some residual bacteria may be inactivated or killed by the antibacterial and entombing effects of the sealer (Sundqvist and Figdor, 1998; Saleh et al., 2004; Siqueira and Rocas, 2008).

Among endodontic sealers, zinc oxide eugenol-based (ZOE) and AH Plus (Dentsply De Trey GmbH, Konstanz, Germany), which is a resin based sealer, were shown to have an antibacterial effect on bacteria in the root canal and dentinal tubules (Sedgley et al., 2005). However, root dentine specimens collected from endodontically treated teeth still contained viable bacteria (Wang et al., 2014) that could recover in nutrient-containing media (Sedgley et al., 2005; Shin et al., 2008). This finding implies that the residual bacteria in a root canal system, including the dentinal tubules, could survive the antibacterial effect of a root canal sealer, and repopulate the canal when nutrients were available. However, it has not been clearly demonstrated whether the residual bacteria in the dentinal tubules, which are either patent or partially/completely sealed by sealer tags, could migrate back into the root canal space after obturation.

When endodontically treated teeth receive occlusal loading, separation can occur at the interface between the root canal sealer and root canal wall (Bishop et al., 2008; Karina et al., 2012). An interfacial space between the sealer and dentine can allow the penetration of saliva from coronal leakage and tissue fluid from apical leakage, providing nutrients for the residual bacteria (Bouillaguet et al., 2004; Roth et al., 2012). These bacteria may repopulate, migrate, and reinfect the interfacial and main root canal space. Subsequently, if these bacteria gain access to the peri-radicular tissue via the apical foramen/lateral canals, they can lead to an unfavorable treatment outcome (Siqueira and Rocas, 2008; Vieira et al., 2012).

It has been shown that AH Plus, an epoxy resin based root canal sealer with superior flowability, penetrated and adapted into the dentinal tubules better than a ZOE sealer (Mamootil and Messer, 2007; Balguerie et al., 2011). The formation of sealer tags in the tubules establishes a physical barrier that may isolate the residual bacteria in dentinal tubules from available nutrients, prevent regrowth or entomb these bacteria (Sundqvist and Figdor, 1998; Siqueira and Rocas, 2008). However, the effect of sealers with different flowability on preventing the residual bacteria in dentinal tubules from accessing the root canal has not been clearly demonstrated.

The aim of this study was to investigate the ability of two root canal sealers with different flowability, ZOE and AH Plus, to prevent the residual bacteria in dentinal tubules from accessing the main root canal space. The hypotheses of this study were 1) the residual bacteria in dentinal tubules could recover, migrate, and regrow in the main root canal space where nutrients were available and 2) AH plus, one of the sealers with high flowability, had superior ability to inhibit or delay residual bacteria from accessing the root canal space than ZOE sealer.

Materials and Methods

The overall process flow of this study is depicted in Fig. 1.

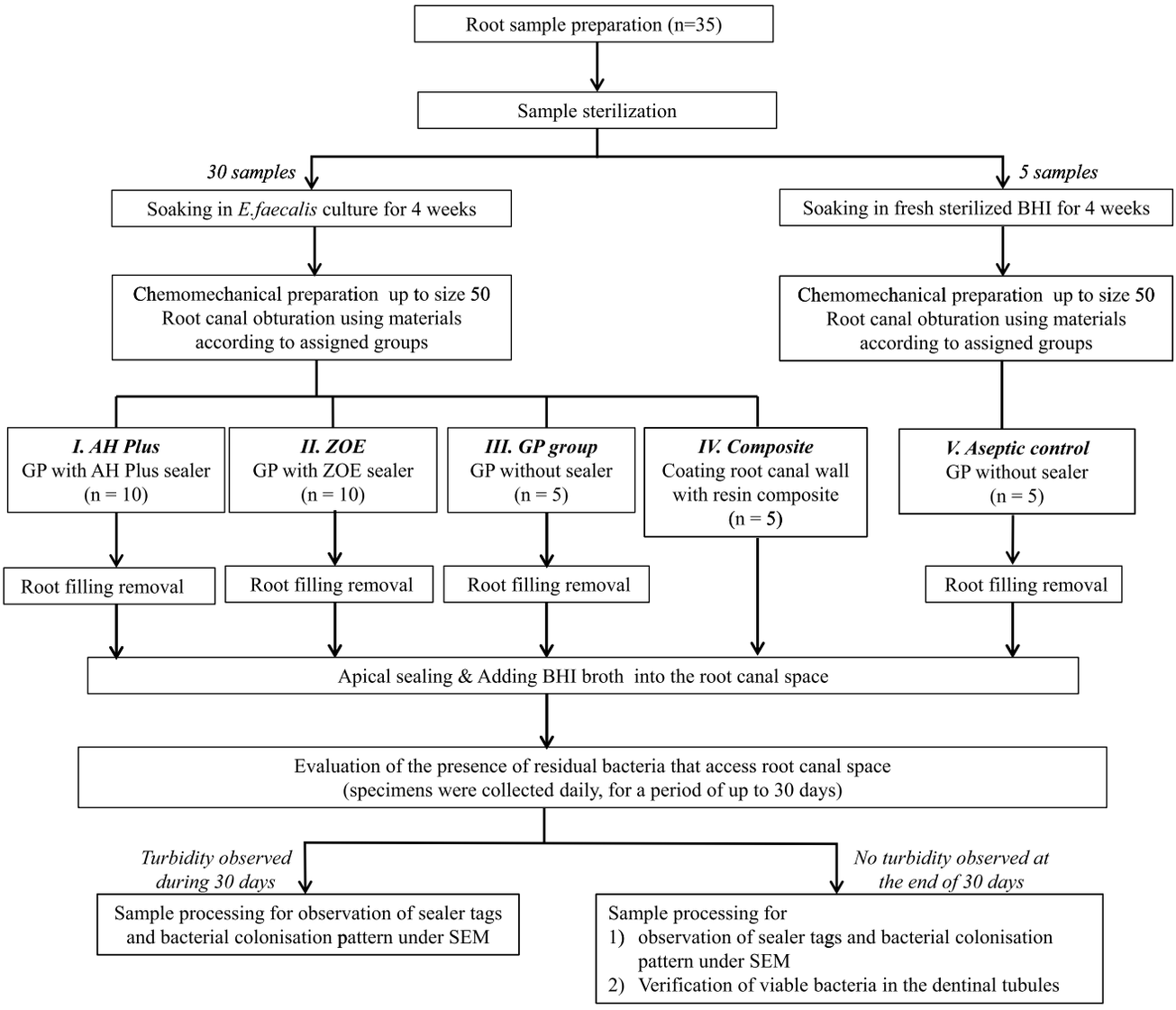

Fig. 1 The overall experimental flow

Root sample preparation

The study protocol was approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University. Intact mandibular premolars with a single straight root canal, extracted for orthodontic reasons from patients aged less than 25 years old, were collected. The teeth were stored in 0.1% thymol solution at room temperature for a maximum of 2 months. Root dentine samples were prepared by sectioning the tooth at the cementoenamel junction and 3 mm from the root apex using a water-cooled diamond saw, to generate 5-mm long root dentine cylinder samples. The apical size of each sample was gauged by inserting K-files (Dentsply Maillefer, Ballaigues, Switzerland) size 15-35 into the root canal toward the apical end. Samples with an apical diameter larger than 0.35 mm were excluded, resulting in 35 samples selected for use in the present study.

To standardize the initial size of the root canal and remove the pulp tissue content, root canals were prepared up to size 40 using Nickel-Titanium rotary files (Profile, Dentsply Maillefer) with a 0.04 taper. The canal was irrigated with 5 mL of 2.5% sodium hypochlorite (NaOCl) between successive files. After

the final file, to make dentinal tubules patent for bacterial invasion, the smear layer was removed from the canal wall by irrigating the canal with 5 mL of 17% ethylene diamine tetra-acetic acid for 3 min and with 5 mL of 2.5% NaOCl for 3 min. Finally, the samples were rinsed three times with 10 mL of distilled water. The samples were secured in an upright position in a customized silicone mould that fit into the wells of 96-well plates and stored for 1 day in a container with water at the bottom to create a humidified atmosphere. The plates and samples were sterilized by gamma irradiation (25 kGy) and stored in a humidified atmosphere until used.

Bacterial preparation

Enterococcus faecalis (E. faecalis) (erythromycin resistant strain, JH2-2 carrying plasmid pGh9:ISS1, derived from the parental strain JH2) (Jacob and Hobbs, 1974) was used as the test microorganism. Brain heart infusion (BHI) broth (HiMedia, Mumbai, India) supplemented with 6.5 μg/mL erythromycin (Sigma, St Louis, MO, USA) was used as the culture medium. Cultures were prepared from glycerol stocks and incubated overnight at 37°C. The cultures were then diluted with fresh BHI to achieve an optical density at 570 nm (OD_{570nm}) of 0.4 (≈ 4.4 x 10^8 CFU/mL).

Experimental procedures

Thirty-five sterile root samples were divided to the infected samples (n=30) and the non-infected samples (n=5). Each of the infected samples was soaked in 5 mL of bacterial culture and incubated for 4 weeks whereas the non-infected samples were soaked in 5 mL of fresh sterile BHI broth. During the incubation period, 4.5 mL of the bacterial culture was replaced with fresh medium every two days. At the end of the incubation period, each root sample was rinsed with 20 mL of 1% phosphate-buffered saline. The outer surface of the root samples was disinfected using a cotton pellet soaked with 1.5% potassium iodide, followed by 70% alcohol. Root canals of all root samples were then chemo-mechanically prepared using #45 and #50 Profile files with a 0.04 taper. The canals were irrigated with 5 mL of 2.5% NaOCl between successive files, and the smear layer was removed as described above. After that, they were obturated according to the assigned groups (Fig. 1). The details of the samples and obturating materials used in each group were summarised as follows:

Group I: Infected sample obturated with Gutta percha and AH Plus sealer (AH Plus, n=10)

Group II: Infected sample obturated with Gutta percha and ZOE sealer (CU sealer, Faculty of Dentistry, Chulalongkorn University, Thailand) (ZOE, n=10)

Group III: Infected sample obturated with Gutta percha without root canal sealer (GP, n=5)

Group IV: Infected sample with flowable resin composite coating on root canal wall. (Composite, n=5)

Group V: Non-infected samples filled with gutta percha without root canal sealer (aseptic control, n=5). This group was used to verify that no cross-contamination occurred during the experiment.

For group I, II, III and V, root canals were obturated with a lateral condensation technique. For group IV composite, the root canal walls were etched with 37.5% phosphoric acid gel (Kerr gel etchant, Kerr Corporation, Orange, CA, USA) for 15 seconds, rinsed with sterile water for 20 seconds and dried with air. A dentine adhesive agent (Optibond Solo Plus, Kerr Corporation, Orange, CA, USA) was applied on the root canal surface using a microbrush, left for 15 seconds, dried with air for 3 seconds and light-activated with a light-emitting diode unit (LED curing light S10, 3M ESPE, St Paul, USA) at an intensity of 1000 mW/cm^2 for 20 seconds. The root canal walls were thinly coated with flowable resin composite (Tetric

N-flow®, Ivoclar Vivadent, Liechtenstein), applied using an endodontic explorer as a carrier. The resin composite layer was polymerized using a light-emitting diode unit at a 1000 mW/cm^2 power density for 20 seconds, leaving a lumen space patent for the next step of the experiment.

After root canal obturation, the coronal and apical ends of all samples were sealed with Cavit (Cavit G, 3M ESPE, Seefeld, Germany) and incubated in a humidified atmosphere at 37°C for 1 week. The Cavit was then removed from the samples. For group I, II, III, and V, to simulate the condition of root dentine after the detachment of root filling materials from root canal wall, the gutta percha and root canal cement were removed from the canal space of the root samples using a size 60 Profile file with a 0.04 taper. The absence of cement coated on the root canal walls was confirmed by visual observation under a stereomicroscope at 10X magnification. The smear layer was removed from the root canal wall and the outer root surface was disinfected as described above. For group IV, the flowable resin composite coating on the root canal wall was not removed. To create an apical barrier for further experiment, the apical ends of all root samples were covered with flowable resin composite.

Evaluation of the presence and regrowth of residual bacteria that access root canal space

Each root sample was placed and secured upright in each well of the sterile 96-well plate. Based on previous study, *E. faecalis* located in deep dentine has ability to survive after obturation and can regrow when they receive nutrients (Sedgley et al., 2005) With this information, 10 µL of BHI broth was added into the root canal space of each sample in order to mimic the availability of nutrients to the residual bacteria in dentinal tubules. The plate was then incubated at 37°C in a humidified atmosphere for 30 days. During the incubation period, the presence of residual bacteria that access and regrow in root canal space was evaluated daily by culturing the added-BHI broth from the root canal. A sterile absorbable paper point was inserted into each root canal to collect the added-BHI broth, and the empty root canal was refilled with 10 µL of fresh BHI broth. Each paper point was then transferred into 5 mL of BHI and incubated at 37°C for 72 hours. If the residual bacteria in dentinal tubules can gain access to the root canal space and regrow in the media, the culture will demonstrate turbidity. If the culture is turbid, Gram staining and sub-culturing on BHI agar will be performed to determine the purity of *E. faecalis.*

The number of root samples demonstrating the presence of residual bacteria regrowing in the root canal space and the number of days required for the regrowth to occur in root canal were recorded. When the presence of residual bacteria in root canals was detected in any sample prior to the 30-day endpoint, those samples were split and further investigated under scanning electron microscopy (SEM) in order to assess the appearance of sealer tags and colonisation pattern of bacteria. For the root samples that the regrowth of the residual bacteria in root canal was not detected at the end of 30 days, the samples were longitudinally split into 2 pieces; the first piece was processed for the investigation under SEM as same as the samples with the bacterial regrowth, while the other one was processed for verification of the viable bacteria in the dentinal tubules.

Observation of sealer tags and bacteria in the dentinal tubules

To observe the appearance of the sealer tags and colonisation pattern of the residual bacteria in the dentinal tubules, all samples were processed and examined by SEM (JSM-5410 LV, JEOL, Japan). Root samples were split in a bucco-lingual direction using a no. 15 blade and a hammer, fixed in 2.5%

glutaraldehyde in phosphate-buffered saline, immersed in serial dilutions of alcohol, critical point dried, and gold-sputter-coated for examination. Using the SEM, ten non-overlapping pictures of the area from the root canal wall to a distance of 250 micrometers from the root canal wall were taken at random locations.

Verification of the viable bacteria in the dentinal tubules of samples without the presence of the residual bacteria in root canal

The presence of viable bacteria in the dentinal tubules of the root samples showing no bacterial regrowth within 30 days was verified. The objective of the verification was to confirm that the absence of residual bacteria in root canal space was not caused by the absence of viable bacteria in dentinal tubules. The inner dentine of the root canal wall was ground using Gates Glidden drills from size 4 to size 6. The dentine powder was incubated in 5 mL of BHI broth at 37°C for 72 hours. Turbidity of the medium served as evidence of bacterial growth, and if present, Gram staining and subculturing by plating on BHI agar were performed to determine the purity of *E. faecalis*.

Statistical analysis

The cumulative percentage of the sample showing the regrowth of residual bacteria that access root canal space at each observation time point was calculated. The difference in the number of root canals with bacterial regrowth between groups was evaluated by the Fisher exact test. The Kruskal-Wallis test with the Conover-Inman post-hoc test were used to evaluate the difference in the number of days when the regrowth of residual bacteria in root canal space was detected among the groups. The data analyses were performed using SPSS 14.0 software (SPSS Inc, Chicago, IL) and the significant level was set at $p < 0.05$.

Results

The cumulative percentage of the sample demonstrating the regrowth of residual bacteria that access root canal space and the number of days required for the regrowth to occur were illustrated in Fig. 2. The residual bacteria could not be detected in the root canal of aseptic control and the composite groups within 30 days. In contrast, the regrowth of residual bacteria that access root canal space was detected in 30% (3/10) of the AH Plus, 70% (7/10) of the ZOE, and 100% (5/5) of the GP groups, starting at day 23, 13 and 10, respectively. The number of samples with the regrowth of residual bacteria accessing the root canal in the AH Plus group was significantly less than that in the GP group ($p < 0.05$). However, no significant difference was detected between the AH Plus and ZOE groups ($p = 0.18$) or between the ZOE and GP groups ($p = 0.50$).

The number of days required for the bacterial regrowth in root canal of AH Plus, ZOE, and GP groups was [median (interquartile range)] 23 (23, 25), 16 (15, 18) and 13 (12, 14), respectively (Fig. 3). Statistical analysis using the Kruskal-Wallis test showed that the number of days required for the bacterial regrowth to occur was significantly different among the three groups ($p < 0.01$). The post-hoc Conover-Inman test indicated that the bacterial regrowth in root canal of AH Plus group significantly required longer time than those in the ZOE ($p < 0.01$) and GP groups ($p < 0.001$). The ZOE group also significantly required longer time for the bacterial regrowth than the GP group ($p < 0.01$) (Fig. 3).

The root samples without the bacterial regrowth were further analysed whether the residual bacteria still survive in the dentinal tubules. The ground dentine from these samples was incubated in culture media. The cultures of all samples, except the aseptic control group, were turbid. The results from gram staining and sub-culturing of the turbid cultures indicate the pure culture of *E. faecalis* (data not shown).

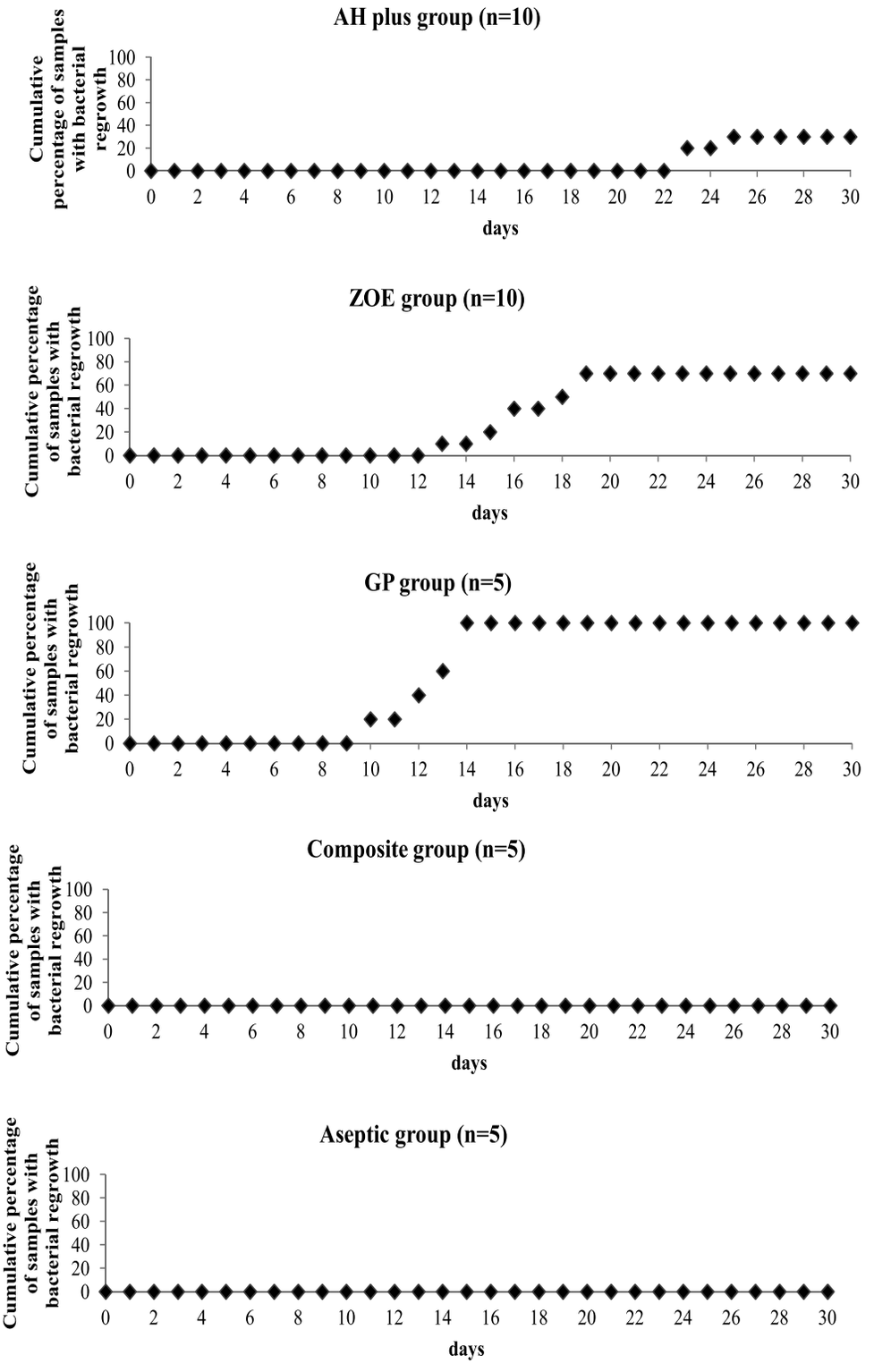

Fig. 2 The cumulative percentage of samples presenting bacterial regrowth in the root canal, at different time points. At the end of the 30-day observation period, the regrowth of residual bacteria that access root canal was detected in 30% of the samples in the AH Plus group, 70% of the samples in the ZOE group and none of the samples in the composite group. All GP samples demonstrated the regrowth of residual bacteria that access root canal within 14 days.

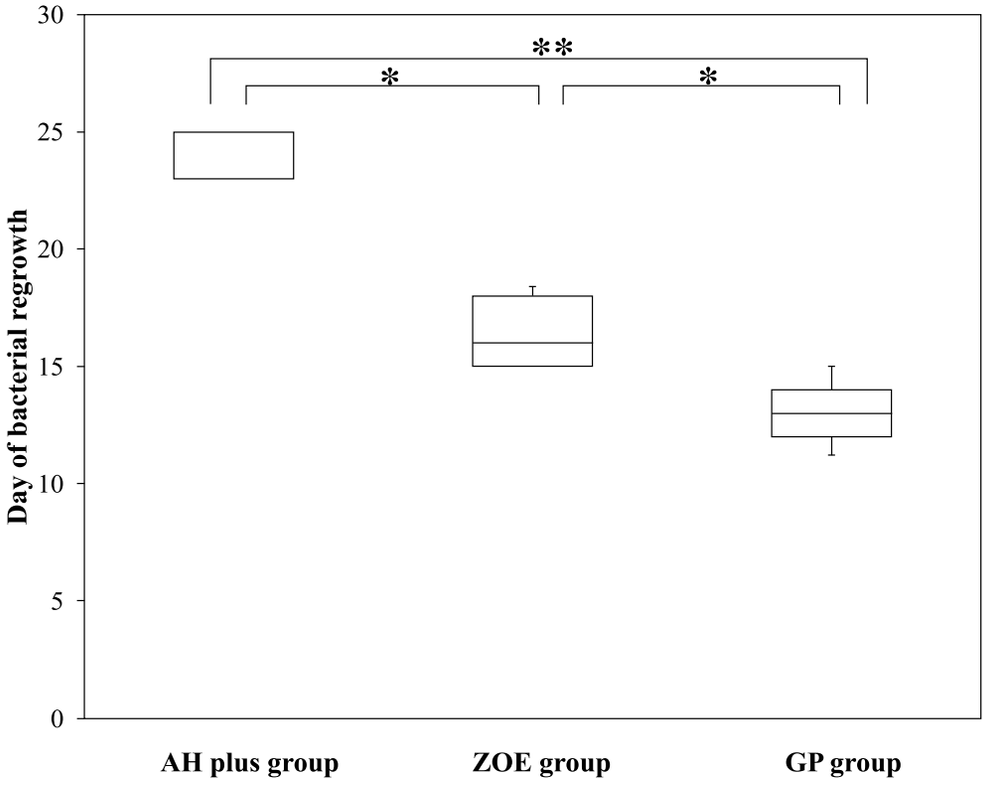

Fig. 3 Box plot of the median and interquartile range of days required for residual bacteria in dentinal tubules to access and regrow in the root canal of AH plus, ZOE and GP groups. Asterisks * and ** represent statistical significant difference at $p < 0.01$ and at $p < 0.001$, respectively, by Kruskal-Wallis with the Conover-Inman post-hoc test.

SEM images of the canal wall revealed that AH Plus occupied most, but not all, of the tubules (Fig. 4A). Moreover, at higher magnification, AH Plus sealer tags appeared homogeneous and almost completely occluded the tubules (Fig. 4C). In addition, the lengthwise image demonstrated that the AH Plus sealer tags were deeply penetrated into the tubules (Fig. 5A). In contrast, many of the tubules in the ZOE group remained patent (Fig. 4B). Even if a few tubules were occupied, the granulated ZOE sealers just dispersed and partially occluded the opening of those tubules (Fig. 4D). In the same way, the ZOE sealer did not form long sealer tags. They just accumulated at the opening of the tubules (Fig. 5B). Bacteria were found to inconsistently penetrate the dentinal tubules in both groups. The presence of bacteria in the dentinal tubules did not seem to block the penetration of sealers. In addition, in case of the AH Plus, bacteria were found entombed in the tubules below the AH Plus sealer tags (Fig. 5A), while this was not seen in the ZOE group (Fig. 5B).

Discussion

The ability of bacteria to survive in the deep layer of root dentine after root canal obturation has been reported in many studies (Sedgley et al., 2005; Shin et al., 2008). In the present study, we investigated whether the bacteria in the dentinal tubules could access the root canal space and regrow when nutrients were provided in the root canal space. We also assessed the capability of root canal sealers differing in flowability to prevent residual bacteria in dentinal tubules from accessing the root canal space. Our results showed that *E. faecalis* that survived in the dentinal tubules after chemomechanical preparation and obturation could

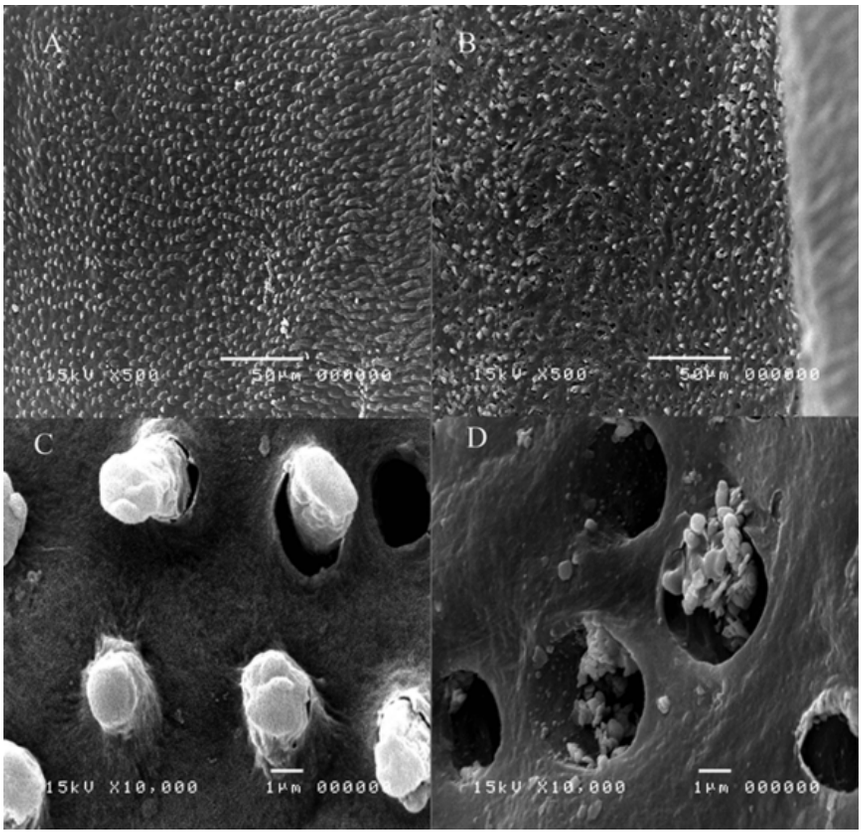

Fig. 4 Scanning electron micrographs of the root canal wall represent the distribution of dentinal tubules occupied by AH Plus (A,C) and ZOE (B,D) sealers. A) Extensive distribution of AH Plus sealer tags. B) Scant penetration of ZOE sealer tags. (Bar=50 µm) C) Higher magnification of the AH Plus sealer tags presenting a homogeneous structure. D) Higher magnification of the ZOE sealer tags demonstrating a granular-like appearance with some bacterial cells attached. (Bar=1 µm)

repopulate, migrate and regrow in the root canal. The absence of bacterial regrowth in the root canal of the composite group demonstrated the benefit of using a material that completely sealed the tubule openings. Root canal obturation using gutta percha and AH plus/ ZOE sealer could not completely prevent the residual bacteria in dentinal tubules from accessing and regrowing in the root canal space. However, the results demonstrated the benefit of using AH Plus, one of sealers with high flowability, in root canal obturation.

This experiment was designed to mimic the situation when space was created at the sealer-root canal wall interface and nutrients were provided. Therefore, the GP and the sealer on the root canal walls were removed and BHI was added into the root canal. Although the available nutrients and the volume of space in the root canal were exaggerated, this method was designed to generate clear results. Although multi-species bacterial infection is involved in teeth with persistent apical periodontitis (Moller et al., 2004; Siqueira and Rocas, 2004; Subramanian and Mickel, 2009), *E. faecalis* was chosen as a representative bacteria in our study because this species is commonly isolated from infected root canals (Siqueira and Rocas, 2004; Subramanian and Mickel, 2009) with the ability to regrow after long-term starvation (Sedgley et al., 2005). The use of *E. faecalis* is sufficient to examine the role of root canal sealers in preventing residual bacteria in dentinal tubules from accessing root canal.

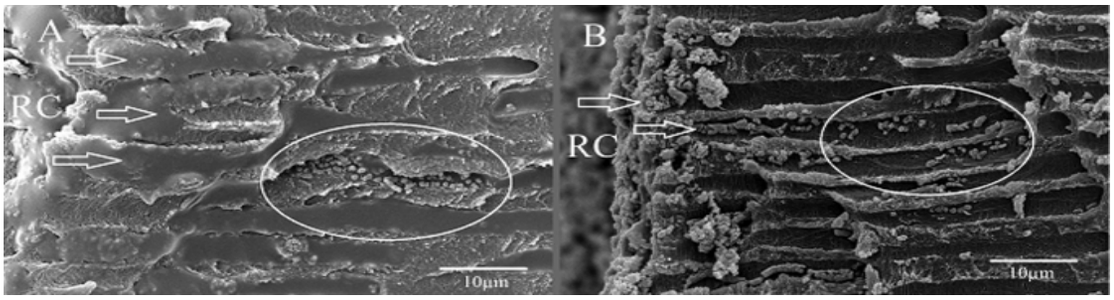

Fig. 5 Scanning Electron Micrographs represent the dentinal tubules of the sealer-treated root canals. Circles indicate bacteria in the dentinal tubules. A) Sealer tags of AH Plus. Arrows indicate the homogeneous sealer tags in the dentinal tubules, especially at the tubule opening area. B) Minimal penetration of short, granular sealer tags are present in the ZOE group as indicated by arrows. (Bar = 10 μm), RC = root canal.

Our SEM findings and culturing results indicated the presence of viable bacteria in the deep dentine of root canal filled samples. These findings are in agreement with previous studies, emphasizing the limitation of endodontic procedures in eliminating bacteria from the root canal system (Fabricius et al., 2006; Siqueira and Rocas, 2008; Harrison et al., 2010). In addition, we confirmed that the residual bacteria in the dentinal tubules could access the root canal space and regrow. Interestingly, although viable bacteria were present in the dentinal tubules, bacterial regrowth was not detected in the root canal of some samples in the AH Plus and ZOE groups. Factors possibly related to the ability of sealer to prevent the residual bacteria from accessing root canal were the antibacterial effect of the sealers, extent and depth of sealer penetration, morphology of the sealer tags, and their solubility.

AH Plus and ZOE sealers contain epoxy resin/amine and eugenol, respectively, which have antibacterial properties (Markowitz et al., 1992; Saleh et al., 2004). However, this properties may only have a minimal effect on preventing root canal reinfection. Both sealers could effectively killed bacteria when their antibacterial components were in direct contact with bacteria (Cobankara et al., 2004; Pizzo et al., 2006). Therefore, the application of these sealers in root canal filling only partially killed or inhibited the growth of bacteria in dentinal tubules (Sedgley et al., 2005; Shin et al., 2008; Wang et al., 2014). Although the application of AH Plus resulted in greater bacterial reduction compared to ZOE sealer (Wang et al., 2014), viable bacteria were still detected in all infected samples regardless of the type of sealers applied.

The finding of this study indicates the possibility that reinfection can be prevented by the barrier effect of root filling materials. Occlusion of the dentinal tubules by sealer tags creates a barrier preventing viable bacteria in the dentinal tubules from reentering the root canal space. To effectively block bacteria in the dentinal tubules from the main root canal space, a sealer should reliably penetrate the dentinal tubules, seal the openings, and remain intact within the dentinal tubules. Thus, quantity, quality and solubility of the sealer tags should be taken into account.

In terms of quantity of sealer tags, neither AH plus nor ZOE sealers were able to penetrate all the dentinal tubules. However, AH Plus showed a superior ability to penetrate dentinal tubules, as determined by the number of occluded dentinal tubules and the depth of penetration, compared to ZOE (Mamootil and Messer, 2007; Balguerie et al., 2011). In terms of the sealer tag quality, our results agreed with a previous study where the majority of AH Plus sealer tags were homogeneous and well adapted to the peritubular dentine (Bouillaguet et al., 2004). The lower solubility of AH Plus compared to that of ZOE (Schafer and Zandbiglari, 2003) also helps to maintain the sealer tags within the dentinal tubules. The combination of the better antibacterial activity, superior flowability and lower solubility of AH Plus sealer may have led to less

viable bacteria and fewer patent dentinal tubules, resulting in fewer opportunities for the residual bacteria in the AH Plus group to reinfect the root canal. These explanations may be similarly applied for the longer period required for the occurrence of the bacterial regrowth in the root canal space of AH Plus group, compared to the ZOE and GP groups.

The bacterial regrowth in root canal space was first detected on day 10 in the GP group, which no sealer was applied. This length of time indicates that the regrowth of bacteria from the dentinal tubules into the root canal is a slow process (Love and Jenkinson, 2002). During chemomechanical preparation, bacteria located within a short distance from the tubule openings may be inactivated by the antibacterial irrigant diffusing into the dentinal tubules (Berutti et al., 1997). Therefore, time is required for nutrients to diffuse to the viable bacteria located in deep dentine and for the starved bacteria there to recover, migrate and access the root canal. The proposal that *E. faecalis* may grow as a chain when invading the dentinal tubules (Love and Jenkinson, 2002) may also apply to bacteria from the dentinal tubules to migrate and access the root canal.

In this study, the capability of root canal sealers to prevent residual bacteria in dentinal tubules from accessing the root canal space was reported, based on a 30-day observation period. In reality, bacteria in the dentinal tubules can survive long-term entombment (Sedgley et al., 2005), and the interfacial space between the root canal and filling materials may continuously percolate with tissue fluid. Further study on the long term observation of root canal reinfection by multi-species bacteria will provide a good insight into the effect of sealers on bacterial entombment in a more clinically relevant context.

In conclusion, bacteria remaining in the dentinal tubules after root canal obturation had the potential to migrate via dentinal tubules to access the root canal space. Although root canal sealers could not completely entomb bacteria in the dentinal tubules, AH Plus possessed some extent of the ability to prevent or delay residual bacteria in dentinal tubules from accessing the root canal space. AH Plus was more effective in preventing and delaying the root canal reinfection, compared with a zinc-oxide eugenol based sealer. Therefore, in addition to bacterial elimination prior to root canal obturation, the use of AH plus, one of root canal sealers with high flowability, seems to be beneficial for the management of root canal infection.

Acknowledgements

This study was supported by a Chulalongkorn University Graduate School thesis grant and The Special Task force for Activating Research (STAR) under as part of the Chulalongkorn Centenary Fund. The authors thank Dr. Kevin Tompkins for manuscript revision.

The authors deny any conflicts of interest related to this study. We do not have any financial interest in the companies whose materials are included in this article.

References

Balguerie E, van der Sluis L, Vallaeys K, Gurgel-Georgelin M, Diemer F. Sealer penetration and adaptation in the dentinal tubules: a scanning electron microscopic study. J Endod. 2011;37:1576-9.

Berutti E, Marini R, Angeretti A. Penetration ability of different irrigants into dentinal tubules. J Endod. 1997;23:725-7.

Bishop D, Griggs J, He J. Effect of dynamic loading on the integrity of the interface between root canal and obturation materials. J Endod. 2008;34:470-73.

Bouillaguet S, Wataha JC, Lockwood PE, Galgano C, Golay A, Krejci I. Cytotoxicity and sealing properties of four classes of endodontic sealers evaluated by succinic dehydrogenase activity and confocal laser scanning microscopy. Eu J Oral Sci. 2004;112:182-7.

Cobankara FK, Altinoz HC, Ergani O, Kav K, Belli S. In vitro antibacterial activities of root-canal sealers by using two different methods. J Endod. 2004;30:57-60.

Fabricius L, Dahlen G, Sundqvist G, Happonen RP, Moller AJ. Influence of residual bacteria on periapical tissue healing after chemomechanical treatment and root filling of experimentally infected monkey teeth. Eu J Oral Sci. 2006;114: 278-85.

Harrison AJ, Chivatxaranukul P, Parashos P, Messer HH. The effect of ultrasonically activated irrigation on reduction of *Enterococcus faecalis* in experimentally infected root canals. Int Endod J. 2010;43:968-77.

Jacob AE, Hobbs SJ. Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. *zymogenes*. J Bacteriol. 1974;117: 360-72.

Karina A, Shimon F, Celine M L, Bettina R B, Yoav F. Microbial biofilm proliferation within saeler-root dentin interfaces is affected by sealer type and aging period. J Endod. 2012;38:1253-6.

Katebzadeh N, Hupp J, Trope M. Histological periapical repair after obturation of infected root canals in dogs. J Endod. 1999;25:364-8.

Love RM, Jenkinson HF. Invasion of dentinal tubules by oral bacteria. Crit Rev in Oral Biol Med. 2002;13:171-83.

Mamootil K, Messer HH. Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and in vivo. Int Endod J. 2007;40:873-81.

Markowitz K, Moynihan M, Liu M, Kim S. Biologic properties of eugenol and zinc oxide-eugenol. A clinically oriented review. Oral Surg Oral Med Oral pathol Oral radiol Endod. 1992;73:729-37.

Moller AJ, Fabricius L, Dahlen G, Sundqvist G, Happonen RP. Apical periodontitis development and bacterial response to endodontic treatment. Experimental root canal infections in monkeys with selected bacterial strains. Eur J Oral Sci. 2004;112:207-15.

Nair PN. Apical periodontitis: a dynamic encounter between root canal infection and host response. Periodontol 2000. 1997;13:121-48.

Nair PN. On the causes of persistent apical periodontitis: a review. Int Endod J. 2006;39:249-81.

Pizzo G, Giammanco GM, Cumbo E, Nicolosi G, Gallina G. In vitro antibacterial activity of endodontic sealers. J Dent. 2006;34:35-40.

Roth KA, Friedman S, Levesque CM, Basrani BR, Finer Y. Microbial biofilm proliferation within sealer-root dentin interfaces is affected by sealer type and aging period. J Endod. 2012;38:1253-6.

Saleh IM, Ruyter IE, Haapasalo M, Orstavik D. Survival of *Enterococcus faecalis* in infected dentinal tubules after root canal filling with different root canal sealers in vitro. Int Endod J. 2004;37: 193-8.

Schafer E, Zandbiglari T. Solubility of root-canal sealers in water and artificial saliva. Int Endod J. 2003;36:660-9.

Sedgley CM, Lennan SL, Appelbe OK. Survival of *Enterococcus faecalis* in root canals ex vivo. Int Endod J. 2005;38:735-42.

Shin SJ, Jee SW, Song JS, Jung IY, Cha JH, Kim E. Comparison of regrowth of *Enterococcus faecalis* in dentinal tubules after sealing with gutta-percha or Resilon. J Endod. 2008;34:445-8.

Siqueira JF Jr, Rocas IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. Oral Surg Oral Med Oral pathol Oral radiol Endod. 2004;97:85-94.

Siqueira JF Jr, Rocas IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod. 2008;34:1291-301.

Subramanian K, Mickel AK. Molecular analysis of persistent periradicular lesions and root ends reveals a diverse microbial profile. J Endod. 2009;35:950-7.

Sundqvist G, Figdor D. Endodontic treatment of apical periodontitis. In: Ørstavik D, Pitt Ford T, editors. Essential Endodontology. Oxford: Blackwell Science Ltd; 1998. p. 242-77.

Vieira AR, Siqueira JF Jr, Ricucci D, Lopes WS. Dentinal tubule infection as the cause of recurrent disease and late endodontic treatment failure: a case report. J Endod. 2012;38:250-4.

Wang Z, Shen Y, Haapasalo M. Dentin Extends the Antibacterial Effect of Endodontic Sealers against *Enterococcus faecalis* Biofilms. J Endod. 2014; 40:505-8.