Journal of Dental Sciences xxx (2018) 1–5

Contents lists available at ScienceDirect

Journal of Dental Sciences

journal homepage: www.e-jds.com

Original Article

Comparison of antimicrobial activity of traditional and new developed root sealers against pathogens related root canal

Joo-Hee Shin [a], Dong-Yul Lee [b], Sung-Hoon Lee [c, *]

[a] Department of Conservative Dentistry, Korea University Medical Center Guro Hospital, Republic of Korea
[b] Department of Orthodontics, Korea University Medical Center Guro Hospital, Seoul, Republic of Korea
[c] Department of Oral Microbiology and Immunology, College of Dentistry, Dankook University, Cheonan, Republic of Korea

ARTICLE INFO

Article history:
Received 23 January 2017
Received in revised form
24 October 2017
Accepted 25 October 2017
Available online xxx

Keywords:
Root canal sealer
Antimicrobial activity
Oxide compound
E. faecalis

ABSTRACT

Background/purpose: Bacterial infection is closely associated with the failure of endodontic treatment, and use of endodontic sealer with antimicrobial activity and biological compatibility is necessary for the success of root canal treatment. The purpose of this study was to investigate and to compare the antibacterial effect of two calcium silicate-based root canal sealers (Endoseal and EndoSequence BC sealer) as recent development sealers and with three conventional root canal sealers (AH Plus, Sealapex, and Tubli-Seal), before or after setting, on *Porphyromonas endodontalis, Porphyromonas gingivalis*, and *Enterococcus faecalis.*

Materials and methods: The sealers were soaked in phosphate buffered saline to elute its compositions after and before setting, and the elutes were performed the antimicrobial assay. Also, X-ray fluorescence analysis was carried out to compare compositions of two calcium silicate-based sealers.

Results: The conventional root canal sealers have strong antibacterial activity against the Gram-negative bacteria, *P. endodontalis* and *P. gingivalis*. Endoseal sealer showed antibacterial activity against not only the Gram-negative bacteria, but also against the Gram-positive bacteria, *E. faecalis*. However, Endosequence BC sealer exhibited a weak antibacterial effect on all bacteria in this study. X-ray fluorescence analysis exhibited that Endoseal contained more types and more amount of the oxide compound known to have strong antimicrobial activity such as Al_2O_3, Fe_2O_3, MgO, Na_2O, NiO, and SO_2 than Endoseqeunce BC.

Conclusion: Endoseal, which contains various types of oxide compounds, seems to be a suitable sealer for preventing bacterial infection in both treated and untreated root canals.

1. Introduction

Bacterial infection into the root canal plays an important role in the induction of pulpal and periapical inflammation and is closely associated with the failure of endodontic treatment.[1] Although individual cases differ, averages of five to seven different species per canal have been detected, and the bacterial species most frequently isolated from necrotic pulps are *Porphyromonas gingivalis* and *Porphyromonas endodontalis.*[2–4] *P. gingivalis* and *P. endodontalis* are associated with initial infection of the root canal, and *Enterococcus faecalis* has been detected in apical periodontitis lesions in root canal-treated teeth.[5] Because the root canal system varies in the anatomical features including fins, isthmi, and accessory canals, complete elimination of the bacteria in the root canals is difficult. In treating the root canal, along with mechanical cleaning, various intracanal irrigants and medicaments, such as calcium hydroxide, sodium hydroxide, and chlorohexidine, are used in attempts to eradicate bacteria in the infected root canal. However, some bacteria may remain in the root canal systems.[6] Therefore, a hermetic seal of the root canal space is required to entomb any residual bacteria and ultimately kill them in the filled root canal.

Root canal sealers are used to overcome the limitations of gutta-percha (GP) cones and obturation techniques by filling the space between the GP and the dentinal wall. Hence, root canals sealers

* Corresponding author. Department of Oral Microbiology and Immunology, College of Dentistry, Dankook University 119 Dandae-ro, Dongnam-gu, Cheonan 31116 Republic of Korea.
E-mail address: dennisyi@dankook.ac.kr (S.-H. Lee).

https://doi.org/10.1016/j.jds.2017.10.007

that possess superior sealing ability and antibacterial activity would be clinically beneficial by preventing bacteria from re-entering the canal and by inactivating bacteria remaining in the canal after root canal obturation. Traditional root canal sealers are categorized as zinc oxide eugenol (ZOE), epoxy resin (ER), or calcium hydroxide (CH) on the basis of their composition.[7–9] Recently, calcium silicate-based cement with the addition of various oxide compounds have been developed for root sealer and are called mineral trioxide aggregation (MTA).[10] This cement is known to bioactive properties that have stimulation of tissue repair and induction of mineralization.[11,12] For these reasons, the cement has been considered suitable for application to root canal sealer and have led to the development of root canal sealers. Antimicrobial activity is also an important factor in investigating dental materials for application to root sealer because bacterial infection is closely associated with the failure of endodontic treatment. Although the antimicrobial activity of these products against *Lactobacillus acidophilus, Staphylococcus aureus,* and *E. faecalis* has been studied,[13] the evaluation has been limited to the antibacterial effect on Gram-positive bacteria notwithstanding the isolation of *P. endodontalis*, *P. gingivalis*, and *E. faecalis* from necrotic pulps, and the antibacterial activity has been examined only before setting of the sealer. Therefore, we investigated and compared the antibacterial activity of two calcium silicate-based root canal sealers (Endoseal and EndoSequence BC sealer) as recent development sealers and with three conventional root canal sealers (AH Plus, Sealapex, and Tubli-Seal), before or after setting, against *P. endodontalis, P. gingivalis*, and *E. faecalis*.

2. Materials and methods

The bacteria in this study were purchased from American Type Culture Collection. *E. faecalis* ATCC 29221 was aerobically cultivated in brain heart infusion (BHI) broth (BD Bioscience, Sparks, MD, USA) at 37 °C, and *P. endodontalis* ATCC 35406, and *P. gingivalis* ATCC 33277 were cultured in BHI broth supplemented with hemin (1 μg/mL) and vitamin K (0.2 μg/mL) at 37 °C in an anaerobic condition (5% H_2, 10% CO_2, 85% N_2).

Table 1 shows the composition of the root canal sealers. Sealers tested for antibacterial activity were prepared according to the manufacturers' directions. Each sealer was dispensed into each well of 12-well polystyrene microplates (SPL Life Science, Gyeonggi, South Korea), and phosphate buffer solution (PBS) was then added, for a sealer concentration of 200 mg/mL. The microplates were agitated on a shaker (50 rpm) for 4 h at room temperature. To compare the antibacterial activity between set and unset materials, eluates from each sealer were also collected after setting. The sealers were placed into the inside wells of the 12-well microplates, and PBS was added in the outside wells of the microplates to ensure stable humidity levels. The sealers were solidified for 24 h at 37 °C, and PBS was then added into the wells. Based on the initial mass, the concentration of the sealer was adjusted to 200 mg/mL by adding PBS into each well. The microplates were agitated on a shaker for 4 h at room temperature. Each eluate was transferred to a fresh 15-mL conical tube, which was then centrifuged at 5000 × g for 10 min to remove any remaining insoluble particles.

Table 1
The used root canal sealers in this study and its characterization.

Materials	Corporation/Country	Product information
Sealapex	Kerr/USA	calcium hydroxide based sealer
Tubli-Seal	Kerr/USA	zinc oxide eugenol based sealer
AH plus	Dentsply/USA	epoxy resin based sealer
EndoSequence BC	Brasseler/USA	calcium silicate based sealer
Endoseal	MARUCHI/Korea	calcium silicate based sealer

Antimicrobial assays were performed according to the protocol of Clinical and Laboratory Standards Institute (CLSI). The incubated bacteria level was assessed using a bacterial counting chamber (Marienfeld, Lauda-Konigshofen, Germany). The concentration of *E. faecalis* was adjusted to a density of 1×10^6 cell/mL by adding fresh BHI broth. The BHI broth supplemented with hemin and vitamin K was added to adjust the level of *P. endodontalis* and *P. gingivalis* to 1.5×10^6 cell/ml. Subsequently, 180 μL of the specific media for each test microorganism was dispensed into each well of 96-well polystyrene plate, and 160 μL of the specific media plus 20 μL of the prepared sealer eluate were added to the first row of the plate, and serial two-fold dilution was performed using a multichannel micropipette. Next, 20 μL of each bacterial suspension was inoculated to the wells containing the eluates from the sealers. The plates were incubated for 24 h at 37 °C, aerobically for *E. faecalis,* and anaerobically for *P. endodontalis and P. gingivalis*. Bacterial growth was monitored by measuring the absorbance at 600 nm in a microplate reader (BioTek, Winooski, VT, USA).

To investigate the difference in the antimicrobial activity between the two calcium silicate-based sealers, Endoseal and EndoSequence BC sealer, the chemical compositions of the sealers were analyzed using an X-ray fluorescence (XRF) spectrometer (ZSX primus II, Rigaku Co., Tokyo, Japan). The sealers were loaded on micro-carry paper and dried at 55 °C. The XRF spectrometer was outfitted with X-ray tubes with Rh anodes and was operated at 60 kV and 150 mA. The XRF patterns for the sealers were obtained using SC and F-PC diode detectors and analyzed using EZ Scan (Rigaku Co., Tokyo, Japan).

The data were analyzed non-parametrically by using the Kruskal-Wallis and Mann-Whitney tests. IBM SPSS Statistics Ver. 23 (IBM, Armonk, NY, USA) was used for statistical analysis. Statistical significance was defined by a P value of less than 0.05.

3. Results

Fig. 1 shows the growth of *E. faecalis* as a function of the sealers' concentration. The antibacterial activity against *E. faecalis* was the greatest in Endoseal, followed by Sealapex, Tubli-Seal, AH Plus and EndoSequence BC sealer. Endoseal exerted an inhibitory effect at 25 mg/mL, whereas Sealapex, Tubli-Seal, and AH Plus inhibited the bacterial growth at 50 mg/mL. All the sealers had less inhibitory effect against *E. faecalis* after the materials were set, and EndoSequence BC sealer was found to have no antibacterial activity.

As shown Fig. 2, the growth of *P. endodontalis* was significantly inhibited when the concentration of AH Plus and Sealapex was greater than 6.4 mg/mL ($P < .05$). Tubli-Seal and Endoseal showed the bacterial growth at 25 mg/mL. When the materials were set, the antibacterial activity of Tubli-Seal was greater, whereas AH Plus, Sealapex, and Endoseal showed less antibacterial activity. EndoSequence BC sealer exhibited the least antibacterial activity regardless of whether or not the material was set.

The inhibitory effects of unset sealers against *P. gingivalis* decreased in the order of AH Plus, Sealapex, Tubli-Seal, Endoseal, and EndoSequence BC sealer. When the materials were set, the antibacterial effect of AH Plus was significantly reduced (Fig. 3).

Endoseal sealer contained more types and larger amount of the oxide compound known to have strong antimicrobial activity such as Al_2O_3, Fe_2O_3, MgO, Na_2O, NiO, and SO_3 than Endoseqeunce BC sealer in XRF analysis (Table 2). The main compounds were zirconium dioxide, calcium oxide, and silicon dioxide according for approximately 97% of the total mass of EndoSequence BC sealer and 86% of Endoseal. Both EndoSeqeunce BC sealer and Endoseal are the sealer on the basis of calcium oxide and zirconium dioxide and have large amount of the two molecules. However, higher levels of metal

Please cite this article in press as: Shin J-H, et al., Comparison of antimicrobial activity of traditional and new developed root sealers against pathogens related root canal, Journal of Dental Sciences (2018), https://doi.org/10.1016/j.jds.2017.10.007