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01

Comparison of **bacterial leakage resistance** of various root canal filling materials and methods: Confocal laser - scanning microscope study

This study evaluated the bacterial leakage resistance and root canal lining efficacy of various root canal filling materials and methods by using confocal laser - scanning microscope (CLSM). Sixty extracted human premolars with mature apex and single root canal were randomly divided into 2 control groups and 4 experimental groups. Group CW was filled with continuous wave technique using gutta - percha and AH Plus sealer. Group GC was coated with AH - Plus sealer and then obturated with soften GuttaCore. Group GF was obturated using GuttaFlow and gutta - percha. Group EM was filled with EndoSeal MTA and gutta - percha using ultrasonic vibration. The AH - Plus, GuttaFlow, and EndoSeal were labeled with Hoechst 33342 to facilitate fluorescence. The obturated root tip was incubated with Carboxyfluorescein diacetate succinimidyl ester (CFSE) - stained E. faecalisfor 14 days. CLSM was performed to evaluate the sealer distribution and bacterial leakage for the apical 1 - , 2 - , 3 - mm specimens. Statistically significant differences were determined by 1 - way ANOVA with Tukey' s post - hoc test and Pearson' s correlation analysis. Group EM showed the better sealer distribution score than the other groups ($p<0.05$). Group CW and group GC exhibited the less bacterial leakage than the group GF, while group EM showed the similar bacterial leakage score with the groups CW and GC. There was no significant correlation between the sealer distribution and bacterial leakage ($p>0.05$). Under the conditions of this study, different root canal filling materials and methods showed different efficacy for canal distribution and bacterial leakage resistance.

02

Dynamic **intratubular biomineralization** following root canal obturation with **pozzolan - based mineral trioxide aggregate sealer cement**

The application of mineral trioxide aggregates (MTA) cement during the root canal obturation is gaining concern due to its bioactive characteristic to form an apatite in dentinal tubules. In this regard, this study was to assess the biomineralization of dentinal tubules following root canal obturation by using pozzolan - based (Pz -) MTA sealer cement (EndoSeal MTA, Maruchi). Sixty curved roots (mesiobuccal, distobuccal) from human maxillary molars were instrumented and prepared for root canal obturation. The canals were obturated with gutta - percha (GP) and Pz - MTA sealer by using continuous wave of condensation technique. Canals obturated solely with ProRoot MTA (Dentsply Tulsa Dental) or Pz - MTA sealer were used for comparison. In order to evaluate the biomineralization ability under different conditions, the PBS pretreatment before the root canal obturation was performed in each additional samples. At dentin - material interfaces, the extension of intratubular biomineralization was analyzed using scanning electron microscopy (SEM) and energy dispersive spectroscopy. When the root canal was obturated with GP and Pz - MTA sealer, enhanced biomineralization of the dentinal tubules beyond the penetrated sealer tag was confirmed under the SEM observation ($p<0.05$). Mineralized apatite structures (calcium/phosphorous ratio, 1.45–1.89) connecting its way through the dentinal tubules were detected at 350–400 μm from the tubule orifice, and the pre - crystallization seeds were also observed along the intra - and/or inter - tubular collagen fiber. Intratubular biomineralization depth was significantly enhanced in all PBS pretreated canals ($p<0.05$). Pz - MTA cement can be used as a promising bioactive root canal sealer to enhance biomineralization of dentinal tubules under controlled environment.

03

Physical properties and biocompatibility of an injectable calcium-silicate-based **root canal sealer:** in vitro and in vivo study

BACKGROUND

The aim of this study was to investigate the physical properties and biological effects of an experimentally developed injectable premixed calcium-silicate root canal sealer (Endoseal) in comparison with mineral trioxide aggregate (MTA) and a resin-based sealer (AHplus).

METHODS

The pH, solubility, dimensional change, flow, and radiopacity of the materials were evaluated. Biocompatibility was evaluated on the basis of cell morphology and a viability test using MC3T3-E1 cells. For evaluate inflammatory reaction, the tested sealers were implanted into dorsal subcutaneous connective tissue of Sprague Dawley rats. After 7 days, the implants with the surrounding tissue were retrieved, and histological evaluation was performed.

RESULTS

Endoseal showed high alkalinity similar to that of MTA. The solubility of the tested materials was similar. The dimensional change and flow of Endoseal was significantly higher than that of other materials ($P<0.05$). The radiopacity of Endoseal was lower than that of AHplus ($P<0.05$). The biocompatibility was similar to those of MTA. Inflammatory reaction of Endoseal was similar with that of MTA, but lower than that of AHplus ($P<0.05$).

CONCLUSIONS

The present study indicates that Endoseal has favorable physical properties and biocompatibility. Therefore, we suggest that Endoseal has the potential to be used as a predictable root canal sealer.

04

Tooth discoloration induced by a novel mineral trioxide aggregate-based **root canal sealer**

OBJECTIVES

The aim of this study was to evaluate tooth discoloration caused by contact with a novel injectable mineral trioxide aggregate (MTA)-based root canal sealer (Endoseal; Maruchi, Wonju, Korea) compared with a widely used resin-based root canal sealer (AHplus; Dentsply De Trey, Konstanz, Germany) and conventional MTA (ProRoot; Dentsply, Tulsa, OK, USA).

MATERIALS AND METHODS

Forty standardized bovine tooth samples were instrumented and divided into three experimental groups and one control group (n = 10/group). Each material was inserted into the cavity, and all specimens were sealed with a self-adhesive resin. Based on CIE Lab system, brightness change (ΔL) and total color change (ΔE) of each specimen between baseline and 1, 2, 4, and 8 weeks were obtained.

RESULTS

At all time points, Endoseal showed no significant difference in ΔL and ΔE compared to AHplus and control group ($P > 0.05$), whereas the ProRoot group showed significantly higher ΔL and ΔE values than the Endoseal group at 2, 4, and 8 weeks ($P < 0.05$). Therefore, Endoseal showed less discoloration than conventional MTA and a similar color change to AHplus.

CONCLUSIONS

Within the limitations of this study, our data indicate that the MTA-based sealer produces a similar amount of tooth discoloration as AHplus which is considered to be acceptable.

05

Push-out Bond Strength of Injectable **Pozzolan-based** Root Canal Sealer

- AH Plus showed superior resistance to dislodgment compared with EndoSeal or MTA Fillapex.
- MTA Fillapex presented the lowest push-out values as compared with other sealers.
- EndoSeal presents satisfactory bond strength performance.
- EndoSeal displays a new alternative of injectable bio-tight root canal sealer.

06

Cytocompatibility of calcium silicate-based sealers in a three-dimensional cell culture model

OBJECTIVES

The aim of the present study was to evaluate cytotoxic effects and cytokine production of calcium silicate-based sealers (EndoSeal, EndoSequence BC Sealer, and MTA Fillapex) using an in vitro root canal filling model and three-dimensional (3D) cell culture. AH Plus as a reference was compared to contemporary calcium silicate cements regarding cell viability and cytokine production.

MATERIAL AND METHODS

Root canals of 30 human maxillary incisors were prepared using a single-file reciprocating technique. The samples were randomly distributed and canals filled with either AH Plus, EndoSeal, EndoSequence BC Sealer, and MTA Fillapex (n = 6). In the negative control group, the root canal remained unfilled. Sealers were placed into the canals along with a gutta-percha cone placed to working length. Balb/c 3T3 fibroblasts, cultured in a type I collagen 3D scaffold, were exposed to filling material and the respective root apex for 24 h. Cytocompatibility of the materials was evaluated using the methyl-thiazoldiphenyl-tetrazolium (MTT) assay. The production of IL-1β, IL-6, and IL-8 was analyzed using enzyme-linked immunosorbent assay (ELISA). One-way analysis of variance was performed, and when the F-ratios were significant, data were compared by Duncan' s multiple-range test. The alpha-type error was set at 0.05.

RESULTS

EndoSeal, Endosequence BC Sealer and AH Plus showed cell viability that was similar to the negative control group ($P > 0.05$), while MTA Fillapex sealer was cytotoxic ($P < 0.05$). Varying production of IL-1β, IL-6, and IL-8 was detected in all samples.

CONCLUSIONS

In an in vitro root canal filling model with 3D cell culture, AH Plus, EndoSeal, and EndoSequence BC Sealer were cytocompatible.

CLINICAL RELEVANCE

These results may suggest that AH Plus, EndoSeal and EndoSequence BC Sealer may achieve better biological response when compared to MTA Fillapex.

07

Physicochemical Properties of Epoxy Resin-Based and Bioceramic-Based Root Canal Sealers

Three bioceramic sealers (EndoSequence BC sealer, EndoSeal MTA, and MTA Fillapex) and three epoxy resin-based sealers (AH-Plus, AD Seal, and Radic-Sealer) were tested to evaluate the physicochemical properties: flow, final setting time, radiopacity, dimensional stability, and pH change. The one-way ANOVA and Tukey' s post hoc test were used to analyze the data ($P = 0.05$). The MTA Fillapex sealer had a highest flow and the BC Sealer presented a flow significantly lower than the others ($P < 0.05$). The BC Sealer and MTA Fillapex samples were not set in humid incubator condition even after one month. EndoSeal MTA had the longest setting time among the measurable materials and Radic-Sealer and AD Seal showed shorter setting time than the AH-Plus ($P < 0.05$). AH-Plus and EndoSeal MTA showed statistically higher values and MTA Fillapex showed statistically lower radiopacity ($P < 0.05$). BC Sealer showed the highest alkaline pH in all evaluation periods. Set samples of 3 epoxy resin-based sealers and EndoSeal MTA presented a significant increase of pH over experimental time for 4 weeks. In conclusion, the bioceramic sealer and epoxy resin-based sealers showed clinical acceptable physicochemical properties, but BC Sealer and MTA Fillapex were not set completely.

08

Comparative evaluation of **Fracture Resistance** of Endodontically Treated Teeth Obturated with Pozzolan-based MTA Sealer and Epoxy Resin-based Sealer: An in vitro Study

AIM

To evaluate and compare the effect of epoxy resin-based sealer and a pozzolan-based mineral trioxide aggregate (MTA) sealer on the fracture resistance of endodontically treated teeth.

MATERIALS AND METHODS

Thirty single-rooted mandibular premolars were decoronated to a standardized root length of 15 mm. ProTaper rotary files up to a master apical file size of F3 were used for cleaning and shaping the root canals followed by 2.5% sodium hypochlorite irrigation. The teeth were randomly divided into three groups (n = 10 each), and the obturation was completed using gutta-percha with Endoseal MTA (group I) and AH Plus (group II) as root canal sealers. Group III served as control (instrumented and unfilled). Each specimen was then subjected to fracture testing by using a universal testing machine at a crosshead speed of 1.0 mm/minute until fracture. The force required to fracture each specimen was recorded and the data were subjected to statistical analysis using one-way analysis of variance (ANOVA), followed by pairwise comparison using post hoc Games-Howell test ($p < 0.05$).

Results

The fracture resistance of groups I and II were significantly higher than those of group III. No significant difference in the fracture resistance was observed between group I (Endoseal MTA) and group II (AH Plus) groups.

Conclusion

It can be concluded that the new root canal sealer, Endoseal MTA, is able to reinforce the tooth against fracture.

09

Root Canal **Filling Quality** of a Premixed **Calcium Silicate** Endodontic Sealer Applied Using **Gutta-percha Cone-mediated Ultrasonic Activation**

- Endoseal MTA, a new paste-type calcium silicate sealer, was recently introduced.
- Ultrasonic application through a gutta-percha (GP) cone is suggested to achieve the best filling quality of the sealer.
- The filling quality of the sealer was assessed using 2 different methods, micro–computed tomography and observation of sectioned samples with a stereomicroscope.
- The filling quality of Endoseal MTA is better when used with GP cone-mediated ultrasonic activation.
- Microscopic observations should be included as a supportive method for evaluating the quality of root canal fills.

10

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

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 Al2O3, Fe2O3, MgO, Na2O, NiO, and SO2 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.

11

Influence of environment on testing of **hydraulic sealers**

In vitro material testing is undertaken by conducting a series of tests following procedures outlined in international standards. All material properties are measured in water; however biological behavior is undertaken in alternative media such as Dulbecco' s modified eagle medium (DMEM) or simulated body fluid. The aim of this study was to characterize four dental root canal sealers and study their properties in different media. Four dental root canal sealers were assessed. They were characterized by a combination of techniques and the sealer properties were tested as specified by ISO 6876 (2012) and also in alternative media. The sealer biocompatibility was measured by cell function and proliferation assays of elutions. All sealers complied with ISO specifications. The material properties were effected by the type of soaking medium used and the surface micromorphology and elemental composition were dependent on the soaking solution type. Both BioRoot and MTA Fillapex showed cytotoxicity which reduced at higher dilutions. The material chemistry, presentation, environmental conditions and testing methodology used affected the sealer properties. Standards specific to sealer type are thus indicated. Furthermore the methodology used in the standard testing should be more relevant to clinical situations.

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01

Comparison of **bacterial leakage resistance of** various root canal filling materials and methods: Confocal laser-scanning microscope study

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Comparison of Bacterial Leakage Resistance of Various Root Canal Filling Materials and Methods: Confocal Laser-Scanning Microscope Study

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Summary: This study evaluated the bacterial leakage resistance and root canal lining efficacy of various root canal filling materials and methods by using confocal laser-scanning microscope (CLSM). Sixty extracted human premolars with mature apex and single root canal were randomly divided into 2 control groups and 4 experimental groups. Group CW was filled with continuous wave technique using gutta-percha and AH Plus sealer. Group GC was coated with AH-Plus sealer and then obturated with soften GuttaCore. Group GF was obturated using GuttaFlow and gutta-percha. Group EM was filled with EndoSeal MTA and gutta-percha using ultrasonic vibration. The AH-Plus, GuttaFlow, and EndoSeal were labeled with Hoechst 33342 to facilitate fluorescence. The obturated root tip was incubated with Carboxyfluorescein diacetate succinimidyl ester (CFSE)-stained *E. faecalis* for 14 days. CLSM was performed to evaluate the sealer distribution and bacterial leakage for the apical 1-, 2-, 3-mm specimens. Statistically significant differences were determined by 1-way ANOVA with Tukey's post-hoc test and Pearson's correlation analysis. Group EM showed the better sealer distribution score than the other groups ($p < 0.05$). Group CW and group GC exhibited the less bacterial leakage than the group GF, while group EM showed the similar bacterial leakage score with the groups CW and GC. There was no significant correlation between the sealer distribution and bacterial leakage ($p > 0.05$). Under the conditions of this study, different root canal filling materials and methods showed different efficacy for canal distribution and bacterial leakage resistance. SCANNING 9999:XX–XX, 2015. © 2015 Wiley Periodicals, Inc.

Key words: bacterial leakage, confocal laser scanning microscope, GuttaCore, GuttaFlow, root canal sealer, sealer distribution

Introduction

Root canal fillings aim to seal the root canal system to prevent reinfection of the periapex. Obturation should eliminate all routes of leakage from the oral cavity and the periradicular tissues into the root canal system by creating a fluid-tight seal. Therefore, ideally, root canal filling materials, and endodontic sealers should seal the canal laterally and apically and have good adaptation to the root canal dentin (Özcan *et al*., 2011; Chandra *et al*., 2012). The materials and methods used for root canal obturation are one of the critical determinants for the success or failure of endodontic treatment (Bodrumlu and Tunga, 2007).

Obturation of the root canal space has been performed using various techniques. The most commonly used technique is cold lateral condensation using gutta-percha (GP) cones which allows good length control and is predictable (Gutmann *et al*., '93). The "continuous wave of condensation" which was designed to simplify the vertical condensation method has been found superior or similar to that provided by the lateral condensation (Buchanan '98; DuLac *et al*., '99; Smith *et al*., 2000; Özcan *et al*., 2013). It is claimed that the heat source allows sufficient heat for the apical GP to be softened and adapted to the irregularities of the intracanal anatomy (Kytridou *et al*., '99; Gilbert *et al*., 2001). Thermoplasticized GP techniques have been advocated for root canal obturation because they may provide a more homogeneous obturation and better adaptation to the canal walls (Kytridou *et al*., '99) which might result in a lower leakage compared with lateral condensation. GuttaCore (DENTSPLY Tulsa Dental Specialties, Tulsa, OK) is one of the carrier-based GP obturation system. As an improvement over the previous Thermafil technique, GuttaCore obturator carriers are not made from plastic, but from a GP elastomer with intermolecular cross links (Li *et al*., 2014). This makes the procedures not only rapid and high-quality three-dimensional root canal obturaion, but also easy post space preparation and root filling removal in a case where retreatment is required. EndoSeal MTA (Maruchi, Wonju, Korea) was recently introduced in a premixed paste type MTA-based sealer with the characteristics of hardening even at the complex and moist canal environment. The company also claims that the ultrasonic method using the EndoSeal MTA may reduce lateral and vertical forces applied to the root dentin during filling procedure. GuttaFlow (Coltene Whaledent, Alstatten, Switzerland), a new flowable root canal filling paste, was introduced as a non-heated flowable obturation system that combines both the sealer and the gutta-percha in 1 injectable system (Li *et al*., 2014). The manufacturer claims that this material has good homogeneity and adaptation to the root canal walls owing to its better flow properties.

As above, new obturation biomaterials and methods have been introduced over the past decades to improve the seal of the root canal system. However, it is not clear whether they really produce a three-dimensional impervious seal that is important for reducing root canal reinfection. Therefore, the purpose of this study was to evaluate the bacterial leakage resistance and root canal lining of various root canal filling materials and methods by using confocal laser-scanning microscope (CLSM).

Materials and Methods

Preparation of Teeth and Canal Obturation

Sixty extracted human premolars with mature apex and single root canal were used in this study. After preparing a conventional access cavity, a size 10 K-file was inserted into the canal until it was just visible at the apical foramen. Working length was determined by subtracting 0.5 mm from this length. The root canal of each tooth was cleaned and shaped using sizes S1, S2, F1, and F2 ProTaper Universal nickel-titanium files (Dentsply Maillefer, Ballaigues, Switzerland) and finally with the size #35/0.04 taper nickel-titanium file (BLX; B&L Biotech, Ansan, Korea). The root canals were irrigated profoundly using 2.5% sodium hypochlorite between each instrument. Then canals were rinsed with 17% EDTA solution (MD-cleanser; Meta Biomed, Chungju, Korea) for 1 min to remove smear layer, followed by flushing again with 2 ml of sodium hypochlorite. The root canals were dried with paper point. After biomechanical preparation, all the teeth were sterilized in an autoclave for 20 min at 121°C. The teeth were randomly divided into two control groups and four experiment groups using continuous wave of condensation technique, GuttaCore obturation system, GuttaFlow system, and EndoSeal MTA, respectively ($n = 10$). To facilitate fluorescence under confocal microscopy, AH Plus, GuttaFlow, and EndoSeal were labeled with Hoechst 33342 (Sigma, St. Louis, MO).

Group CW (Continuous wave of condensation technique with GP and AH Plus sealer): Each canal was filled with epoxy resin-based sealer (AH Plus; Dentsply Caulk, Milford, DE) and a size #35/0.04 taper GP master cone using Duo-Alpha and Duo-Beta system (B&L Biotech). The GP was down-packed with a Duo-Alpha heat source to the 4 mm from the working length and backfilled using a Duo-Beta.

Group GC (GuttaCore obturation system with AH Plus sealer): The canals were coated with a thin layer of AH Plus sealer using K-file. The size 30 GuttaCore obturator was softened in the dedicated GuttaCore oven and slowly inserted to the working length. The carrier was twisted off and the filled material was compacted at the orifice.

Group GF (GuttaFlow obturation system with GP): Teeth were obturated using the GuttaFlow and GP as the manufacturer's instructions. GuttaFlow provided in a special capsule was mixed in the triturator for 30 s. The capsule was then loaded on the dispenser with attached canal tip. GuttaFlow was layered slowly into the apical canal part. The master GP cone was coated with GuttaFlow and inserted in the canal. The master GP cone was cut at the orifice level.

Group EM (Ultrasonic condensation with EndoSeal MTA and GP): The EndoSeal MTA was prepared as the manufacturer's instruction and loaded in a metal tip (Centrix; Centirx, Shelton, CT). EndoSeal MTA was then injected into coronal and middle root canal. By using lentulo spiral, EndoSeal MTA was adjusted to middle and apical root canal. Then the master GP cone was inserted into the canal. By ultrasonic vibration to the pincette which hold the master cone, the GP cone could reach all the way to working length. The master GP cone was cut at the orifice level.

Group PC (Positive control; $n = 10$): The canals of this group were not filled.

Group NC (Negative control; $n = 10$): The canals of this group were filled with GP and AH plus sealer. The negative control group was sealed around entire root surface with colored nail varnish.

Except the Group NC, the roots of 50 teeth were sealed with colored nail varnish, except for the apical 1 mm around the apical foramen.

Conflict of interest: None.
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Bacterial Leakage Experiment

Enterococcus faecalis (*E. faecalis* strain ATCC 19433) were grown in Brain Heart Infusion (BHI) broth (DIFCO, BD science, San Jose, CA) under aerobic conditions at 37˚C. The bacteria were resuspended in phosphate buffer saline (PBS) at 5×10^7 CFU/ml and stained with cellTrace Carboxyfluorescein diacetate succinimidyl ester (CFSE, Molecular probes, Life technology, Carlsbad, CA) following the manufacture's instruction. Briefly, *E. faecalis* were stained with 10 μM of CFSE at 37˚C for 10 min. After incubation, cold PBS was added to stop the staining and *E. faecalis* were thoroughly washed. The prepared CFSE-stained *E. faecalis* were inoculated in BHI broth. After cutting the tapered end of 2 ml Eppendorf plastic tube (Eppendorf-Elkay, Shrewsbury, MA), the obturated root tip was inserted into the tube containing BHI broth inoculated with CFSE-stained *E. faecalis* until the root tip protruded through the end. The entire assembly was then incubated at 37˚C for 14 days.

Specimen Preparation and Confocal Laser-Scanning Microscope for Scoring

The samples were washed with PBS and specimens were sectioned at every 1 mm from 1 to 3 mm level of apex using a low speed diamond disk (Horico, Berlin, Germany). The procedure was done with minimum pressure under water cooling to minimize the smearing of the GP.

All samples were observed by CLSM (LSM 700; Carl Zeiss, Oberkochen, Germany) at each 1 mm specimen. Each 10 samples were evaluated for a blue fluorescent ring around the canal wall, indicating the sealer-dye distribution. The ratio of bacterial penetration was detected for a green spot around the canal wall, indicating the CFSE-stained *E. faecalis*. At each level, tooth surface was divided into eight parts of same arc and if there was fluorescence (Blue or Green) in a eighth, 0.125 point was given (Fig. 1). Because the score given in each level is not clinically available to evaluate the efficiency of the materials and methods, the final NET score for each specimen was given by summing up of the points from the levels of 1 mm to 3 mm of apical root.

Statistics

Quantitative data were tabulated and analyzed for 1-way analysis of variance (ANOVA) and Tukey's post-hoc tests using Graphpad Prism software (GraphPad Software, San Diego, CA). The correlation between sealer distribution and bacterial leakage was presented by Pearson's correlation analysis. The statistical significance was set at a confidence level of 95%.

Results

The representative CLSM results of sealer distribution and bacterial leakage of the tested groups are shown in Figure 2. The specimens of positive control group show a consistent green fluorescent ring around the canal wall, indicating the bacterial penetration into the canal (Fig. 2(A)). The blue fluorescent ring around the canal wall of negative control group represents the sealer distribution in canal wall (Fig. 2(B)).

The results of the quantitative evaluation of the penetration of AH Plus, GuttaFlow, and EndoSeal MTA stained with Hoechst 33342 are shown in Table I and Figure 3 (A and B). Group EM showed the better sealer distribution score than the other groups ($p < 0.05$). The results of bacterial leakage scores at 1, 2, and 3 mm levels from the apex and net score are presented in Table I and Figure 3 (C and D). Group CW and group GC exhibited the less bacterial leakage than the group GF, while group EM showed the similar bacterial leakage score with the groups CW and GC (Table I). As shown in Figure 3E, there was no significant correlation between the sealer penetration and bacterial leakage ($p > 0.05$).

Discussion

The elimination of bacteria from the root canals by cleaning and shaping procedures and the prevention of recontamination of the obturated root canal are fundamental for the successful treatment of apical periodontitis (Ray and Trope, '95). Healing of periapical disease involves a combination of bacterial eradication during treatment through chemomechanical means along with sealing of the root canal (Ray and Trope, '95). Recently, new materials for root canal filling and methods for these materials were introduced. This study evaluated the various root canal filling materials and methods by comparing the sealer distribution to canal wall and bacterial leakage resistance under the CLSM.

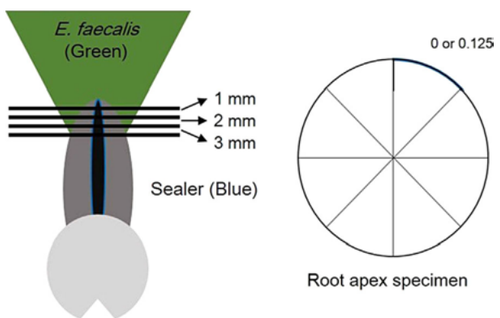

Figure 1. The experimental designs for specimen preparation and scoring of fluoresences.

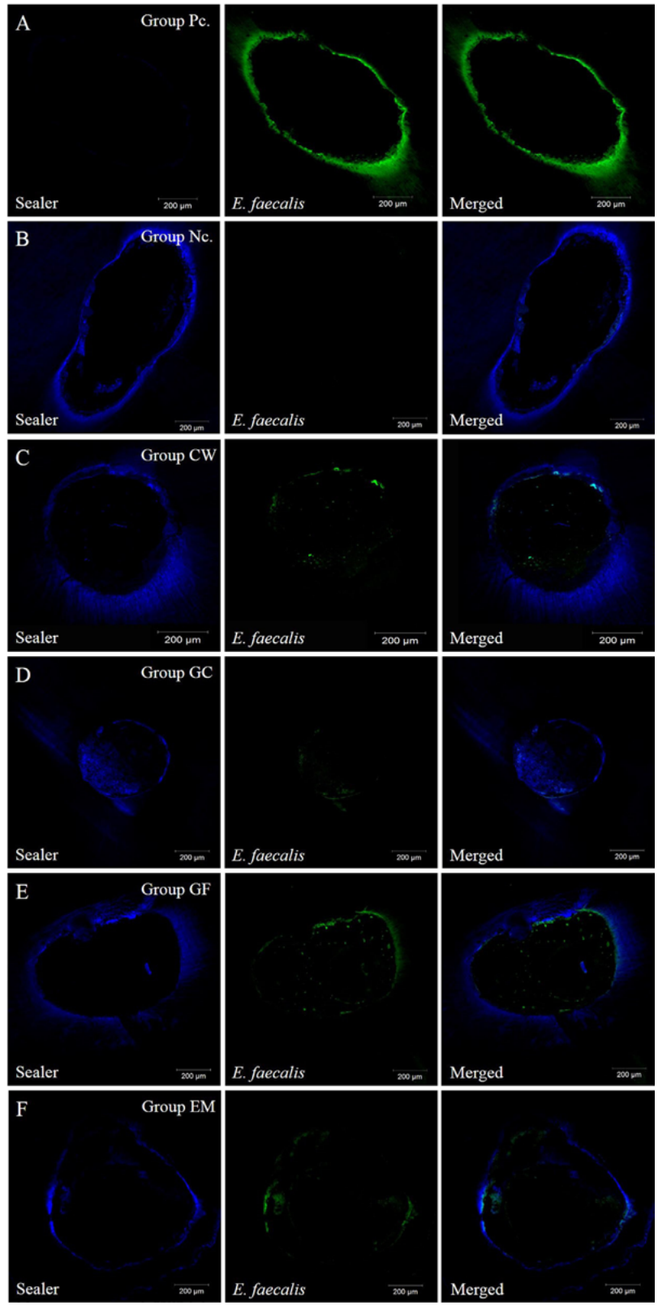

Figure 2. The representative CLSM results of control groups and experimental groups: A. Group Pc (Positive control). B. Group Nc (Negative control). C. Group CW (continuous wave of condensation technique with GP and AH Plus sealer). D. Group GC (GuttaCore system with AH Plus sealer). E. Group GF (GuttaFlow obturation system with GP). F. Group EM (Ultrasonic condensation with EndoSeal MTA and GP).

TABLE I The sealer distribution score (%) and bacterial leakage score (mean ± SD)

Group	Level Score	1 mm	2 mm	3 mm	Net
Group CW	sealer distribution	85 ± 8	91 ± 8	91 ± 6	89 ± 4A
	bacterial leakage	0.35 ± 0.15	0.33 ± 0.11	0.29 ± 0.12	0.33 ± 0.07a
Group GC	sealer distribution	86 ± 9	86 ± 9	90 ± 5	88 ± 3A
	bacterial leakage	0.46 ± 0.15	0.27 ± 0.05	0.29 ± 0.10	0.32 ± 0.09a
Group GF	sealer distribution	85 ± 9	90 ± 5	88 ± 7	88 ± 4A
	bacterial leakage	0.54 ± 0.06	0.46 ± 0.05	0.36 ± 0.04	0.44 ± 0.07[b]
Group EM	sealer distribution	90 ± 8	93 ± 6	97 ± 5	93 ± 4[B]
	bacterial leakage	0.38 ± 0.12	0.39 ± 0.12	0.32 ± 0.06	0.37 ± 0.08a[b]

CLSM technique may be useful as a complement to the established microbiological, histologic, standard electron microscopy, and PCR-based techniques for the identification of viable bacteria (Scivetti *et al*., 2007). In the present study, CLSM offers advantages to evaluate clearly the distribution and interfacial adaptation of root canal sealers and the bacterial infiltration (Gharib *et al*., 2007). Meanwhile, because *E. faecalis* is also one of the most commonly isolated microbes from the root canal in secondary infections (Baumgartner and Falkler, '91; Timpawat *et al.*, 2001), this bacterial species was used in this study.

Ideally, a root canal sealer should be capable of producing a bond between the core material and the root dentin for preventing leakage effectively. A wide variety of root canal sealers are commercially available, however

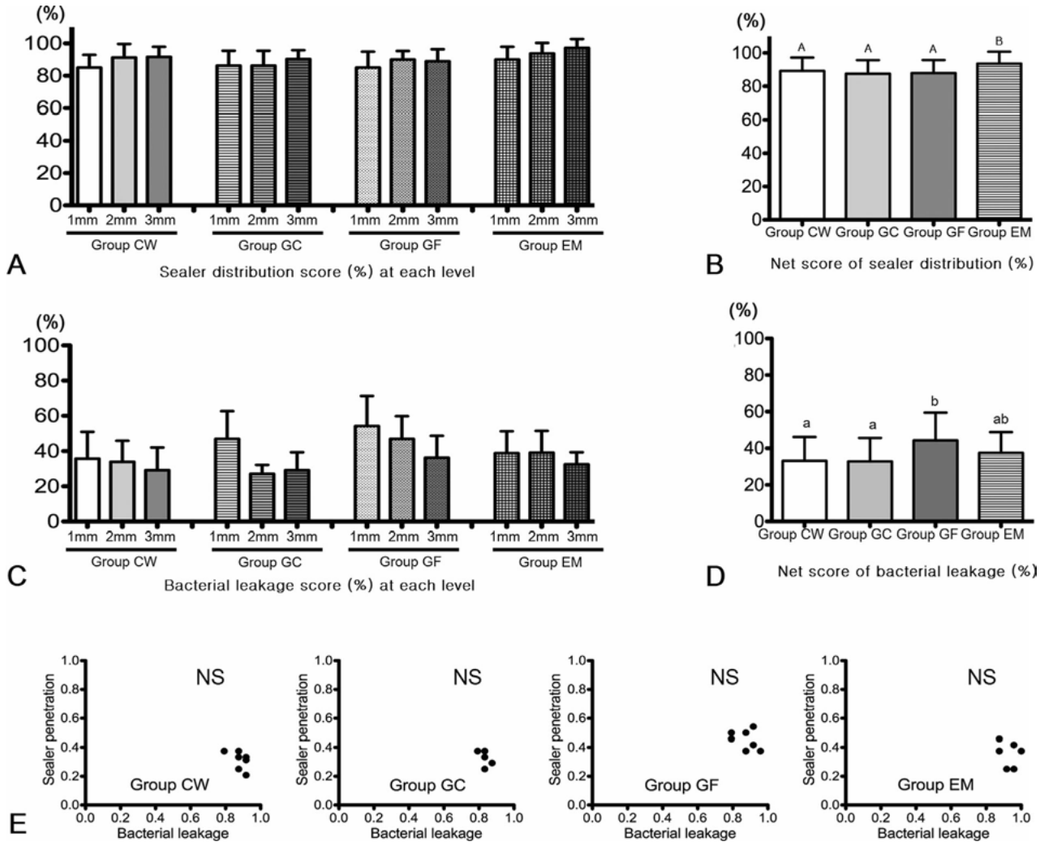

Figure 3. A. The ratio of sealer distribution (%) in the 3 different levels. B. The net score of sealer distribution (%) of each group (groups with different superscripts have significant difference, $p < 0.05$). C. The score of bacterial leakage in the 3 different levels. D. The net score of bacterila leakage of each group (groups with different superscripts have significant difference, $p < 0.05$). E. The correlation between sealer penetration and bacterial leakage has no statistical significance (Pearson analysis, NS: not significant).

there is lack of consensus on which material seals the most effectively. AH Plus is an epoxy resin-based sealer. Some studies reported the apical seal of resin-based sealers are superior to the sealing ability of other sealers and shown to effectively seal the root canal (Oguntebi and Shen, '92; Cobankara *et al*., 2002; Wu *et al*., 2002; Gençoglu *et al*., 2003). These were reported as to be related to the slight expansion during their setting reactions (Ørstavik *et al*., 2001). In present study, it could be a same reason that the two groups of CW and GC had the less bacterial leakage than the groups which were AH Plus used. These results are coincidence with some previous reports regarding the efficacy of filling techniques (DuLac *et al*., '99; Silver *et al*., '99; Smith *et al*., 2000; Wu *et al*., 2001). The continuous wave of condensation technique has been found to be superior to some other canal filling techniques in terms of less apical leakage (DuLac *et al*., '99; Smith *et al*., 2000). However, some studies have found poor adaptation of GP to the canal wall by this technique (Silver *et al*., '99; Wu *et al*., 2001). Wu *et al*. (2001) reported that the apical GP ratio was low in some specimens and the sealer was too thick.

Meanwhile, the present results showed that group GF had significantly higher bacterial leakage than the continuous wave of condensation technique and GuttaCore system. However, on the contrary to the present results, Brackett *et al.* (2006) found no significant difference in sealing ability between Gutta-Flow and vertically compacted GP used with AH Plus sealer. In a study by Hammad *et al.* (2008) GuttaFlow also showed good penetration into the dentinal tubules, and thus to help in preventing leakage. The good sealing ability exhibited by the GuttaFlow root-canal filling material could be attributed to its ability to flow into lateral grooves and depressions (Zielinski *et al.*, 2008).

On the other hand, considering the sealer distribution score, group GF had the similar efficacy with the groups CW and GC to line the canal wall but lower than the group EM which had the highest distribution score. The use of "ultrasonic" condensation method seemed to make the higher distribution score in the group EM. Ultrasonic vibration may have improved the flowability to ease in filling procedure or adjusting for varying working lengths and complex anatomy.

The EndoSeal MTA is similar to MTA Fillapex (Angelus, Londrina, PR, Brazil) which is a new calcium silicate-based sealer containing MTA and is known to possess favorable biocompatibility, antimicrobial activity, and good sealing ability (Torabinejad and Parirokh, 2010). EndoSeal MTA has a special concept of ultrasonic condensation (12). The ultrasonic vibration for sealer condensation may have brought positive effects to seal the canal and make higher sealer distribution scores and lining efficacy in this study. MTA Fillapex which has the similar composition mainly with MTA was also found to have greater flow values than AH Plus (Zhou *et al*., 2013).

GuttaCore represents the latest generation of carrier-based root canal filling material that uses thermoplasticised GP as the core materials. In this present study, GuttaCore obturation system used with AH Plus sealer exhibited the less bacterial leakage than group GF and similar with the others. The reason for this finding might be similar with that found in group CW used with AH Plus sealer. The core-carrier may have enhanced the adaptation of GP to the canal wall and the flow of the molten GP material into irregular canal spaces. Previous studies reported that canals obturated with core-carrier techniques had the highest GP content within the filled canal space (Gençoglu, 2003; De-Deus *et al*., 2006).

The flow of a sealer determines how effectively it fills accessory canals, irregularities on the dentinal wall, and spaces between the core filling materials (Zhou *et al*., 2013). However, as found in Figure 3E in this study, the correlations of sealer distribution and bacterial leakage were not significant. It might be due to the technical sensitivity in filling procedures, although sufficient preliminary tests were done to minimize the procedural errors before the experimental tests. Probably the chemical and mechanical properties may have brought the different bacterial leakage resistances which were not correlated with the sealer distribution and penetration extents. This phenomenon should be studied further to see any potential correlations. Clinically, it is highly recommended to follow the manufacturers' instructions and do some sub-clinical practices using extracted teeth.

It is important to assess microbial leakage not only immediately after root canal filling but also with some time elapsed, because root canal sealing needs to be long lasting for clinical effectiveness. Thus, further studies are required to test the bacterial leakage with some time elapsed as well as thermos-mechanical loading considering clinical effects.

Conclusion

Within the limitations of present study, four different root canal filling materials and methods showed significant differences in their canal sealing efficiency and bacterial leakage resistance without correlations between the two variables.

Acknowledgment

Ji Hee Hwang and Jin Chung contributed equally to this work and have the first authorship shared.

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Dynamic intratubular biomineralization following root canal obturation with pozzolan-based Mineral Trioxide Aggregate sealer cement

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Dynamic Intratubular Biomineralization Following Root Canal Obturation With Pozzolan-Based Mineral Trioxide Aggregate Sealer Cement

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Summary: The application of mineral trioxide aggregates (MTA) cement during the root canal obturation is gaining concern due to its bioactive characteristic to form an apatite in dentinal tubules. In this regard, this study was to assess the biomineralization of dentinal tubules following root canal obturation by using pozzolan-based (Pz-) MTA sealer cement (EndoSeal MTA, Maruchi). Sixty curved roots (mesiobuccal, distobuccal) from human maxillary molars were instrumented and prepared for root canal obturation. The canals were obturated with gutta-percha (GP) and Pz-MTA sealer by using continuous wave of condensation technique. Canals obturated solely with ProRoot MTA (Dentsply Tulsa Dental) or Pz-MTA sealer were used for comparison. In order to evaluate the biomineralization ability under different conditions, the PBS pretreatment before the root canal obturation was performed in each additional samples. At dentin-material interfaces, the extension of intratubular biomineralization was analyzed using scanning electron microscopy (SEM) and energy dispersive spectroscopy. When the root canal was obturated with GP and Pz-MTA sealer, enhanced biomineralization of the dentinal tubules beyond the penetrated sealer tag was confirmed under the SEM observation ($p < 0.05$). Mineralized apatite structures (calcium/phosphorous ratio, 1.45–1.89) connecting its way through the dentinal tubules were detected at 350–400 μm from the tubule orifice, and the pre-crystallization seeds were also observed along the intra- and/or inter-tubular collagen fiber. Intratubular biomineralization depth was significantly enhanced in all PBS pretreated canals ($p < 0.05$). Pz-MTA cement can be used as a promising bioactive root canal sealer to enhance biomineralization of dentinal tubules under controlled environment. SCANNING 38:50–56, 2016. © 2015 The Authors. *Scanning* Published by Wiley Periodicals, Inc.

Key words: biomineralization, dentinal tubule, pozzolan-based MTA sealer, pre-crystallization seeds, scanning electron microscopy

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Conflicts of interest: None.

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Introduction

Endodontic treatment is an ongoing process to eliminate infection source and to create a fluid-tight seal of the root canal system (Siqueira, 2001). Theoretically a root canal filling material which can completely seal the root canal system would be of ideal in practice. However, several studies confirmed that currently available root canal obturation materials such as gutta-percha (GP) and/or polymer-based materials showed incomplete sealing even with the aid of sealers or dentin bonding systems (Zmener *et al*., 2008; Santos *et al*., 2010; Brosco *et al.*,2010; Punia *et al.*, 2011).

Mineral trioxide aggregate (MTA) has been widely used in variety of applications including root-end filling, perforation repair, or apical/coronal sealing material during regenerative endodontic procedures (Parirokh and Torabinejad, 2010). The most favorable property, leaving its biologic properties aside, is the superior sealing ability

which comes from the water-resistant final product after hydration. This sealing ability of MTA is largely attributable to its bioactive capacity to form an apatite layer when it is in contact with phosphate-containing physiological fluids (Tay *et al*., 2007; Reyes-Carmona *et al*., 2009; Gandolfi *et al.*, 2010; Han *et al.*, 2010). Such characteristic features of MTA appear to be important in biomineralization of dentinal tubules for enhanced sealing of the root canal system (Tay *et al*., 2007; Reyes-Carmona *et al.*, 2010; Yoo *et al*., 2014), thus makes it a good candidate for root canal filling material of choice.

However, MTA cannot be recommended as a routine orthograde root canal filling material because the sandy property and irretrievability of the substance (Bogen and Kuttler, 2009) have made it challenging to be used in a complicated root canal system. Inadequate water-to-powder ratio, insufficient packing also impedes adaptation of MTA to the canal wall (Fridland and Rosado, 2003; El-Ma'aita *et al*., 2012; Saghiri *et al*., 2012). In order to overcome such limitations of MTA as a root canal filling material, recent study has utilized MTA sealer cement during the root canal filling procedure (Camilleri *et al*., 2011).

EndoSeal MTA (Maruchi, Wonju, Korea), a finely pulverized pozzolan-based MTA was recently introduced. The pozzolan cement, the main component of this sealer, gets cementitious properties after pozzolanic reaction which includes calcium hydroxide and water, and enables sufficient flow of the pre-mixed substrate though injection tip with adequate working consistency. The favorable mechanical characteristics such as fast setting time (around 4 min), higher washout resistance than other commercially available MTAs, and biologic effects including biocompatibility, mineralization potential, and odontogenic effect of the pozzolan cement had been previously reported (Choi *et al*., 2013; Jang *et al*., 2013a,b; Park *et al*., 2014; Song *et al*., 2014). However, no study has confirmed the intratubular biomineralization ability of this sealer material when it is applied in the root canal yet. Therefore, this study was aimed to investigate and compare the biomineralization ability of this pozzolan-based (Pz-) MTA sealer cement under various root canal obturation conditions.

Materials and Methods

Tooth Preparation

The protocol of this study was approved by the Institutional Review Board of the Seoul National University Dental Hospital, Seoul, Korea. A total sample size of 56 roots was calculated to be sufficient to detect significant differences (alpha at level 0.05, 90% power). Sixty curved roots (mesiobuccal and distobuccal roots) less than 30 degree (Schneider, '71) with fully formed apices from human maxillary molars were used in this study. Teeth with root cracks or defects confirmed under a microscopic evaluation (OPMI Pico; Carl Zeiss, Germany) were excluded from the study. All teeth were radiographically examined to evaluate the canal curvature, by measuring the angle between the long axis of the root and the line connecting the point that begins to move away from the long axis of the root to the apex (Schneider, 1971). The overall mean canal curvature was 15.28 ± 7.08 degree, and the roots were assigned to 6 sets (10 canals each) according to the canal curvature by block randomization (Fig. 1(A)).

The teeth were accessed with #4 round carbide burs and Endo-Z burs. The canal patency was gained using a size #10 stainless-steel (SS) K-file (Dentsply Maillefer, Ballaigues, Switzerland) until the tips were visible at the apical foramen. The working length was determined as 1 mm short from the measured length. After coronal flaring using Gates Glidden burs #2 to #4 (Komet, Rock Hill, SC), root canals were instrumented to an apical size of #35 and 0.06 taper with a crown-down technique using ProFile 0.04 and 0.06 Ni-Ti rotary instruments (Dentsply Maillefer). The root canals were irrigated with 2 ml of 5.25% sodium hypochlorite (NaOCl) solution between instrumentation, and immersed with 17% ethylenediaminetetraacetic acid (EDTA) solution (pH 7.2) for 1 min before final flush with 2.5 ml of 5.25% NaOCl solution. All irrigants were activated by using ultrasonic devices (P5 Newtron® XS; Satelec, Acteon group, Mérignac, France). Then, the canals were copiously rinsed with sterile distilled water and dried with sterile paper points.

For the root canal obturation procedure, 10 canals were obturated with MAF size- and taper- tailored GP cone and Pz-MTA sealer (EndoSeal MTA) by using continuous wave of condensation technique (CW). For the comparison control, another 10 canals were obturated solely with Pz-MTA per se. The pre-mixed Pz-MTA sealer cement was released via injection syringe and tip system, and the final coronal portion was tidied up with SS hand pluggers. The canals ($n = 10$) filled with ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK) according to the obturation technique suggested by Bogen and Kuttler (2009) were also used as a positive control. The ProRoot MTA was mixed with distilled water according to the manufacturer's instructions and placed incrementally with a carrier gun. An SS K-file, 1 or 2 sizes smaller than master apical file (MAF) was used to compact the apical 3–4 mm, then a progression of K-files sizing upward incrementally were used for further compaction. The final coronal portion was tamped by using SS hand pluggers to complete the root canal obturation.

For the phosphate buffered saline (PBS) pretreatment, additional 10 canals were assigned to each experimental group. They were immersed in sterile PBS solution for a minute and dried with sterile paper points before root canal filling.

Following the obturation, the teeth were stored at 37°C with 100% humidity for a day to allow complete

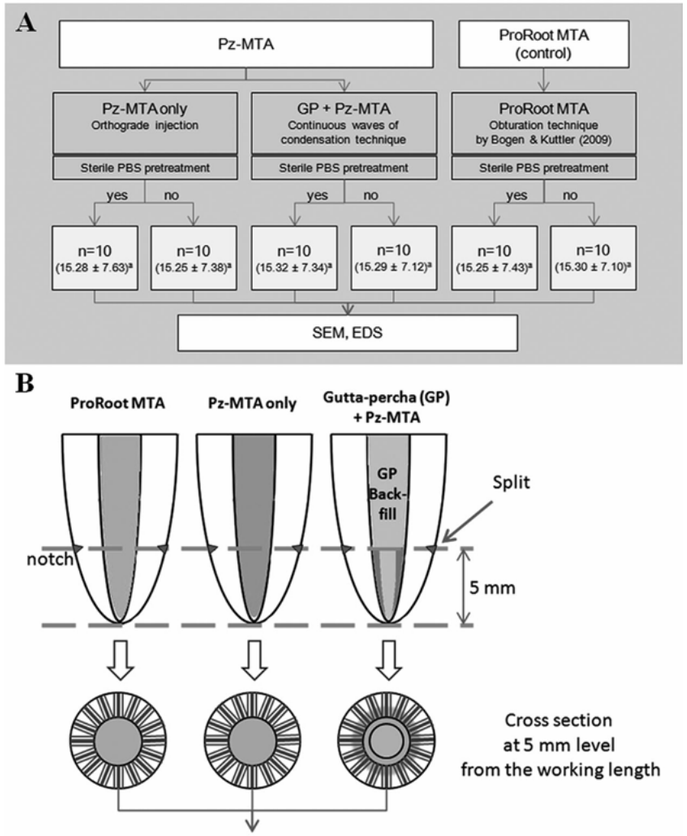

Fig 1. A: Schematic diagram of the experimental groups. The number in parenthesis shows the mean and standard deviation of the canal curvature of each set of roots, and their same lowercase superscripts show no significant differences among the mean values of the canal curvatures ($p > 0.05$). B: Schematic diagram of the root specimen preparation for SEM evaluation. MTA, mineral trioxide aggregate; Pz-MTA, pozzolan-based mineral trioxide aggregate; GP, gutta-percha; PBS, phosphate buffered saline; SEM, scanning electron microscopy; EDS, energy dispersive spectroscopy.

setting of the filling materials, and sealed the access cavities using intermediate restorative material (IRM; Dentsply Caulk, Milford, DE). The teeth were stored at 37˚C with 100% humidity until further analysis.

Assessment of Dentinal Tubule Biomineralization

After 12 weeks, the specimens were evaluated by scanning electron microscopy (SEM; S-4700, Hitachi, Tokyo, Japan) to characterize microstructural variations of the dentinal tubules. Each tooth was embedded in an acrylic block, and each mesiobuccal or distobuccal root was separated from the teeth with a slow-speed, water-cooled diamond saw (Isomet Low Speed Saw; Buehler, Lake Bluff, IL). The separated roots were split in horizontal direction for cross-section analysis at 5 mm level from the apex (Fig. 1(B)). Root segments were briefly washed in distilled water and sputter coated with platinum for SEM observation at an accelerating voltage of 15 kV. At the interface of main canal and obturation material, the depths of material penetration into dentinal tubules and intratubular mineralization were recorded. The elemental composition of intratubular mineralized precipitates were analyzed by using energy dispersive spectroscopy (EDS; 7200-H, Horiba, Northampton, England).

Statistical Analyses

The data were analyzed with one-way ANOVA and Tuckey *post hoc* test with SPSS software (SPSS Inc., Chicago, IL) to assess the differences among

experimental groups. For each group, the effect of PBS pretreatment on the intratubular biomineralization was investigated using a two-sample t-test. The significance level was set at $\alpha = 0.05$.

Results

The scanning analysis of GP with Pz-MTA sealer obturated samples showed the direct tubular penetration of Pz-MTA, and further formation of apatite crystals densely packing the dentinal tubules were detected (Fig. 2(A–C)). On the other hand, a close adaptation of the material to the main canals and intratubular biomineralization near the tubule orifices were observed in the ProRoot MTA obturation samples (Fig. 2(E–G)). The mineralized structure connecting the material-dentin interface was confirmed at the orifice level, although there was a lacking of direct penetration of ProRoot MTA into the tubules. The patterns of apatite crystallization appeared similar at the entrance of dentinal tubules in both GP with Pz-MTA sealer and ProRoot MTA obturated samples (Fig. 2(B and F)). However, they simultaneously changed along the tubule pathway; the agglomerated precipitates sparsely clogged the dentinal tubules in ProRoot MTA obturated samples (Fig. 2(G)), whereas continuous and successive crystallization along the tubule pathway was observed in GP with Pz-MTA sealer group (Fig. 2(C)). EDS evaluation indicated that the intratubular mineralized precipitates from all groups contained primarily calcium (Ca), phosphorous (P), oxygen, and trailed amount of silica with similar Ca/P ratio (1.45–1.89) (Fig. 2(D and H)).

In GP with Pz-MTA sealer group, there was a variety of precipitate nanostructures, mainly petals and flakes, in stratified or organized spherical form, or mixed (Fig. 3(A–D)). Interestingly, mineralized apatite structures connecting its way through the dentinal tubules were confirmed at 350–400 μm from the tubule orifice, and the pre-crystallization seeds were also observed along the intra- and/or inter-tubular collagen fiber (Fig. 3(E–H)).

The depth of intratubular mineralization is presented in Table I. The roots in GP with Pz-MTA sealer group demonstrated the direct tubular penetration of the sealer with significantly greater depth of dentinal tubule biomineralization than the other groups ($p < 0.05$). Pretreatment with PBS significantly promoted the biomineralization depth in all groups ($p < 0.05$). The roots solely obturated with either of ProRoot MTA or Pz-MTA sealer showed the minimum biomineralization depth without penetration of the materials into the tubules.

Discussion

Seeking for the better root canal filling material is the utmost concern of the clinicians. However the technological advancement of the obturation method had not been shown to have a statistically relevant impact on

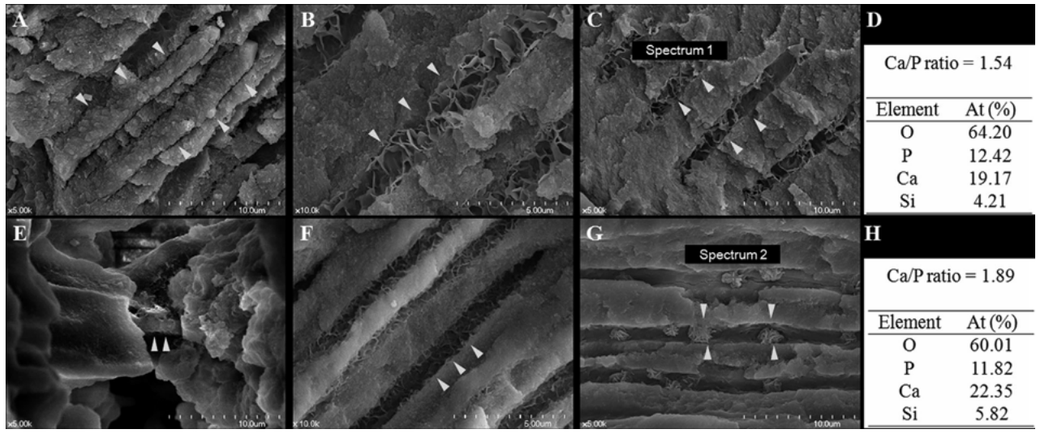

Fig 2. Representative scanning electron microscope images of gutta-percha with pozzolan-based (Pz-) MTA sealer cement- (A–D) or ProRoot MTA- (E–H) filled roots with phosphate buffered saline pretreatment. A: The Pz-MTA sealer cement penetrated into the dentinal tubules (arrowheads) at orifice level (×5,000). B: Further biomineralized dentinal tubules (arrowheads) beyond penetrated Pz-MTA cement at 50–100 μm distance showing densely packed dentinal tubules with organized apatite nanoprecipitates (×10,000). C: Successively biomineralized dentinal tubules (arrowheads) at 100–150 μm distance (×5,000). D: The semiquantitative chemical composition showing Ca/P ratio of the pointed area (red) of (C). E: Intermediate layer of ProRoot MTA connecting the material and dentinal tubule (arrowheads) at orifice level (×5,000). F: Biomineralized dentinal tubules at 50–100 μm distance (×10,000). G: Biomineralized dentinal tubules at 100–150 μm distance (×5,000). The agglomerated precipitates induced by ProRoot MTA (arrowheads) sparsely clogged the dentinal tubules. H: The semiquantitative chemical composition showing Ca/P ratio of the pointed area (red) of (G).

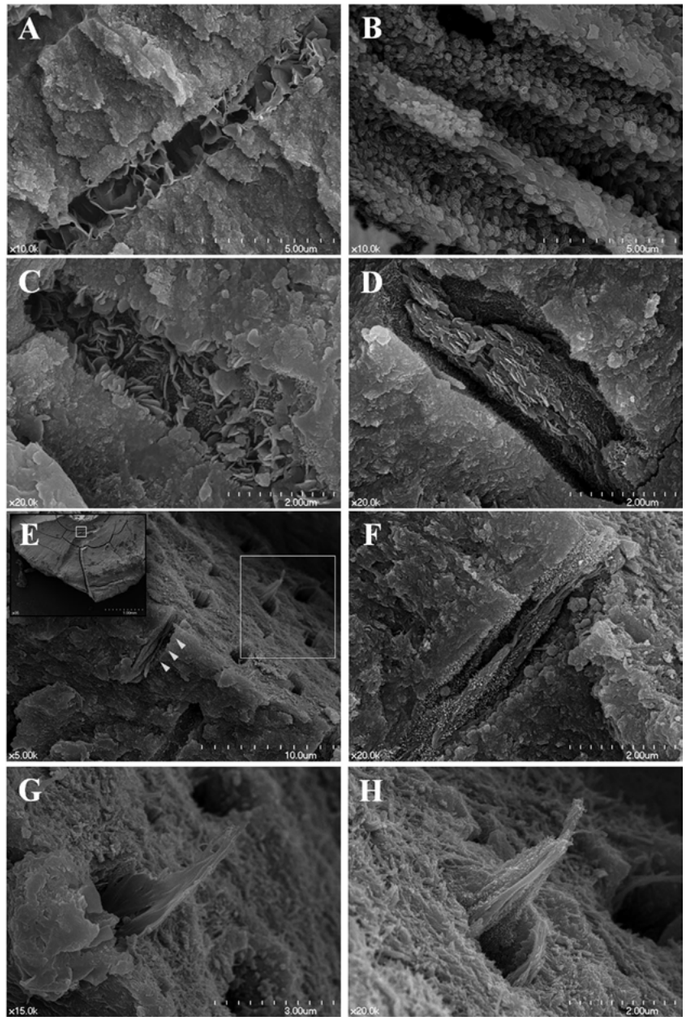

Fig 3. A–D: Diverse nanocrystallographs of pozzolan-based (Pz-) MTA sealer cement induced intratubular biomineralization. A: Organized nanoflakes (×10,000), (B) microsphere (×10,000), (C) mixed (×20,000), or (D) organized plates (×20000) are intergrown and exploited to seal the dentinal tubules. (E-F) Successive intratubular biomineralization of gutta-percha and Pz-MTA sealer filled canals. E: Boxed area (yellow) of left upper lower magnification image (×35) from the horizontal split specimen obturated with gutta-percha and Pz-MTA sealer cement showing the interface at 350–400 μm distance from dentinal tubule orifice (×5,000). (F) The pre-crystallization seeds observed along the collagen fiber (arrowheads of (E), ×20,000). (G, H) A higher magnification of upper right boxed area of (E) showing successive intratubular biomineralization in either (G) plate-like form or (H) agglomeration of precipitation seeds along the collagen fibrils (×20,000). Scanning electron microscope images.

treatment outcome (Peng *et al*., 2007). The clinical radiographs with densely filled root canals do not support the biologically sealed root canal system from surrounding periapical tissue. In fact, several methodologies such as micro-computed tomography proposed to assess the sealing ability of root canal filling materials did not fully provide sufficient amount of information about the sealing of the root canal system. In this regard, confirmation of the dentinal tubule mineralization may provide the secondhand evidence showing the possible ultimate sealing of root canal system, and for this purpose, the present study was conducted to extrapolate and compare the biomineralization capacity of currently available Pz-MTA cement with enhanced clinical conveniences.

In the present study, the Pz-MTA sealer cement showed further intratubular biomineralization up to significantly deeper level of the tubules. This provides compelling evidence of Pz-MTA as a bioactive root canal sealer when it was coupled with core material (GP)

TABLE I Depths of material penetration into the dentinal tubules and intratubular mineralization (mean ± standard deviation)

Root canal obturation material	GP with Pz-MTA sealer	Pz-MTA sealer only	ProRoot MTA only
Tubular penetration depth (μm)	23.77 ± 2.48	Not detected	Not detected
Intratubular mineralization depth (μm)			
PBS pretreatment			
No	350.25 ± 36.50^{Ab}	62.55 ± 9.56^{Bb}	68.20 ± 11.20^{Bb}
Yes	392.69 ± 39.43^{Aa}	98.12 ± 14.45^{Ba}	130.51 ± 20.21^{Ba}

GP, gutta percha; MTA, mineral trioxide aggregate; PBS, phosphate buffered saline; Pz-MTA, pozzolan-derived mineral trioxide aggregate. Same uppercase alphabet superscripts in row show no significant differences among the mean values of experimental groups ($p > 0.05$). Same lowercase alphabet superscripts in column show no significant effect of PBS pretreatment on the mean values of intratubular mineralization within each group ($p > 0.05$).

and vertical condensation pressure. In addition, PBS pretreatment before final obturation enhanced intratubular mineralization of both Pz-MTA sealer- (110–150%) and ProRoot MTA- (130–190%) obturated canals. Although the intratubular mineralization depth of MTA was rather limited when compared to previous researches (Reyes-Carmona *et al*., 2009; Reyes-Carmona *et al*., 2010) in which the specimens were immersed continuously in regularly refreshed PBS solution, the strategic importance to boost biomineralization ability is the incorporation of phosphate ion as an initial precipitation seed. Preconditioning with the phosphate ions derived from PBS soaking sequence might have enhanced the nucleation formation, which is known as polymer-induced liquid precursor (PILP) process (Gower, 2008). Phosphate anions in PBS are considered to make the intratubular environment even more labile in PILP process, and enhance the formation of the prenucleation cluster and its subsequent crystal growth. The collagen fibers exposed after smear layer removal with EDTA pretreatment also might have been directed the crystallization process once it is infiltrated with the amorphous precursors. However, there have not been any reports on the clinical use and tubular biomineralization inducing ability of PBS as a final soaking solution before root canal obturation. In this regard, the results of our SEM analysis (Fig. 3) is the first report of biomineralized apatite confirmed at 350-400 μm level of dentinal tubules with variety of precipitate nanostructures, such as petals and flakes, in stratified or organized spherical form.

It is noteworthy that although bulk obturated materials were closely adapted to the canal wall, occasionally clogging the tubule orifices as previously reported (Bird *et al*., 2012), they could not penetrate into the tubules regardless of their different particle sizes. Rather, they showed the mineralized tag-like structures connecting the material-dentin interface at the orifice level. These structures are supposed to be the flocculated crystals formed on the material surface, which have been grown from the precursor–precipitation phase (Reyes-Carmona *et al*., 2009; Reyes-Carmona *et al*., 2010). In fact, precedent researches reported that such tag-like structures were the result of biomineralization potential of Portland cement and MTA, which could be enhanced by the interaction with phosphate-containing solution (Reyes-Carmona *et al*., 2009; Reyes-Carmona *et al*., 2010). However, the tags found in dentinal tubules of GP with Pz-MTA sealer group are the results of material penetration aided by the vertical condensation pressure transmitted via thermoplasticized gutta-percha, and are clearly distinct from such "tag-like structures" in the other groups.

Further, the small particle size would have contributed to induce more stable precursors for guiding an effective diffusion of the ions than the higher molecular weight particles of ProRoot MTA (Huang *et al*., 2008). The mean particle size for white ProRoot MTA is 10 μm, with all particles being smaller than 50 μm (Komabayashi and Spangberg, 2008). The slurry made from such aggregates of particles become rheopectic when orthograde-filled in root canals. In contrast, finely pulverized Pz-MTA cement, with a mean particle size of 1.5 μm, becomes thixotropic when the material is released via needle tip and further compressed by vertical pressure. It then infiltrates or grouts toward dentinal tubules to form sealer tags and apatite precursors for further intratubular biomineralization. Such stable precursors may induce the propagation of crystallization along the dentinal tubules by secondary nucleation among individual nanoparticles of the disordered phase, providing successive biomineralization densified into deeper tubules. In fact, ProRoot MTA treated teeth showed sparsely clogging discrete agglomerates in limited depth.

The crystallographs of Pz-MTA cement-induced precipitates were also notable. They were in various shapes, relatively smaller in size than those induced by ProRoot MTA, within elemental composition of Ca/P ratio similar to hydroxyapatite. Among the various apatite structures, the particles with a grain size less than 100 nm in at least one direction have higher surface activity and ultrafine structure, resulting in enhanced bioactivity than coarser crystals (Vallet-Regi and Gonzalez-Calbet, 2004). We could confirm the seamless flow of mineralization precipitates in GP with Pz-MTA sealer group, extended beyond 300 μm-depth regardless

of the PBS pretreatment. In that, Pz-MTA cement as a sealer presented a favorable biomineralization pattern for the sealing of root canal system, while the canals solely filled with Pz-MTA sealer or ProRoot MTA lacked such fine structures. The in-depth investigation on the correlation among crystallography and elemental composition of Pz-MTA cement-induced biomineralization of dentinal tubules requires further researches.

Within the limitations of this study, the use of Pz-MTA cement as a sealer in conjunction with well-fit gutta-percha cone and vertical pressure resulted in consistent dentinal tubule biomineralization. Preconditioning with PBS before root canal obturation promoted PILP process, and led to enhanced biomineralization of the dentinal tubules beyond the penetrated Pz-MTA sealer tag in various crystallographs. The Pz-MTA cement as a root canal sealer thus figuratively renders a new possibility of bio-tight sealing of the root canal system.

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Physical properties and biocompatibility of an injectable calcium-silicate-based root canal sealer: in vitro and in vivo study

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RESEARCH ARTICLE Open Access

Physical properties and biocompatibility of an injectable calcium-silicate-based root canal sealer: *in vitro* and *in vivo* study

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Abstract

Background: The aim of this study was to investigate the physical properties and biological effects of an experimentally developed injectable premixed calcium-silicate root canal sealer (Endoseal) in comparison with mineral trioxide aggregate (MTA) and a resin-based sealer (AHplus).

Methods: The pH, solubility, dimensional change, flow, and radiopacity of the materials were evaluated. Biocompatibility was evaluated on the basis of cell morphology and a viability test using MC3T3-E1 cells. For evaluate inflammatory reaction, the tested sealers were implanted into dorsal subcutaneous connective tissue of Sprague Dawley rats. After 7 days, the implants with the surrounding tissue were retrieved, and histological evaluation was performed.

Results: Endoseal showed high alkalinity similar to that of MTA. The solubility of the tested materials was similar. The dimensional change and flow of Endoseal was significantly higher than that of other materials ($P < 0.05$). The radiopacity of Endoseal was lower than that of AHplus ($P < 0.05$). The biocompatibility was similar to those of MTA. Inflammatory reaction of Endoseal was similar with that of MTA, but lower than that of AHplus ($P < 0.05$).

Conclusions: The present study indicates that Endoseal has favorable physical properties and biocompatibility. Therefore, we suggest that Endoseal has the potential to be used as a predictable root canal sealer.

Keywords: Injectable, Calcium silicate, Root canal sealer, Physical, Biological

Background

Endodontic sealers are used for the obturation of root canal systems in order to achieve a fluid-tight seal between the dentinal wall and core filling material throughout the entire canal [1]. A root canal sealer must demonstrate appropriate physicochemical and biological properties. Grossmann stated that an ideal root canal sealer should possess excellent sealing ability, dimensional stability, a slow setting time, insolubility, and biocompatibility [2]. There are many types of root canal sealers available in the endodontic market; resin-based sealers, zinc oxide-eugenol sealers, calcium hydroxide-containing sealers, glass ionomer-based sealers, and mineral trioxide aggregate (MTA)-based calcium-silicate sealers. All of the currently used sealer systems consist of a powder/liquid or base/catalyst, and the two components should be mixed at chairside and then applied to the root canal system. Recently, an injectable calcium-silicate-based root canal sealer (Endoseal; Maruchi, Wonju, Korea) that is preserved in an air-tight syringe and applied in the root canal by injection was developed (Fig. 1a). Interestingly, Endoseal sets slowly by itself without any mixing when exposed to air by absorbing the ambient moisture.

According to the manufacturer, this calcium-silicate cement is considered an MTA-derived material because it contains similar chemical elements as MTA. Therefore, it is expected to have favorable physical and biological effects like those of various MTA-derived materials demonstrated in previous studies [3–5]. Furthermore, many studies showed that MTA-derived root canal sealers have higher biocompatibility compared to resin-based sealers [6–9]. However, to our knowledge, there is little information regarding the self-setting calcium-silicate-based root canal sealer. Therefore, the aim of this study was to investigate the physical properties and biocompatibility of this root canal sealer in comparison with MTA (ProRoot; Dentsply, Tulsa, OK, USA) and a resin-based sealer (AHplus; Dentsply-De Trey, Konstanz, Germany).

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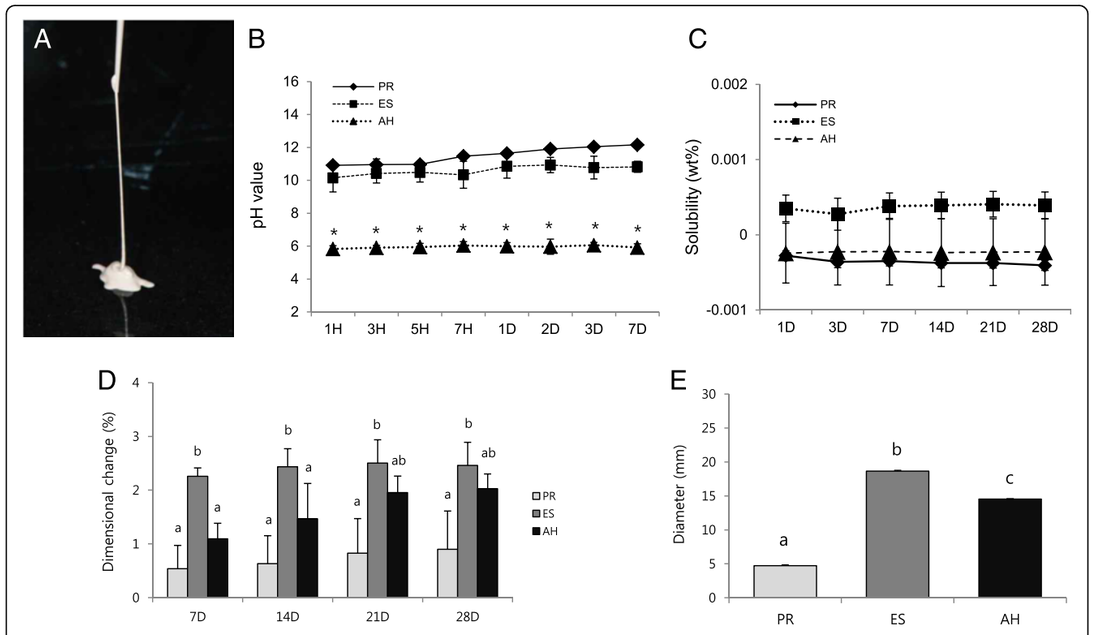

Fig. 1 Physicochemical properties of the tested materials. **a** The injectable calcium-silicate-based root canal sealer used in this study. **b** The changes in pH value during the experimental period. Groups identified by the same symbols were not significantly different in the same gene group ($P > 0.05$). Solubility (**c**), dimensional change (**d**), and **e** flow of the tested materials. Different letters/symbols represent significant differences between the different endodontic sealers ($P < 0.05$). *PR; ProRoot, ES; Endoseal, AH; AHplus*

Methods

Measurement of pH

The pH was measured according to the criteria used in a previously published study [10]. Specimens (1-mm thickness and 5-mm diameter) of the tested materials were prepared and allowed to set for 1 day ($n = 3$). After setting, one tablet was added to 10 mL of deionized water. Then, the pH value was measured using a pH meter (Orion 3 Star; Thermo Scientific, Singapore). The apparatus was previously calibrated with pH 7.0 and 4.0 solutions.

Evaluation of solubility

The solubility was measured by using the method recommended by ISO 6876/2012. Samples of each material were placed in a paraffin wax mold 1.5 mm thick and 20 mm in diameter ($n = 3$). Each sample was weighed using an analytical balance, and the weight was recorded as W_1. The samples were then immersed in tubes containing 10 mL of distilled water. Samples were removed at 1, 3, 7, and 14 days, dried with absorbent paper, and placed in a desiccator. The samples were dried to a constant weight (±0.001 g), which was recorded as W_2. The solubility (S) was calculated using the following formula: $S = (W_1 - W_2)/W_1 \times 100$.

Dimensional change

The dimensional change was measured by using the method recommended by ISO 6876/2012. Each material was placed into a cylindrical silicon mold with an internal diameter of 6 mm and a height of 12 mm ($n = 5$). After setting, we measured the distance between the flat ends (M1) to an accuracy of 10 μm by using a digital caliper (Absolute Digimatic, Mitutoyo, Kawasaki, Japan). The materials were then stored in distilled water at 37 ± 1 °C. After 7, 14, and 21 days, the distance (M_2) was re-measured to an accuracy of

10 μm. The test was carried out three times, and the mean change in length was recorded as the dimensional change (D) using the following formula: $D = (M_2 - M_1)/M_1 \times 100$.

Flow test

The flow was tested by using the method recommended by ISO 6876/2012. A total of 50 mg of sealer was placed onto a glass plate ($n = 3$). After 180 s, another glass plate was applied centrally on top of the material, to make a total mass on the plate of 120 g. Ten minutes after the application, the load was removed, and the average of the major and minor diameters of the compressed discs was measured using a digital caliper. The mean of three measurements for each sealer was taken as the flow of the material.

Radiopacity

The radiopacity was measured by using the method recommended by ISO 6876/2012. The specimens were placed on occlusal X-ray film (Kodak Insight, Rochester, NY, USA) along with an aluminum (99.5 % pure) step wedge with step heights ranging from 1 to 10 mm in increments of 1 mm ($n = 5$). A Kodak-2200 X-ray machine (Kodak) operating at 70 kV, 10 mA, 18 pulses/s and with a focus-sensor distance of 30 cm was used. After the films were developed, they were transformed into digital images (Fig. 2a) at a resolution of 300 dpi using a scanner. Then, the radiographic images were analyzed using a densitometer (GS-800; Bio-Rad, Hercules, CA, USA). In brief, we created a calibration curve for the aluminum step wedge, then the optical density of each specimen was expressed in terms of the equivalent thickness of the wedge in accordance with the following formula: $y = a\ln x + b$ (y: optical density, x: thickness of aluminum, 'a' and 'b': coefficients, ln: natural log value).

Preparation of material extracts

The tested material was placed into a paraffin wax mold (1-mm thickness and 5-mm diameter). After setting, the cement was removed from the mold and stored in 10 mL of minimal essential medium-α (MEM-α; HyClone Laboratories, Logan, UT, USA) containing 10 % fetal bovine serum (FBS; HyClone Laboratories) for 3 days.

Cell viability test

MC3T3-E1 cells were seeded in 24-well culture plates (SPL Life Sciences, Pocheon, Korea) at a density of 2×10^4 cells per well and pre-incubated in growth medium for 24 h ($n = 5$). Then, the cells were treated with the prepared extracts for 1, 3, 7, and 14 days. Cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, 200 μL of MTT solution (0.5 mg/ml in PBS) (Amresco, Solon, OH, USA) was added to each well, and the wells were incubated for 2 h. Subsequently, 200 μL of dimethyl sulfoxide (DMSO; Amresco) was added to each well. Reduced MTT was then measured spectrophotometrically at 540 nm in a dual-beam microtiter plate reader (SPECTROstar Nano; BMG Labtech, Ortenberg, Germany).

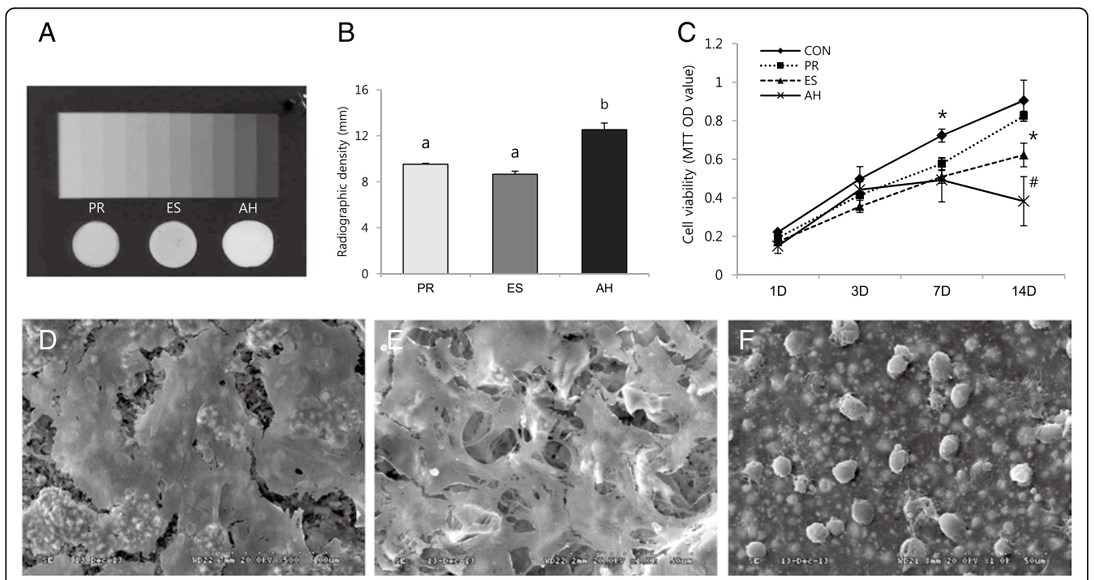

Fig. 2 Radiopacity and biocompatibility of the tested materials. **a** Radiograph showing the radiopacity of each material and its equivalence to that of the aluminum step wedge. **b** Relative radiographic density of each material in comparison with that of a 10-step aluminum step wedge. **c** Cell viability tested by the MTT assay. **d**-**f** SEM micrographs of MC3T3-E1 cells grown on ProRoot, Endoseal, and AHplus, respectively (×1000). Different letters/symbols represent significant differences between the different materials ($P < 0.05$). *PR; ProRoot, EC; Endoseal, AH; AHplus*

Cell morphological observations using SEM

Under aseptic conditions, materials were condensed into 1 × 5-mm round wax molds. The materials were allowed to set for 24 h in a humidified incubator at 37 °C. Then, the disks were placed at the bottom of 24-well tissue culture plates (SPL Life Sciences). MC3T3-E1 cells were seeded at 1×10^5 cells per well on the prepared materials. After a 72-h incubation period, the dishes were fixed with 2.5 % glutaraldehyde (Sigma-Aldrich, St. Louis, MO, USA) for 2 h. Samples were then dehydrated in increasing concentrations of ethanol (70 %, 80 %, 90 %, 95 %, and 100 %) for 20 min at each concentration and immersed in n-butyl alcohol (Junsei Chemical Co., Tokyo, Japan) for 20 min. SEM was performed using an SN-3000 system (Hitachi, Tokyo, Japan) operated at 10 kV.

Histological evaluation of inflammatory reaction

The inflammatory reactions of animal tissue to ProRoot, Endoseal, and AHplus were evaluated ($n = 6$). The sealers were inserted into sterile polyethylene tubes approximately 10 mm height and 3 mm in inner diameter. After setting, the materials were implanted in the Sprague Dawley rats' dorsal subcutaneous tissue. An empty tube was used as the negative control. In brief, the animals were anesthetized with 0.33 mL/100 g xylazine hydrochloride (Rompun, Bayer, Leverkusen, Germany) and 0.2 mL/100 g zolazepam (Zoletil 50; Virbac SA, Carros, France), followed by shaving of dorsal fur, disinfection, incision, and divulsion of the subcutaneous tissue to insert the testing materials. Each animal received 4 materials. The position in which each sealer was implanted was standardized. The incisions were closed using a 5–0 Vicryl suture material (Johnson & Johnson, Lenneke Marelaan, Belgium).

After 7 days, the animals were euthanized by CO_2 inhalation. An excisional biopsy of the implant area was performed with a safety margin of 1 cm. The samples were fixed in 4 % paraformaldehyde for 24 h, and the materials were removed from the samples. Then, the samples were set in paraffin blocks, and processed for histologic analysis. Sections with a thickness of 5 μm were stained with hematoxylin-eosin. Three representative sections were examined under a light microscope by a single-blinded, calibrated examiner. Quantitative evaluations of inflammatory cells (lymphocytes and polymorphonuclear leukocytes) were made in ten separate areas of sections at ×400 magnifications. An average value for each material was obtained from the sum of cells counted in ten separate areas. Inflammatory reactions were scored and evaluated according to the criteria used in a previously published study with slight modification as follows [11]; 0, none or few inflammatory cells and no reaction; 1, <25 cells and mild reaction; 2, between 25 and 125 cells and moderate reaction; 3, ≥ 125 cells and severe reaction. These experimental procedures were approved by the Institutional Animal Care and Use Committees (IACUC) of Chonbuk National University Hospital (Jeonju, Korea).

Statistical analysis

Statistical analysis was performed by one-way ANOVA followed by Tukey's test for physical properties, cell viability, and gene expression assay ($P = 0.05$). For histological evaluation, the data were evaluated using one-way nonparametric Kruskal–Wallis for a 5 % significance level.

Results

Measurement of pH, solubility, dimensional change, flow, and radiopacity

The pH values of ProRoot and Endoseal showed high alkalinity (pH between 10 and 12), whereas AHplus showed mild acidity around pH 6 (Fig. 1b). The solubility values of the tested materials were similar throughout the experimental period ($P > 0.05$) (Fig. 1c). As shown in Fig. 1d, the dimensional change of Endoseal was significantly higher than that of the other materials at all experimental time points ($P < 0.05$). The flow of Endoseal was significantly higher than that of other materials ($P < 0.05$) (Fig. 1e). The radiopacity of AHplus was higher than those of ProRoot and Endoseal ($P < 0.05$). However, all the materials evaluated presented the minimum radiopacity required by the ISO standard (Fig. 2b).

Biocompatibility

To evaluate cell viability in the presence of the material extracts, an MTT assay was performed. As shown in Fig. 2c, ProRoot showed significantly higher cell viability compared to the other groups on 14-day ($P < 0.05$). Further, the viability of Endoseal-treated cells was significantly higher than that of AHplus-treated cells on 14-day ($P < 0.05$). The cell growth and morphology on each material were evaluated by SEM. As shown in Fig. 2d and e, well-spread and flattened cells were observed in contact with the surfaces of ProRoot and Endoseal. On the contrary, round but dead cells were observed on the surface of AHplus (Fig. 2f). Moreover, in histological evaluation, inflammatory scores of ProRoot and Endoseal group were significantly lower than that of AHplus group ($P < 0.05$) (Fig. 3).

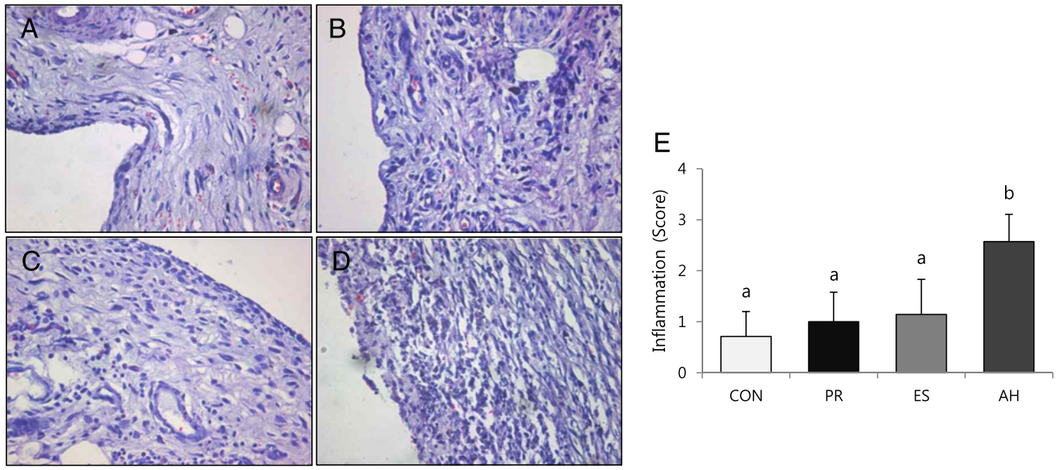

Fig. 3 Reaction of rat subcutaneous connective tissue to the tested sealers and the control group after 7 days (H & E staining, ×100); **a** control, **b** ProRoot, **c** Endoseal, **d** AHplus. **e** Mean and standard deviation of histological scores. Different letters represent significant differences between the different materials ($P < 0.05$)

Discussion

According to Grossman, an ideal root canal sealer should provide various physical properties [2]. Among them, we evaluated pH, solubility, dimensional change, flow and radiopacity. In our study, Endoseal showed high alkalinity (pH 10–11) similar to that of ProRoot (Fig. 1b). The base material of Endoseal is calcium-silicate with a chemical composition very similar to that of MTA. It is generally believed that MTA and its derivatives dissolve into calcium hydroxide when coming into contact with soft tissue, which results in a high pH [12]. The high pH of root canal sealers may provide several biological advantages. First, high pH of the sealer can promote hard tissue formation such as apical obliteration with calcified tissue [13]. Second, high sealer alkalinity changes the environment in the dentin to a more alkaline pH, possibly interfering with osteoclastic activity and promoting alkalinization in the adjacent tissues, which favors healing [14, 15]. Furthermore, there have been several studies demonstrated that calcium-hydroxide itself inhibited osteoclast activity by various molecular mechanisms [16–19]. Therefore, the high pH of Endoseal may exert an advantageous effect through the aforementioned mechanism compared to conventional resin-based sealers.

In the current study, water solubility of the tested sealers was evaluated because there is a strong link between sealer solubility and periapical reinfection [20]. In our study, the water solubility of Endoseal was the highest among the tested materials although there was no significant difference among the three experimental groups ($P > 0.05$) (Fig. 1c).

Dimensional change demonstrates the shrinkage or expansion of the material after setting. In this study, all the tested materials showed expansion. In previous reports, expansion was also verified for ProRoot and AHplus [21–23]. It is interesting to note that Endoseal expanded significantly more than the other tested materials ($P < 0.05$) (Fig. 1d). Slight expansion may contribute to superior sealing ability, but excessive expansion is undesirable when the material is employed as a root canal filling material as it may elicit cracks in the root [21]. Thus, further tests are required to ascertain if Endoseal effectively seals root canals without increasing the risk of development of cracks or root fracture.

Flow allows a sealer to penetrate into the irregularities and accessory canals of the root canal system [24]. In this study, Endoseal showed significantly higher flow values compared with AHplus ($P < 0.05$) (Fig. 1e). In this respect, Endoseal would have advantage in terms of penetrating into the ramifications and irregularities of root canal system than AHplus. The flow ability is generally influenced by the size of the sealer particles. According to the manufacture, Endoseal contains small particles of calcium-silicate cement to increase the flow. However, if the flow is excessive, the risk of sealer extrusion beyond apical foramen is increased, which could damage periodontal tissues or important anatomical structures such as inferior alveolar nerve or maxillary sinus [25]. Because Endoseal is injectable material which is susceptible to be extruded, clinicians should be careful not to try to fill whole root canal space with it. In this respect, further *in vitro* or *in vivo* study should be performed to conclude the adequate flow of Endoseal.

The addition of radiopaque agents to root canal filling materials should ideally enable their visualization and assessment on a radiograph without altering their chemical properties. According to the ISO standards, root canal sealing materials should be at least 3 mm in aluminum thickness. In the present study, the radiopacity of Endoseal was lower than that of AHplus ($P < 0.05$) (Fig. 2b). However, Endoseal showed much higher radiopacity (over 8 mm/Al) than that required by the ISO standards, similar to ProRoot and AHplus.

Endodontic sealers are often placed in close contact with periapical tissues. Thus, we investigated the biocompatibility of Endoseal in comparison with ProRoot and AHplus. As shown in Fig. 2c, the cell viability was also higher in cells treated with an extract of Endoseal than in cells treated with AHplus on 14-day ($P < 0.05$). However, the cell viability was significantly lower than that of ProRoot. Similarly, SEM observations in this study showed that the cells were attached and had proliferated on the surface of Endoseal and ProRoot, whereas dead cells were found on the surface of AHplus (Fig. 2d–f). These findings indicate that calcium-silicate-based Endoseal has higher biocompatibility compared to epoxy resin-based AHplus and permits adhesion and proliferation of cells.

We also investigated the tissue response to verify whether the materials induce inflammatory reaction *in vivo*. Several *in vivo* studies have shown that most of root canal sealers might induce inflammatory reactions when they contact with connective tissues intimately [26–29]. However, in this study, ProRoot and Endoseal did not show severe inflammatory reaction compared to control group. Calcium-silicate cements such as MTA is believed to induce less inflammation tissue reaction compared to other root canal filling materials [30–34]. In this respect, Endoseal, calcium-silicate cement, might show favorable tissue response comparable to ProRoot although it may contain various chemical ingredients.

We requested the chemical composition of Endoseal from the manufacturer in order to understand in detail the physical properties and biological effects determined in our experiments. According to the manufacturer, Endoseal contains various constituents including hydroxypropyl methylcellulose (HPMC), N-methyl-2-pyrrolidone (NMP), bentonite, bismuth oxide (Bi_2O_3), and zirconium oxide (ZrO_2). HPMC is a non-toxic thickening agent and can react vigorously with oxidizing agents. Use of viscosity agents is suggested for sealer development in order to penetrate into the complex root canal space. NMP is used as a solvent for various chemical agents but has been identified as a toxicant [35]. In this study, Endoseal showed significantly lower cell viability compared to ProRoot ($P < 0.05$) (Fig. 2d), and the presence of NMP in Endoseal might have affected this result. Bentonite is a useful adsorbent of ions in solution as well as fats and oils. It is the main active ingredient of fuller's earth, probably one of the earliest industrial cleaning agents. It is mainly recommended as an ingredient of preparations for dermatologic ointments because its colloidal nature confers detergent properties [36]. Therefore, bentonite is added to the formula to absorb moisture and contamination from the mixture. Bi_2O_3 and ZrO_2 are components in Endoseal that act as radiopacifiers and are widely used in MTA and other endodontic materials [37–39].

Conclusions

Collectively, the present study indicates that Endoseal has comparable physical properties to MTA, a biocompatible root-end filling material. In addition, Endoseal had favorable biocompatibility/odontogenicity compared to AHplus, a widely used resin-based sealer. Furthermore, this injection-type, self-setting root canal sealer has a clinical advantage in terms of dentist-friendly application. Therefore, within the limitations of this study, we suggest that Endoseal has the potential to be used as a predictable root canal sealer.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
Min KS, Shon WJ and Lee KW contributed to planning and designing the study, in the data analysis and submission of the manuscript. Lim ES performed most of the laboratory work. Park YB and Kwon YS performed the animal study. All authors have read and approved the final manuscript.

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Tooth discoloration induced by a novel Mineral Trioxide Aggregate-based root canal sealer

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Original Article

Tooth discoloration induced by a novel mineral trioxide aggregate-based root canal sealer

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ABSTRACT

Objectives: The aim of this study was to evaluate tooth discoloration caused by contact with a novel injectable mineral trioxide aggregate (MTA)-based root canal sealer (Endoseal; Maruchi, Wonju, Korea) compared with a widely used resin-based root canal sealer (AHplus; Dentsply De Trey, Konstanz, Germany) and conventional MTA (ProRoot; Dentsply, Tulsa, OK, USA). **Materials and Methods:** Forty standardized bovine tooth samples were instrumented and divided into three experimental groups and one control group (n = 10/group). Each material was inserted into the cavity, and all specimens were sealed with a self-adhesive resin. Based on CIE Lab system, brightness change (ΔL) and total color change (ΔE) of each specimen between baseline and 1, 2, 4, and 8 weeks were obtained. **Results:** At all time points, Endoseal showed no significant difference in ΔL and ΔE compared to AHplus and control group ($P > 0.05$), whereas the ProRoot group showed significantly higher ΔL and ΔE values than the Endoseal group at 2, 4, and 8 weeks ($P < 0.05$). Therefore, Endoseal showed less discoloration than conventional MTA and a similar color change to AHplus. **Conclusions:** Within the limitations of this study, our data indicate that the MTA-based sealer produces a similar amount of tooth discoloration as AHplus which is considered to be acceptable.

Key words: Mineral trioxide aggregate, root canal sealer, spectrophotometry, tooth discoloration

INTRODUCTION

Root canal sealers are generally used in combination with Gutta-percha to seal the root canal system. These materials are categorized according to their main chemical composition such as zinc oxide eugenol, calcium hydroxide, epoxy resin, or glass ionomer.[1] As the root canal sealers are put into the canal during the filling procedure, there is a possibility that some portion of the filler remains smeared in the coronal access cavity despite cleaning with alcohol pellets or careful preparation of the cavity. Therefore, it is important to predict how much discoloration would occur if root canal sealer is left in the access cavity. Furthermore, the color of the root canal sealer itself may produce tooth discoloration. This discoloration may be seen in the cervical third of the crown where the overlying enamel, which is a translucent and colorless structure, is thinner.[2] Therefore, improper coronal extension of the root canal filling above the gingival margin should be avoided.

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Mineral trioxide aggregate (MTA) is a useful material that was first introduced for the purpose of root-end filling.[3] Numerous *in vitro* and *in vivo* studies have confirmed its superior properties such as biocompatibility, bioactivity, and sealability.[4-6] Therefore, MTA has been advocated for use in various clinical procedures including pulp capping, pulpotomy, apexification, and perforation repair. Furthermore, there have been attempts to use MTA as a root canal filling material and some MTA-based products have been introduced into the endodontic market. Regardless of the composition, most of the currently used sealer systems consist of a powder/liquid or base/catalyst and these two components must be mixed at chairside and then applied to the root canal coated with Gutta-percha. During this procedure, the sealer may contaminate the pulp chamber and any remaining sealer may induce tooth discoloration.

Recently, a novel root canal sealer based on MTA (EndoSeal; Maruchi, Wonju, Korea) has been developed in an attempt to introduce the useful features of MTA into the root canal sealer. Endoseal is a premixed and injectable endodontic sealer that uses moisture in the air to initiate the setting reaction [Figure 1]. Consequently, it sets slowly by itself without any mixing procedure. A recent study indicates that Endoseal has comparable physical properties to MTA and superior biocompatibility compared to AHplus.[7] However, many studies show that MTA, which is mainly composed of calcium silicate and bismuth oxide, has discoloration potential.[8-10] Naik and Hegde reported that when MTA was used for pulpotomy in primary molars, discoloration occurred in 60% of all cases.[11] Belobrov and Parashos also presented a case report of a complicated crown fracture treated by partial pulpotomy with white MTA that resulted in tooth discoloration.[12] Therefore, when dealing with any MTA-based sealer the potential of discoloration of the tooth cannot be excluded. However, limited information is available regarding the effect of this new root canal sealer on tooth discoloration. The purpose of this *in vitro* study was to evaluate the tooth discoloration effect of Endoseal in comparison with a commonly used root canal sealer (AHplus) and conventional MTA (ProRoot). The null hypothesis was as follows: There is no difference between the tested materials regarding tooth discoloration.

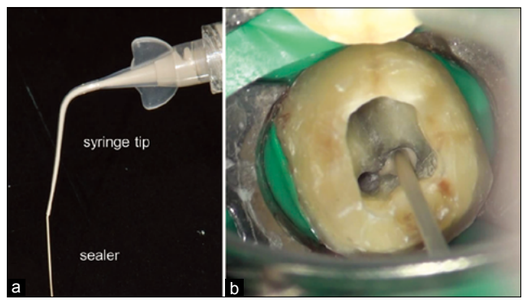

Figure 1: Endoseal (a) and its clinical application (b)

MATERIALS AND METHODS

Sample preparation

A total of 40 intact bovine incisors were used. Exclusion criteria were the presence of caries, coronal staining, observable structural defects, and narrow crown width and height (each should be longer than 10 mm). The samples were prepared as shown in Figure 2a with reference to the model introduced in previous studies.[8,13] In brief, bovine teeth were disinfected in 1% chloramine-T solution (Sigma-Aldrich, St. Louis, MO, USA) and stored in normal saline at room temperature for 30 days. After resection of the roots with a diamond-coated disc, an ultrasonic scaler was used to remove the extrinsic stains and calculus on the coronal labial surface.

Using a microtome (ISOMET; Buehler, Lake Bluff, IL, USA), a cuboid enamel-dentin block (10 mm × 10 mm × 3.5 mm) was obtained from the middle third of each crown. The labial enamel surface was finished and polished with successive use of 220, 600, 1200, and 2000 grit abrasive papers (CC261; DEERFOS, Seoul, Korea). A box-form cavity

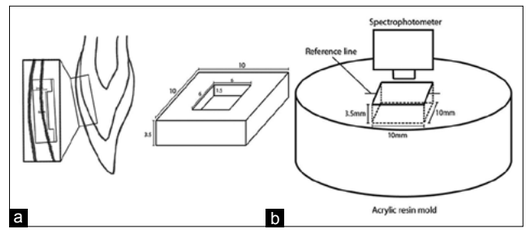

Figure 2: (a) Standardized cuboid enamel-dentin block prepared by removing the middle third of a bovine incisor. (b) Standardized acrylic resin mold used to repeatedly measure the same position in each sample

(6 mm × 6 mm × 1.5 mm) was prepared with a diamond bur in the middle of each specimen, leaving approximately 2 mm thickness of the labial tooth structure (1 mm each of enamel and dentin). Solutions of 2% sodium hypochlorite (NaOCl) and 17% ethylene diamine tetraacetic acid (EDTA; PrevestDentpro, Jammu, India) were applied to the specimens for 30 min and 2 min, respectively. A final rinse was performed with 1% NaOCl and saline. All specimens were stored at room temperature and 100% relative humidity.

Experimental and control groups

The specimens were randomly assigned to three experimental groups and one negative control group (n = 10). Each material was mixed according to the manufacturers' instructions and placed into the tooth cavity of the relevant group; nothing was placed in the cavity for the control group. A resin material (RelyX Unicem; 3M ESPE, Seefeld, Germany) was used to seal all of the cavities. All specimens were stored at room temperature and 100% relative humidity.

Tooth color measurement

A standardized acrylic resin mold was constructed for measurement with a spectrophotometer (Color i5; GretagMacbeth, Martinsried, Germany) [Figure 2b]. This mold allowed each specimen to be measured in the same position each time. The sample was positioned in the mold and the spectrophotometer was adjusted to the reference line. The tooth color measurement was taken at baseline (W_0; immediately after tooth preparation and placement of materials) and at 1 (W_1), 2 (W_2), 4 (W_4), and 8 weeks (W_8) with a spectrophotometer. All measurements were repeated three times and averaged.

The difference in brightness (ΔL) at each time point was calculated by subtracting the corresponding L value from the baseline L value. The color difference (ΔE) between the baseline and the W_1, W_2, W_4, and W_8 measurements was calculated using the following formula:

$$\Delta E = ([L^*2 - L^*1]^2 + [a^*2 - a^*1]^2 + [b^*2 - b^*1]^2)^{1/2}$$

where L^* represents the degree of lightness and ranges from 0 (black) to 100 (white), a* represents degree of greenness (negative a^*) or redness (positive a^*), and b^* represents degree of blueness (negative b^*) or yellowness (positive b^*).[14]

Statistical analysis

SPSS software (SPSS 12.0K for Windows; SPSS Inc., Chicago, IL, USA) was used to evaluate the data. One-way analysis of variance and Tukey's *post hoc* test were used to evaluate significant differences between the tested materials at each time point (P = 0.05).

Stereomicroscopic examination

A representative sample was randomly selected for each group and sectioned horizontally at 1 mm thickness with a low-speed microtome (ISOMET). The slice in the center of the sample was selected and the cross section was examined under a stereomicroscope (Leica MZ16FA; Leica, Wezler, Germany).

RESULTS

The tooth color measurement data are summarized in Figures 3 and 4. The Endoseal group showed a similar amount of brightness change (ΔL) and color change (ΔE) as the AHplus group. At 1 week, the ProRoot group showed significantly higher ΔL compared to the ES group (P < 0.05). However, the AHplus and control group showed no significant difference from the other groups (P > 0.05). At 2 weeks, the ΔL and ΔE values of the ProRoot group increased and as a result, the ProRoot group showed a significant difference from all the other groups (P < 0.05). At 4 weeks, the ProRoot group still showed a significantly higher ΔL and ΔE than all other groups (P < 0.05), whereas the Endoseal group showed no significant difference from the AHplus and control group (P > 0.05). At 8 weeks, the ProRoot group showed significantly higher ΔL and ΔE than the Endoseal and control group (P < 0.05). The Endoseal group was not significantly different from the AHplus and control group for both ΔL and ΔE (P > 0.05). On stereomicroscopic examination, a dark discolored area was shown in dentin in contact with ProRoot, but not in any other group [Figure 5].

Overall, whereas ProRoot tended to show the greatest brightness or total color change, the change in the Endoseal group tended to remain relatively low, comparable to that in the AHplus and control groups. At all time points, Endoseal showed no significant difference from the control group in both brightness difference and total color difference. Endoseal also showed no significant difference from the AHplus group at all time points. Therefore, Endoseal shows a similarly small amount of tooth discoloration to AHplus, and was comparable to the control where no sealer was applied on the cavity.

DISCUSSION

Although MTA has favorable physical and biological properties, attempts to insert MTA as a root canal sealer have been hampered by its poor manipulability. Recently, a premixed, injectable endodontic sealer (Endoseal) was introduced to the endodontic field. This injectable MTA-based sealer is preserved in an airtight syringe and applied into the root canal by injection. Consequently, clinicians can easily apply the sealer directly into the root canal without contaminating the access cavity. Furthermore, it was recently demonstrated that Endoseal has favorable physical characteristics and biocompatibility.[7] However, a controversy arose regarding tooth discoloration because the base material of Endoseal is MTA, which is known to induce discoloration.

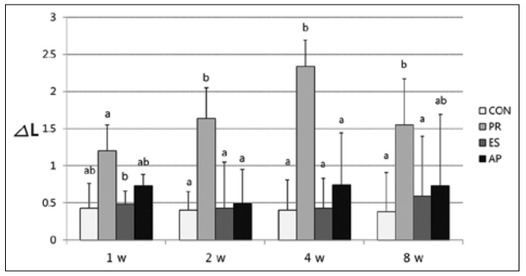

Figure 3: ΔL values (mean ± standard deviation) for each group at five different time points. The same letters indicate no significant difference between the groups (Tukey test, P = 0.05). CON: Control, PR: ProRoot, ES: Endoseal, AP: AHplus

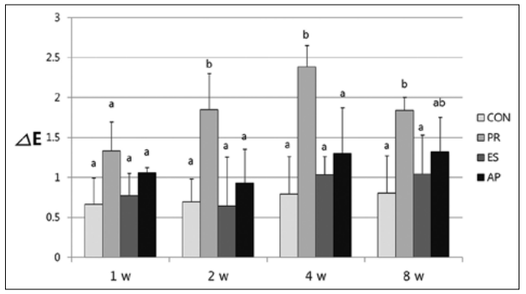

Figure 4: ΔE values (mean ± standard deviation) for each group at five different time points. The same letters indicate no significant difference between groups (Tukey test, P = 0.05). CON: Control, PR: ProRoot, ES: Endoseal, AP: AHplus

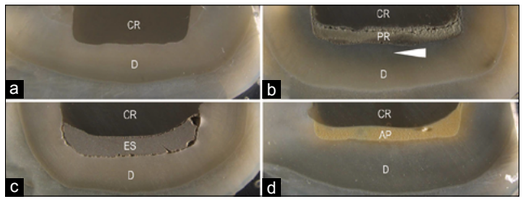

Figure 5: Stereomicroscopic appearance of a representative sample from each group after 8 weeks. (a) Control, (b) ProRoot, (c) Endoseal, and (d) AHplus. CR: Composite resin, D: Dentin, PR: ProRoot, ES: Endoseal, AP: AHplus. The white arrow indicates a discolored area induced by ProRoot

In the present study, Endoseal showed significantly lower ΔL and ΔE values compared to ProRoot, a widely used conventional MTA (P < 0.05). Furthermore, Endoseal did not show any difference in ΔL and ΔE compared with AHPlus, and even with the control (P > 0.05). Consequently, our null hypothesis was rejected. Several mechanisms of the discoloration induced by MTA have been proposed. The first was that the gray color of the material itself is responsible for the discoloration.[15] To address this concern, white MTA was introduced into the endodontic market; however, several reports indicated that even white MTA induces tooth discoloration.[12,15,16] It was also proposed that metal oxides (Fe, Mn) could be responsible for the discoloration.[17] Another suggestion is that the discoloration is due to chemical interaction of bismuth oxide (Bi_2O_3) with dentin.[18,19] Bi_2O_3 is added to MTA to provide radiopacity.[20] The discoloration induced by MTA is attributed to its progressive mass darkening due the presence of reduced black crystals of bismuth atoms.[21,22] Among possible alternatives to Bi_2O_3, zirconium oxide (ZrO_2) was investigated as a candidate because of its adequate radiopacity and cost-effectiveness. Recent studies showed that a ZrO_2-containing MTA induced less discoloration than MTAs containing Bi_2O_3.[13,23] According to the manufacturer, Endoseal contains both Bi_2O_3 and ZrO_2 as radiopacifiers. It can be postulated that although Endoseal still has Bi_2O_3 as a constituent a considerable amount of Bi_2O_3 has been substituted by ZrO_2 and as a result Endoseal showed little tooth discoloration in our study, comparable to that of AHplus. AHplus, a resin-based sealer, showed less discoloration than ProRoot, as expected (P < 0.05). In fact, AH26, an early version of AHplus, is well known to induce tooth darkening and is not recommended when aesthetics are important.[24,25] This can be explained by the fact that AH26 contains Bi_2O_3 as a filler and radiopacifier whereas AHplus contains ZrO_2. Taken together, these findings indicate that Bi_2O_3 can be considered a major cause of tooth discoloration, and it is best to avoid adding this radiopacifier to root canal sealers.

In the analysis of L^*, a^*, b^* data, it was evident that a and b values were not affected by the application of root canal sealer. This may mean that the discoloration induced by sealers is not relevant to red/green color tendency or to yellow/blue tendency; rather, the discoloration induced by sealers seems to only

influence the lightness of the tooth. Since *a*, *b* values remained relatively stable over all time points, Δ*L* values are directly proportional to Δ*E* values.

In this study, we used bovine incisors to evaluate discoloration because of their many advantages over human teeth. First, we could easily obtain a sufficient number of intact bovine incisors. Second, the number of dentinal tubules per mm^2 and diameter of tubules in coronal dentin of bovine incisors are similar to those of human teeth.[26] Moreover, bovine incisors are wide enough to easily obtain standardized tooth samples. Although bovine incisors are widely used as specimens for *in vitro* studies, there are still limitations to their use and further investigations using human incisors are recommended.

CONCLUSIONS

Within the limitations of the present study, we conclude that a novel MTA-based root canal sealer, Endoseal, showed discoloration that is comparable to that of AHplus, and significantly lower than that with ProRoot. Although Endoseal appears to have little effect on tooth discoloration, further studies should be conducted to confirm its long-term color stability.

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Conflicts of interest
There are no conflicts of interest.

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05

Push-out bond strength of injectable pozzolan-based root canal sealer

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Push-out Bond Strength of Injectable Pozzolan-based Root Canal Sealer

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Abstract

Introduction: The present study aimed to rank the bond strength to root dentin of a new injectable pozzolan-based root canal sealer, EndoSeal MTA, as compared with MTA Fillapex and AH Plus. **Methods:** Eighteen dentinal slices (1 ± 0.1 mm) were obtained from the middle third of 6 maxillary incisors previously selected. Three canal-like holes with 0.8 mm diameter were drilled perpendicularly on the axial surface of each slice. Then, a standardized irrigation was applied for all holes that were subsequently filled with 1 of 3 test root canal sealers. After that, slices were stored in contact with phosphate-buffered saline solution (pH 7.2) for 7 days at 37°C before the push-out assay. Friedman test and Wilcoxon signed rank test with a Bonferroni correction were used to rank the results. Significance boundary was set at α = 5%. **Results:** Friedman test confirmed a significant dissimilarity in push-out ranks among the tested cements ($P < .01$). Wilcoxon signed rank test demonstrated AH Plus had significant superior resistance to dislodgment compared with Endo Seal ($P < .01$) or MTA Fillapex ($P < .01$), whereas MTA Fillapex presented the lowest push-out values as compared with Endo Seal ($P < .01$) or AH Plus ($P < .01$). **Conclusions:** EndoSeal presents satisfactory bond strength performance for application in endodontic therapy compared with MTA Fillapex, and although it displays a new alternative of injectable bio-tight root canal sealer, it is not able to improve adhesion compared with AH Plus. *(J Endod 2016;42:1656–1659)*

Key Words

MTA, pozzolan, push-out, root canal obturation, root canal sealer

Significance

EndoSeal MTA was previously demonstrated to induce dentinal tubule biomineralization, favorable cytocompatibility, and superior sealer distribution, as well as satisfactory biological and physical properties. The present finding adds to EndoSeal satisfactory bond strength performance for application in endodontic therapy and increases the overall knowledge about a material that has potential to become a clinical alternative of injectable bio-tight root canal sealer.

Mineral trioxide aggregate (MTA) is a Portland cement–derived hydraulic material that has been widely used in a variety of applications in endodontics as supported by a broad body of evidence (1, 2). MTA has excellent physical and biological properties such as biocompatibility, bioactivity, and sealing ability (1, 3). This sealing capacity is largely attributed to MTA's bioactivity and ability to release calcium ions and produce an apatite layer in the presence of phosphate-containing physiological fluids (4–7). The crystalline deposits produced by the interaction of MTA and physiological fluids positively influence the push-out bond strength of MTA. Conversely, MTA cement fails to present the physical properties required for a root canal sealer, which leads to increasing efforts to create an ideal MTA-based sealer with remarkable balance between its biological and physicochemical characteristics (1, 3, 8).

EndoSeal MTA (Maruchi, Wonju, Korea), a pozzolan-based MTA sealer, was recently introduced. It consists of a premixed and pre-loaded material confined into an air-tight syringe that permits its direct application into the root canals. During the injection, EndoSeal absorbs the environmental moisture from atmospheric air and sets without the need of previous powder/liquid or base/catalyst mixing (9, 10). This sealer contains pozzolan cement, which gets cementitious properties after the pozzolanic reaction with calcium hydroxide and water, allowing efficient flow of the pre-mixed substrate with adequate working consistency and reduced setting time (4, 11). The incorporation of small particle pozzolan cement, which is a mineral aggregate with watery calcium silicate hydration, resulted in fast-setting MTA without the addition of a chemical accelerator (4, 12).

Previous reports have demonstrated the capacity of EndoSeal MTA to induce dentinal tubule biomineralization (11), satisfactory biological and physical properties (10), favorable cytocompatibility (13), and superior sealer distribution (9). To date, however, no study has ranked the push-out bond strength of EndoSeal. Therefore, the present study aimed to investigate the bond strength to root dentin of this new injectable pozzolan-based root canal sealer by using MTA Fillapex (Angelus, Londrina, Brazil) and AH Plus (Dentsply, DeTrey, Konstanz, Germany) as control materials for comparison. The following null hypotheses were tested:

1. There is no difference in dentin bond strength between the tested root canal sealers.
2. There is no difference in bond strength to root dentin between both MTA-derived materials (EndoSeal and MTA Fillapex).

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Materials and Methods

Sample Size Calculation

According to a previous study (14), an effect size of 0.74 was added to power β = 95% and α = 5% inputs into F test family for repeated-measures analysis of variance (G*power 3.1 for Macintosh). A total size of 9 slices samples was necessary to identify differences among the tested materials.

Sample Preparation

Local Ethics Committee approved the study. Six maxillary incisors were selected and cleaned by removing the hard deposits and the soft tissues with the aid of curettes and 5.25% NaOCl immersion for 10 minutes. After that, coronal and apical segments were removed from each tooth to obtain the middle third. Three horizontal cross sections (1 ± 0.1 mm thick) were obtained from this segment by using a low-speed saw (ISOMET; Buehler Ltd, Lake Buff, IL) with a diamond disk (Ø 125 × 0.35 × 12.7 mm; Buehler Ltd) under continuous water irrigation. The final thickness of each slice was checked with the aid of a digital caliper with accuracy of 0.001 mm (Avenger Products, North Plains, OR). Eighteen root slices were produced following this protocol (Fig. 1).

Preparation of Canal-like Holes for the Push-out Assay

Samples were drilled by using a 0.8-mm cylindrical carbide bur. Three canal-like holes were made parallel to the root canal in each root slice. The perforations were performed under constant water irrigation by using a vertical drill stand (Dremel Workstation 220, Mount Prospect, IL) to standardize the holes drilled perpendicular to the surface. A minimum distance of 1 mm was established between the holes drilled, the external cementum, and the root canal walls.

Thereafter, all samples were immersed in 2.5% sodium hypochlorite (NaOCl) solution for 15 minutes and further immersed for 1 minute in bidistilled water to neutralize the NaOCl solution. The smear layer was removed by using 17% EDTA for 3 minutes, bidistilled water for 1 minute, 2.5% NaOCl for 1 minute, and a final flush with bidistilled water for 1 minute. The holes were dried with absorbent paper points, and each of the 3 holes of every root slice was filled with 1 of the selected materials: EndoSeal, MTA Fillapex, or AH Plus. All the materials were mixed according to the manufacturers' instructions and were delivered into the holes. Bubble formation was avoided by gentle vibration while placing the material. Finally, the filled root slices were stored in contact with phosphate-buffered saline solution (pH 7.2) at 37°C for 7 days before the push-out assessment (Fig. 1).

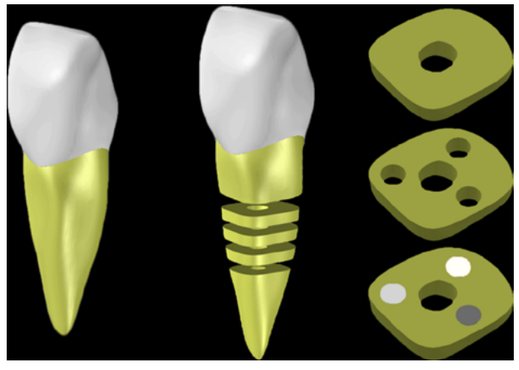

Figure 1. Schematic representation of sample preparation.

Push-out Assessment

A plunger tip of 0.6-mm diameter was set up over the tested material, avoiding touching the surrounding dentin wall. Loading was performed on a universal testing machine (EMIC DL200 MF, São José dos Pinhais, PR, Brazil) at a head-speed of 0.5 mm/min^{-1} until the displacement of the material. The load was applied only in a coronal-apical direction. A load × time curve was plotted during the test by using a real-time software program. The maximum load at failure, recorded in newtons, was divided by the area of the bonded interface, resulting in a bond strength expression in MPa^2. The adhesion area of the root canal material was calculated by using the following formula: area – $2\pi r \times h$, where π = the constant 3.14, r = radius of the cavity with the root canal material (0.4 mm), and h = height of the material (1.0 mm)[3].

Data Presentation and Analysis

After testing for data skewing (Shapiro-Wilk test, $P < .05$), the push-out from paired artificial holes was ranked by using a nonparametric Friedman procedure. Multiple comparisons were performed with the aid of a Wilcoxon signed rank test with Bonferroni correction. Significance boundary was set at α = 5% (SPSS 17.0; SPSS Inc, Chicago, IL).

Results

Friedman test confirmed a significant dissimilarity in push-out ranks among the tested cements ($P < .01$). Wilcoxon signed rank test demonstrated AH Plus had significant superior resistance to dislodgment compared with Endo Seal ($P < .01$) or MTA Fillapex ($P < .01$), whereas MTA Fillapex presented the lowest push-out values as compared with Endo Seal ($P < .01$) or AH Plus ($P < .01$). Figure 2 displays a graphic representation of the findings.

Discussion

The use of endodontic sealers during root canal obturation yields the sealing between gutta-percha and dentinal walls, acting against bacterial leakage that may lead to endodontic failures (15, 16). Every currently available root canal sealer presents limitations regarding the ideal properties of an endodontic sealer (17). Therefore, new sealers are constantly being developed, especially calcium silicate–based or bioceramics materials—MTA and BioAggregates—because of their biological and sealing properties (3, 8, 18). A relevant physical aspect of a newly developed injectable pozzolan-based sealer (EndoSeal MTA), the dentinal bond strength, was ranked in comparison with MTA Fillapex and AH Plus.

The first null hypothesis was not accepted because a significant difference in push-out was observed among the materials. Both MTA-based sealers, EndoSeal and MTA Fillapex, produced inferior dentinal bond strength values compared with AH Plus. The present result is in accordance with several previous studies in which AH Plus was also associated with significantly higher bond strength values when compared with other sealers (19–23). The superior bond strength property of AH Plus may be largely attributed to the capacity of

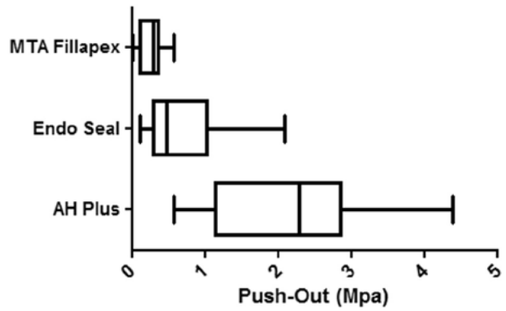

Figure 2. Horizontal box plot of the central tendency push-out values displayed by the 3 cements. Significant difference among the 3 tested sealers was detected by Friedman and Wilcoxon signed rank tests ($P < .05$).

producing a covalent bond by mixing diepoxide compounds and polyamine paste during its manipulation so that each amine group in the collagen network reacts with an open epoxide ring, resulting in a heavily cross-linked polymer that is rigid and strong (24, 25). In addition, low polymerization stress, long-term dimensional stability, and efficient cohesion between molecules had already been reported to increase root dentin micro retention of AH Plus (19, 20, 24).

EndoSeal displayed significantly superior bond strength performance than MTA Fillapex. Hence, the second null hypothesis was also rejected. The chemical composition of a root canal sealer has substantial impact over adhesion capacity. The base material of EndoSeal is calcium silicate, which presents a similar chemical composition to that of MTA cement. Hence, it is expected that EndoSeal would display comparable biological and physical properties to this cement. Indeed, recent studies confirmed that EndoSeal produces consistent dentinal tubule biomineralization beyond the sealer tag (11) and presents biocompatibility, solubility, radiopacity, and high alkalinity in similar levels as commercially available MTA (10, 13). In this context, it seems that EndoSeal contains a significant percentage of MTA that yields bioactivity and may explain its satisfactory bond strength results. In addition, according to the manufacturer, EndoSeal presents outstanding flowability, which may be related to the sealer penetration into dentinal tubules, anatomic irregularities, or accessory canals and thus results in increase in sealing ability and bond strength. EndoSeal also has excellent dentinal wall distribution (9) that may result from its injection-type, self-setting use that has a clinical advantage in terms of application. It is important to point out that it was not possible to compare the values of dentin bond strength of EndoSeal with previous results because this is a pioneer evaluation of this subject.

MTA Fillapex produced the worst results in the present study. This is in agreement with previous research that also observed poor bond strength values for MTA Fillapex (16, 22, 23). This is a paste-to-paste sealer that, whenever mixed, establishes 2 important chemical reactions that are responsible for the material's setting and physical-mechanical characteristics, the progressive hydration of the orthosilicate ions and the reaction between MTA and salicylate resin (26). A 1:1 ratio of MTA:-salicylate resin would be required to achieve an appropriate setting. This allows the formation of an ionic polymer containing calcium silicate particles that reacts with water (1, 8, 27). The final reaction leads to the formation of calcium hydroxide and a nanoporous amorphous calcium silicate hydrate gel that polymerizes and creates a solid network (1, 27). However, it has been speculated that MTA Fillapex displays a higher ratio of salicylate resin than MTA, which affects the chemical reaction among these components and explains the extended setting time of this material (8). During setting time, endodontic sealers with salicylate resin experience an initial volumetric shrinkage that increases the contraction factor (28). Hence, higher amounts of salicylate resin associated with extended setting time increase dimensional changes and formation of gaps between root canals and filling materials. This affects the bond strength of a root canal sealer both directly and indirectly because it may interfere with other properties such as flowability and solubility that are also associated with dentinal wall sealing. Therefore, the extended setting time consequent of an unbalanced resin/MTA ratio may explain the unenthusiastic MTA Fillapex results regarding push-out bond strength.

Calcium silicate–based sealer bioactivity represents the spontaneous production of an apatite layer, dentin intratubular calcium, and silicon ions incorporation (29), as well as an intrafibrillar apatite deposition (5) and production of tag-like structures at sealer-dentin interface (5, 6, 29) that lead ultimately to dentin remineralization (30) when in contact with phosphate-containing physiological fluids (27, 31). Apatite nucleation resulting from bioactivity processes improves the displacement resistance of filling materials by producing a micromechanical bonding system to dentin that decreases gaps at the interface (7). The ability to nucleate calcium phosphate is strictly correlated to environmental conditions, the material's chemical composition, and calcium-releasing properties (32). Although MTA Fillapex is an MTA-based sealer, the particle size and composition of several types of MTA differ. MTA Fillapex contains higher amounts of resins and less than 20% MTA particles, which may be an insufficient proportion to demonstrate the full biological and sealing characteristics of this cement (25, 33). Furthermore, MTA Fillapex has bismuth oxide as the radiopacifier, which is associated with increase in deterioration of mechanical strength, porosity, and material degradation, which was mostly replaced by zirconium oxide in EndoSeal (13, 34).

The present study was set to improve the reliability of the push-out model proposed. To reduce confounding factors such as mineralization and tooth age and hardness (14, 35), the same dentin source was provided to all root canal sealers. A cylindrical final shape was established for all canal-like holes, providing the standardization of size and internal root canal anatomy that ultimately improves biological standardization of the holes at the baseline that allows a paired mathematical evaluation to be taken, which reduces the amount of samples required for a test. The root canal space was filled only with the sealer to obtain an application of the compressive load over the sealer without the presence of a resilient gutta-percha material and to provide better control of the type of failure mode that could lead to misinterpretations of the results.

On the basis of the results, EndoSeal MTA presents superior bond strength performance compared with MTA Fillapex, although it is not comparable to a traditional epoxy resin–based sealer and thus may be taken as a new alternative of injectable bio-tight root canal sealer.

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The authors deny any conflicts of interest related to this study.

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06

Cytocompatibility of calcium silicate-based sealers in a three-dimensional cell culture model

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ORIGINAL ARTICLE

Cytocompatibility of calcium silicate-based sealers in a three-dimensional cell culture model

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Abstract
Objectives The aim of the present study was to evaluate cytotoxic effects and cytokine production of calcium silicate-based sealers (EndoSeal, EndoSequence BC Sealer, and MTA Fillapex) using an in vitro root canal filling model and three-dimensional (3D) cell culture. AH Plus as a reference was compared to contemporary calcium silicate cements regarding cell viability and cytokine production.
Material and methods Root canals of 30 human maxillary incisors were prepared using a single-file reciprocating technique. The samples were randomly distributed and canals filled with either AH Plus, EndoSeal, EndoSequence BC Sealer, and MTA Fillapex (n = 6). In the negative control group, the root canal remained unfilled. Sealers were placed into the canals along with a gutta-percha cone placed to working length. Balb/c 3T3 fibroblasts, cultured in a type I collagen 3D scaffold, were exposed to filling material and the respective root apex for 24 h. Cytocompatibility of the materials was evaluated using the methyl-thiazoldiphenyl-tetrazolium (MTT) assay. The production of IL-1β, IL-6, and IL-8 was analyzed using enzyme-linked immunosorbent assay (ELISA). One-way analysis of variance was performed, and when the F-ratios were significant, data were compared by Duncan's multiple-range test. The alpha-type error was set at 0.05.
Results EndoSeal, Endosequence BC Sealer and AH Plus showed cell viability that was similar to the negative control group ($P > 0.05$), while MTA Fillapex sealer was cytotoxic ($P < 0.05$). Varying production of IL-1β, IL-6, and IL-8 was detected in all samples.
Conclusions In an in vitro root canal filling model with 3D cell culture, AH Plus, EndoSeal, and EndoSequence BC Sealer were cytocompatible.
Clinical relevance These results may suggest that AH Plus, EndoSeal and EndoSequence BC Sealer may achieve better biological response when compared to MTA Fillapex.

Keywords Cytotoxicity · Calcium silicate-based materials · Root canal sealer

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Introduction

Laboratory and in vivo studies have demonstrated that mineral trioxide aggregate (MTA) has suitable biological and physical-chemical properties for the use in endodontics [1, 2]. As a consequence, this endodontic reparative material could be considered close to the ideal in terms of biocompatibility [1–3]. Nevertheless, despite its favorable characteristics, MTA does not exhibit the physical properties for its use as an endodontic sealer because of its working time, setting time, and difficult handling. EndoSeal (Maruchi, Wonju, Korea) and EndoSequence BC Sealer (Brasseler, Savannah, GA, USA) and MTA Fillapex (Angelus, Londrina, PR, Brazil) are examples of calcium silicate-containing endodontic materials recently developed for permanent root canal filling, with improved physico-chemical properties when compared to conventional MTA.

Root canal sealers may come in intimate contact with the periapical tissues for an extended period because of extrusion over the apex or because degradation products that may leach through lateral and accessory canal or apical foramina, reaching the surrounding tissues [4, 5]. Thus, for safety reasons, each sealer must have its biological properties comprehensively and independently first screened by in vitro tests before its unlimited clinical use in order to minimize the incidence of local and/or systemic adverse effects. Generally, cytotoxicity tests are evaluated using traditional two-dimensional (2D) culture [6].

It could be argued that although some sealers have significant toxic behavior in vitro, this may be of little relevance in the clinical situation mostly because of the difference between in vitro and in vivo conditions. Conventional 2D culture systems form a monolayer that might have contact inhibition among cells and hence not duplicate original characteristics of cell morphology and functionality [7]. Three-dimensional (3D) cell models, on the other hand, can mimic in vivo cellular conditions more appropriately because the 3D scaffold supports cell growth and cell functions, including morphogenesis, cell metabolism, and cell-to-cell interactions [8].

Therefore, the present study intended to assess, using such a 3D cell culture associated with an in vitro root canal filling model, the cytocompatibility of these calcium silicate-based sealers on Balb/c 3T3 fibroblasts. An often used root canal sealer, AH Plus (Maillefer Dentsply, Ballaigues, Switzerland), was used as reference material for comparison. Inflammatory cytokine expression was also evaluated. The null hypotheses tested were that there was no significant difference in cytotoxicity and cytokines synthesis between the different calcium silicate-based sealers.

Materials and methods

Selection and preparation of specimen

This study was approved by an internal review board. Thirty human maxillary incisor teeth were selected. Only roots with angle of curvature <10° and an initial apical size equivalent to a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) were selected for the study. Straight-line access cavities were performed and apical patency was determined by inserting a size 10 K-file into the root canal until its tip was visible at the apical foramen. The working length (WL) was set 1 mm shorter of this measurement. After that, the foramen diameter of all specimens was standardized to a size 15 K-file (Dentsply Maillefer).

Root canals were shaped using Reciproc R40 files (VDW, Munich, Germany) 1 mm coronal to the foramen. The canals were irrigated with 10 mL of freshly prepared 5.25 % NaOCl and received a final flush of 3 mL of 17 % EDTA (pH 7.7) for 3 min. The canals were dried with R40 paper points (VDW) and sterilized at 135 °C for 35 min. Thereafter, roots were randomly distributed with the aid of a computer algorithm (http://www.random.org) in order to create three equal experimental groups and two control groups, with six teeth each.

Root canal filling procedures

Root canals were filled under sterile conditions in a laminar flow hood. All procedures were performed by the same operator using a single-cone technique with one of the three following sealers (n = 6): EndoSeal, Endosequence BC sealer, and MTA Fillapex. AH Plus was used as a reference material and teeth with unfilled root canals were used as negative controls. Sealers were prepared following the manufacturers' instructions. A R40 (VDW) gutta-percha cone was coated with one of the tested sealers and placed in the canal to the full working length. Then, a heated instrument was used to remove excess coronal gutta-percha. Filled roots were immediately exposed to cell culture (Fig. 1).

3D cell culture

Balb/c 3T3 fibroblasts cells (ATCC®, Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10 % fetal bovine serum (FBS) (Sigma Chemical Co., St. Louis, MO), 100 μg/mL of streptomycin, and 100 mg/mL of penicillin at 37 °C at 100 % humidity in an incubator under air

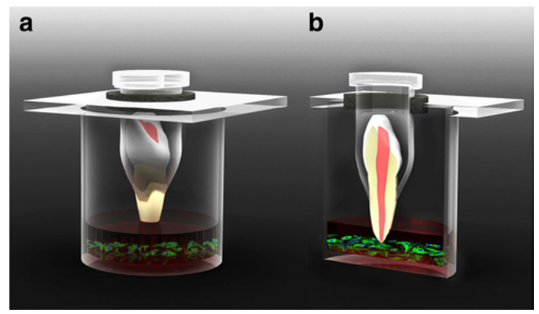


Fig. 1 In vitro root filling model apparatus. The bottom of Eppendorf tubes was cut to allow the protrusion of the tooth apex through this opening and placed into a six-well cell culture plate using a rubber O-ring (**a**). Three millimeters of the root apex was placed in contact with the 3D cell culture for 24 h (**b**)

atmosphere containing 5 % CO_2 at ambient pressure. When cells reached confluence, trypsin/ethylenediaminetetraacetic solution was used to remove adherent cells.

For the 3D cell culture, rat tail collagen type I (Gibco) was diluted to 1 mg/mL at 4 °C and neutralized with 0.1 M NaOH and 10× DMEM as recommended by the manufacturer. Six-well plates culture dishes were prechilled and then precoated with 1 mL collagen solution mixed with 3×10^1 cell. Then, plates were immediately transferred to an incubator for 20 min to allow polymerization of the collagen. After polymerization of the collagen, culture media (2 mL) was added on the top of the collagen gel. Cells were cultured for 7 days and then exposed to the filled roots. The medium was changed daily.

In vitro root canal filling model

The root model applied on this work has been described previously in detail [9]. In brief, 24 1.5-mL polypropylene Eppendorf tubes were cut 3 mm from the bottom to receive the roots with the apex protruding 5 mm through the opening. A rubber O-ring (Ø 0.8 cm) was inserted into the apex of each tube and its position adjusted so that the calcium silicate-based sealers dipped into a six-well cell culture plate well containing the 3D cell culture. With this apparatus, 3 mm of the root apex stayed in contact with the cells for 24 h (Fig. 1).

Cytotoxicity evaluation

Cytotoxicity of endodontic sealers in 3D culture was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay after 24 h of contact. After the removal of culture medium from each well, the 3D structures were gently washed two times with 1.0 mL phosphate-buffered saline. The wash was replaced with 1 mL MTT-succinate solution (1 mg/mL; Sigma-Aldrich, St Louis MO) for 4 h at 37 °C. Then, the 3D structures were washed two times with 1.0 mL phosphate-buffered saline. The blue formazan precipitate was solubilized using 0.3 mL dimethyl sulfoxide on a shaker at room temperature for 30 min. Three aliquots (100 μL) of the solution were then transferred from each well to a 96-well plate, and the absorbance was measured at 490 nm using a microplate reader (Urit 660, Urit, Guillin Guanxi, China). Percentage cell viability was calculated by dividing the absorbance values of experimental wells by those of negative control group wells and multiplying by 100.

Cytokine production

After 24 h of cell exposure to the root model, the production of IL-1β, IL-6, and IL-8 was analyzed. Cytokines were measured in triplicate in culture supernatants by enzyme-linked immunosorbent assay kit (BD Biosciences, San Jose, CA, USA).

Statistical analysis

One-way analysis of variance (ANOVA) was performed, and when the F-ratios were significant, data were compared by Duncan's multiple range test. The alpha-type error was set at 0.05. SPSS 11.0 (SPSS Inc., Chicago) and Origin 6.0 (Microcal Software, Inc., Northampton, MA) were used as analytical tools.

Results

Figure 2a shows cell viability measured by MTT after 24 h in 3D culture with AH Plus, EndoSeal, EndoSequence BC sealer and MTA Fillapex. AH Plus, EndoSeal and Endosequence BC Sealer had similar cell activity to the negative control group ($P > 0.05$), indicating no cytotoxic effects. A significantly stronger cytotoxicity effect was identified for MTA Fillapex sealer ($P < 0.05$). The production of IL-1β, IL-6, and IL-8 was detected in all samples; while there were no differences for IL-8, both IL-1β and IL-6 were significantly elevated with MTA Fillapex compared to the other sealers and the negative control (Fig. 2b).

Discussion

The first results of the present study revealed that MTA Fillapex showed higher cytotoxic effects when compared to AH Plus, EndoSeal, and EndoSequence BC sealer ($P < 0.05$). Therefore, the first null hypothesis was rejected. Cytotoxic effects of MTA Fillapex have indeed been well documented in endodontic literature [10–12]. Components present in MTA Fillapex formulation, such as salicylate resin, diluting resin, and silica, may be related to the results. Moreover, MTA Fillapex probably has an unbalanced ratio among resin and MTA, with higher values for salicylate resin. It has been observed that to take set, a ratio of 1:1 is necessary (MTA:salicylate resin) [13]. Thus, this unbalanced resin/MTA ratio and the difficulty to set may explain the higher cytotoxicity and the properties of extended setting time and working time, excessive flow ability, and solubility as disturbance in calcium silicate properties, which has been reported in previous studies [10, 13–16].

The cytotoxicity results of AH Plus, EndoSeal, and EndoSequence BC sealer did not differ from those observed for the control group (unfilled canal), suggesting no cytotoxic effects for these sealers under the present experimental

conditions. These results are in apparent contrast with some previously published studies [10–12, 17–21] that showed some amount of cytotoxicity for those sealers; however, it is important to emphasize that in the present study, a 3D cell culture and an in vitro root model was used; these methodological differences could explain the differences in the studies.

Another consideration for the level of cytotoxicity is that, under clinical conditions, the concentration of toxic substances may be reduced by tissue fluid [22]. This fact reduces the direct transferability of the current results to the clinic but could be mitigated but pretreatment of the set test material with simulated tissue fluid according to Widbiller et al. 2016 [23]. Using this approach, further research may provide additional data points regarding the cytotoxicity of MTA Fillapex.

The cytotoxicity potential of dental materials is commonly assessed using 2D cell monolayer structures, in line with the International Organization for Standardization 10-993 [24]. In comparison with the traditional 2D cell culture model, considered a simple model without most in vivo characteristics and natural tissue architecture, the 3D model may be a more physiologically relevant, with more typical cell behavior, greater stability and longer cell lifespans [25].

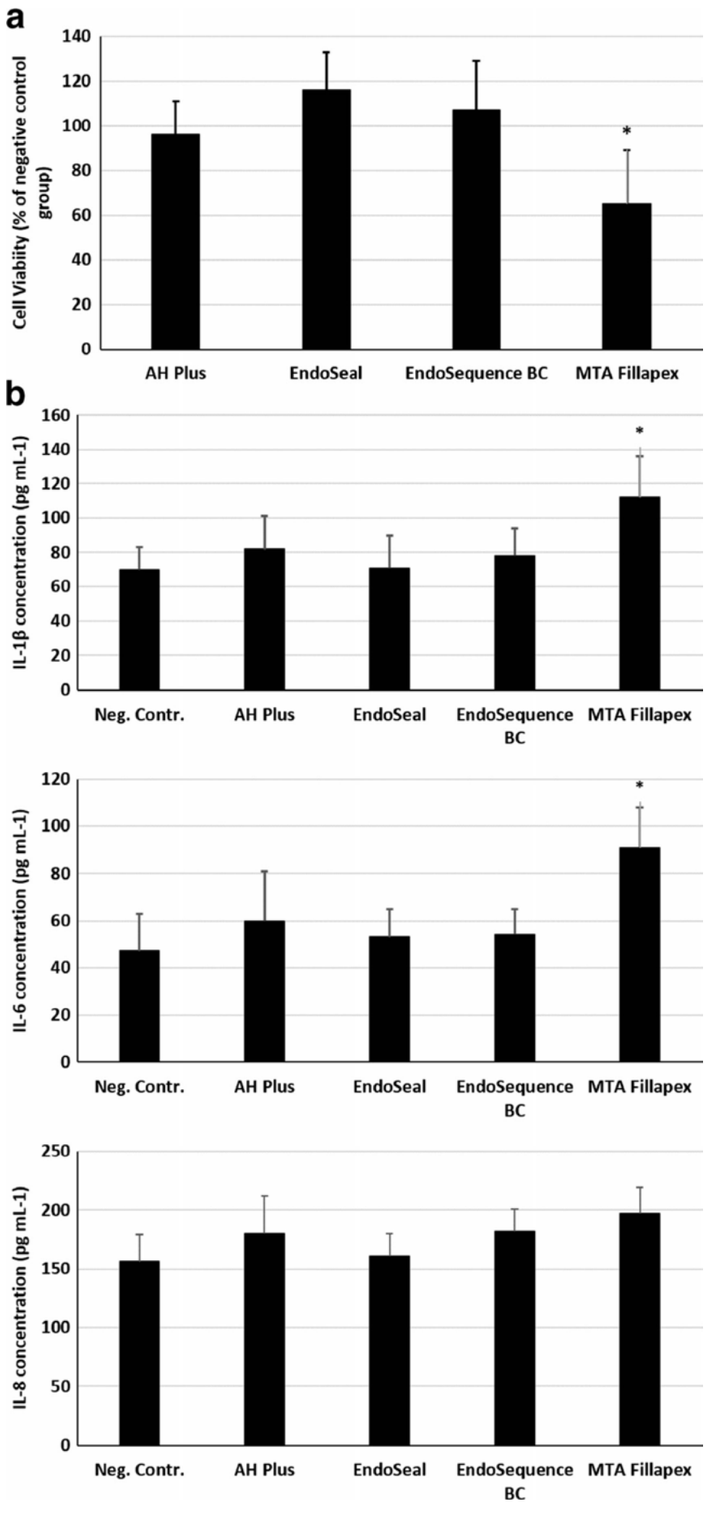
It has been recently demonstrated that endodontic sealers have higher cytotoxic effects in the 2D cell culture model than the 3D cell culture model [26]. This discrepancy was put down to the use of non-physiological 2D cell monolayers which does not simulate the 3D structure of the in vivo tissue [27]. These differences could be explained by the extensive cell-cell and cell-to-matrix interactions occurring in the 3D cell aggregates and the decreased capability of sealers extracts to penetrate within the 3D cell aggregates. Longer time points should be considered in further studies. Another argument for less cytotoxicity in 3D culture compared to 2D is the fact that biocompatibility testing in conventional cultures mostly uses material eluates and therefore provides a continuous stimulus, whereas most 3D models are created with only an initial stimulus. Collagen type I was chosen as scaffold material as it represents the major component of extracellular matrix in dental pulp. The material is highly cytocompatible and biodegradable, promotes cell adhesion, and allows for cell migration. A recent study confirmed using confocal images the equal distribution of cells within this carrier material [23]. Regardless of the type of cell culture, any dentin in contact with cultured cells in vitro must be sterilized first to avoid microbial contamination. In the present study, this was done with heat sterilization which is likely to denaturize some of the proteins contained in dentin such as TGF-β; it has been shown that acids and alkaline compounds such as EDTA and $Ca(OH)_2$ may release these sequestered compounds and thus modify the biologic response [23]. Future studies should be conducted using gamma-ray and ethylene oxide sterilized samples and compare the results with those obtained by heat sterilization.

Another methodological issue that could contribute to the nontoxic results of AH Plus, EndoSeal, and EndoSequence BC sealer is the use of root model in the present study, simulating the in vivo situation for endodontic sealers. In this root model, the contact area of the root canal sealer with the cell culture is small compared to standard 2D tests, likely decreasing the toxic effects of the root canal sealers. This model has been previously recommended and has several advantages when compared to assessments performed with sealers alone: (a) in this model, more realistic material amounts are used, and (b) the interaction between sealer and the surrounding dentin is also taken into consideration [9, 28, 29]. Based on this, the combination of 3D cell culture and the root model may be used as an alternative in vitro experimental model to provide reliable information on endodontic sealer toxicity under conditions more closely related to the physiological scenario found in real-life tissue microenvironments.

The inflammatory process is initiated and maintained by upregulation of a network of chemokines (e.g., IL-8) and proinflammatory mediators (e.g., IL-1β and IL-6) that play distinct or shared biological activities [30]. IL-1β and IL-6 are pivotal in periapical disease development, stimulating osteoclastic differentiation and bone resorption as well as contributing to inflammation by inducing synthesis of other cytokines [31].

In the present study, contact to MTA Fillapex led to upregulation of IL-1β and IL-6 expression compared to the other sealers and the negative control. This observation suggests that MTA Fillapex may be associated with acute inflammation after its use during root canal filling. However, chemokine IL-8 was not upregulated by exposition to either sealer. This may be related to the fact that no interaction with immune cells occurred in this model. Future studies could include a co-culture system with neutrophils or macrophages, to study a possible interaction [32].

Fig 2. **a** Cytotoxic effects after 24 h exposure to in vitro root models in 3T3 fibroblast cells ($n = 6$). Optical density measurements of negative control group (0.477 ± 0.024) served as reference and were set to 100 %. Results are expressed as means and standard deviations. The *asterisk* indicates significant differences ($P < 0.05$) to control group; **b** mean production of IL-1β, IL-6, and IL-8 after 24 h exposure to root models in 3T3 fibroblasts cells ($n = 6$). Results are expressed as means and standard deviations. *Asterisks* indicate significant differences ($P < 0.05$) to the control group



Collectively, the data suggest that in an in vitro model with 3D cell culture, AH Plus, EndoSeal, and EndoSequence BC Sealer showed better cytocompatibility compared to MTA Fillapex.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent For this type of study, formal consent is not required.

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Physicochemical properties of epoxy resin-based and bioceramic-based root canal sealers

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Research Article

Physicochemical Properties of Epoxy Resin-Based and Bioceramic-Based Root Canal Sealers

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Three bioceramic sealers (EndoSequence BC sealer, EndoSeal MTA, and MTA Fillapex) and three epoxy resin-based sealers (AH-Plus, AD Seal, and Radic-Sealer) were tested to evaluate the physicochemical properties: flow, final setting time, radiopacity, dimensional stability, and pH change. The one-way ANOVA and Tukey's post hoc test were used to analyze the data ($P = 0.05$). The MTA Fillapex sealer had a highest flow and the BC Sealer presented a flow significantly lower than the others ($P < 0.05$). The BC Sealer and MTA Fillapex samples were not set in humid incubator condition even after one month. EndoSeal MTA had the longest setting time among the measurable materials and Radic-Sealer and AD Seal showed shorter setting time than the AH-Plus ($P < 0.05$). AH-Plus and EndoSeal MTA showed statistically higher values and MTA Fillapex showed statistically lower radiopacity ($P < 0.05$). BC Sealer showed the highest alkaline pH in all evaluation periods. Set samples of 3 epoxy resin-based sealers and EndoSeal MTA presented a significant increase of pH over experimental time for 4 weeks. In conclusion, the bioceramic sealer and epoxy resin-based sealers showed clinical acceptable physicochemical properties, but BC Sealer and MTA Fillapex were not set completely.

1. Introduction

Endodontic sealers are used to attain a fluid-proof seal throughout root canal system [1]. An ideal root canal sealer should offer an excellent seal when set, dimensional stability, a sufficient setting time to ensure working time, insolubility against tissue fluids, proper adhesion with canal walls, and biocompatibility [2, 3].

The commercially available sealers are categorized according to chemical components: zinc-oxide eugenol, calcium hydroxide containing, resin-based, glass-ionomer-based, silicone-based, and bioceramic-based sealers [4–6]. Epoxy resin-based sealers were introduced in endodontics by Schroeder [7], and current modifications of the original formula are widely used for root canal filling procedures [8, 9]. Recently, bioceramic-based materials such as EndoSequence BC Sealer (Brasseler USA, Savannah, GA), EndoSeal MTA (MARUCHI, Wonju, Korea), and MTA Fillapex (Angelus Soluções Odontológicas, Londrina, PR, Brazil) have received considerable attention because of their favorable physicobiological properties [10, 11]. Among them, EndoSequence BC Sealer and EndoSeal MTA are supplied in a premixed injectable paste and thus give clinicians easy manipulation. These currently introduced calcium silicate based sealers still have few reports about their chemical and physical properties [3, 10, 12].

This study aimed to evaluate the physicochemical properties of 3 epoxy resin-based sealers and 3 bioceramic-based sealers according to the international standards such as ISO 6876/2012 standards [13] and ANSI/ADA's specifications number 57 [14] (Table 1).

2. Materials and Methods

Three epoxy resin-based root canal sealers of AH-Plus, AD Seal, and Radic-Sealer and 3 bioceramic-based sealers of EndoSequence BC Sealer, EndoSeal MTA, and MTA Fillapex were used as the experimental materials (Figure 1, Table 2).

Table 1: ISO 6876/2012 and ANSI/ADA specification number 57 standards.

	ISO standards	ANSI/ADA standards
Setting time	When ≤30 min, ≤110% stated by the manufacture When >30 min, <72 hours, within the range (min)	Within 10% of setting time stated by the manufacturers
Flow	≥20 mm	≥25 mm
Solubility	≤3% for 24 hours	≤3% for 24 hours
Dimensional change	Shrinkage (contraction) ≤1% for 30 days Expansion ≤ 0.1% for 30 days	
Radiopacity	≥3 mm aluminum thickness	

(a) (b) (c)

(d) (e) (f)

Figure 1: Six root canal sealers tested in the present study: (a) Radic-Sealer, (b) AD Seal, (c) AH-Plus, (d) EndoSequence BC Sealer, (e) MTA Fillapex, and (f) EndoSeal MTA.

AH-Plus (Dentsply DeTrey, Konstanz, Germany) is the most popular hydrophobic epoxy resin-based sealer that has been used as the gold standard material [3]. AD Seal (Meta Biomed, Cheongju, Korea) and Radic-Sealer (KM, Seoul, Korea) are the epoxy resin-based sealers with few reports in literature [15, 16].

The physicochemical properties including flow, final setting time, radiopacity, dimensional stability, and pH change were examined according to modified ISO 6876/2012 standards [13] and ANSI/ADA's specifications number 57 [14]. All sealers were mixed and manipulated depending on the manufacturers' instructions.

2.1. Flow. A volume of 0.05 mL mixed sealer was dropped on a glass plate. At 3 minutes after the onset of mixing, a second glass plate was placed on the sealer and a 100 g weight was added to make a total mass of 120 g. The 120 g weight was unloaded after 10 minutes from the start of mixing. The minimum and maximum diameters of the sealer disc were measured by a digital caliper (Mitutoyo Corp, Tokyo, Japan) with a resolution of 0.01 mm. If the disks were not uniformly circular, the test was repeated. Ten tests were taken for each sealer.

2.2. Final Setting Time. Stainless steel ring molds (inner diameter 10 mm, height 2 mm) were placed on a glass plate, and then the sealer materials were mixed and packed into the molds. The whole assembly was then stored in an incubator (37°C, >95% relative humidity) for at least 1 hour. To measure the setting time, the needle of a custom-made Vicat apparatus was adjusted vertically onto the surface of the sealer. The setting time was determined as the time when the indenter needle failed to create an indentation. The measurement interval was adjusted from 1 hour at the beginning to 5 min in accordance with the setting process. The time from the onset

Table 2: Chemical compositions of the root canal sealers investigated in the present study.

Sealer		Components	
		Base	*Catalyst*
Epoxy resin-based sealer	*Radic-Sealer*	Poly epoxy resin Zirconium oxide	TEA (triethanolamine) Zirconium oxide Calcium oxide
		Base	*Catalyst*
	AD Seal	<20% epoxy resin NS calcium phosphate NS zirconium dioxide NS calcium oxide NS ethylene glycol salicylate	2.5%–10% N, n-dibenzyl-5-oxanonandiamin-1,9 2.5%–10% amantadine
		Paste A	*Paste B*
	AH-Plus	25%–50% bisphenol A 10%–25% zirconium dioxide NS calcium tungstate NS iron oxide	2.5%–10% N, n-dibenzyl-5-oxanonandiamin-1,9 2.5%–10% amantadine
Bioceramic-based sealer	*EndoSequence BC Sealer*	Zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler, thickening agents	
	EndoSeal MTA	Calcium silicates, calcium aluminates, calcium aluminoferrite, calcium sulfates, radiopacifier, thickening agents	
	MTA Fillapex	Salicylate resin, diluting resin, natural resin, bismuth trioxide, nanoparticulated silica, MTA	

of mixing to the sealer setting was taken as the setting time. Ten measurements were made for each sealer.

2.3. Radiopacity. Ten cylindrical samples were fabricated from each sealer by placing the handled sealers into metallic rings with 8 mm internal diameter and 1 mm thickness. Then the filled rings were stored at 37°C until sealers were completely set. The samples were radiographed on a digital X-ray sensor (Schick Technology Inc., Long Island City, NY) with an aluminum step-wedge graduated from 1 mm to 10 mm (in 1 mm increment), which was used with exposures set at 60 kV, 2 mA, 0.08 seconds, and a focus-film distance of 10 cm. The aluminum wedge equivalent thickness (mm Al) of each sealer was analyzed by using Photoshop (Adobe photo shop 7.0; Adobe systems Incorporated, San Jose, CA).

2.4. Dimensional Stability. Dimensional stability was measured for the settable sealers of 3 epoxy resin-based sealers and EndoSeal MTA. Cylindrical Teflon molds (inner diameter 6 mm, height 12 mm) were filled with mixed sealer and backed by a glass plate on each side. The whole assembly was transferred to an incubator and kept for at least 3 times the measured setting time. After deciding the complete setting, the ends of the molds containing the specimens were ground by using 600-grit sandpaper with water supply. Then the specimens were removed from the mold and measured for length (L_0) using a digital caliper with a resolution of 0.01 mm. The specimens were stored in distilled water and kept in an incubator throughout the study period (6, 24, and 72 hours and 7, 14, and 30 days). After being immersed in water for the assigned periods, the dimensions of 4 tested sealers were compared to their initial dimension. The samples were then blotted dry with tissue paper and measured again for length (L_1). The test was implemented ten times for each sealer, and the change in length was recorded as the dimensional change (D) using the following formula: $D(\%) = (L_1 - L_0)/L_0 \times 100$.

2.5. pH Change. The sealer samples mixed immediately after manipulation were denoted as fresh samples, and the samples stored in the incubator until setting were denoted as set samples. Teflon molds (inner diameter 5 mm, thickness 1 mm) were used to shape the set samples. Both the set sample and fresh sample were dropped in distilled water in a polypropylene conical tube and then stored at 37°C throughout the study period. After predetermined periods (3, 30, and 60 minutes and 2, 12, and 24 hours for fresh samples and 12 hours, 3 days, 7 days, 2 weeks, and 4 weeks for set samples), the pH of the solution was measured by using a digital pH meter (STARTER 2100 Bench pH Meter; Ohaus). Ten measurements were made for each sealer and condition.

2.6. Statistical Analysis. The one-way ANOVA test and Tukey's post hoc test were used to compare the physicochemical property results by using SPSS software 10.0 (SPSS Inc., Chicago, IL). The significance level was set at $P < 0.05$.

3. Results

The physical properties of the sealers are summarized in Table 3.

All the tested sealers except BC Sealer showed the flow greater than 20 mm, which is in agreement with the ISO standards [13]. MTA Fillapex had a highest flow and BC Sealer presented a significantly lower flow than the other sealers ($P < 0.05$) (Figure 2).

BC Sealer and MTA Fillapex were not set in humid incubator condition even after one month. EndoSeal MTA had the longest setting time (mean: 1223 min) among the measurable materials and Radic-Sealer and AD Seal showed shorter setting time than the AH-Plus ($P < 0.05$).

For the radiopacity test, AH-Plus and EndoSeal MTA showed statistically higher values and MTA Fillapex showed statistically lower values in comparison to the other evaluated sealers ($P < 0.05$) (Figure 3). All the tested sealers showed radiopacity values complying with the ISO standards [13].

Dimensional stability was measured for the settable sealers of 3 epoxy resin-based sealers and EndoSeal MTA. After being immersed in water for 30 days, 4 tested sealers expanded compared to their initial dimension. At 30 days, AD Seal had a significantly greater expansion than the others ($P < 0.05$) (Table 4, Figure 4).

Fresh samples of the tested sealers showed significant differences of pH change among themselves at all evaluation time points and BC Sealer showed the highest alkaline pH in all evaluation periods (Table 5, Figure 5). Set samples of 3 epoxy resin-based sealers and EndoSeal MTA presented a significant increase of pH over experimental time for 4 weeks.

Table 3: The flow (mm), setting time (min), and radiopacity (mmAl thickness) of tested sealers (mean ± standard deviation).

	Radic-Sealer	AD Seal	AH-Plus	EndoSequence BC Sealer	EndoSeal MTA	MTA Fillapex
Flow (mm)	20.80 ± 0.84^{b}	21.87 ± 1.40^{b}	21.87 ± 1.40^{b}	18.45 ± 1.31^{c}	20.21 ± 1.57^{b}	34.13 ± 2.91^{a}
Setting time (min)	114.1 ± 2.8^{c}	115.7 ± 2.8^{c}	959.6 ± 79.0^{b}	—	1223.4 ± 156.3^{a}	—
Radiopacity (mmAl)	7.67 ± 0.38^{b}	4.70 ± 0.33^{d}	10.00^{a*}	6.68 ± 0.99^{c}	9.50 ± 0.84^{a}	3.01 ± 0.20^{e}

^{a,b,c,d,e}Different letters in each line indicate significant difference ($P < 0.05$).
*All of the AH plus samples had 10 mmAl thickness or the higher values.

Table 4: The dimensional stability (%) of the tested sealers at various time periods (mean ± standard deviation).

Ratio (%)	6 hours	24 hours	72 hours	7 days	14 days	30 days
Radic-Sealer	0.42 ± 0.33^{AB}	0.62 ± 0.35^{aBC}	0.98 ± 0.28^{aC}	1.73 ± 0.39^{aD}	2.22 ± 0.28^{bDE}	2.69 ± 0.32^{bE}
AD Seal	0.21 ± 0.27^{A}	0.55 ± 0.32^{aAB}	1.20 ± 0.39^{aB}	2.07 ± 0.56^{aC}	2.88 ± 0.54^{aD}	3.41 ± 0.76^{aD}
AH-Plus	0.10 ± 0.64	0.13 ± 0.65^{b}	0.23 ± 0.55^{b}	0.24 ± 0.64^{b}	0.25 ± 0.54^{c}	0.35 ± 0.51^{c}
EndoSeal MTA	0.36 ± 0.32	0.33 ± 0.28^{ab}	0.25 ± 0.34^{b}	0.14 ± 0.33^{b}	0.23 ± 0.29^{c}	0.21 ± 0.31^{c}

^{a,b,c}Different letters in each column indicate significant difference between groups at the same period ($P < 0.05$).
^{A,B,C,D,E}Different capital letters indicate significant difference during the time periods in the same material ($P < 0.05$).

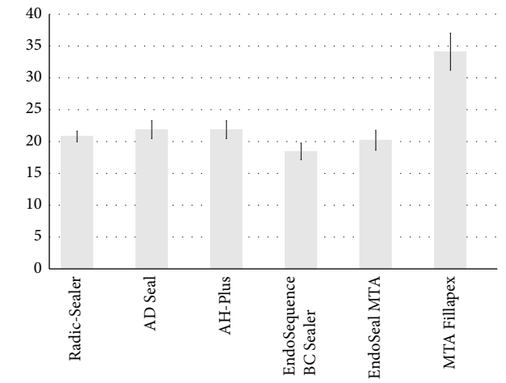

Figure 2: Flow values from each sealer evaluated (in mm). ^{a,b,c}Different letters present significant difference between groups ($P < 0.05$).

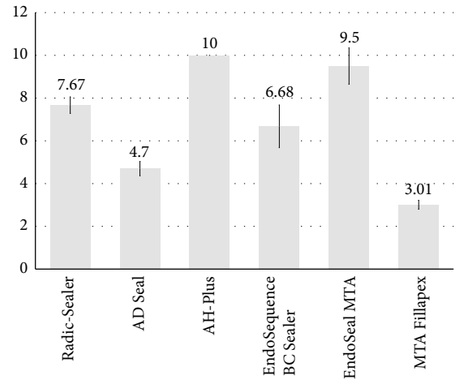

Figure 3: Radiopacity values from each sealer evaluated (in mm Al). ^{a,b,c,d,e}Different letters present significant difference between groups ($P < 0.05$).

Table 5: pH change of freshly mixed samples during 24 hours.

	3 min	30 min	60 min	2 hours	12 hours	24 hours
Radic-Sealer	8.84 ± 0.25^{d}	8.59 ± 0.27^{e}	8.34 ± 0.13^{e}	7.77 ± 0.34^{e}	7.72 ± 0.17^{e}	7.70 ± 0.26^{e}
AD Seal	9.06 ± 0.47^{d}	8.91 ± 0.55^{d}	8.65 ± 0.67^{d}	8.41 ± 0.92^{d}	7.87 ± 0.68^{e}	7.46 ± 0.77^{e}
AH-Plus	9.33 ± 0.28^{c}	9.45 ± 0.26^{c}	9.37 ± 0.23^{c}	9.18 ± 0.37^{c}	8.91 ± 0.46^{d}	8.68 ± 0.60^{d}
BC Sealer	11.64 ± 0.03^{a}	11.60 ± 0.02^{a}	11.67 ± 0.03^{a}	11.7 ± 0.03^{a}	11.78 ± 0.03^{a}	11.78 ± 0.03^{a}
EndoSeal MTA	10.41 ± 0.05^{b}	10.42 ± 0.06^{b}	10.42 ± 0.07^{b}	10.45 ± 0.07^{b}	10.77 ± 0.06^{b}	10.90 ± 0.05^{b}
MTA Fillapex	8.50 ± 0.26^{e}	8.93 ± 0.13^{d}	9.30 ± 0.15^{c}	9.52 ± 0.18^{c}	9.90 ± 0.11^{c}	10.02 ± 0.23^{c}

[a,b,c,d,e]Different letters in each column indicate significant difference between sealer groups at the tested period ($P < 0.05$).

Table 6: pH change of set samples during 4 weeks.

	Initial	12 hours	3 days	7 days	2 weeks	4 weeks
Radic-Sealer	5.79 ± 0.06	6.35 ± 0.09^{c}	6.51 ± 0.12^{c}	6.40 ± 0.13^{c}	6.59 ± 0.51^{d}	6.34 ± 0.39^{c}
AD Seal	5.84 ± 0.57	6.95 ± 0.83^{b}	7.30 ± 0.75^{b}	7.15 ± 0.74^{b}	7.51 ± 0.86^{c}	7.49 ± 0.74^{b}
AH-Plus	5.84 ± 0.04	5.85 ± 0.35^{d}	5.87 ± 0.47^{d}	5.96 ± 0.44^{d}	6.10 ± 0.94^{b}	6.40 ± 0.47^{c}
EndoSeal MTA	5.76 ± 0.11	10.58 ± 0.06^{a}	10.90 ± 0.05^{a}	11.02 ± 0.04^{a}	11.26 ± 0.04^{a}	11.29 ± 0.07^{a}

[a,b,c,d]Different letters in each column indicate significant difference between sealers at the tested period ($P < 0.05$).

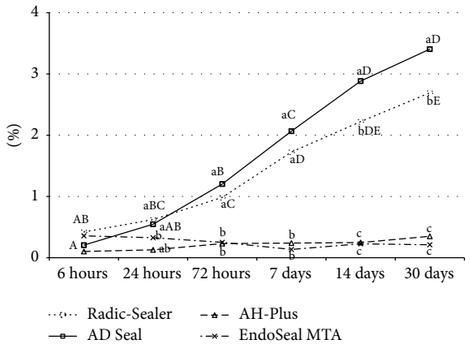

Figure 4: Dimensional stability (%) of the test sealers at various time periods. Different letters present significant differences between groups (a, b, and c) at the same period and between the time periods (A, B, C, D, and E) in the same material ($P < 0.05$).

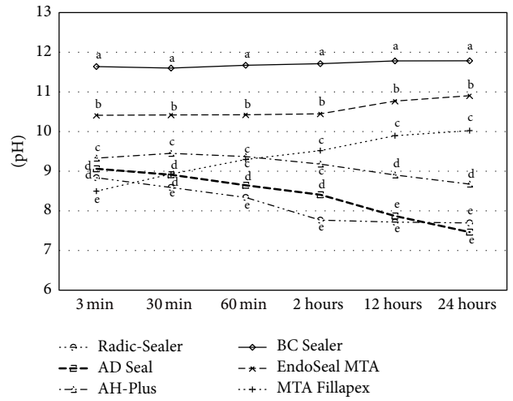

Figure 5: pH change of freshly mixed samples during 24 hours. [a,b,c,d,e]Different letters present significant difference between sealers at the tested period ($P < 0.05$).

The pH of EndoSeal MTA was significantly higher than that of 3 epoxy resin-based root canal sealers at all experimental time points. Radic-Sealer and AH-Plus showed mild acidity around pH 6 and AD Seal presented neutral pH at 4 weeks ($P < 0.05$) (Table 6, Figure 6).

4. Discussion

Among the clinically available root canal sealers, epoxy resin-based sealers are widely used for root canal filling due to their resorption resistance and dimensional stability [4–6]. Most recently introduced bioceramic-based materials have attractive physical, chemical, mechanical, and biological properties [10, 11, 17]. Therefore, the representative 3 epoxy resin-based sealers and 3 bioceramic-based sealers were compared for physical and chemical properties, in this study.

The flow of endodontic sealers may have an effect on obturation of accessory canals and microspaces between master and accessory cones [3]. Various factors such as composition, shear rate, particle size, temperature, and time from mixing are related to the flowability of sealers [3]. MTA Fillapex sealer had the highest flow and BC Sealer presented the lowest flow in this study. The flow value of MTA Fillapex was similar to the value obtained by Silva et al. [18]. A high resin/MTA ratio may be one of the reasons why a high flow rate occurs [19].

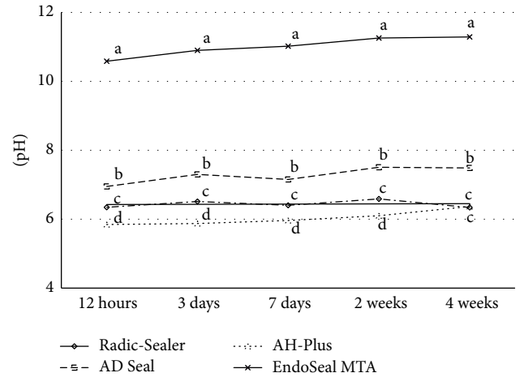

Figure 6: pH change of set samples during 4 weeks. [a,b,c,d]Different letters present significant difference between four sealers at the same test time periods ($P < 0.05$).

Setting time is also important to provide adequate working time and proper consistency enough to fill the root canal system completely [20]. Setting times of evaluated sealers in this study were different from that given by the manufacture. Only AH-Plus was in agreement with the ISO standards and it showed a significant higher mean setting time value, almost 8 times greater than the other epoxy resin-based root canal sealers. AH-Plus is comprised of base and catalyst in which a slow polymerization reaction of epoxy resin amines with a high molecular weight including bisphenol A and bisphenol F occurs [21]. This chemical composition could explain significantly higher setting time of it. On the other hand, Radic-Sealer and AD Seal are the kind of resin composites containing a catalyst component that accelerates the process [22]. In the meanwhile, BC Sealer and MTA Fillapex were not set in humid incubator condition, and this result was different from several reports that final setting of these materials occurred [4–6, 10, 23, 24]. Depending on Loushine et al. [10], water is essential for this sealer to reach its final set because the inorganic and radiopacifier components of the sealer are premixed with water-free liquid-thickening carriers, and the manufacturer suggests that there is a prolonged setting time in overly dry canals. However, the authors concluded that overly wet canals may affect the setting time and, in particular, adversely affect the microhardness of the sealer after setting [10]. They also pointed out that a more porous matrix would be present when the sealer sets in the wet canals, which, in turn, may result in increased leaching of tissue-irritating substances from the sealer [10]. The delayed setting time of sealers may also affect biocompatibility and the sealers may have the potential to release cytotoxic byproducts before the final setting [10]. Silva et al. [18] reported that MTA Fillapex showed severe cytotoxicity when cells were exposed to the fresh sealer and the toxicity was not decreased over the tested time periods. These findings are in agreement with other previous studies [25, 26] that showed strongly affected cell viability with MTA Fillapex.

Radiopacity is an essential property of endodontic sealing materials. Among other physical, chemical, and biological properties, the ideal root canal sealing material should have a certain level of radiopacity [27]. Sufficient radiopacity allows clinicians to make a clear distinction between the materials and the surrounding anatomic structures and to evaluate the quality of the root fillings [28]. International standards require a minimal radiopacity equivalent to 3.00 mmAl [29]. In the present study, AH-Plus and EndoSeal MTA showed statistically higher radiopacity values ($P < 0.05$), but all the tested sealers exhibited values complying with the international standards. Vitti et al. [19] suggested that the differences between radiopacities of root canal sealers probably were caused by the presence of different radiopacifying agents in each material. According to Duarte et al. [30], radiopacity of AH-Plus is provided by zirconium oxide and calcium tungstate and suggested that its radiopacity could vary in different published studies because of the deposition of radiopacifying agents at the lower end of the tube, whereas the upper portion can present a lower quantity of its substance [19].

In this study, 4 tested sealers expanded compared to initial dimension and AD Seal had a significant increase of height (i.e., expansion) than the others. This increase of mass and height presented by 3 epoxy resin-based sealers probably occurred as a result of the water absorption and a high expansion of resin-based sealers, which was also verified by Versiani et al. [31]. AH-Plus maintained the most constant mass, presenting mass change rate within −0.5% (minus value means water sorption) for 30 days in this study. Dimensional change values ranging from 0.62% to 1.28% for AH-Plus obtained in previous investigations were also explained by water sorption after polymerization [31, 32]. It has been demonstrated that polymerized materials from mixtures of hydrophilic monomers had high water sorption [33]. And the dimensional change of EndoSeal MTA was not significant with the minimal change of specimens' height in this study. However, all the tested materials showed bigger expansion rate than the favorable rate suggested by the international standards (Table 1). Therefore, it is highly recommended to study the potential risk of inducing the vertical root fractures by the sealer expansion.

An alkaline pH may contribute to their osteogenic potential, biocompatibility, and antibacterial ability [3, 34–37]. It has been reported that an alkaline pH of root canal sealers could neutralize the lactic acid from osteoclasts and prevent dissolution of mineralized components of teeth. Therefore, root canal sealers can contribute to hard tissue formation by activating alkaline phosphatase [38]. In this study, the pH value of 3 freshly prepared bioceramic-based root canal sealers remained significantly higher than that of 3 epoxy resin-based sealers for 24 hours, with the highest alkaline pH measured from BC Sealer for the entire period of evaluation. Considering the setting time required, BC Sealer with prolonged high pH (up to 12) before its setting may cause damage to the periapical tissue via the loss of cell viability and membrane integrity, similar to cellular responses observed in chemical burns. Such complications thus need to be carefully considered, along with bactericidal effect of the sealers. In

case of set samples, the pH of EndoSeal MTA was significantly higher than that of 3 epoxy resin-based root canal sealers at all experimental time points ($P < 0.05$).

5. Conclusion

Based on the present results, the tested epoxy resin-based sealers as well as the bioceramic-based sealers except the BC Sealer and MTA Fillapex are showed to fulfill the required chemical and physical properties as ideal root canal sealers. The EndoSequence BC Sealer and MTA Fillapex should be improved to be set finally within clinically acceptable time limit. Clinical trial tests and long term follow-up studies using various types of the sealers would be highly valuable to evaluate the sealers' clinical performances.

Competing Interests

The authors deny any conflict of interests related to this study.

Authors' Contributions

Ju Kyung Lee and Sang Won Kwak contributed equally to this work and share the first authorship.

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Comparative evaluation of **fracture resistance** of endodontically treated teeth obturated with pozzolan-based MTA sealer and epoxy resin-based sealer: An in vitro study

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ORIGINAL RESEARCH

Comparative evaluation of Fracture Resistance of Endodontically Treated Teeth Obturated with Pozzolan-based MTA Sealer and Epoxy Resin-based Sealer: An *in vitro* Study

[1]Sree Theja Upadhyay, [2]Tina Puthen Purayil, [3]Kishore Ginjupalli

ABSTRACT

Aim: To evaluate and compare the effect of epoxy resin-based sealer and a pozzolan-based mineral trioxide aggregate (MTA) sealer on the fracture resistance of endodontically treated teeth.

Materials and methods: Thirty single-rooted mandibular premolars were decoronated to a standardized root length of 15 mm. ProTaper rotary files up to a master apical file size of F3 were used for cleaning and shaping the root canals followed by 2.5% sodium hypochlorite irrigation. The teeth were randomly divided into three groups (n = 10 each), and the obturation was completed using gutta-percha with Endoseal MTA (group I) and AH Plus (group II) as root canal sealers. Group III served as control (instrumented and unfilled). Each specimen was then subjected to fracture testing by using a universal testing machine at a crosshead speed of 1.0 mm/minute until fracture. The force required to fracture each specimen was recorded and the data were subjected to statistical analysis using one-way analysis of variance (ANOVA), followed by pairwise comparison using *post hoc* Games-Howell test ($p < 0.05$).

Results: The fracture resistance of groups I and II were significantly higher than those of group III. No significant difference in the fracture resistance was observed between group I (Endoseal MTA) and group II (AH Plus) groups.

Conclusion: It can be concluded that the new root canal sealer, Endoseal MTA, is able to reinforce the tooth against fracture as good as AH Plus.

Clinical significance: Endoseal MTA is a sealer for the reinforcement of endodontically treated teeth.

Keywords: AH Plus, Endodontically treated teeth, Fracture resistance, Sealer.

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INTRODUCTION

Endodontically treated teeth are structurally different from unrestored vital teeth, and their strength is affected by several factors like excessive loss of tooth structure because of caries or trauma, dehydration of dentin, access cavity preparation, instrumentation and irrigation of the root canal, excessive pressure during root obturation, and preparation of intraradicular post space.[1] All these factors interact cumulatively to influence the stress distribution or concentration, ultimately increasing the possibility of catastrophic failures.

As endodontically treated teeth are weaker and more prone to fracture than vital teeth,[2] the obturating material that strengthens the root is mandatory.[3] Therefore, the use of a root canal sealer possessing an additional quality of strengthening the root against fracture would be of obvious value.[4]

Several root canal sealers like zinc oxide eugenol sealers, calcium hydroxide-containing sealers, glass ionomer-based sealers, resin-based sealers, and mineral trioxide aggregate (MTA)-based sealers have been used along with gutta-percha (GP) for the obturation of root canals. Among them, the most widely investigated and reported material in the recent past is AH Plus sealer. AH Plus is an epoxy resin-based sealer containing bisphenol-A and bisphenol-F epoxy resins, dibenzyldiamine, aminoadamantane, calcium tungstate, tricyclodecane diamine, zirconium oxide, silica, and iron oxide pigments. Conflicting reports have been reported regarding the effect of root canal sealers on the fracture resistance of roots. Some studies have indicated that neither zinc oxide eugenol-based sealers nor epoxy resin-based sealers were able to strengthen the endodontically treated roots significantly,[5,6] while other studies have reported positive results for epoxy resin-based sealers and glass ionomer sealers.[4,7]

Endoseal MTA is a new endodontic sealer containing calcium silicates, calcium aluminates, calcium aluminoferrite, and calcium sulfates. It is a premixed, paste-type root canal sealer based on pozzolan cement that has excellent physical and biological properties of MTA. It is preloaded in a syringe that allows direct application of the sealer into the root canal. According to the manufacturer, it has fast setting time, antibacterial effect, biocompatibility, adequate flowability, excellent film thickness, and also promotes hard tissue formation. But, the ability of this MTA sealer in enhancing the fracture resistance of endodontically treated teeth has not been investigated.

Therefore, the purpose of this study is to evaluate the effect of Endoseal MTA and AH Plus sealers on the fracture resistance of endodontically treated teeth.

MATERIALS AND METHODS

Specimen Selection and Preparation

Ethical clearance was obtained from the ethical committee (IEC 337/2016) of Manipal University, Manipal, Karnataka, India. Thirty single-rooted human mandibular premolar teeth, with completely formed apex with approximately similar buccolingual and mesiodistal dimensions, extracted for periodontal reasons were selected and stored in 0.2% sodium azide (Sigma Chemical Co, St Louis, MO, USA) at 4°C until the experiment. The teeth with calcified canals, cracks or fractures, development defects, multiple canals, root caries, and endodontically treated teeth were excluded.

The crowns of all the teeth were removed by using a diamond disk to adjust the length of the roots to a standardized length of 15 mm. The working length was determined by subtracting 1 mm from the length of an inserted #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) with its tip visualized at the apical foramen. All teeth were instrumented up to a master apical file size of F3 with ProTaper rotary files (Dentsply Maillefer, Ballaigues, Switzerland) by using torque- and speed-controlled electric motor (X Smart; Dentsply, Maillefer, Ballaigues, Switzerland) as per the manufacturer's instructions.

A 3 mL of 2.5% Sodium hypochlorite solution (KMC Pharmacy, Manipal, Karnataka, India) was used between each file size. After completion of instrumentation, the smear layer was removed by flushing the root canals with 3 mL of 17% ethylenediaminetetraacetic acid (EDTA) solution (Merck, Darmstadt, Germany). The canals were finally rinsed with 5 mL distilled water and dried with ProTaper paper points (Dentsply Maillefer, Switzerland).

The teeth were then randomly divided into two experimental groups and one control group (n = 10).

Grouping Method

- *Group I*: Received canal preparation and were obturated with Endoseal MTA sealer (Maruchi, Wonju, South Korea).
- *Group II*: Received canal preparation and obturated with AH Plus sealer (Dentsply DeTrey, Konstanz, Germany).
- *Group III*: Received instrumentation but no obturation (Control).

In group II, AH Plus (Dentsply DeTrey, Konstanz, Germany) base and catalyst were mixed according to the manufacturer instructions and introduced into the root canal using lentulospiral (Dentsply, Maillefer, Ballaigues, Switzerland) at 300 rotations per minute, whereas in group I, Endoseal MTA (Maruchi, Wonju, South Korea) was introduced into the root canal via intracanal tip, inserted into the canal, not less than the coronal one-third. An F3 master GP cone (Dentsply Maillefer, Switzerland) with good tug-back was coated with sealer and slowly inserted into the canal until the working length was reached. Excess GP was removed with a hot instrument and all the filled root specimens were subsequently sealed with temporary filling material (Cavit-G, Espe Dental, Seefeld, Germany).

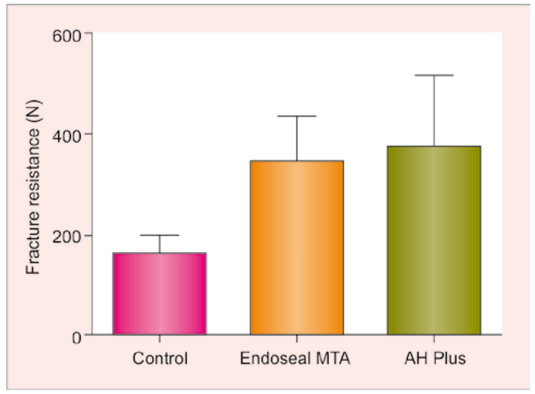
Mesiodistal and buccolingual radiographs were taken to ensure homogeneous adequate root filling without voids and then the teeth were stored at 37°C at 100% humidity for 7 days to allow the sealer to set.

Mechanical Testing

To simulate a periodontal membrane, the apical 10 mm of all roots was covered with wax before embedding the roots into acrylic resin. Each tooth was then mounted vertically in cold cure acrylic resin (Imicryl, Konya, Turkey) using a metal mold of dimensions 2.5 × 2.5 × 3 cm exposing 5 mm of the coronal part of the roots.

As soon as polymerization of the acrylic resin started, the roots were removed from the resin, and the wax was cleaned from the root surfaces by using a curette. The cleaned root surfaces were coated with a thin layer of medium body polyvinylsiloxane impression material (Coltene/Whaledent AG, Altstatten, Switzerland), and then they were again embedded back into acrylic resin which was allowed to polymerize overnight.

A universal testing machine (Instron 3366, Inston corp, Canton, MA, USA) was used for testing the fracture resistance. The acrylic blocks were fixed on the lower plate of the machine and the upper plate consisted of a spherical steel tip with a diameter of 1.5 mm. The tip was centered over the canal orifice, and a slowly increasing vertical force was exerted at a crosshead speed of 1 mm/minute until fracture. The maximum force required to fracture each specimen was recorded in Newtons.



Graph 1: Mean fracture resistance and standard deviations for all the groups

All the analysis was done using Statistical Package for the Social Science (SPSS) version 18. Comparison among the three groups was done using one-way analysis of variance (ANOVA) with *post hoc* Games-Howell test at a confidence interval of 95%.

RESULTS

The mean values and their respective standard deviations of the force required to fracture the roots are presented in Graph 1.

Among the groups, group II (AH Plus sealer) had the highest fracture resistance, followed by group I (Endoseal MTA), and then group III (control). Both test groups exhibited significantly higher fracture resistance compared to control group ($p < 0.001$). However, no significant difference in the fracture resistance among the test groups was observed ($p > 0.05$).

Two fracture modes were detected, a split vertical fracture that extended along the long axis of the root and a comminuted fracture that shattered the root into fragments. The most common fracture mode observed was the split vertical fracture in buccolingual direction.

DISCUSSION

The primary goal of endodontics is not only to restore the tooth structure but also to increase the inherent strength of the remaining tooth structure. As the endodontically treated teeth are weaker compared to natural tooth, fatigue failures might result even from normal functional stresses and from increased functional and parafunctional stresses.[8] In order to avoid such situations, various endodontic filling materials can be used to reinforce the endodontically treated tooth and improve its fracture resistance.[9,10]

In the present study, the effectiveness of Endoseal MTA sealer on fracture resistance was compared with that of AH Plus sealer. The results showed that the fracture resistance of AH Plus and Endoseal MTA sealer reinforced teeth was superior when compared to the fracture resistance of unreinforced teeth (control group). The highest mean fracture value was found in the teeth obturated with GP and AH Plus (Group II). This is because of greater adhesion of AH Plus to root dentin than Endoseal MTA. Sagsen et al[11] showed that AH Plus sealer increased the fracture resistance of instrumented root canals. AH Plus has already been proven to have better penetration into the microirregularities because of its creep capacity and long polymerization period.[12] The retention of the filling material may be improved by mechanical locking between the canal walls and the sealers resulting in greater resistance to fracture.[11]

In the previous research study by Mandava et al,[8] teeth obturated with AH Plus showed the highest fracture resistance compared to those with Meta SEAL and MTA Fillapex. The results of our study are in accordance with these findings.

Mineral trioxide aggregate-based root canal sealers have been recently used in root canal obturation because of their high biological compatibility and favorable biological response obtained in laboratory tests and clinical applications.[13,14] An earlier study by Tanalp et al,[15] it was reported that MTA Fillapex did not improve the fracture resistance of immature teeth. Contrary to that, another study demonstrated that MTA Fillapex did increase the fracture resistance of endodontically prepared teeth.[12]

The ability of an obturating material to reinforce the tooth depends on its ability to flow or penetrate into the dentinal tubules, which in turn depends on the size of the dentinal tubules, the particle size of the material, and the rate of reaction of the material.[16] The greater fracture resistance values obtained for Endoseal MTA in the present study can be attributed to its higher flow rates and biomineralization of dentinal tubules.[17] Lim et al reported that Endoseal MTA exhibited significantly higher flow compared to AH Plus sealer. Endoseal MTA is a premixed material supplied in syringes and the freshly extruded mix exhibits thixotropic behavior. Its flow is further facilitated by low mean particle size of 1.5 µm,[18] which allows it to penetrate into ramifications and irregularities of root canal system there by reinforcing the tooth.[19] Though, Endoseal MTA does not bond to dentin, it causes interfacial deposition of hydroxyapatite, which increases the frictional resistance of the obturating material, thus enhancing the fracture resistance of the tooth. This was confirmed in an earlier study which showed that Endoseal MTA enhanced biomineralization of the dentinal tubules beyond the penetrated sealer tag (350–400 µm from the tubule orifice) as observed under scanning electron microscope.[18]

Topcuoglu et al[20] used Tech Biosealer Endo, an MTA-based sealer in the form of a powder and liquid for obturation and reported that it did not increase the root fracture resistance of the teeth, indicating that powder/liquid formulation exhibit less flow compared to paste formulations. In the present study, both AH Plus and Endoseal MTA are supplied as pastes and showed increased fracture resistance possibly due to higher flow and better penetration into dentinal tubules than sealers with a powder/liquid formulation.

As in other mechanical studies,[5,6] the force in the present study was applied along the long axis of the root resulting primarily in a splitting stress applied over the access opening. This is more clinically relevant as it better simulates the support given to healthy teeth by alveolar bone and results in less catastrophic stress buildups caused by unrealistic bending movements.[3]

CONCLUSION

Within the limitation of this *in vitro* study, it can be concluded that the new root canal sealer Endoseal MTA has the potential to reinforce endodontically treated teeth, and it showed no significant difference in the fracture resistance as compared to AH Plus.

CLINICAL SIGNIFICANCE

Endoseal MTA can be considered as a sealer of choice to improve the fracture resistance of endodontically treated teeth.

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09

Root Canal Filling Quality of a Premixed Calcium Silicate Endodontic Sealer Applied Using Gutta-percha Cone-mediated Ultrasonic Activation

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Root Canal Filling Quality of a Premixed Calcium Silicate Endodontic Sealer Applied Using Gutta-percha Cone-mediated Ultrasonic Activation

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Abstract

Introduction: The aim of this study was to investigate the filling quality of a recently developed premixed calcium silicate–based endodontic sealer (Endoseal MTA) with a single gutta-percha (GP) cone technique compared with a resin-based sealer (AH plus) with warm vertical compaction. We also explored the effect of GP cone-mediated ultrasonic activation on the filling quality of Endoseal MTA. **Methods:** Thirty human single-rooted maxillary premolars with ribbon-shaped canals were prepared and assigned to 3 experimental groups according to filling method: EMS group was Endoseal MTA + single-cone; EMSU group was Endoseal MTA + single-cone with ultrasonic activation; and the APW group was AH plus + warm vertical compaction. Each tooth was scanned using micro–computed tomography (μ-CT), and the proportions of sections with void and the volume percentages of void were calculated. Then, the tooth was sectioned transversely, and the presence of void in the slices was scored under a stereomicroscope. The data were statistically analyzed using 1-way analysis of variance and Tukey tests to detect any significance ($\alpha = 0.05$). **Results:** In the μ-CT assessment, there was no significant difference among the groups regarding the proportion of sections with void and the void volume percentage ($P > .05$). However, in the stereomicroscopic evaluation, the EMS group showed a higher number of voids and a higher void score compared with the other groups ($P < .05$). **Conclusion:** Endoseal MTA performs best when used with GP cone-mediated ultrasonic activation. Furthermore, stereomicroscopic observation of sections of the specimens should be performed when evaluating root canal filling quality. *(J Endod 2017;■:1–6)*

Key Words

Calcium silicate, filling quality, gutta-percha, micro–computed tomography, premixed, ultrasonic

Significance

Endoseal MTA, the premixed calcium silicate sealer, is preferred to be used with gutta-percha cone-mediated ultrasonic activation

Calcium silicate cement (eg, mineral trioxide aggregate [MTA]) has been widely used adjacent to pulp and periradicular tissues for perforation repair (1), root-end filling (2), direct pulp capping (3), and regenerative endodontics (4). Some have suggested using calcium silicate cement to obturate the entire root canal system due to its favorable sealing ability and biocompatibility. However, these attempts were limited to particular clinical situations, such as primary teeth (5), 1-visit apexification (6), and internal root resorption repair (7). Furthermore, some studies showed that MTA exhibited significantly lower filling quality than conventional filling technique with gutta-percha (GP) and sealer (8, 9). One possible explanation for the inferior results was that it is difficult to place the cement up to the apical area of the root canal due to handling difficulty (9). Therefore, some studies suggested the use of ultrasonication to improve filling quality (10–12). Ultrasonic activation may generate compressive force that leads to rearrangement of the cement particles, facilitating the escape of entrapped air. However, there is still some discussion regarding the effectiveness of this technique (13, 14).

Recently, calcium silicate paste endodontic sealers, such as Endosequence BC (Brasseler USA, Savannah, GA), iRoot SP (Innovative BioCeramix, Inc, Vancouver, BC), and Endoseal MTA (Maruchi, Wonju, Korea) were introduced in an attempt to overcome the difficulty of placing the cement throughout the root canal space. The sealers are premixed, and they are ready-to-use calcium silicate–based materials that are stored in an air-tight syringe. This permits their direct application into the root canals without mixing. This type of sealer absorbs moisture during the setting reaction, and sets slowly by itself without any mixing procedure. Among these, Endoseal MTA was launched most recently, and it has been investigated more frequently than other mentioned sealers. Previous studies indicated that Endoseal MTA showed satisfactory physical properties (15), biocompatibility (15, 16), good bond strength performance (17), fracture resistance of root dentin (18), minimal discoloration (19), and superior sealer distribution (20); conversely, there have been no studies regarding the root canal filling quality of the sealer.

Premixed injectable sealers are designed to be used with a single-cone technique, and several previous studies described the technique in detail (9, 21–25). The single-cone technique is considered to be less operator-dependent and potentially less damaging to the root canal dentin (26). To facilitate obturation, this technique relies on a sealer with good physicochemical properties that allows the sealer to flow and fill any space between the cone and dentin to provide a tight seal (26). In this respect, it is necessary to find a method, such as ultrasonication, to improve the filling quality of the sealers. As a result, the manufacturer of Endoseal MTA has proposed a new method in which ultrasonic power is applied directly to the master GP cone so that the cone transfers energy to the preplaced sealer to achieve better filling quality with fewer voids (Fig. 1). Previously, Hwang et al. (20) used this technique to fill the canal with Endoseal MTA, and they reported favorable results regarding sealer distribution and bacterial leakage. In this respect, the aim of this *ex vivo* study was to investigate the filling quality of Endoseal MTA with a single-cone technique and to compare this method with AH plus vertically compacted with a heated plugger. In addition, the study aimed to explore the effect of the GP cone-mediated ultrasonic application on the filling quality. Additionally, the evaluation was assessed by using micro–computed tomography (μ-CT) scans and stereomicroscopic observation of mechanically sectioned specimens to verify the validity of the methods.

Materials and Methods

Sample Preparation

Thirty intact, caries-free human single-rooted maxillary premolars with ribbon-shaped canals in the cross section were obtained with patient informed consent under a protocol approved by the Institutional Review Board of Chonbuk National University Hospital. Each tooth underwent a μ-CT scan (SkyScan, Kontich, Belgium) to ensure that it contained a ribbon-shaped canal, and the ribbon-shaped canal was prepared containing 2 canals.

After preparing an access cavity, a size 10 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) was inserted into the canal until it was just visible at the apical foramen. The working length was determined by subtracting 0.5 mm from this length. The root canals were instrumented with nickel-titanium reciprocating files (Reciproc; VDW, Munich, Germany) in the presence of a 5.25% sodium hypochlorite (NaOCl) solution. A new instrument was used for the preparation of each tooth to ensure optimal shaping efficacy. After completing the instrumentation, the canal was irrigated with 5 mL of 17% ethylenediaminetetraacetic acid and 5 mL of a NaOCl solution. After shaping and cleaning, the teeth were randomly divided into 3 groups ($n = 10$) before obturating the instrumented canal spaces.

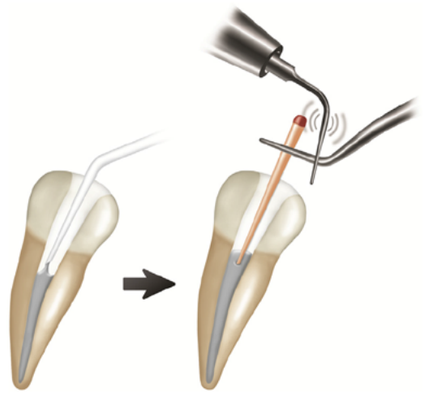

Figure 1. Illustration of gutta-percha cone-mediated ultrasonic activation with Endoseal MTA.

EMS group: Endoseal MTA + single-cone technique: The sealer was dispensed directly into the canal from a premixed syringe via a disposable canal tip. The selected master GP cones (0.04 taper, #25 or 30 size) exhibited good apical tug back, and the cones were slowly inserted into the canals. The excess cone was trimmed off at the canal orifice level and no additional cones were used.

EMSU group: Endoseal MTA + single-cone technique with ultrasonic activation: An ultrasonic tip (StartX #3, Dentsply-Maillefer) was connected to an ultrasonic device (P-5 Newtron XS; Satelec, Mount Laurel, NJ), which was set on "8" in the yellow code (ie, indicated as suitable for endodontics by the manufacturer). After placing the sealer into the canal, ultrasonic vibration was applied to a cotton plier that held the GP cone 20 mm from the tip (Fig. 1). Then, the cone slowly reached the working length during continuous ultrasonic activation. The ultrasonic application time during GP cone placement was 2 to 3 seconds, and the excess cone was cut at the orifice level.

APW group: AH plus + warm vertical compaction technique: Each canal was filled with AH plus (Dentsply DeTrey, Konstanz, Germany) and a GP cone using a warm vertical compaction technique. Briefly, the inserted GP cone was down packed with a Dia-Pen heat source (Diadent, Cheongju, Korea) to within 3 to 5 mm of the working length, and the canal was backfilled using Dia-Gun (Diadent).

The access cavities were filled with a flowable composite resin (G-ænial Flo; GC, Tokyo, Japan), and the teeth were maintained at 100% humidity for 7 days at 37°C to allow the sealer to completely set.

μ-CT Evaluation

A SkyScan 1076 high-resolution μ-CT scanner was used to scan the teeth, and the μ-CT scanner had a pixel size of 30 μm. The X-ray source voltage was 100 kV, the beam current was 100 μA, and the aluminum filter thickness was 0.5 mm. The rotation was 0.4° per step, and the exposure time was 316 ms. To minimize ring artifacts, air calibration of the detector was carried out before each scan. Other settings included the beam-hardening correction and input of optimal contrast limits according to the manufacturer's instructions. Images obtained from the scan were reconstructed using the NRecon (SkyScan) software, and CT-An (version 1.12.9; SkyScan) was used to measure the volume of the void.

The presence of void was assessed in 2-dimensional slices using a previous study's protocol (27). Starting at the apical end of the root, new cross-sectional images were prepared perpendicular to the long axis of the root. The sections had an interval of 50 μm, and the average number of cross-sections was 243. The μ-CT images of the sections were then converted to tagged image file format (tiff) and coded. The presence of void was assessed in each section on a diagnostic screen by 2 observers (Fig. 1*A–D*). The observers were unaware of the root canal filling technique. For each section, measurements were repeated 2 times, and the mean was calculated. Then, the proportion of sections with void was computed for each root.

To calculate the void in the 3-dimensional volumes, the original grayscale images were processed using a Gaussian low-pass filter for noise reduction. An automatic segmentation threshold was used to subtract dentin using CT-An. A thresholding (binarization) process was used

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in which the range of gray levels was processed to obtain an imposed image of black/white pixels only. Then, a region of interest was chosen separately for each slice to allow calculation of void volumes (Fig. 2*E* and *F*). Then, the percentage of void (*V*%) was calculated as *Vv*/(*Vv* + *VM*) × 100. Here, *VM* is the volume of the filling material, and *Vv* is the volume of the void. Three-dimensional images of the filling material were visualized using CT-Vol (SkyScan) to evaluate the overall filling quality (Fig. 2*G*).

Stereomicroscopic Observation

After μ-CT evaluation, the teeth were sectioned perpendicular to the longitudinal axis of the root using a low-speed diamond-coated saw (Isomet, Buehler, IL) under water cooling. Six slices per root were obtained at a thickness of 1.5 ± 0.1 mm, and then each 2 slices were assigned as coronal, middle, or apical part, respectively. All slices were observed under a digital stereomicroscope (MZ16FA; Leica Microsystems, Wetzlar, Germany), and pictures were taken of each slice. The digital images of each segment were then used to estimate the presence of void (Fig. 3). The number of voids was counted, and then the presence of void was scored using the scoring system described in Table 1. The scoring was performed by 2 independent observers who were unaware of the specific treatment protocol of each specimen. For each section, measurements were repeated 2 times, and the mean was calculated.

Statistical Analysis

The data were statistically analyzed using Kolmogorov-Smirnov test for determination of normal distribution, and then by 1-way analysis of variance and Tukey tests to detect any significance ($P < .05$). These analyses were performed with the SPSS software (SPSS 12.0 K for Windows; SPSS Inc., Chicago, IL).

Results

μ-CT Evaluation

Table 2 summarizes the mean percentage of μ-CT sections with void and the volume percentage of void. A high frequency of void was found in all specimens, but there was no significant difference between the groups ($P > .05$) in relation to the proportion of μ-CT sections with void and volume of void.

Stereomicroscopic Evaluation

There were significant differences between the groups in terms of the average number of void and void score ($P < .05$) (Table 3). The EMS group showed a higher number of void and void score compared with the other groups ($P < .05$). There were significantly fewer voids when the Endoseal MTA was used with ultrasonic activation ($P < .05$). Furthermore, significant differences were observed between the EMS group and other groups at similar locations along the root ($P < .05$). Meanwhile, there was no significant difference between the EMSU and APW groups ($P > .05$).

Discussion

The main function of an endodontic sealer is to inhibit intracanal leakage, and its sealability should be assessed to evaluate this requirement. Generally, the sealability can be evaluated by using 2 methodologies: (1) measuring the leakage and (2) visually assessing the filling quality. The former, however, does not attract research attention anymore because of some crucial limitations. In December 2007, the *Journal of Endodontics* placed a moratorium on leakage tests and sealability studies that directly compare one endodontic technique with another (28). Instead, the journal encouraged investigators to study the validity of the methods themselves. Therefore, in this study, we evaluated the filling quality of calcium silicate–based endodontic sealers using 2 different methods. One method was nondestructive (μ-CT), and the other was destructive (sectioning). For this purpose, we evaluated the number of voids or gaps because it is a relevant parameter for assessing the quality of a root canal obturation system (29).

There have been some studies regarding the sealability of premixed, ready-to-use calcium silicate–based root canal sealers. Celikten et al (25) reported in the μ-CT study that bioceramic sealers (EndoSequence BC and Smartpaste bio) produced similar voids compared with AH plus. We also found some studies that evaluated the sealability of calcium silicate–based sealers using a microleakage model. In their fluid filtration test, Zhang et al (30) indicated that iRoot SP was equivalent to AH plus sealer in apical sealing ability. Pawar et al (23) also reported a dye penetration test that showed that Endosequence BC sealed the root canal better than AH plus. A recent review article that considered laboratory experiments and clinical studies of bioceramic sealers (including both calcium silicate and calcium phosphate) indicated that the sealability of the sealers was satisfactory and comparable to other commercially available sealers (31). On the other hand, most previous studies used microleakage models, which are considered to be nonreproducible, and they show large standard deviations (32). Furthermore, there have been a very limited number of studies regarding the assessment of filling quality. Therefore, we chose to investigate the filling quality by using void or gap detection methods.

In this study, interestingly, the 2 methods showed different results for void differences between EMS and other groups. In the μ-CT analysis, there was no significant difference among the 3 experimental groups ($P > .05$), but in the stereomicroscopic evaluation of sectioned samples, there was a significant difference between EMS and other groups ($P < .05$). The μ-CT analysis used in the present study is known to provide a clear understanding of the location and volumetric measurements of gaps and internal voids because of its highly accurate and nondestructive characteristics (33). Sealers, though, are often radiopaque, and they may affect the μ-CT detection of voids within the bulk of the root filling. In this respect, we performed a stereomicroscopic analysis using the same specimen by preparing transverse sections. In the stereomicroscopic analysis, both the average number of voids and void scores were higher in the EMS group compared with other groups ($P < .05$). This can be

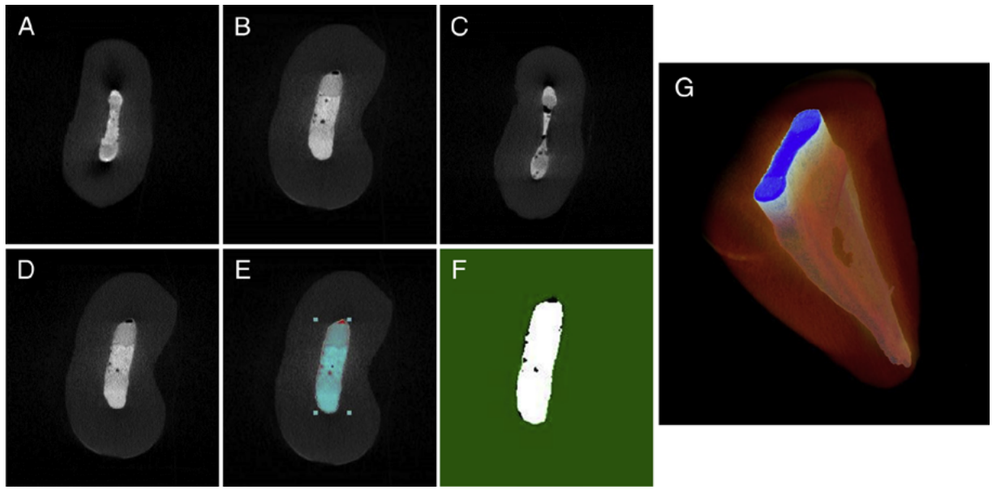

Figure 2. Representative micro–computed tomography images showing (*A*) no void, (*B–D*) marked defects, (*E*) region of interest (ROI) selection on images, and (*F*) void detection within the ROI. (*G*) Three-dimensional reconstructed models of the obturated root canals.

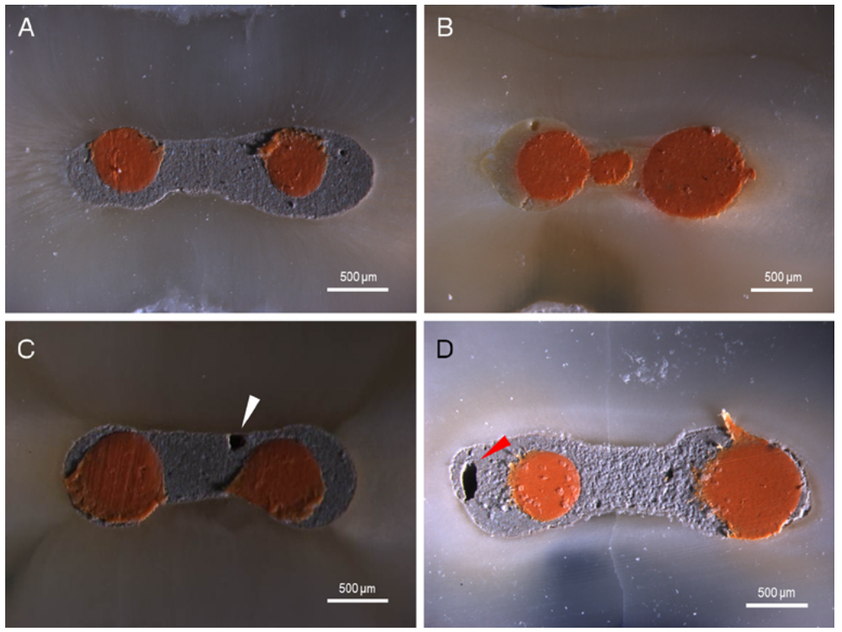

Figure 3. Representative stereomicroscopic images. (*A*) Score 1 of EMSU (Endoseal MTA + single-cone technique with ultrasonic activation) group, (*B*) score 1 of APW (AH plus + warm vertical compaction technique) group, (*C*) score 2 of EMSU group, (*D*) score 3 of EMS (Endoseal MTA + single-cone technique) group. *White* and *red arrows* indicate mid- and large-sized air bubbles, respectively.

TABLE 1. Scores Used for Evaluating the Filling Quality by Void Detection

Score	Definition
1	Well-condensed filling that showed only a few, minor air bubbles (less than 0.1 mm in diameter).
2	An imperfectly condensed filling that showed some minor air bubbles (more than 3 defects) or medium-sized air bubbles (0.1 mm to 0.2 mm in diameter).
3	Inadequately condensed filling that showed many minor air bubbles (more than 5 defects) or large air bubbles (more than 0.2 mm in diameter).
4	Poorly condensed filling that showed many minor air bubbles (more than 7 defects) or empty space connecting separate canal walls.

TABLE 2. Mean Percentage and SD of Sections with Void in 2-dimensional Slices and the Volume Percentage of Void in Root Canal Filling Materials in 3-dimensional Images

Group	Criteria	Mean ± SD
EMS	Proportion of section with void (%)	70.79 ± 14.3
EMSU		66.07 ± 13.1
APW		68.94 ± 11.3
EMS	Volume of void (%)	9.67 ± 3.22
EMSU		7.87 ± 2.89
APW		7.31 ± 3.74

APW, AH plus + warm vertical compaction technique; EMS, Endoseal MTA + single-cone technique; EMSU, Endoseal MTA + single-cone technique with ultrasonic activation.
Values are shown as the mean ± SD. There was no significant difference between the groups ($P > .05$).

TABLE 3. The Average Number of Void and Void Score in Stereomicroscopic Images of Each Sectioned Specimen

Location	Average number of voids			Void score		
	EMS	EMSU	APW	EMS	EMSU	APW
Apical	3.50 ± 1.08^{a}	2.40 ± 1.20^{b}	1.40 ± 0.82^{b}	1.87 ± 0.64^{A}	1.23 ± 0.32^{B}	0.87 ± 0.35^{B}
Middle	5.60 ± 2.07^{a}	3.40 ± 1.74^{b}	2.75 ± 1.28^{b}	1.93 ± 1.08^{A}	1.39 ± 0.51^{B}	1.18 ± 0.45^{B}
Coronal	7.30 ± 1.70^{a}	5.05 ± 1.40^{b}	3.73 ± 1.31^{b}	2.45 ± 0.73^{A}	1.88 ± 0.53^{B}	1.58 ± 0.41^{B}
Overall	5.47 ± 1.38^{a}	3.62 ± 1.11^{b}	2.86 ± 1.34^{b}	2.25 ± 0.61^{A}	1.50 ± 0.43^{B}	1.22 ± 0.59^{B}

APW, AH plus + warm vertical compaction technique; EMS, Endoseal MTA + single-cone technique; EMSU, Endoseal MTA + single-cone technique with ultrasonic activation.
Values are shown as the mean ± standard deviation. Groups identified by the same letter are not significantly different ($P > .05$).

explained in 2 ways: (1) the loss of material that occurred during sectioning and affected the score (34), and (2) the resolution of μ-CT was not sufficient to detect small voids. The former is the limitation of the sectioning method, but differences were found between the 2 groups using the same sealer, EMS and EMSU. This difference might depend on the materials, and the former possibility can be considered. A method-dependent difference was observed between the EMS and EMSU. Although μ-CT provides information along the entire root canal system, we speculated that μ-CT observations might be less sensitive compared with the sectioning method in terms of void detection.

In the current stereomicroscopic analysis, the EMS group showed significantly more voids compared with EMSU and APW groups at all locations along the root ($P < .05$). Voids in root canal sealer are a great concern because they create porosity, reduce the quality of filling, serve as hubs for microbial housing, and may even link up to tunnel and transport contaminants along the filled root canal (35). Several types of flaws may occur in Portland cement concrete. Among these, honeycomb refers to a structure containing small cavities that are the result of entrapped air bubbles in the cement during placement and consolidation. In concrete science, these voids can be avoided by applying a vibrator into the unset cement (36). During the application of calcium silicate cement to the root canal, vibration minimizes flaws by producing a series of rapid compressive impulses that reduce the surface friction between the cement particles that enabled the cement to support itself in a honeycombed condition (11). Based on these principles, we applied ultrasonic vibration to the sealer through a master GP cone, and we obtained better filling quality, which produced fewer voids in the sealer mass. On the contrary, Parashos et al (12) reported that excessive ultrasonication adversely affected MTA properties and void formation. Therefore, we suggested that gentle ultrasonic activation may be necessary to achieve more favorable results. According to the manufacturer's information, the ultrasonic vibration frequency generated from the device (P-5 Newtron XS) ranges from 28 to 36 kHz (37). Amplitude is an important parameter, and it is related to the strength of the ultrasonic wave. Amplitude is defined as the difference between the peak value and the average value of the waveform. Generally, amplitude is attenuated as the ultrasonic wave moves through the substrate when the frequency is constant (38). Here, the ultrasonic wave generated from the device should pass through 3 media, including the ultrasonic tip, the cotton plier, and the GP cone. Therefore, we speculated that the actual amplitude transmitted to the GP cone was reduced so that the ultrasonic vibration was just enough to remove the entrapped air bubbles without affecting the integrity of the sealer.

Collectively, Endoseal MTA, a premixed calcium silicate sealer, performs better when used with GP cone-mediated ultrasonic activation. Furthermore, we suggest that the microscopic observations of the specimen obtained by sectioning should be included as a supportive method for evaluating the quality of root canal filling.

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The authors deny any conflicts of interest related to this study.

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

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Original Article

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

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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.

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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 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*.

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 multi-channel 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.

Results

Figure 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 ($P < 0.05$). 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 oxide such as soidum oxide, aluminum oxide, ferric oxide, and silcon dioxide in Endoseal were detected than EndoSequence BC sealer.

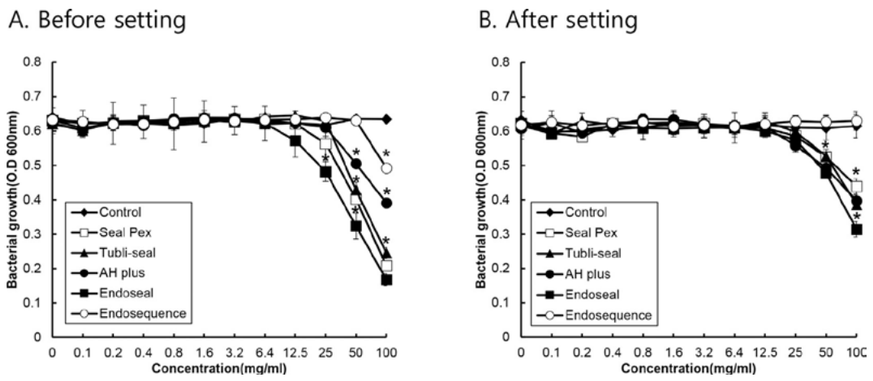

Figure 1 The antibacterial activity of traditional and calcium silicate-based root canal sealers against *E. faecalis*. The eluate from traditional and calcium silicate-based root canal sealers before (A) or after setting (B) was prepared using a PBS, and *E. faecalis* was cultivated with and without the prepared eluate of various sealers at various concentrations in a 96-well polystyrene plate. The growth of *E. faecalis* was measured using a microplate reader at 600 nm. The experiments were conducted three times in duplicate, and data are represented as the mean ± S.D. * Statistically significant differences compared with cultures not treated with the spent culture medium ($p < 0.05$).

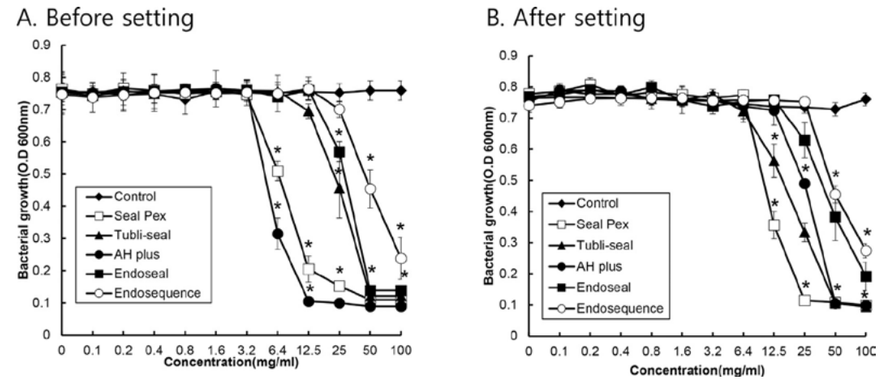

Figure 2 The antibacterial activity of traditional and calcium silicate-based root canal sealers against *P. endodontalis*. The eluate from traditional and calcium silicate-based root canal sealers before (A) or after setting (B) was prepared using a PBS, and *P. endodontalis* was cultivated with and without the prepared elute of various sealers at various concentrations in a 96-well polystyrene plate under anaerobic conditions. The growth of *P. endodontalis* was measured using a microplate reader at 600 nm. The experiments were conducted three times in duplicate, and data are represented as the mean ± S.D. * Statistically significant difference compared with cultures not treated with the spent culture medium ($p < 0.05$).

Discussion

Control of the bacteria determines the success or failure of root canal treatment. Although, chemomechanical procedures, cleaning, and disinfectant treatment are performed to reduce the number of bacteria when treating the root canal, some bacteria often remain in the root canal systems.[6] Therefore, the root-filling materials with antibacterial activity are required and are advantageous. Recently, calcium silicate-based root canal sealers have been developed, and their antibacterial activity against some Gram-positive bacteria, including *E. faecalis*, has been examined.[13,14] However, other bacteria, such as *P. gingivalis* and *P. endodontalis*, are related to pulpal inflammations, and the antibacterial activity of the sealers after setting has not been evaluated. Therefore, this study investigated and compared the antibacterial activity of traditional sealers and calcium silicate-based sealers against *P. gingivalis*, *P. endodontalis*, and *E. faecalis* before and after setting.

In the susceptibility test, before setting, the traditional sealers showed stronger antimicrobial activity on *P. gingivalis* and *P. endodontalis* than the calcium silicate-based. Endoseal showed the strongest antibacterial activity against *E. faecalis*, whereas, Endosequence BC sealer showed weak

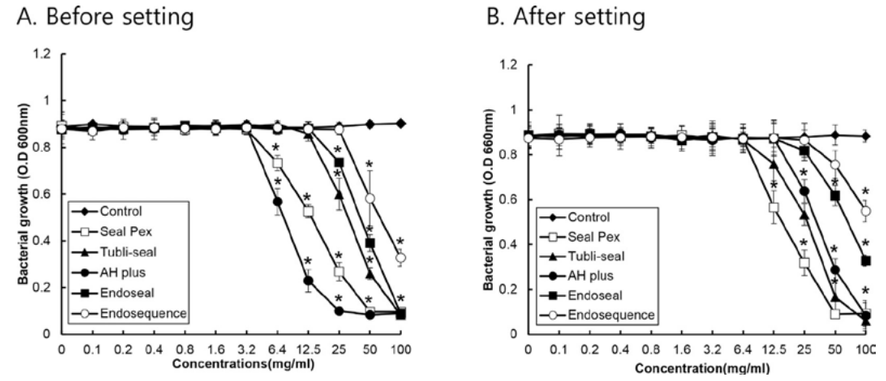

Figure 3 The susceptibility of *P. gingivalis* for various root canal sealers. The eluate from traditional and calcium silicate-based root canal sealers before (A) or after setting (B) was prepared using a PBS, and *P. gingivalis* was cultivated with and without the prepared eluate of various sealers at various concentration in a 96-well polystyrene plate under anaerobic conditions. The growth of *P. gingivalis* was measured using a microplate reader at 600 nm. The experiments were conducted three times in duplicate, and data are represented as the mean ± S.D. * Statistically significant differences compared with cultures not treated with the spent culture medium ($p < 0.05$).

Table 2 Comparison of compositions of calcium silicate-based root canal sealers by X-ray fluorescence analysis.

EndoSequence BC		Endoseal	
Components	Mass %	Components	Mass %
Al_2O_3	0.0035	Na_2O	0.0706
SiO_2	5.77	MgO	1.20
P_2O_5	1.80	Al_2O_3	2.84
K_2O	0.0358	SiO_2	7.56
CaO	37.8	SO_3	1.27
MnO	0.0157	K_2O	0.574
SrO	0.0046	CaO	25.1
ZrO_2	53.3	TiO_2	0.141
HfO_2	1.04	Cr_2O_3	0.105
		MnO	0.0365
		Fe_2O_3	1.29
		NiO	0.0029
		SrO	0.0503
		Y_2O_3	0.0592
		ZrO_2	53.0
		HfO_2	1.05
		Bi_2O_3	5.66

antibacterial activity against all bacteria. The comparison of antimicrobial activity among the sealers against the bacteria or the comparison of susceptibility among the bacteria for the sealer is possible by performing broth method using the elute. The used elutes were extracted after measuring same weight of the sealer at one time and then carried out the test of the antimicrobial activity. These data may be not obtained by the experiment of agar diffusion assay.

In the present study, AH plus, an epoxy resin-based sealer, showed the strongest antibacterial activity against *P. gingivalis* and *P. endodontalis* but was weaker against *E. faecalis*. Epoxy resin-based sealers exhibit antibacterial activity through bisphenol A diglycidyl ether and formaldehyde during polymerization.[15] Therefore, Gram-negative bacteria, which have thin cell walls, are sensitive to chemicals because of easy penetration into the bacterial cytosol. Formaldehyde penetrates into the interior of bacteria and inhibits metabolism of bacteria by reacting with cytosolic proteins, RNA, and DNA.[16] Endoseal is a calcium silicate-based sealer and showed an antibacterial effect against *E. faecalis* before or after setting. Endoseal showed the strongest antimicrobial effect against bacteria under alkaline conditions because of calcium silicate.[17] However, Endosequence BC sealer, another calcium silicate-based sealer, showed weak antimicrobial activity against *E. faecalis*. Endosequence BC sealer and Endoseal commonly exhibit antibacterial activity because of $Ca(OH)_2$ (calcium hydroxide) reaction, which is bactericidal against Gram-negative bacteria through damage of bacterial membrane or DNA, and denaturation proteins.[18] We performed X-ray fluorescence analysis to investigate the difference between the two sealers. Endoseal contained more types and more amount of the oxide compound known to have antimicrobial activity such as Na_2O, MgO, Al_2O_3, SO_2, and Fe_2O than EndoSequence BC. Among oxide compounds, these oxide compounds damage the cell wall of Gram-positive bacteria and increase the permeability of molecules into the cytoplasm through electrostatic interaction.[19–22] Finally, various oxide compounds with antimicrobial activity in Endoseal may damage the cell wall of bacteria and help the penetration of $Ca(OH)_2$ into the cytosol, and then $Ca(OH)_2$ may denature DNA and protein. Because Endosequence BC contained relatively low amount of oxide compounds with antimicrobial activity, Endosequence BC may weakly damage the cell walls of bacteria, and $Ca(OH)_2$ may penetrate less. This indicates that calcium silicate-base sealers containing oxide compounds may show the strong antimicrobial activity against Gram-negative and Gram–positive bacteria.

P. gingivalis and *P. endodontalis* are related to untreated root canal infection,[23] and *E. faecalis* is associated with re-infection of treated root canals.[4,23] According to the results of this study, traditional sealers may be effective in treating primary root canal infections, whereas Endoseal, a calcium silicate-base sealer that contains oxide compounds, is more effective in preventing re-infection with *E. faecalis*. However, considering that traditional sealers have a cytotoxic effect on human pulp cell in vitro.[17,24,25] Endoseal may be useful for preventing bacterial infection in untreated and treated root canals.

Within the limitations of the present study, all of the freshly mixed sealers exhibited higher antibacterial activity than the set sealers. The antibacterial activity of the tested sealers was found to be material- and bacteria-dependent. Endoseal continues to exhibit antibacterial activity after setting and may be the most effective in eliminating *E. faecalis* in the root canal. Finally, Endoseal may be the most useful sealer for preventing bacterial infection when treating the root canal.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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Influence of environment on testing of hydraulic sealers

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Influence of environment on testing of hydraulic sealers

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In vitro **material testing is undertaken by conducting a series of tests following procedures outlined in international standards. All material properties are measured in water; however biological behavior is undertaken in alternative media such as Dulbecco's modified eagle medium (DMEM) or simulated body fluid. The aim of this study was to characterize four dental root canal sealers and study their properties in different media. Four dental root canal sealers were assessed. They were characterized by a combination of techniques and the sealer properties were tested as specified by ISO 6876 (2012) and also in alternative media. The sealer biocompatibility was measured by cell function and proliferation assays of elutions. All sealers complied with ISO specifications. The material properties were effected by the type of soaking medium used and the surface micromorphology and elemental composition were dependent on the soaking solution type. Both BioRoot and MTA Fillapex showed cytotoxicity which reduced at higher dilutions. The material chemistry, presentation, environmental conditions and testing methodology used affected the sealer properties. Standards specific to sealer type are thus indicated. Furthermore the methodology used in the standard testing should be more relevant to clinical situations.**

A number of materials are used in various fields of dentistry. These materials should comply with the norms defined in international standards. Root canal sealer cements are used in obturation of root canals during root canal therapy. They are used in conjunction with solid gutta-percha points. The aim of using this material combination is the hermetic seal[1,2] to avoid bacterial recontamination of the root canal space and thus treatment failure. The use of root canal sealers is mandatory to enhance the three dimensional compact sealing of gutta-percha in complex root canal systems. The properties of the ideal root canal sealer as suggested by Grossman[3] include an excellent seal when set, dimensional stability, a slow setting time to ensure sufficient working time, insolubility to tissue fluids, adequate adhesion to canal walls, and biocompatibility. These sealers interact with the root dentine by mechanical interlocking and the formation of resin tags, which bind the sealer mechanically to the dentinal tubules[4–6].

The conventional root canal sealer cements are classified according to the material chemistry and are tested following norms defined in ISO 6876; 2012[7]. During the last decade root canal sealers based on building material, Portland cement have been introduced and are known as hydraulic calcium silicate-based sealers which are also tested using the ISO 6876; 2012[7]. The popularity with these sealers is their hydraulic nature and their interaction with blood, tissue fluids and tooth tissue. Most of the properties of these materials depend on the formation of calcium hydroxide as a by-product of material hydration[8,9]. The release of this calcium hydroxide in solution is responsible for a number of properties that make this material popular for clinical use[10,11]. The release of the calcium hydroxide in solution renders the material soluble[12] which in turn affects the other material properties such as biocompatibility, antimicrobial properties and physical and chemical characteristics[13,14].

As indicated, the chemistry and material morphology of the different root canal sealers changes their physical, chemical and biological properties. When tested *in vitro* and when in use, the materials are subjected to different environments. The different environments have been shown to affect the material chemistry[15,16] thus they can be postulated to also change the other material properties. The aim of this study was to characterise sealers based on tricalcium silicate, assess their properties according to ISO 6876; 2012[7] specifications and re-evaluate if the sealer properties change in contact with different fluids used in biocompatibility testing and simulated body fluid. The cell proliferation and expression of the sealers was also evaluated.

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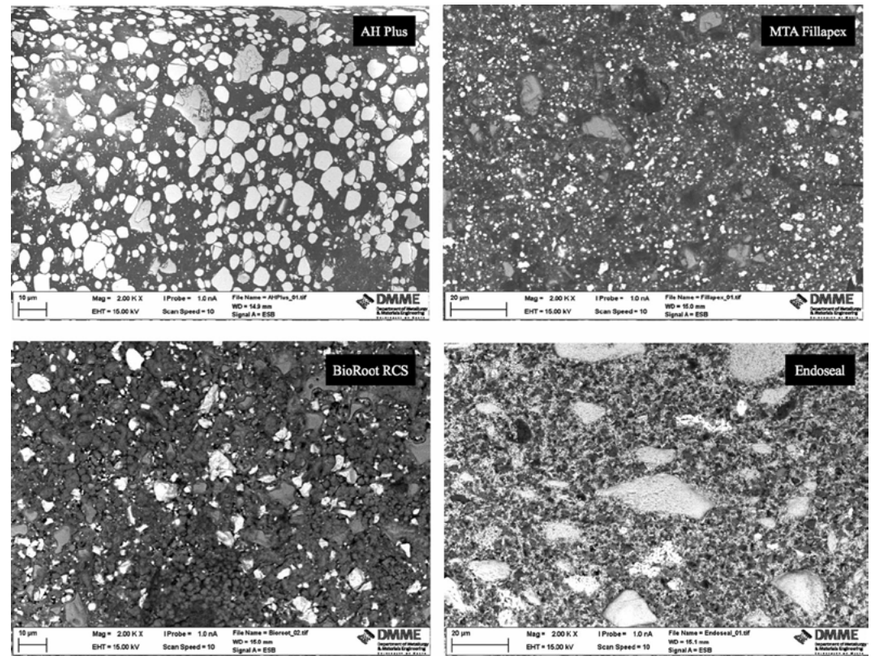

Figure 1. Back-scatter scanning electron micrographs of polished sections of test sealers showing micro-structural components.

Results

Material characterisation. The scanning electron micrographs and EDS plots of all sealers tested are shown in Figs 1 and 2. The XRD plots in Fig. 3. Although the materials were all based on tricalcium silicate, the characterisation showed a different microstructure and presence of diverse radiopacifiers. The AH Plus included calcium tungstate (ICDD: 01-085-0443) and zirconium oxide (ICDD: 00-037-1484), the MTA Fillapex had calcium tungstate (ICDD: 00-041-1431), zirconium oxide in BioRoot RCS (ICDD: 04-015-6852) and zirconium oxide (ICDD: 04-015-4188) and bismuth oxide (ICDD: 04-003-2034) in Endoseal.

Measurement of sealer physical properties. The results for the measurement of the physical properties described ISO 6876; 2012[7] and the relevant modifications discussed in methodology are shown in Table 1.

Flow and film thickness. The results for sealer flow, and film thickness are shown in Table 1 and compared to ISO norms. All sealers complied with the standards as they exhibited a flow greater than 17 mm and a film thickness smaller than 50 mm.

Radiopacity. All the sealers exhibited a radiopacity greater than 3 mm aluminium thickness (Table 1) specified by ISO 6876; 2012[7].

Setting time. The MTA Fillapex did not set indefinitely. Although it was not completely unset an indentation could still be seen on the materials surface. Immersion in HBSS and DMEM did not affect the setting of this sealer (Table 1). The other tricalcium silicate-based sealers exhibited a lower setting time than AH Plus when allowed to set in air ($P < 0.001$) while immersion in HBSS and DMEM led to a longer setting time when compared to AH Plus ($P < 0.001$). Immersion in HBSS and DMEM reduced the setting time of AH Plus considerably ($P < 0.001$) while that of BioRoot RCS and Endoseal was extended when sealers were immersed in solution (Table 1).

Fluid uptake, sorption, solubility and porosity measurements. The results for the fluid uptake are shown in Table 2. The AH Plus exhibited the lowest fluid uptake of all the sealers tested. The solution type did not affect the fluid uptake of AH Plus which was low and increased slightly throughout the period of study. The BioRoot RCS exhibited slightly higher fluid uptake compared to AH Plus with a trend to increase in HBSS and DMEM. The one-day fluid uptake was high and reduced slightly after 7 days with an increase over the 28 day period. The Endoseal exhibited very high initial fluid uptake, which reduced over the 28-day testing period. The solution type did not affect the results obtained.

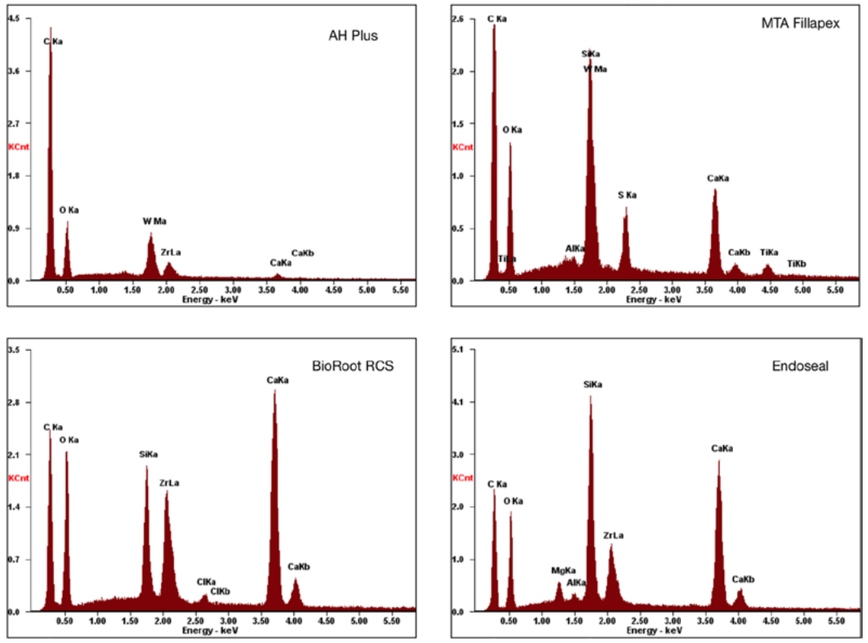

Figure 2. Energy dispersive spectroscopic analysis of the test materials showing the elemental analysis.

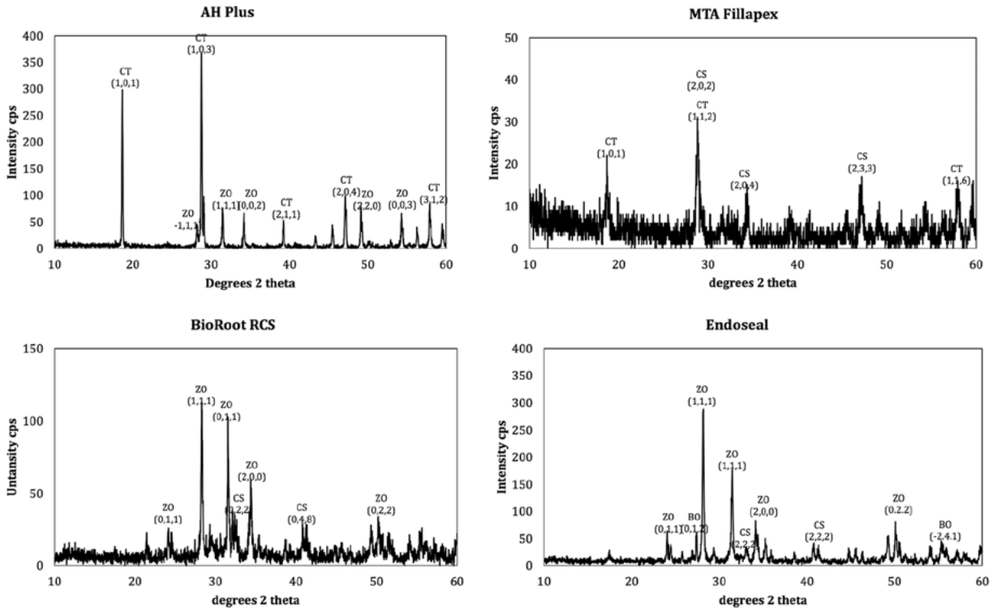

Figure 3. X-ray diffraction plots of test sealers showing the main crystalline phases present (BO: bismuth oxide, CS: calcium silicate, CT: calcium tungstate, ZO: zirconium oxide).

Material	Media	Test			
		Flow	Film Thickness	Radiopacity	Setting Time
		mm	*μm*	*mm Al*	*mins*
AH Plus	as specified	24 ± 2	15 ± 5	16 ± 1	688
	HBSS	/	/	/	433
	DMEM	/	/	/	485
MTA Fillapex	as specified	30 ± 1	19 ± 4	4 ± 1	unset
	HBSS	/	/	/	unset
	DMEM	/	/	/	unset
BioRoot RCS	as specified	25 ± 1	46 ± 7	7 ± 1	235
	HBSS	/	/	/	667
	DMEM	/	/	/	650
Endoseal	as specified	25 ± 1	35 ± 5	12 ± 1	435
	HBSS	/	/	/	662
	DMEM	/	/	/	645
ISO Standard		6876	6876	6876	6876
value		>17	<50	<3	/

Table 1. Results for testing of physical properties of test sealers in different environmental conditions ± SD.

Material	Media	TEST				
		Fluid Uptake (%)				
		1 day	*7 days*	*14 days*	*21 days*	*28 days*
AH Plus	water	0.2 ± 0.16	0.7 ± 0.21	1.1 ± 0.33	0.9 ± 0.18	2.2 ± 0.15
	HBSS	0.1 ± 0.05	0.5 ± 0.24	0.8 ± 0.30	0.5 ± 0.07	2.0 ± 0.15
	DMEM	0.3 ± 0.12	1.0 ± 0.23	0.9 ± 0.12	1.0 ± 0.37	2.2 ± 0.16
BioRoot RCS	water	2.4 ± 0.62	0.9 ± 1.62	1.2 ± 1.52	1.5 ± 1.37	5.5 ± 1.62
	HBSS	2.3 ± 0.54	1.2 ± 0.44	1.9 ± 0.83	2.1 ± 0.98	7.2 ± 0.71
	DMEM	1.7 ± 0.55	1.7 ± 1.48	2.3 ± 1.13	2.9 ± 1.05	9.8 ± 0.95
Endoseal	water	11.7 ± 2.14	1.4 ± 3.54	1.1 ± 3.94	1.1 ± 4.21	3.6 ± 3.92
	HBSS	10.5 ± 2.29	2.4 ± 1.96	1.9 ± 1.81	1.8 ± 1.78	4.5 ± 1.79
	DMEM	11.8 ± 1.58	1.3 ± 3.51	0.7 ± 3.22	0.7 ± 3.33	0.3 ± 3.33

Table 2. Results for testing fluid uptake of test sealers in different environmental conditions ± SD. *MTA Fillapex did not set so the fluid uptake could not be measured.

Material	Media	Test				
		Sorption 28 days	Solubility 28 days	Sorption 1 day	Solubility 1 day	Solubility
		mg/mm³	*mg/mm³*	*mg/mm³*	*mg/mm³*	%
AH Plus	water	1.7 ± 0.4	0.3 ± 0.2	1.1 ± 0.1	0.6 ± 0.3	−0.04 ± 0.01
	HBSS	2.2 ± 1.0	0.4 ± 0.7	0.6 ± 0.1	0.4 ± 0.2	−2.3 ± 0.9
	DMEM	1.6 ± 0.6	0.4 ± 0.2	1.2 ± 0.3	0.4 ± 0.1	−25.1 ± 10.8
BioRoot	water	32.6 ± 1.8	36.1 ± 4.4	31.1 ± 2.9	26.6 ± 2.0	15.8 ± 5.7
	HBSS	34.1 ± 0.6	39.4 ± 1.9	33.5 ± 1.8	29.1 ± 0.9	30.2 ± 8.5
	DMEM	34.7 ± 3.2	41.4 ± 4.8	34.3 ± 0.6	31.1 ± 1.3	31.1 ± 4.3
Endoseal	water	39.3 ± 2.7	37.5 ± 9.9	40.4 ± 1.5	17.9 ± 4.4	1.4 ± 0.3
	HBSS	39.0 ± 2.7	35.9 ± 3.1	41.4 ± 3.4	20.0 ± 2.7	2.4 ± 1.7
	DMEM	44.1 ± 1.8	44.0 ± 6.6	38.5 ± 3.2	16.5 ± 5.2	20.4 ± 7.3
ISO Standard		4049	4049	4049	4049	6876
value		<40	<7.5	<40	<7.5	>3

Table 3. Sorption and solubility values for test sealers using two standard methodologies (Mean ± SD). *MTA Fillapex did not set so the fluid uptake could not be measured.

The MTA Fillapex could not be tested since it remained partially set. The sorption and solubility data shown in Table 3 shows that both properties for AH Plus were lower than that of the tricalcium silicate-based sealers for all the soaking solutions tested ($P < 0.001$). All the sealers tested complied to ISO 4049 recommendations[17] for sorption except the Endoseal in DMEM after 28 days where the sorption was slightly higher than 40 mg/mm³. Since

Material	Media	% Porosity	
		1 day	*28 days*
AH Plus	water	0.21 ± 0.16	0.73 ± 0.15
	HBSS	0.09 ± 0.05	0.68 ± 0.15
	DMEM	0.35 ± 0.12	0.73 ± 0.16
BioRoot RCS	water	2.42 ± 0.62	−1.82 ± 1.62
	HBSS	2.32 ± 0.54	−2.47 ± 0.71
	DMEM	1.70 ± 0.55	13.26 ± 0.95
Endoseal	water	11.69 ± 2.14	1.21 ± 3.92
	HBSS	10.54 ± 2.29	1.51 ± 1.79
	DMEM	11.85 ± 1.3	0.11 ± 3.33

Table 4. Percentage porosity measured after 1 and 28 day immersion of test sealers in different media. *MTA Fillapex did not set so the percentage porosity could not be measured.

Material	Media	pH					
		pH	*1 day*	*7 days*	*14 days*	*21 days*	*28 days*
AH Plus	water	7.4	9.8 ± 0.2	9.2 ± 0.1	9.1 ± 0.3	9.4 ± 0.3	9.8 ± 0.4
	HBSS	8.5	10.5 ± 0.2	7.5 ± 0.2	7.6 ± 0.3	7.5 ± 0.2	7.6 ± 0.2
	DMEM	7.9	10.5 ± 0.2	8.3 ± 0.5	8.4 ± 0.5	8.4 ± 0.1	6.1 ± 0.4
MTA Fillapex	water	7.4	10.4 ± 0.1	11 ± 0.1	10.4 ± 0.1	10.4 ± 0.0	9.8 ± 0.1
	HBSS	8.5	10 ± 0.1	10.5 ± 0.1	10.2 ± 0.1	10.2 ± 0.1	9.8 ± 0.0
	DMEM	7.9	8.5 ± 0.2	10.2 ± 0.1	10 ± 0.1	10 ± 0.1	9.6 ± 0.1
BioRoot RCS	water	7.4	10.5 ± 0.05	12.5 ± 0.1	12.4 ± 0.1	12.4 ± 0.1	12.4 ± 0.0
	HBSS	8.5	10.5 ± 0.05	12.1 ± 0.5	12.4 ± 0.1	12.3 ± 0.2	12.5 ± 0.0
	DMEM	7.9	10.5 ± 0.1	12.4 ± 0.1	12.5 ± 0.1	12.4 ± 0.1	12.5 ± 0.1
Endoseal	water	7.4	10.5 ± 0.1	12.2 ± 0.1	11.9 ± 0.2	12.2 ± 0.1	12.2 ± 0.1
	HBSS	8.5	10.9 ± 0.2	11.5 ± 0.1	11.6 ± 0.1	11.8 ± 0.1	11.7 ± 0.1
	DMEM	7.9	10.5 ± 0.3	10.7 ± 0.2	10.6 ± 0.1	10.9 ± 0.1	10.9 ± 0.1

Table 5. pH values measured weekly over a 28 day period of test sealers in different media.

the ISO recommendations are for 1 day soaked materials this aberration was not considered to be significant. The solubility was high for both BioRoot RCS and Endoseal compared to ISO recommendations and increased over the 28-day period for all solutions tested. Using the ISO 6876 recommendations[7] the solubility was mostly negative for all the sealers tested in HBSS and DMEM showing that rather than being soluble the sealers allowed deposition of matter on them thus increasing in weight showing a negative solubility values. This was very marked in AH Plus and Endoseal in DMEM when compared to the solubility of both sealers in water which is the liquid recommended for testing in ISO 6876[7]. These sealers are never in contact with water so results for solubility tested according to a specified standard are not significant *in vivo* where the materials are in contact with physiological solution and more so in biological studies where the materials are placed in contact with DMEM and material solubility affects the results of testing. On the other hand the BioRoot RCS exhibited high solubility in water compared to the other sealers ($P < 0.001$) and negative solubility in both the HBSS and DMEM. The results of sealer solubility using different ISO recommendations were not comparable.

The results of the porosity of the test sealers calculated by gravimetric method after 1 and 28 day soaking in different solutions is show in Table 4. The AH Plus exhibited the lowest porosity at both 1 and 28 days in all media compared to the other sealers ($P > 0.001$). The BioRoot showed a ~2% porosity in the initial stages of setting and this value did not vary with the test medium. The porosity reduced to a negative value after 28 days. The Endoseal had the highest porosity after 1 day soaking which reduced considerably after 28 days ($P < 0.001$) for all media. The soaking media had no effect on the porosity values for all sealer types.

pH and chemical analyses of leachates. The results for measurement of pH and elemental analyses of soaking solutions over a period of 28 days are shown in Tables 5 and 6. All the sealers alkalinized the soaking solutions regardless the type of soaking solution used (Table 5). The pH rose over the 28-day period for the tricalcium silicate-based sealers while it decreased for AH Plus. The elemental analyses (Table 6) showed the same trends in both soaking solutions. Aluminium was leached in solution from both Endoseal and MTA Fillapex as both materials use cements which are based on Portland cement. Endoseal also leached bismuth in solution in higher quantities ($P < 0.05$) in DMEM than in HBSS. The other radiopacifiers were more stable particularly the zirconium where the release in solution was very low and independent on the soaking solution used ($P > 0.05$). The calcium ion release was highest in BioRoot RCS when compared to the other tricalcium silicate-based sealers ($P < 0.05$) and also the AH Plus ($P < 0.001$) which exhibited the lowest calcium release. The calcium ion release

Material	Media	Elements detected in leachate mg/L						
		Al	Bi	Ca	P	Si	W	Zr
AH Plus	HBSS	BDL	BDL	2.27	26.23	0.48	4.64	0.01
	DMEM	BDL	BDL	37.18	27.05	0.83	0.09	BDL
MTA Fillapex	HBSS	0.02	BDL	441.50	BDL	91.78	0.07	BDL
	DMEM	0.02	BDL	442.80	2.06	82.70	0.09	BDL
BioRoot RCS	HBSS	BDL	BDL	1533.00	0.79	0.52	BDL	BDL
	DMEM	BDL	BDL	1709.00	2.87	0.29	BDL	0.01
Endoseal	HBSS	8.90	0.39	264.80	0.12	3.94	BDL	0.03
	DMEM	5.82	10.30	636.90	23.66	7.36	BDL	0.04

Table 6. Elements detected in leachate from different sealers after 28 day exposure to different media. BDL: below detection limit.

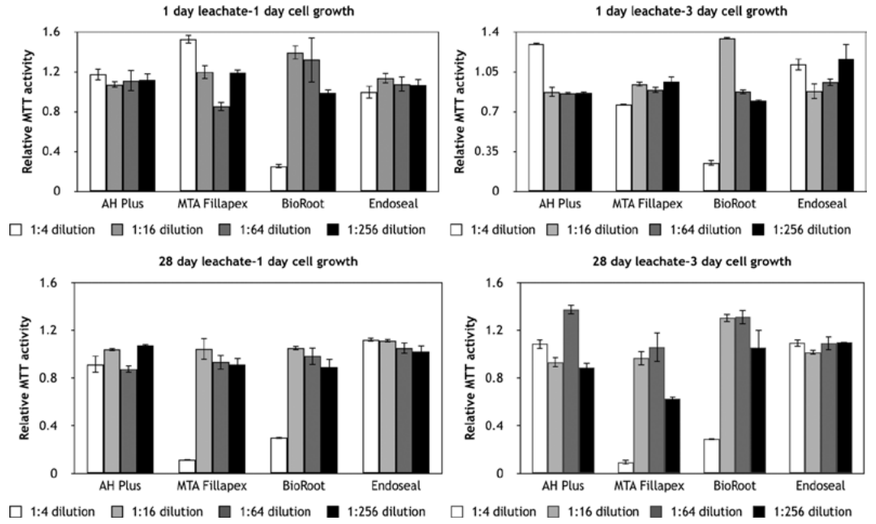

Figure 4. Cell proliferation and expression of gingival fibroblasts in response to exposure of leachates at different dilutions from test sealers in indirect contact test.

was higher in DMEM for all sealers ($P < 0.05$) except MTA Fillapex. Silicon was eluted in low quantities from all sealers except for MTA Fillapex; the release was independent of the solution used.

Investigation of biological activity. The results for the indirect contact test are shown in Fig. 4. The BioRoot RCS leachate was toxic to the gingival fibroblasts both in the 1-day and the 28-day leachates with cell activity enhanced after 3-days of exposure. This effect was reduced at higher dilutions. The same was observed for MTA Fillapex in the 28 day leachate. The MTA Fillapex exhibited optimal cell activity in the 1-day leachate, which deteriorated in the 28-day leachate. Cell activity improved over the 3-day exposure of the 28-day leachate. This suggests that MTA Fillapex initially does not leach anything toxic but over a longer time, other chemicals leach out which, have a more toxic or inhibitory effect on cell growth. This is confirmed by the enhanced cell activity at higher dilutions. The AH Plus and Endoseal showed stable cell activity in both 1 and 28-day leachates after both exposure times.

Surface characterization of materials after contact with different solutions. The surface morphology and elemental analyses of the materials in contact with either DMEM or HBSS is shown in Figs 5–7. This test was performed in order to evaluate the surface morphology of the materials in contact with DMEM (Fig. 6) and whether the surface morphology varies compared to that in contact with HBSS (Fig. 5), which is usually used to evaluate the material bioactivity. The sealer surfaces in contact with DMEM were different to those in contact with HBSS for all sealer types except AH Plus (Figs 5 and 6). The P peak in relation to the Ca peak was higher in DMEM for MTA Fillapex and BioRoot RCS as opposed to the Endoseal where a higher P peak was observed in HBSS (Fig. 7).

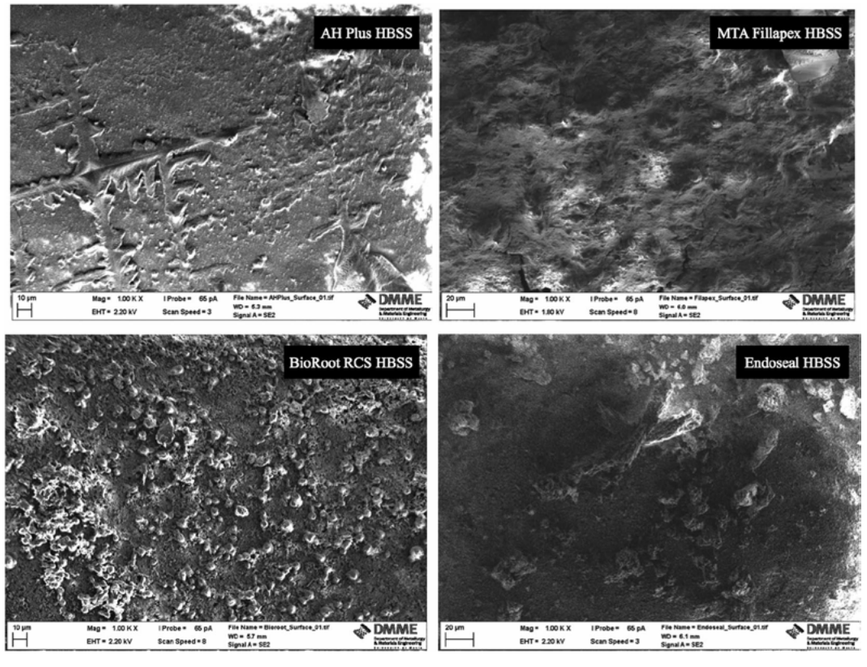

Figure 5. Secondary electron scanning electron micrographs of sealers immersed in HBSS to assess surface microstructure.

Discussion

The current study investigated four sealer types. The sealers were based on tricalcium silicate and thus leach calcium hydroxide making them susceptible to reaction with environmental fluids. AH Plus was used as a control sealer. The MTA Fillapex used in this study was a new version, which was recently launched by Angelus. It is bismuth oxide-free to avoid tooth discoloration as bismuth oxide containing materials were shown to discolour teeth when in contact with sodium hypochlorite which is used in all root canal treatments[18]. The composition of BioRoot RCS was in accordance with previous studies[19,20]. Endoseal is also a relatively new sealer on the market thus not characterized. A combination of scanning electron microscopy, energy dispersive spectroscopy and X-ray diffraction analyses was used to characterize the sealers.

The differences in the material composition and presentation were correlated to the material properties and their behaviour when exposed to different environments. In fact the leaching of calcium ions in solution was less for Endoseal than for BioRoot RCS as indicated in the leachate analyses. BioRoot RCS was shown in previous studies to leach high levels of calcium compared to other tricalcium silicate-based endodontic cements[19].

All sealers complied with ISO 6876; 2012[7] standards for flow, film thickness and radiopacity. These tests were run following the ISO recommendations. The testing of setting time, fluid uptake, sorption, solubility and porosity were conducted in air/water as suggested by the ISO standards but also in DMEM and HBSS. A number of material properties such as biocompatibility and bioactivity depend on these properties. The HBSS is used to simulate *in vivo* conditions while DMEM is used in cytology. Variations of material properties in contact with these fluids can affect the related biological characteristics. In addition where applicable different standards were used and the results obtained compared.

The BioRoot RCS exhibited high fluid uptake, which reduced over the 28-day period and was dependent on the soaking solution as opposed to the Endoseal where the solution type did not affect the fluid uptake. The high solubility exhibited by BioRoot RCS and the different solubility demonstrated in different soaking media has been reported[12]. The high solubility observed for Endoseal in the current study has already been reported[21]. The solubility was high for both BioRoot RCS and Endoseal using the gravimetric method and the formulae suggested in ISO 4049[17]. The results obtained for both materials using the ISO 6876[7] were different. Thus standards specific to the material type are necessary particularly for tricalcium silicate-based materials, which possess particular and distinguishing properties when compared to other sealer types.

The leaching in DMEM was higher than in HBSS for most elements. The leaching depends on the material solubility and solubility was also shown to be dependent on the soaking solution. The MTA Fillapex leachate was shown to enhance cell attachment and proliferation. The BioRoot RCS in comparison was shown to be cytotoxic but the cell growth resumed at higher dilutions. This is in contrast to previous research showing BioRoot RCS to be biocompatible tested using periodontal ligament stem cells[22]. Furthermore the BioRoot RCS was shown

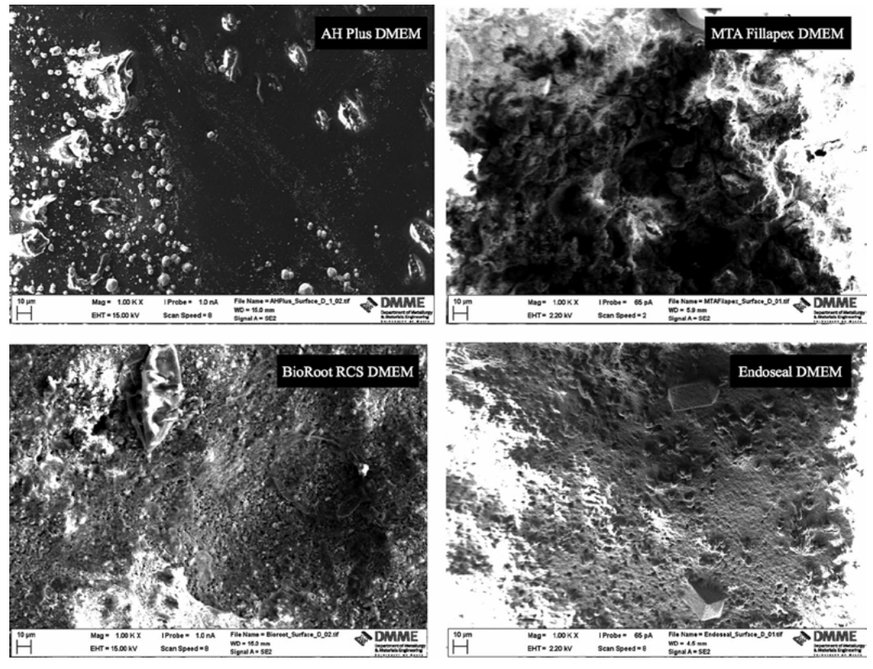

Figure 6. Secondary electron scanning electron micrographs of sealers immersed in DMEM to assess surface microstructure.

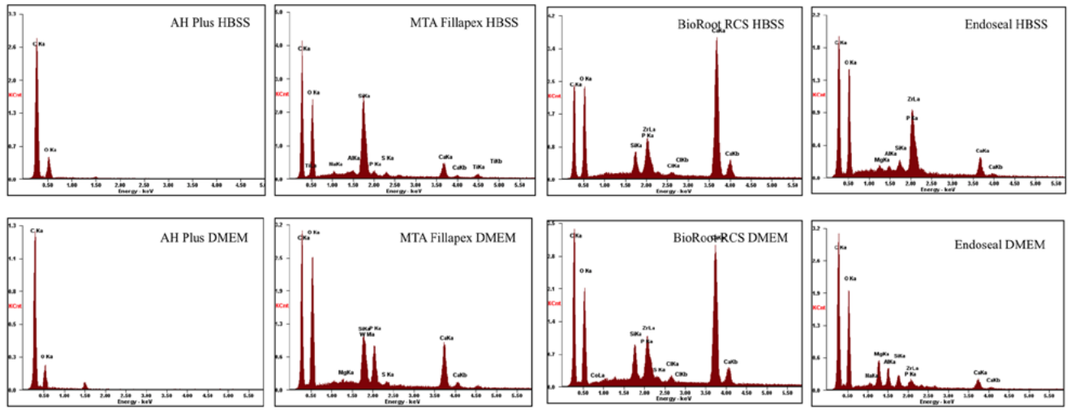

Figure 7. Energy dispersive spectroscopic scans of sealer surfaces in contact with different solutions.

to enhance the stem cells better than the Endoseal also in contrast to the findings in the current study. Previous research on biocompatibility of Endoseal implanted in subcutaneous tissues of rats showed Endoseal to have a similar reaction to MTA and better than AH Plus[21]. This is also inferred in the current study at the cellular level. Furthermore Endoseal was shown to enhance cell activity better than MTA Fillapex[23]. However the data cannot be compared to the current study since the MTA Fillapex used in the previous research may have been the bismuth-containing MTA Fillapex. Material characterization is necessary in every research work to make sure that the materials are well characterized to enable comparison to further research.

The energy dispersive spectroscopic data of the sealer surfaces after exposure to the DMEM and HBSS indicate that the material chemistry changes and the surface morphology as well. Thus data obtained after exposure to simulated body fluid cannot be extrapolated for cytology where DMEM is used.

Name	Presentation	Chemical composition	
		Component 1	*Component 2*
AH Plus	Two tubes	Diepoxide, calcium tungstate, zirconium oxide, aerosil, pigment	1-adamantane amine N,N′-dibenzyl-5-oxa-nonandiamine-1,9 TCD-Diamine, calcium tungstate, zirconium oxide, aerosil, silicone oil
MTA Fillapex	Two tubes	Methyl salicylate, butylene glycol, colophony, calcium tungstate, silicon oxide	Mineral trioxide aggregate, silicon dioxide, titanium dioxide, pentaerythritol, rosinate, P - Toluenesolfonamide
BioRoot	Powder/liquid	Tricalcium silicate, zirconium oxide	Water, calcium chloride, water-soluble polymer
Endoseal	1 tube	Calcium silicates, calcium aluminate, calcium aluminoferrite, calcium sulphate, radiopacifier, thickening agent	/

Table 7. Constituents of sealers tested.

Methods

The following root canal sealers were used in this study:

1. AH Plus (Dentsply, DeTrey GmbH, Konstanz, Germany)
2. MTA Fillapex (Angelus, Londrina, Brazil)
3. BioRoot RCS (Septodont, Saint-Maur-des-Fossés, France)
4. Endoseal (Maruchi, Wonju-si, Gangwon-do, South Korea)

The composition of the sealers as provided by the manufacturers is shown in Table 7. All sealers were mixed and manipulated in accordance with the manufacturers' instructions, except for Endoseal, a premixed root canal sealer that was syringed. The environmental factors were modified as indicated below. The sealers were tested according to the standard specifications but in addition were also immersed in Hank's balanced salt solution (HBSS, Sigma Aldrich, Gillingham UK) and Dulbecco's modified eagle medium (DMEM; Sigma Aldrich, Gillingham UK). This was done in order to investigate the material properties in contact with simulated tissue fluids and fluids used for cell culture.

Material characterization. The set sealers were characterized by scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) and X-ray diffraction analysis (XRD). The micromorphology at different magnifications was assessed on polished specimens using a scanning electron microscope (SEM; Zeiss MERLIN Field Emission SEM, Carl Zeiss NTS GmbH, Oberkochen, Germany). Phase analysis was performed on powdered sealers after 1 or 28 day immersion in HBSS using a Bruker D8 diffractometer (Bruker Corp., Billerica, MA, USA) with Co Kα radiation (1.78 A°). The X-ray patterns were acquired in the 2θ (10–60°) with a step of 0.02° and 0.5 seconds per step. Phase identification was accomplished using a search-match software utilizing ICDD database (International Centre for Diffraction Data, Newtown Square, PA, USA).

Assessment of physical and chemical properties. *Assessment of film thickness, flow, setting time and radiopacity.* Film thickness, sealer flow, setting time and radiopacity were assessed following ISO 6876; 2012[7]. For setting time the testing was performed also with material immersed in HBSS and DMEM.

Investigation of fluid uptake, sorption, solubility and porosity. Fluid uptake, sorption and solubility was performed as specified in ISO 4049; 2009 using water and also Hank's balanced salt solution (HBSS; Sigma Aldrich, St Louis, MO, USA) and Dulbecco's modified eagle medium (DMEM). Weight changes were recorded after 7, 14, 21 and 28 days thus enabling the calculation of fluid uptake at each specified interval. Sorption and solubility were also calculated. In addition the sealer solubility was also assessed using ISO 6876; 2012[7] procedure with water, HBSS and DMEM media used to soak the different sealers.

Porosity was assessed by calculating the porosity for the materials using a gravimetric method as described in Cutajar *et al.*; 2011[24] after 1 and 28 day-immersion in different solutions namely water, HBSS and DMEM.

Assessment of pH and chemistry of leachates. The pH of the soaking solutions before and after immersion (7, 14, 21 and 28 days) of the test sealers was measured with a pH meter (Hanna HI 3221, Hanna Instruments, Woonsocket, RI, USA). For leachate analysis, cylindrical specimens (10 mm in diameter and 2 mm thick) were prepared. They were allowed to set for 24 hours at 37 ± 1 °C, weighed, after which the materials were immersed in 5 mL of HBSS or DMEM at 37 ± 1 °C for 28 days. The sealers were removed from the storage solution and discarded. The storage solution and a blank were assessed using inductively coupled plasma (ICP).

Investigation of sealer biological properties. The cytocompatibility of the test materials was evaluated *in vitro* on human gingival fibroblasts following to ISO 10993-5;2009[25] using an indirect testing method. The 3-(4,5 dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[26] was used to assess cell metabolic function. The leachate extraction was made in cell culture medium without serum and antibiotics using a surface area (sample) to volume (medium) ratio of approximately 150 mm^2/ml. 24 hours after setting the sealers were exposed to medium for either 1 day or 28 days. The extract collected at each time point was serially diluted to 1:2, 1:8, 1:32 and 1:128 with fresh DMEM. DMEM alone served as a negative control. Cells were seeded in 96-well plates at 1.5×10^4 cells/well in 100 µl of DMEM. Five repeats were performed for every solution. After overnight attachment, cells were treated with various extracts of sealers (100 µl /well) resulting in final concentrations of the sealer-conditioned medium of 1:4, 1:16, 1:64 and 1:256.

Surface characterization of materials after contact with different solutions. In addition, the sealer surfaces in contact with different media were assessed by scanning electron microscopy and energy dispersive spectroscopy in order to evaluate the effect of the media used on sealer chemistry and surface morphology after immersion in HBSS or DMEM for 28 days.

Statistical Analysis. The data were evaluated using Statistical Package for the Social Sciences software (PASW Statistics 18; SPSS Inc, Chicago, IL). One-way analysis of variance and Tukey post-hoc tests at a significance level of $P = 0.05$ were used to perform multiple comparison tests.

Conclusions

The material chemistry, presentation, environmental conditions and testing methodology used affected the sealer properties. Standards specific to sealer type are thus indicated. Furthermore the methodology used in the standard testing should be more relevant to clinical situations.

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Author Contributions

Kebudi Benezra M.: Experimental work and editing manuscript Schembri Wismayer P.: Biological work and editing manuscript Camilleri J.: Characterization, writing, editing and originator of idea.

Additional Information

Competing Interests: The authors declare that they have no competing interests.

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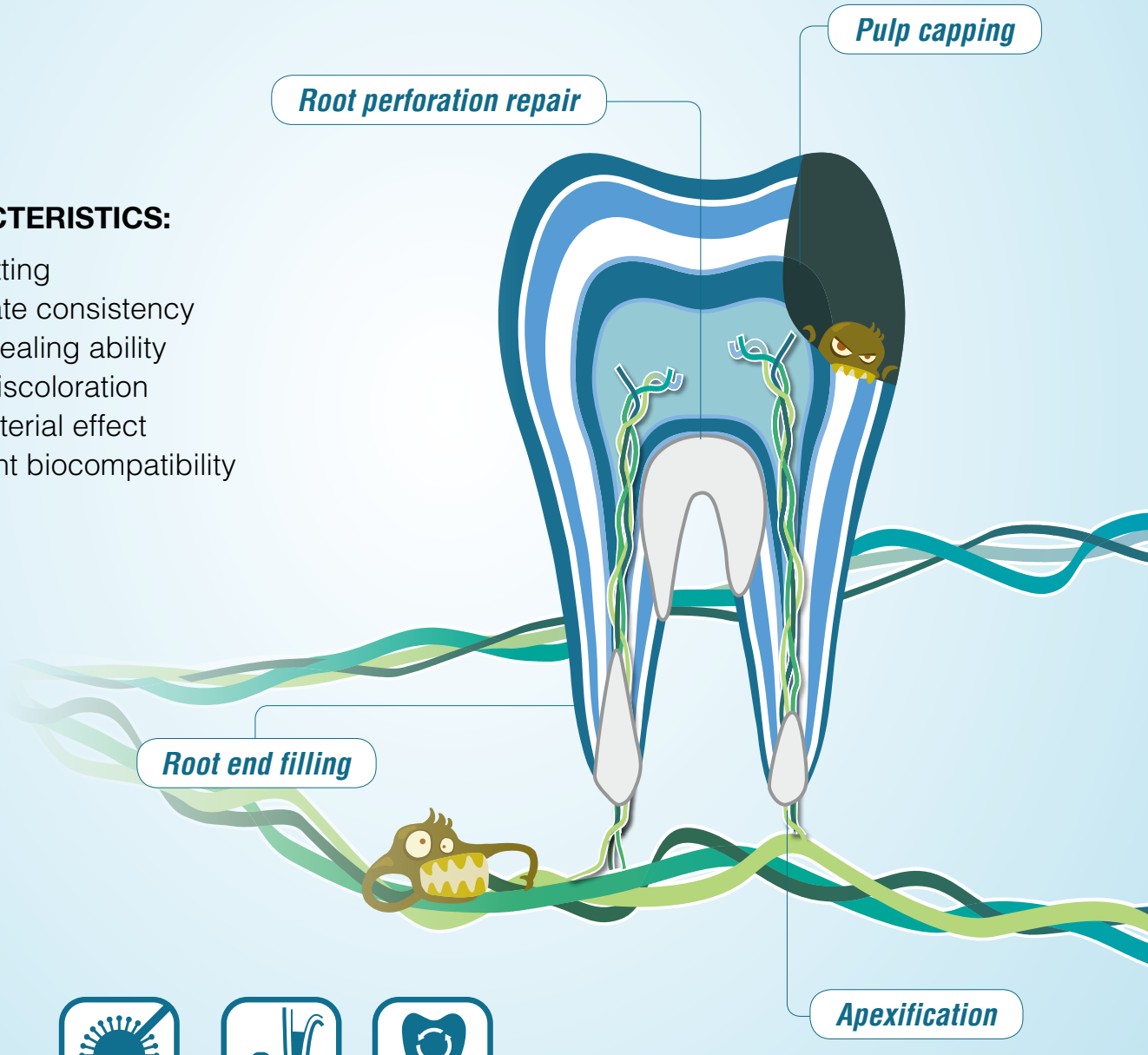

Biocompatible

Antibacterial

Hermetic

Retrievable