Original Article

# Evaluation of sealing ability of four bioceramic root canal sealers and an epoxy resin-based sealer: An *in vitro* study

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## Abstract

**Introduction:** The aim of the study was to evaluate the bacterial leakage of four different bioceramic root canal sealers and to compare it to the conventional epoxy resin-based sealer.

**Materials and Methods:** The study sample consisted of 94 single-rooted human teeth with one round root canal, which were instrumented and randomly distributed into five experimental groups according to the root canal sealer: Group 1: TotalFill bioceramic sealer; Group 2: BioRoot root canal sealer; Group 3: mineral trioxide Aggregate (MTA) Fillapex; Group 4: MTA Plus; Group 5: epoxy resin-based sealer AH Plus and positive and negative control group. The prepared samples were fixed in a model designed for the *Enterococcus faecalis* leakage evaluation and were sterilized in plasma. The bacterial penetration through the filled root canals was checked by the color change in the lower tube during 60 days. The scanning electron microscopy was used to check the bond between the sealer and the dentine wall. Data were statistically analyzed.

**Results:** Total Fill bioceramic sealer (BCS) group presented significantly lower number of samples that leaked (14.2%) compared to the AH Plus group (78.6%) ($P = 0.002$) and MTA Plus group (50%) ($P = 0.046$). There was no statistically significant difference between other groups ($P > 0.05$).

**Conclusion:** The TotalFill BCS root canal sealer provided better sealing ability compared to the AH Plus and the MTA Plus sealers.

**Keywords:** Bacterial leakage, bioceramics, mineral trioxide aggregate, root canal, sealing ability

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**Submission:** 09-10-19 **Revision:** 12-11-19. **Acceptance:** 14-01-20. **Web Publication:** 09-01-21

## INTRODUCTION

The aim of root canal obturation is to prevent the intracanal bacterial recolonization by blocking any communication between the oral cavity and the periradicular tissues and by entombing residual bacteria after chemomechanical preparation.[1] Furthermore, apical obturation prevents seeping of periradicular tissue fluids into the canal, which could maintain the viability of the remaining bacteria, and consequently, prevent the healing of the periapical lesion.[2]

Many studies have shown that root canal fillings leaked coronally when exposed to oral flora. *In vitro* and *in vivo* studies reported penetration of dye and bacteria through filled root canal within 10–73 days[3-6] and bacterial endotoxin within 21 days.[7] Microleakage of filled root

Access this article online

Quick Response Code:

Website: www.saudiendodj.com

DOI: 10.4103/sej.sej_153_19



**How to cite this article:** Antunovic M, Vukmanovic L, Budimir A, Kabil E, Anic I, Bago I. Evaluation of sealing ability of four bioceramic root canal sealers and an epoxy resin-based sealer: An *in vitro* study. Saudi Endod J 2021;11:66-72.

canal occurs between sealer and intracanal wall or between the sealer and a gutta-percha cone.[8] Traditional sealers have few shortcomings in that they shrink on setting and can be washed up in the presence of tissue fluids, leaving a gap with potential for microbial leakage.[9,10]

To achieve better sealing of root canal space, dimensionally stable and hydrophilic calcium silicate and phosphate-based root canal sealers "bioceramic sealers" have been developed.[11] The first commercially available calcium silicate-based sealer is mineral trioxide aggregate (MTA) Fillapex, which is composed of 13% of MTA and salicylate resin. Studies showed deeper and greater penetration of the MTA Fillapex into dentinal tubules compared to the AH Plus,[12,13] what could be probably a result of its better fluidity and its smaller particles.[14,15] However, past few studies, which evaluated the bacterial microleakage of the MTA Fillapex and the AH Plus, reported conflicting results.[16,17]

The newest generation of calcium silicate and phosphate-based root canal sealers is so called "premixing bioceramic sealers" (TotalFill Bioceramic Sealer, iRoot SP, and EndoSequence), which do not require mixing. These bioceramic sealers release more calcium hydroxide during the setting compared to the MTA Fillapex[18] that explains their higher pH[19] and antibacterial properties.[18,20] When used with gutta-percha points that are impregnated and coated with bioceramic nanoparticles, they bring about so-called "intracanal bonded restoration" free from gaps.[11] In addition, the bioceramic root canal sealers can promote physical and chemical bond to dentin by creating hydroxyapatite precipitate at the dentin-sealer bond during the setting.[21] However, in a study of Ersahan and Aydin,[22] the bioceramic sealer (iRoot SP) did not demonstrate any superiority over the AH Plus sealer based on the bacterial leakage test. In addition, Viapiana *et al*.[23] also did not find any superiority of the newest two-component bioceramic sealer and the BioRoot Root Canal Sealer, over the epoxy resin-based sealer (AH Plus). However, given the relatively small number of past studies on this topic, no conclusions can be drawn yet.

The aim of the study was to evaluate the bacterial leakage of four different bioceramic root canal sealers and to compare it to the conventional epoxy resin-based sealer.

## MATERIALS AND METHODS

### Preparation of samples

The study protocol was accepted and approved by the Local Ethics Committee of the University of Zagreb, Croatia, No 05-PA-26-12/2016. The study sample consisted of 94 human single round canal mandibulary premolars. All the selected teeth had closed apex without external resorption, fractures, caries, or previous endodontic treatment. Digital periapical radiographs were taken to confirm the presence of a single straight canal. The teeth were stored in 0.5% chloramine solution for approximately 2 months before the beginning of the study. All the teeth were selected on the basis of cone-beam computed tomography (CBCT) evaluations (Cranex 3DX, Soredex, Tuusula, Finland) performed under the following parameters: field of view, 5 × 5 (5.0 mm) mm; ENDO, 85 μm; 6.3 mA; 90 kV; 8.7 s; and 450.3 mGycm$^2$. The canals were classified as round if buccolingual diameter was not more than 2.5 times larger than the mesiodistal diameter.[24] Only teeth with round root canal were included in the study.

The teeth were prepared according to the protocol described in the study of Fernández *et al*.[25] The teeth were decoronated with a diamond fissure bur no 16 (Komet, Rock Hill, SC, USA) to standardize the working length at 14 mm using K-file size #10 or #15 (Dentsply/Maillefer, Ballaigues, Switzerland). The root canals were instrumented with the ProTaper Next (PTN) rotary technique, (Dentsply, Ballaigues, Switzerland) using WaveOne Gold motor (Dentsply) set at 300 rpm and torque of 2.8/Nm till the instrument PTN X3 (size 0.30, taper. 07). The root canals were irrigated with 1 ml of 2.5% sodium hypochlorite (NaOCl) after each instrument using 30 G needle (BD, Microlance, Becton Dickinson, Madrid, Spain) positioned at 3–5 mm short of the working length and syringe of 2 ml. The smear layer was removed by sequential irrigation with 5 ml 2.5% NaOCl, 5 ml 15% ethylenediaminetetraacetic acid (Calcinase, Lege Artis, Dettenhausen, Germany), which was left in the canal for 1 min, and 15 ml of saline solution as the final irrigant. The irrigants were, in the final irrigation protocol, activated with EndoActivator (Dentsply, Tulsa Dental Specialties, USA). The EndoActivator polymer tip size #25 was placed in the canal 2 mm shorter of the working length, and then, during activation, it was moved vertically in approximately 2 mm amplitude along the canal. The root canals were dried with sterile PTN X3 paper points (Maillefer, Ballaigues, Switzerland). Root canals, which had been planned to be filled with bioceramic sealers, were left slightly moist, according to the manufacturers' recommendation. For canal wetting, 0.02 ml saline was poured into the canal using an insulin syringe (BD Plastipak, Becton Dickinson, Madrid, Spain). Apical patency was verified using a sterile K-file size # 10 (Dentsply/Maillefer, Ballaigues, Switzerland).

## Filling procedure

The prepared samples were randomly distributed into five groups ($n$ = 15/each) according to the sealer used and positive and negative control groups ($n$ = 7/each).

*Group 1: TotalFill bioceramic sealer*

The root canals were filled with gutta-percha cones (taper 0.04) covered by bioceramic nanoparticles and TotalFill Bioceramic Sealer (TotalFill BCS, FKG, La Chaux-de-Fonds, Switzerland). The sealer was filled in the canal with a corresponding plastic extension. Then, the Total-Fill gutta-percha cone size #30 (FKG, Switzerland) coated with the sealer was inserted into the root canal until the working length. For cold lateral condensation, finger spreader size #20 (Anataeos, München, Germany) and additional TotalFill gutta-percha cones size #20 were used. Accessory gutta-percha cones were added until the spreader could not penetrate into the coronal part of the root canal.

*Group 2: Bioceramic bioroot canal sealer*

The root canals were filled with another bioceramic sealer, BioRoot RCS (Septodont, Saint Maur-des-Fosses, France) using a single-cone technique, according to the manufacturer's recommendation. The sealer was mixed and introduced into the canal with PTN X3 gutta-percha cone (Dentsply) until the working length.

*Group 3: Mineral trioxide aggregate fillapex sealer*

The root canals were filled with MTA Fillapex sealer (Angelus Solucoes Odontologicas, Londrina, Brazil) using cold lateral condensation technique. The sealer was mixed and into the canal with PTN X3 gutta-percha cone (PTN, Maillefer, Ballaigues, Švicarska) until it reached the working length. For the lateral condensation, additional gutta-percha cones size # 20 (DiaDent, Seoul, Korea) were added using finger spreader size #20.

*Group 4: Mineral trioxide aggregate plus sealer*

The root canals were filled with MTA Plus sealer (Avalon, Biomed Inc., Bradenton, FL, USA) and PTN gutta-percha cone X3 (Maillefer, Ballaigues, Switzerland) in the same way as described in Group 3.

*Group 5: Epoxy resin-based sealer*

The root canals were filled with an epoxy resin-based sealer (AH Plus, Dentsply Sirona) in the same way described in Group 3.

Excess gutta-percha was removed using a round carbide bur (Komet, Rock Hill, SC, USA). The gutta-percha at the orifice level was additionally compacted with a hand plugger.

The canals in the negative control group were not instrumented (intact), but the root surface, including apical foramen, was sealed with two layers of nail varnish. The root canals in the positive control group were instrumented as in the experimental groups and remained unfilled to demonstrate the leakage of bacteria through the canal space.

The surface of the roots in the experimental groups and the positive controls was sealed with two layers of transparent nail varnish except for the apical 3 mm around the apical foramen.

## Scanning electron microscopy

One sample from each experimental group was prepared for scanning electron microscopy (SEM) to inspect the bond between the sealer and the dentin.

The roots were fixed in chemically polymerization acrylate (Meliodent, Heraeus, Hanau, Germany) and were then cut perpendicularly to the longitudinal axis using a diamond disc on Isomet saw machine (IsoMet, Buehler, Lake Bluff, Illinois, USA) to get 2-mm thick samples. The samples were polished with 600, 800, and 1000 sanding discs (3M ESPE, USA) underwater cooling and were ultrasonically cleaned for 2 min in distilled water. Then, the samples were dehydrated in a series of increasing concentrations of alcohol (40%, 60%, 70%, and 95%) for 30 min each. After 12 h of drying on air, the samples were mounted on aluminum stubs and the sputter was coated with a gold–palladium alloy under a vacuum and was then scanned on the SEM (Tescan Vega TS5136 LS, Tescan, Brno, Czech Republic).

## Preparation of the samples for the evaluation of bacterial leakage

The filled root canals were scanned using CBCT device (Cranex 3DX, Soredex, Tuusula, Finland) at the parameters: field 5 × 5 (5.0 mm), ENDO 85 μm, 6.3 mA, 90 kV, 8.7 s, and 450.3 mGycm$^2$ to check the quality of root canal obturation. All the root canals were well compacted and there were no overfilled or underfilled samples. The access cavities of the canals were covered with a temporary filling (Caviton, GC, Tokyo, Japan) and the samples were stored in 100% humidity at 37°C for 14 days.

Each sample was fixed in two Eppendorf tubes (Eppendorf, Hamburg, Germany). The bottom of the upper tube (upper chamber) was cut perpendicularly with a diamond disc (Filex turbo 505.504 220 HP, Edenta, Switzerland) and the sample was pulled through that opened bottom so the coronal part of the root sample remained in the upper

tube. Then, the apical part of the root was pulled through the holed cap of another threaded tube (lower chamber). The whole system was fixed in that position with the flowable composite material (G-aenial Universal Flo, GC, Japan), which was polymerized for 30 s and cyanoacrylate adhesive (Loctite Super Bond Liquid, Henkel, Germany).

The prepared samples were sterilized in plasma (PLASMA; Sterrad 100S, Johnson and Johnson, Irvine, California, USA).

### Evaluation of the bacterial microleakage of the fillings

The evaluation of bacterial microleakage of the root canals was performed according to the protocol described by Yücel *et al*.[5] The lower chamber was filled in with a solution made of sterile broth (Brain Heart Infusion Broth, Mast Group Ltd., Merseyside, UK) and indicator (Phenol Red, Honeywell Chemicals, USA) at the concentration of 15 mg/l. The upper chamber was filled in with bacterial suspension of *Enterococcus faecalis* (ATCC 29212) which had been previously prepared and standardized at $10^8$ CFU/mL.

All the samples were stored in an incubator at 37°C and 100% humidity for 60 days. The bacterial suspension in the upper chamber was refresh one every 7$^{th}$ day.

Indicator of the bacterial penetration through the root canal was the change of color (from red to yellow) in the lower chamber, which was checked every 5$^{th}$ day. The presence of the bacteria in the lower chamber with changed color was confirmed additionally by the cultivation method, namely the aliquots of the cloudy broth were grown on blood agar plates, and after the incubation period of 48 h, the growth of the colonies was checked. The samples with confirmed bacterial leakage were discarded.

### Statistical analysis

The results were analyzed with Chi-quadrat test and Fisher's exact test. All *P* values lower than 0.05 were considered significant. For statistical analysis, program IBM SPSS Statistics version 23.0 (www.spss.com) was used.

## RESULTS

The samples in the positive control group leaked after 1 day, and the samples in the negative control group did not leak during the observation period of 60 days.

Table 1 shows the number of samples, in each group, which leaked during the period of 30 and 60 days. After 60 days, the TotalFill BCS group presented a significantly lower number of samples that leaked compared to the AH Plus group ($P = 0.002$) and the MTA Plus group ($P = 0.046$). There was no statistically significant difference between other groups in the number of samples that leaked ($P > 0.05$).

Figure 1 shows the time when samples leaked during the observation period of 60 days. Overall, comparison analysis of the time of leakage revealed a significant difference between the groups ($P = 0.01$).

Uniform bond without gaps was observed between the bioceramic sealers "TotalFill BCS and BioRoot RCS" and dentin [Figures 2 and 3].

The bond between MTA Fillapex sealer and the dentin and MTA Plus sealer and dentin was also continuous without gaps [Figures 4 and 5]. A gap between the AH Plus sealer and the dentin was clearly seen [Figure 6].

## DISCUSSION

The sealing ability of endodontic filling materials has been evaluated using different leakage tests: dye leakage test, bacterial leakage test, fluid filtration model, leakage of fluorescent microspheres, and glucose leakage test.[5,23,26,27] Due to the different results reported for the same sealers, there is no clear opinion regarding the most appropriate leakage test for the evaluation of sealing ability of sealers.[23,28,29] In this study, we used a bacterial leakage test

**Table 1: The number of samples which leaked in each group**

| Materials | *n* | 30 days' period (*n*) | | 60 days' period (*n*) | |
|---|---|---|---|---|---|
| | | No | Yes | No | Yes |
| Total fill BCS | 14 | 14 | 0 | 12 | 2 |
| BioRoot RCS | 14 | 13 | 1 | 9 | 5 |
| MTA Fillapex | 14 | 12 | 2 | 9 | 5 |
| MTA Plus | 14 | 9 | 5 | 7 | 7 |
| AH Plus | 14 | 13 | 1 | 3 | 11 |

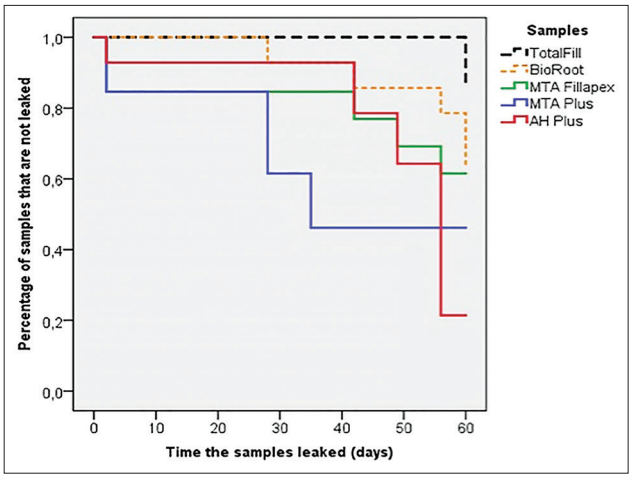


**Figure 1:** Presentation of time when samples leaked during the observation period

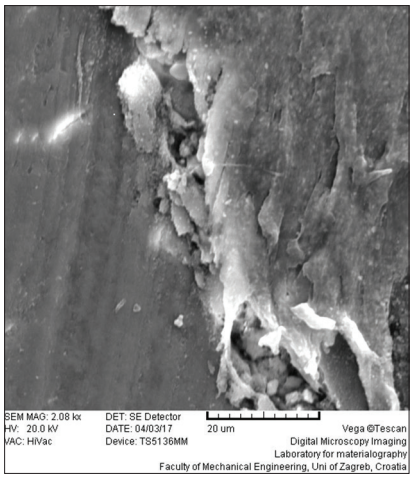


**Figure 2:** Scanning electron microscopy of the bond between BioRoot RCS and dentin

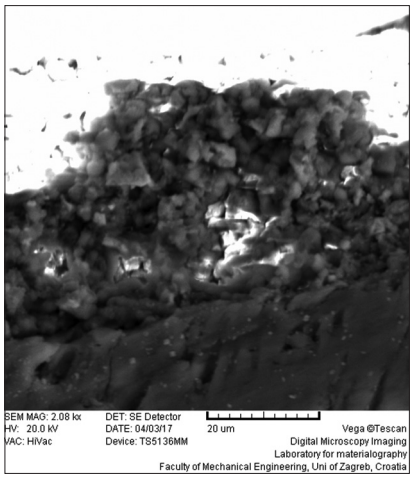


**Figure 3:** Scanning electron microscopy of the bond between total fill BCS and dentin

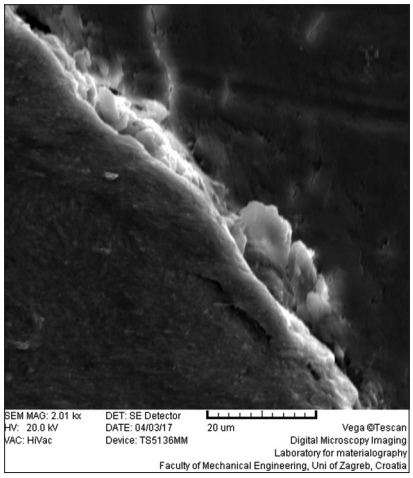


**Figure 4:** Scanning electron microscopy of the bond between mineral trioxide aggregate Fillapex and dentin

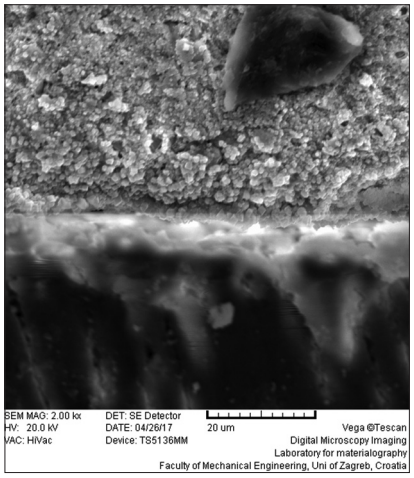


**Figure 5:** Scanning electron microscopy of the bond between mineral trioxide aggregate plus and dentin

for the evaluation and comparison of the sealing ability of four bioceramic sealers and a conventional epoxy resin-based sealer. The bacterial leakage test was first described by Torabinejad *et al*.[4] many years ago. According to some authors, this test is most clinically acceptable for the evaluation of leakage of filling materials.[30-33] In this study, we used a bacterial leakage model described by Yücel *et al*.[5] *E. faecalis* was used as a test bacteria since it is normal part of oral flora[34] and was frequently found in endodontically treated teeth with the persistent periapical lesion.[34]

The results of this study showed the better sealing ability of the TotalFill BCS sealer compared to the MTA Plus sealer and conventional the AH Plus root canal sealer. Interestingly, the BioRoot RCS, which is the newest bioceramic sealer on the market, was not significantly superior over the AH Plus sealer although yielded a less number of leaked samples. Previous studies on bioceramic sealers demonstrated their ability to induce biomineralization when in interaction with dentinal fluid[35,36] and to create mineral infiltration zone in dentin.[37] Furthermore, bioceramic sealers do not shrink during the setting because are monomer free, thus, allow the tight seal of the root canal.[11] This was confirmed also in this study by SEM images, which showed a uniform bond between the bioceramic sealers and the dentin without gaps. The sealing ability of bioceramic endodontic materials in retrograde cavities shows similar or worse results compared to MTA.[38,39] There have been only a few studies published so far on the microleakage of bioceramic sealers. In a study by Ersahan and Aydin,[22] no difference between the bioceramic sealer (iRoot) and the conventional AH Plus sealer was found. Viapiana *et al*.,[23] reported more voids in root canals filled with the BioRoot RCS compared to the AH Plus sealer that was explained by shorter setting time and lower fluidity of the tested bioceramic sealer. Furthermore, consistently with the present study, no difference in the leakage of the two materials was found.

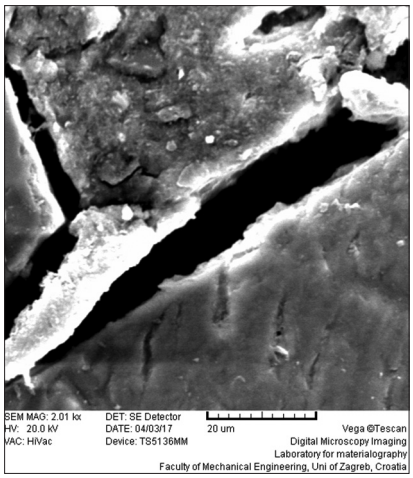


**Figure 6:** Scanning electron microscopy of the bond between AH plus and dentin

However, when comparing these two studies, it has to be taken into consideration that the root canals in the current study were filled using lateral condensation technique, and in the study of Viapiana *et al.,*[23] cold lateral condensation technique was used. In addition, the TotalFill BCS sealer was used with gutta-percha points covered with bioceramic nanoparticles in combination with cold lateral condensation technique that probably assured the creation of monoblock in the root canal. This could be the reason of its superior results.[7] Another factor, which could have influenced the results of this study, is the presence of light moisture in root canals before filling with bioceramic sealers,[25] namely, the setting reaction of bioceramic sealers is influenced by the moisture in the root canal, resulting in the formation of hydroxyapatite along the mineral infiltration zone.[20]

In this study, MTA-based sealers showed a similar sealing ability as the AH Plus sealer. Previous studies showed deeper penetration of MTA Fillapex in dentinal tubules;[13] however, it significantly leakage more compared to the AH Plus or the AH26 sealer.[16,17,40] The reason could be its less release of calcium hydroxide during setting compared to the premixed bioceramic sealers.[18] In addition, MTA Fillapex showed worse bond strength in root canals compared to the AH Plus.[41,42] In this study, there was no difference in bacterial leakage between the MTA Fillapex and the AH Plus sealer.

The limitation of this study is regarding the leakage studies having a questionable scientific significance. All bacterial leakage evaluation techniques have some limitations and may yield different results compared to other microleakage assessment methods (i.e., dye penetration, fluid filtration, or electrochemical tests).[43] In most reviewed studies, the results of SEM were correlated with those of the microbial leakage test.[43] de-Deus[44] published a critical review on leakage methods in endodontic studies saying that there is still no clear positive correlation between dye leakage test and the presence of periapical pathosis as determined from radiographs. Microbial leakage studies cannot estimate the time of occurrence of periradicular infection because it depends on several factors such as the virulence of microorganisms, defense capacity of the periradicular tissues, nutritional status, and bacterial interactions. However, Veríssimo and do Vale[45] showed that the evaluation of coronal leakage by the use of bacteria provided more biologically significant and clinically relevant data than other methods. Future studies should focus on the evaluation of the sealing ability of the bioceramic sealers by push-out test and evaluation of voids with micro-computed tomography analysis to get a more comprehensive analysis of the sealers.

## CONCLUSION

The TotalFill BCS root canal sealer provided better sealing ability compared to the AH Plus and the MTA Plus sealers. There were no significant differences in the sealing ability between the other sealers.

### Acknowledgment

The study was performed under the support of the University of Zagreb, Croatia.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

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