




ORIGINAL RESEARCH

# Physicochemical properties and cytocompatibility of newly developed calcium silicate-based sealers

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## Keywords

calcium silicate, cell viability, cytocompatibility, endodontics, physical property, root canal sealer.

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We declare that all authors have contributed significantly and are in agreement with the manuscript.

## Abstract

The purpose of this study was to compare the physical properties and cytocompatibility of contemporary calcium silicate-based sealers. Four calcium silicate-based sealers (BrightEndo MTA sealer, CeraSeal, EndoSeal TCS and One-Fil) were compared to an epoxy resin-based sealer (AH Plus). Flow, setting time, radiopacity and dimensional change were evaluated according to ISO 6876 standards. Cytotoxicity on human periodontal ligament fibroblast (hPDLF) cells was compared for biological properties using MTT assay. The surface of the sealer was analysed using scanning electron microscopy to evaluate cell attachment. Flow and radiopacity of all sealers met ISO standards, while setting time and dimensional stability did not meet the ISO standards. Calcium silicate-based sealers showed favourable cytocompatibility, and hPDLF cells were well attached to the calcium silicate-based sealers. Calcium silicate-based sealers have clinically acceptable flow and radiopacity, and cytocompatibility. However, these sealers had longer setting time and higher dimensional change than those required by ISO 6876.

## Introduction

The purpose of root canal filling is providing the hermetic sealing of the root canal system to prevent bacterial infection and isolating the remaining irritants in the root canal (1,2). Generally, gutta-percha and root canal sealer have been used as a filling material. Root canal sealer fills the voids and irregularities in the root canal, lateral and accessory canals and spaces between gutta-percha and dentinal wall (3). Proper root canal sealer should have certain physicochemical properties such as high radiopacity, insolubility in tissue fluids, adhesion to the canal wall, dimensional stability, slow setting time for sufficient working time and biocompatibility (3). Since its physicochemical

and biological properties are important factors to root canal treatment, many studies have been conducted (4-8).

There are different types of root canal sealers depending on the main composition: zinc oxide-eugenol, calcium hydroxide, glass ionomer, resin-based and calcium silicate-based sealers. Some of epoxy resin-based sealers, such as AH plus (Dentsply De Trey, Konstanz, Germany), have been used as a gold standard with excellent sealing ability, high radiopacity and long-term dimensional stability (5,9,10). However, these sealers have an adverse effect on periapical tissue due to their cytotoxicity (6,7). Although the sealer should ideally be filled to the apical terminus, in clinical cases, it is often extruded beyond the apical foramen, which causes an inflammatory reaction

to the periapical tissue and adversely affects the results of root canal treatment (8).

Calcium silicate-based sealer has calcium releasing ability, adequate biocompatibility and similar properties and sealing ability to conventional sealer such as AH Plus (9,11,12). Recently, many products with calcium silicate-based sealer in syringe have been developed. These products have the advantage of having easily applied to the root canal, absorbing the moisture of dentinal tubule and omitting the mixing process since the calcium silicate-based sealer sets by itself (13). Calcium silicate-based sealers form calcium hydroxide, hydroxyapatite and mineral infiltration layer at the dentin wall, which improves the ability to bond with dentin (14). In addition, these sealers produce a mechanical interlocking to a dentinal wall by diffusing into the dentinal tubules (4).

BrightEndo MTA sealer (GENOSS, Suwon, Korea), CeraSeal (Meta Biomed, Cheongju, Korea), EndoSeal TCS (Maruchi, Wonju, Korea) and One-Fil (MEDICLUS, Cheongju, Korea) are new root canal sealers containing calcium silicates, zirconium oxide and thickening agent in their composition. According to the manufacturers, these sealers have clinically adequate physical properties and cytocompatibility. While many of these calcium silicate-based sealers have been used clinically, research on physical and biological properties are still lacking, especially for some brand new materials. Therefore, the purpose of this study is to evaluate the physical properties and biocompatibility of newly developed calcium silicate-based sealers.

**Materials and methods**

In this study, newly developed calcium silicate-based sealers, BrightEndo MTA sealer, CeraSeal, EndoSeal TCS and One-Fil, were compared to an epoxy resin-based sealer AH Plus Jet (Dentsply De Trey) (Table 1). AH Plus Jet was selected as a control considering its reputation for studies on sealers and as a gold standard. Flow, setting time, radiopacity and dimensional stability were compared for physical properties, and cytotoxicity on human periodontal ligament fibroblast (hPDLF: Sciencell; Carlsbad, CA, USA) cells was compared for biological properties using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) assay. The experiments to evaluate flow, setting time and radiopacity were performed based on ISO 6876/2012 (Table 2) (15). Dimensional change was evaluated based on ISO 6876/2001 (16). The hPDLF cells were used to evaluate the cytotoxicity of the tested sealers as these cells could be in direct contact with sealer (17). In addition, the surface of the sealer was analysed using scanning electron microscopy (SEM) to evaluate cell attachment.

**Table 1** Chemical compositions of the root canal sealers investigated in the present study

Sealer	Components			
Epoxy resin-based sealer	AH-Plus	<i>Paste A</i>	<i>Paste B</i>	
		Bisphenol-A epoxy resin	Dibenzyl diamine	
		Bisphenol-F epoxy resin	Amino adamantane	
		Calcium tungstate	Tricyclodecane-diamine	
		Zirconium oxide	Calcium tungstate	
		Silica	Zirconium oxide	
		Iron oxide pigments	Silica	
			Silicone oil	
Bioceramic-based sealer	BrightEndo MTA	Calcium silicates		
		Zirconium oxide		
		Bismuth oxide		
		Solvent / thickening agent		
		CeraSeal	Calcium silicates	
			Zirconium oxide	
		EndoSeal TCS	Thickening agent	
			Tricalcium silicate	
		One-Fil	Zirconium dioxide	
			Dimethyl sulphoxide	
Thickening agent				

**Table 2** ISO 6876/2001 and 6876/2012 standards

ISO standards	
Flow**	Not less than 17 mm
Setting time**	If setting time stated by manufacture is less than 30 min, the setting time shall be no longer than 110% stated by manufacture
	If setting time stated by manufacture is more than 30 min, the setting time shall be within the range stated by manufacture
Radiopacity**	Not less than 3 mm of aluminium
Dimensional change*	Shrinkage ≤ 1% for 30 days
	Expansion ≤ 0.1% for 30 days

\*ISO 6876/2001, \*\*ISO 6876/2012.

**Flow**

After dropping the 0.05 ml sealer onto the slide glass, 3 min later, another slide glass was placed over the sealer and a total of 120 grams of weight was loaded to the sealer. Seven minutes later (10 min after dropping the sealer), the weight was removed, and the maximum and minimum diameter of the sealer spread was measured by a digital calliper (Mitutoyo Corp, Tokyo, Japan) with a resolution of 0.01 mm to obtain the average. Fifteen samples per sealer were measured (n = 15 per group).

### Setting time

The stainless steel ring mould, which is 10 mm in diameter and 2 mm in height, was placed on the slide glass and the sealer was filled into the mould. Sealer samples ( $n = 15$  per group) were stored in an incubator with a humidity of 95% at 37°C. Vicat apparatus needle of 300g total weight was carefully placed vertically against the sealer. The final setting time was recorded when the needle no longer forms an indentation on the sealer surface. An hour after the sealer was filled in the mould, measurements were taken every 5 min.

### Radiopacity

The metal ring mould, which is 8 mm in diameter and 1 mm in height, was placed on the slide glass, and the sealer was filled into the mould. Samples ( $n = 15$  per group) were stored in an incubator with a humidity of 95% at 37°C until the sealer was completely set. Radiography of the sealer sample and aluminium step wedge, which increases by 1 mm from 1 mm to 10 mm, was taken (Fig. 1). The settings were as follows: 60 kV, 2 mA, 0.08 s, 10 cm distance from the tube and sensor. Using ImageJ software (National Institutes of Health, Bethesda, MD, USA), the mean grey value of the sealer sample and the aluminium step wedge was compared.

### Dimensional stability

The cylindrical teflon mould, which is 6 mm in internal diameter and 12 mm height, was placed on the slide glass, and the sealer was filled into the mould. Sealer samples ( $n = 15$  per group) were stored in an incubator with a humidity of 95% at 37°C for a period that tripled the final setting time of each sealer. Both sides of the teflon mould were polished with a 600 grit sandpaper. After carefully removing the teflon mould, the height ( $H_0$ ) of the sealer sample was measured by a digital caliper with a resolution of 0.01 mm. Sealer samples were stored in distilled water at 37°C. After storing the sealer sample, the height was measured again for 6, 24, 72 h, 7, 14 and 30 days. The percentage was obtained by dividing the change in height by  $H_0$ .

### Cell viability assay

#### *Fresh material extraction medium sample preparation*

The sealer was mixed with 20 mg ml<sup>-1</sup> of Dulbecco's Modified Eagle's Medium (DMEM; Gibco, NY, USA) and stored for 24 h in an incubator with 5% CO<sub>2</sub> at 37°C. The supernatant liquid was filtered with a 0.2 µm filter and diluted with a 1/4 volume ratio.

#### *Setting material extraction medium sample preparation*

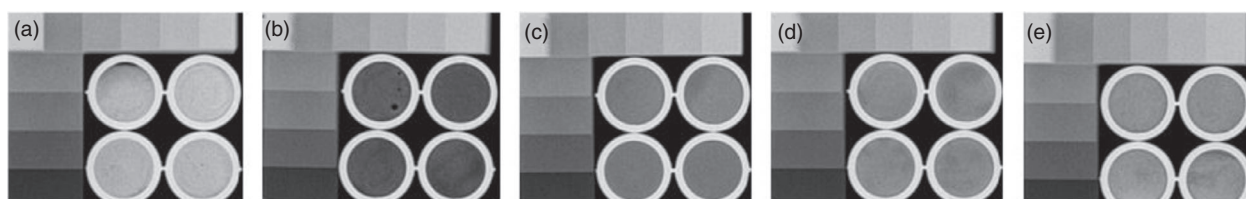
In a sterilised environment, a cylindrical teflon mould, which is 5 mm in diameter and 2 mm height, was placed on a slide glass and filled with a sealer. It was stored in an incubator with a humidity of 95% at 37°C for 48 h. After the sealer was set, the sample was placed in a DMEM solution containing 10% foetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 1% penicillin-streptomycin and stored in an incubator with 5% CO<sub>2</sub> at 37°C for 72 h. The media was changed every 24 h. After 48 and 72 h, the extraction media was obtained and filtered with a 0.2 mm filter.

#### *MTT assay*

hPDLF cells ( $2 \times 10^4$  cells) were seeded in a 24-well plate and stored in extraction media to evaluate cytotoxicity for 7 days. Fresh extraction media samples were evaluated on the 1st, 2nd, 3rd and 7th day, and setting extraction media samples were evaluated on the 1st, 3rd and 7th day. On the date of measurement, 0.5 mg ml<sup>-1</sup> MTT solution was put into the well and kept in an incubator at 37°C for 2 h. After media was removed, 300 µl dimethyl sulphoxide (DMSO) was put into the well and washed for 10 min, then moved to the 96-well plate to measure absorbance with a wavelength of 540 nm.

### Cell attachment evaluation

Sealer disc with a diameter of 5 mm and a height of 2 mm was prepared in the same way as 2.5.2. hPDLF cell ( $5 \times 10^4$  cells ml<sup>-1</sup>) was seeded on the sealer disc in the 96-well plate and kept it in an incubator with 5% CO<sub>2</sub> at



**Figure 1** Radiopacity of each sealers with aluminium step wedge. (a) AH Plus, (b) BrightEndo MTA sealer, (c) CeraSeal, (d) EndoSeal TCS, (e) One-Fil.

37°C for 72 h. After the media was removed, the sealer disc was washed with PBS and fixed with 2% glutaraldehyde at 4°C for 4 h. Sealer disc was washed 3 times for 2 min using PBS, 5 min at 25%, 50%, 75% and 95% EtOH, respectively, 3 times for 10 min at 100% anhydrous EtOH, 15 min with a 1:1 solution of HMDS and EtOH and twice for 15 min with 100% HMDS. Excess liquid was removed with filter paper and dried 4 h at room temperature before the scanning electronic microscopy (SEM; Hitachi SU5000; Hitachi, Tokyo, Japan) evaluation. After the sealer disc was coated with 100 nm platinum, the cell attached to the sealer disc was observed at 500x, 1000x magnification.

**Statistical analysis**

For data analysis, SPSS software (version 20.0; IBM SPSS Statistics, Chicago, IL, USA) was used. Shapiro–Wilk test was used for the normality test and Levene’s test was used for homogeneity of variance test. One-way analysis of variance (ANOVA) and Scheffé test were used for flow test results due to the homogeneity of data. On the other hand, the Kruskal–Wallis H test and pairwise comparison were used for the analysis of setting time, radiopacity and dimensional stability test results. The significance level was set at  $P < 0.05$ .

**Results**

The properties of each sealer are summarised in Tables 3 and 4.

**Flow**

The flow of all the tested sealers was more than 17 mm, which is in agreement with the ISO 6876/2012 (Table 3). One-Fil showed a flow significantly higher than that of other sealers ( $P < 0.05$ ), whereas EndoSeal TCS had the significantly lowest flow than that of other sealers ( $P < 0.05$ ).

**Setting time**

BrightEndo MTA sealer showed a longer setting time than the other sealers ( $P < 0.05$ ) (Table 3). EndoSeal TCS

had the shortest setting time. Significant difference was not found between EndoSeal TCS and CeraSeal ( $P > 0.05$ ).

**Radiopacity**

AH Plus Jet showed the highest radiopacity value and that is significantly different from other sealers ( $P < 0.05$ ) (Table 3). BrightEndo MTA sealer showed the lowest radiopacity value, and no significant difference was observed between BrightEndo MTA sealer and CeraSeal ( $P > 0.05$ ) (Fig. 1).

**Dimensional stability**

While all sealers expanded after 30 days, significant difference was not found among the sealers ( $P > 0.05$ ) (Table 4). CeraSeal showed the largest dimensional change, whereas BrightEndo MTA sealer showed the smallest. All sealers did not differ significantly over time ( $P > 0.05$ ).

**Cell viability**

In the MTT assay using fresh extraction media, AH Plus Jet showed lower absorbance than other sealers in all experimental periods ( $P < 0.05$ ) (Fig. 2). The BrightEndo MTA sealer showed significantly lower absorbance than the control group after 3 days ( $P < 0.05$ ). At day 7, CeraSeal and EndoSeal TCS showed significantly higher absorbance than the control group ( $P < 0.05$ ). The absorbance of BrightEndo MTA sealer, CeraSeal, EndoSeal TCS and One-Fil increased over time.

In the MTT assay using 48h extraction media, the absorbance of all sealers increased over time (Fig. 3). Until day 3, all sealers were not significantly different from the control group ( $P > 0.05$ ). On the 7th day, AH Plus Jet and One-Fil showed significantly higher absorbance than the control group ( $P < 0.05$ ).

In the MTT assay using 72h extraction media, AH Plus Jet showed significantly higher absorbance than the control group on day 1 and significantly lower absorbance on day 3 ( $P < 0.05$ ) (Fig. 4). One-Fil showed significantly lower absorbance than the control group on day 3 and significantly higher absorbance on day 7 ( $P < 0.05$ ). The

**Table 3** Physical properties of tested sealers (mean ± standard deviation)

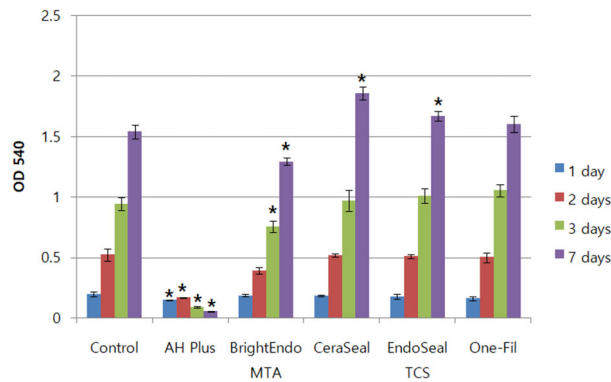
	AH Plus	BrightEndo MTA	CeraSeal	EndoSeal TCS	One-Fil
Flow (mm)	23.08 ± 0.34 <sup>e</sup>	21.91 ± 1.28 <sup>d</sup>	25.02 ± 0.55 <sup>b</sup>	19.33 ± 0.86 <sup>a</sup>	26.51 ± 0.42 <sup>a</sup>
Setting time (min)	402.00 ± 8.82 <sup>b</sup>	1420.67 ± 22.11 <sup>a</sup>	312.67 ± 13.21 <sup>c</sup>	268.33 ± 15.20 <sup>c</sup>	370.33 ± 16.85 <sup>b</sup>
Radiopacity (mmAl)	10.00 <sup>a*</sup>	4.31 ± 0.10 <sup>c</sup>	5.94 ± 0.25 <sup>c</sup>	7.41 ± 0.40 <sup>b</sup>	6.82 ± 0.65 <sup>b</sup>

\*All samples had the mmAl value of 10 or higher. Different superscript lowercase letters (a,b,c,d,e) indicate significant differences ( $P < 0.05$ ).

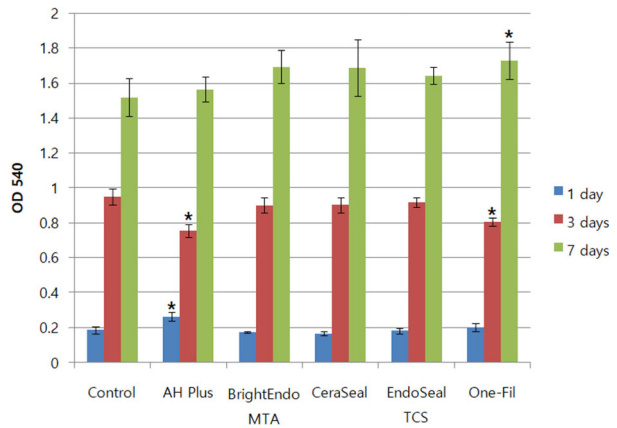
**Table 4** Dimensional stability of tested sealers at the different time period (mean ± standard deviation)

Ratio (%)	6 h	1 day	3 days	7 days	14 days	21 days	30 days
AH Plus	0.13 ± 0.30 <sup>aA</sup>	0.22 ± 0.34 <sup>bCA</sup>	0.29 ± 0.35 <sup>abA</sup>	0.28 ± 0.41 <sup>abA</sup>	0.42 ± 0.49 <sup>aA</sup>	0.35 ± 0.42 <sup>aA</sup>	0.37 ± 0.41 <sup>aA</sup>
BrightEndo MTA	0.02 ± 0.25 <sup>aA</sup>	0.06 ± 0.18 <sup>cA</sup>	0.07 ± 0.30 <sup>bA</sup>	-0.03 ± 0.42 <sup>bA</sup>	0.00 ± 0.40 <sup>bA</sup>	0.11 ± 0.55 <sup>aA</sup>	0.16 ± 0.51 <sup>aA</sup>
CeraSeal	0.07 ± 0.18 <sup>aA</sup>	0.41 ± 0.37 <sup>aA</sup>	0.59 ± 0.68 <sup>aA</sup>	0.50 ± 0.62 <sup>aA</sup>	0.53 ± 0.60 <sup>aA</sup>	0.46 ± 0.51 <sup>aA</sup>	0.51 ± 0.59 <sup>aA</sup>
EndoSeal TCS	0.14 ± 0.21 <sup>aA</sup>	0.23 ± 0.26 <sup>aA</sup>	0.53 ± 0.17 <sup>aA</sup>	0.43 ± 0.28 <sup>aA</sup>	0.35 ± 0.45 <sup>abA</sup>	0.28 ± 0.41 <sup>aA</sup>	0.37 ± 0.43 <sup>aA</sup>
One-Fil	0.10 ± 0.15 <sup>aA</sup>	0.42 ± 0.26 <sup>aA</sup>	0.46 ± 0.30 <sup>aA</sup>	0.48 ± 0.36 <sup>aA</sup>	0.57 ± 0.55 <sup>aA</sup>	0.31 ± 0.43 <sup>aA</sup>	0.41 ± 0.44 <sup>aA</sup>

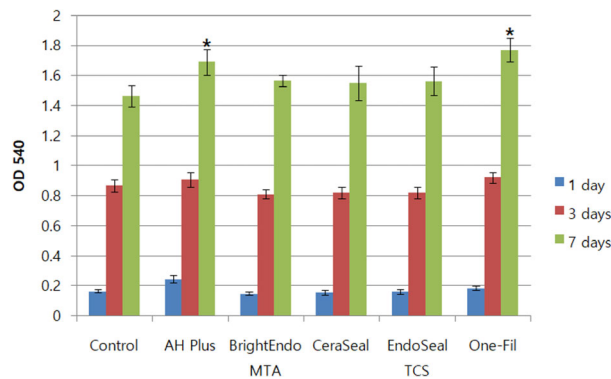
Different superscript lowercase letters (a,b,c) indicate significant differences depending on the type of sealer at the same experimental time ( $P < 0.05$ ). Different superscript uppercase letters (A,B) indicate significant differences over the time of the experiment in the same sealer ( $P < 0.05$ ). Different superscript uppercase letters (A,B) indicate significant differences over the time of the experiment in the same sealer ( $P < 0.05$ ).



**Figure 2** The graph shows the absorbance measured in 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using fresh extraction media and human periodontal ligament fibroblasts (hPDLFs). Asterisk (\*) means that there is a statistically significant difference between the experimental group and the control group in the same period.



**Figure 4** The graph shows the absorbance measured in 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using 72 h extraction media and human periodontal ligament fibroblasts (hPDLFs). Asterisk (\*) means that there is a statistically significant difference between the experimental group and the control group in the same period.



**Figure 3** The graph shows the absorbance measured in 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using 48 h extraction media and human periodontal ligament fibroblasts (hPDLFs). Asterisk (\*) means that there is a statistically significant difference between the experimental group and the control group in the same period.

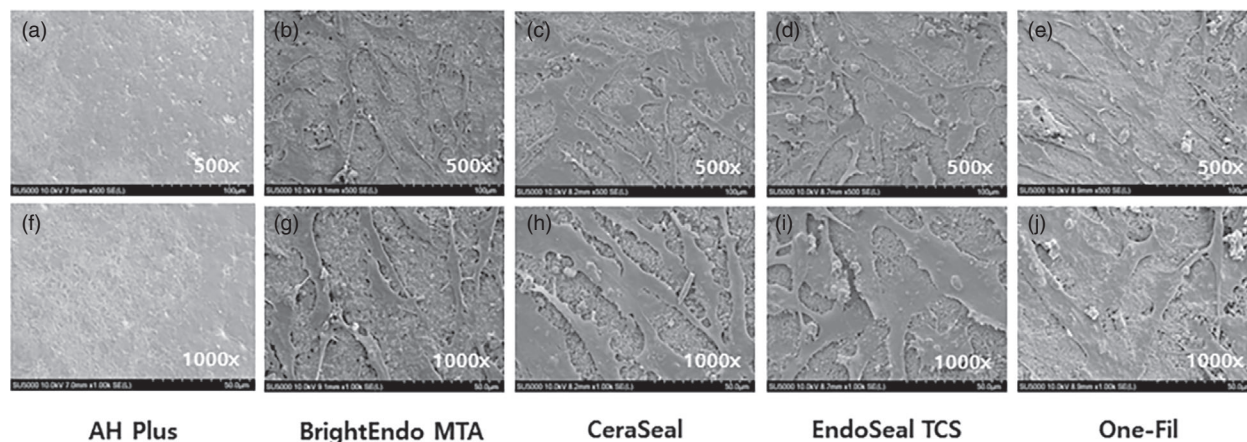
BrightEndo MTA sealer, CeraSeal and EndoSeal TCS showed no significant difference from the control group in all experimental periods ( $P > 0.05$ ).

**Cell attachment**

As a result of analysing the surface of the sealer disc by SEM, hPDLF cells spread widely in all calcium silicate-based sealer discs, whereas no attached living cells were observed in the AH Plus Jet disc (Fig. 5).

**Discussion**

With the development of bioceramic technology, it has been widely used in endodontics. Calcium silicate-based sealer is a bioceramic material that is known to have excellent cytocompatibility, mineralisation activity and osteogenic potential (8,18,19). This study compared the



**Figure 5** Surface of sealer disc observed by SEM. (a, f) SEM images of AH Plus Jet discs observed at a magnification of 500x and 1000x, respectively. (b, g) SEM images of BrightEndo MTA sealer disc observed at a magnification of 500x and 1000x, respectively. (c, h) SEM images of the CeraSeal disc observed at a magnification of 500x and 1000x, respectively. (d, i) SEM images of EndoSeal TCS disc observed at a magnification of 500x and 1000x, respectively. (e, j) SEM images of One-Fil disc observed at a magnification of 500x and 1000x, respectively.

physicochemical properties and cytotoxicity of four newly developed calcium silicate-based sealers with an epoxy resin-based sealer. The physicochemical properties experiments, except for dimensional change, were conducted according to ISO 6876/2012 standards. The dimensional change test was performed based on ISO 6876/2001 standards, because ISO third edition removed the dimensional changes. Human periodontal ligament fibroblast (hPDLF) was used for MTT assay for cytotoxicity evaluation. hPDLF is a multi-differentiated cell that differentiates into osteoblast or cementoblast, and has been widely used in studies on the cytotoxicity of sealer (7,17,20).

As a result of the flow test, all sealers met ISO 6876/2012 standards. Flow is an important property for root canal filling and is related to penetration ability into irregular root canal system such as isthmus and accessory canal (21,22). The sealer with high flowability is prone to extrude beyond the apical foramen (23). In that case, extruded sealers contact with periapical tissue directly, which causes an inflammation (8). Therefore, sealers with high flowable behaviour should be used with caution.

The setting time of the root canal sealer should be sufficient to be easily manipulated until the canal is filled with gutta-percha and sealer. A prolonged setting time may be considered a critical issue in clinical situation, because excessively long setting time may increase the solubility of materials and form gaps, which cause the reproduction of microorganisms and reinfection in the root canal (3,24). According to ISO 6876/2012, the setting time shall be no more than 10 % longer than that claimed by manufacturer (15). In the current study, all

the tested sealers showed longer setting time required by the ISO standard. However, the setting time of the AH Plus Jet was similar to the previous study (10). Calcium silicate-based sealers except the BrightEndo MTA sealer had a shorter setting time than the AH Plus Jet. Setting time is dependent on the composition of the sealers (25). It seemed that some components in BrightEndo MTA might be related to the increased setting time. Since moisture in the dentinal tubule also induces the setting reaction of calcium silicate-based sealer, setting time may increase in dry root canals (4,13).

The radiopacity of the sealer should be sufficient to distinguish the filling material from the adjacent tooth structure as an essential factor for evaluating the quality of the canal filling (11). The radiopacity of all sealers used in the experiment was higher than 3.00 mmAl, which satisfies the ISO 6876/2012 standard. The previous study reported that radiopacity varies depending on the amount and type of radiopacifier included in the sealer (26). In this study, the radiopacity of AH Plus Jet was significantly higher than that of calcium silicate-based sealers. According to the manufacturer, CeraSeal, EndoSeal TCS and One-Fil contain zirconium oxide for the radiopacifier, while BrightEndo MTA sealer contains bismuth oxide and zirconium oxide. AH Plus Jet contains calcium tungstate and zirconium oxide. Differences in the types and amounts of radiopacifier in the endodontic sealers may have influenced on the results.

Dimensional stability is a parameter that evaluates the stability of volume change such as shrinkage or expansion after setting. As the result of this study, all sealers expanded more than 0.1% on the 30th day after setting, which was inadequate for the ISO 6876/2001 standards.

When the calcium silicate-based sealer is set, calcium hydroxide is produced as a reaction product (11), and it is known that the sealer expands by absorbing moisture due to the hygroscopic effect of calcium hydroxide (27). These sealers should be used with caution because excessive expansion may cause crack formation in the roots (28,29).

In this study, the biocompatibility of the sealer was analysed using MTT assay, and SEM was used to evaluate cell attachment. Biocompatibility is one of the essential factors for the root canal sealer because the sealer may contact directly with apical tissue through the apical foramen. As a result of MTT assay, when using a fresh extraction medium, the calcium silicate-based sealer had significantly higher absorbance values than that of AH Plus. As absorbance is related to the number of living cells, higher absorbance value implies a larger number of living cells (19). On the other hand, when using 48h and 72h extraction medium, AH Plus and the calcium silicate-based sealer showed a similar tendency. The above results are consistent with previous studies that reported the initial toxicity of AH Plus caused by the amine and resin components of the epoxy resin-based sealer (8,21,30). Calcium silicate-based sealers showed superior biocompatibility in all experimental groups. In addition, they induce osteoblastic differentiation of hPDLF cells and have an osteogenic potential to induce bone regeneration when unintentionally extruded beyond the apical foramen (4,31,32).

As a result of SEM analysis, no attached hPDLF cells were observed in the epoxy resin-based sealer. The hPDLF cells might not have been able to adhere to the surface of resin sealer from the beginning or they might have died after being attached and/or washed out during the sample preparation. On the contrary, hPDLF cells were well attached to the four calcium silicate-based sealers used in this experiment (Fig. 5). This was consistent with previous studies that showed better cell attachment for calcium silicate-based sealer than epoxy resin-based sealer (19,21). These results suggest that the epoxy resin-based sealer may be toxic to hPDLF cells even after setting, while the four calcium silicate-based sealers have excellent cytocompatibility for hPDLF cells.

There is a limitation that solubility is not included in this study. Although many studies have included solubility, the results are controversial (4,10,22,23,28,33). The results of Bronzel *et al.* showed that TotalFill BC and Bio-C sealers have high solubility than AH Plus yet disqualified for the ISO standards (10). On the other hand, in the study of Prüllage *et al.*, BioRoot RCS showed reasonable solubility and met the requirements of ISO 6876/2012 (34). Overall, the solubility test showed various results

depending on the test method and materials. Since the solubility of the sealer has a strong association with the reinfection of the apical region (35), further study is needed.

BrightEndo MTA sealer, CeraSeal, EndoSeal TCS and One-Fil are the new sealers available in the market. Physicochemical properties and cytocompatibility are utmost important characteristics for broad clinical application. From the results of this study, it can be seen that the four newly developed calcium silicate-based sealers have similar properties and superior biocompatibility compared to AH Plus, which was used as a gold standard. Based on these results, it is worth considering using a calcium silicate-based sealer rather than an epoxy resin-based sealer for immature permanent teeth as well as for routine root canal treatment. However, since it is a recently developed product, there are limitations in the lack of research results, and further study including long-term clinical results is needed.

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Dr. Hyeon-Cheol Kim and Dr. Sang Won Kwak contributed equally to this work and have the corresponding authorship shared.

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