

# Compressive Strength and Surface Morphology of Premixed and Conventional Calcium Silicate Cement in Presence of Blood Serum

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**Purpose:** The objective of this study was to investigate the impact of blood contamination on the compressive strength and surface morphology of both conventional and newly developed calcium silicate cements (CSCs). **Materials and Methods:** Compressive strengths of Endocem MTA Premixed Regular (EMPR) and ProRoot MTA (PMTA) were assessed after immersion in fetal bovine serum (FBS), saline, and deionized water (DW). Surface morphology was examined using scanning electron microscopy (SEM). **Results:** The compressive strength of EMPR samples immersed in FBS for both 1 and 7 days was significantly lower compared to those in saline and DW, with no significant differences between the saline and DW groups. The PMTA group exhibited the lowest compressive strength after 1 day in FBS, although it did not significantly differ from that of saline and DW groups. SEM images revealed significant differences in crystalline formation between FBS and the other experimental groups. **Conclusion:** Minimizing blood contamination during vital pulp therapy (VPT) is crucial to ensure optimal CSC setting. PMTA may be preferred over EMPR for resisting high occlusal forces in the presence of blood contamination. [J Korean Dent Sci. 2024;17(3):112-20]

**Key Words:** Calcium silicate cement; Blood contamination; Compressive strength; Scanning electron microscopy

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## Introduction

Vital pulp therapy (VPT) seeks to preserve the health and functionality of teeth with compromised dental pulp due to caries or trauma, allowing them to remain in their natural position within the dental arch<sup>1,2</sup>. These procedures are commonly performed on both primary and permanent teeth<sup>3,4</sup>. VPT techniques include indirect pulp capping, where a biomaterial is placed over a thin layer of dentin to prevent pulp exposure; direct pulp capping, where the exposed pulp is directly covered with a biomaterial to stimulate healing; and pulpotomy, which involves the partial or complete removal of the coronal pulp followed by covering the remaining pulp with a biomaterial to facilitate healing<sup>5</sup>.

Various dental materials have been employed in VPT<sup>6</sup>. Traditionally, calcium hydroxide-based medications have been predominantly used. However, several retrospective studies have shown a decline in long-term success rates<sup>7-9</sup>. Mineral Trioxide Aggregate (MTA), a calcium silicate-based cement, was initially introduced in dentistry for repairing root perforations<sup>10</sup>. MTA is proposed as an alternative to calcium hydroxide for VPT due to its superior physical properties, improved sealing ability, and biocompatibility<sup>3,9,11-13</sup>.

ProRoot MTA (PMTA), an early version of MTA also known as White Mineral Trioxide Aggregate, is recognized for its beneficial properties and is considered the gold standard for VPT<sup>14</sup>. However, it has drawbacks, including tooth discoloration, high cost, and a prolonged setting time<sup>15</sup>. To address these issues, modified formulations of calcium silicate-based cements (CSCs) with varying compositions have been introduced<sup>16</sup>. One such premixed CSC is Endocem<sup>®</sup> MTA premixed regular (EMPR), which offers practical advantages for dental practitioners by enabling precise injection, minimizing wastage, improving efficiency, and reducing setting time<sup>16-18</sup>.

In clinical settings, CSCs may be exposed to blood contamination, particularly when applied to exposed

pulp. An *in vitro* study comparing the dye leakage of an MTA prototype with amalgam, Super EBA, and IRM in the presence of blood found that blood did not significantly affect dye leakage<sup>19</sup>. However, several studies have indicated that blood contamination can negatively impact the mechanical properties of CSCs and alter the surface morphology of PMTA<sup>20-24</sup>. Despite this, limited research has explored the behavior of newly introduced premixed CSCs under blood contamination scenarios. In this study, we aimed to assess the response of conventional PMTA and the newly developed EMPR to blood contamination. Fetal Bovine Serum (FBS) was used to simulate blood contamination. The investigation included measuring compressive strength to evaluate physical differences and using Scanning Electron Microscopy (SEM) to observe surface characteristics.

## Materials and Methods

### 1. Sample preparation

This study utilized ProRoot<sup>®</sup> MTA (PMTA; Dentsply Tulsa Dental, Tulsa, OK, USA) and Endocem<sup>®</sup> MTA Premixed Regular (EMPR; Maruchi, Wonju, Korea). The storage media used were fetal bovine serum (GIBCO, Grand Island, NY, USA), 0.9% saline solution (JW Pharmaceutical, Seoul, Korea), and deionized water (DW; Clean Guy, Suwon, Korea) (Table 1).

### 2. Compressive strength

Compressive strength was measured using a protocol adapted from ISO 9917-1:2007<sup>25</sup>. The material was placed into split stainless-steel molds of 4.0 mm diameter and 6.0 mm height, and then stored in a 95% relative humidity environment at 37°C for 1 hour. Wet P600-grit abrasive paper was used to grind both ends of the specimens. After removal from the molds, samples were divided into three groups immersed in conical tubes containing 10.0 mL of FBS, saline, or DW (n=20). The specimens were incubated in these

**Table 1.** Materials used in the study

Trade name	Category	Code	Composition	Manufacturer
Endocem MTA® Premixed Regular	Premixed type MTA	EMPR	Zirconium dioxide, Calcium silicate, Calcium aluminate, Calcium sulfate, Dimethyl sulfoxide, Lithium carbonate, Thickening agents	Maruchi, Wonju, Korea
ProRoot® MTA	Powder-liquid mix type MTA	PMTA	Tricalcium silicate, Dicalcium silicate, Bismuth oxide, Tricalcium aluminate, Calcium sulfate dehydrate, Tetra calcium aluminoferrite, Gypsum, Calcium oxide	Dentsply, Tulsa, OK, USA
Fetal bovine serum	Storage media	FBS	Bilirubin, Cholesterol, Creatinine, Urea, Glucose, Protein, Albumine, a-Globulin	GIBCO, Grand Island, NY, USA
Saline	Storage media	Saline	Sodium chloride 9 g	JW Pharmaceutical, Seoul, Korea
Deionized water	Storage media	DW	-	Cleanguy, Suwon, Korea

solutions at 37°C. Compressive strength was measured after 1 day and 7 days of immersion (n=10). Compressive strength was measured using a universal testing machine (KUM-3A, KMNT, Incheon, Korea) with a crosshead speed of 0.75 mm/min. The maximum compressive load was recorded in Newtons (N). Compressive strength (MPa) was calculated using the following formula:

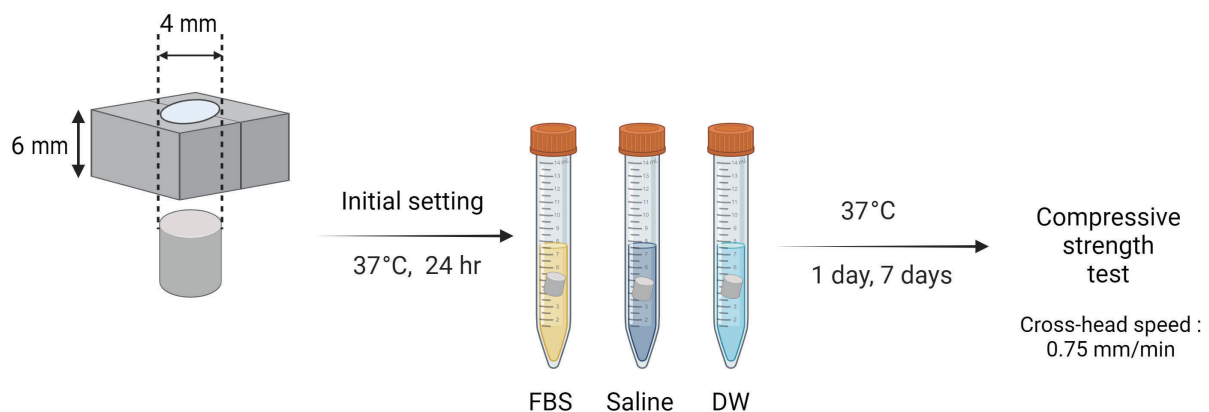
$$MPa = \frac{4 \times P}{\pi \times D^2}$$

where P (N) is the maximum load applied at failure and D (mm) is the mean diameter of the specimens. Fig. 1 shows a schematic of the compressive strength

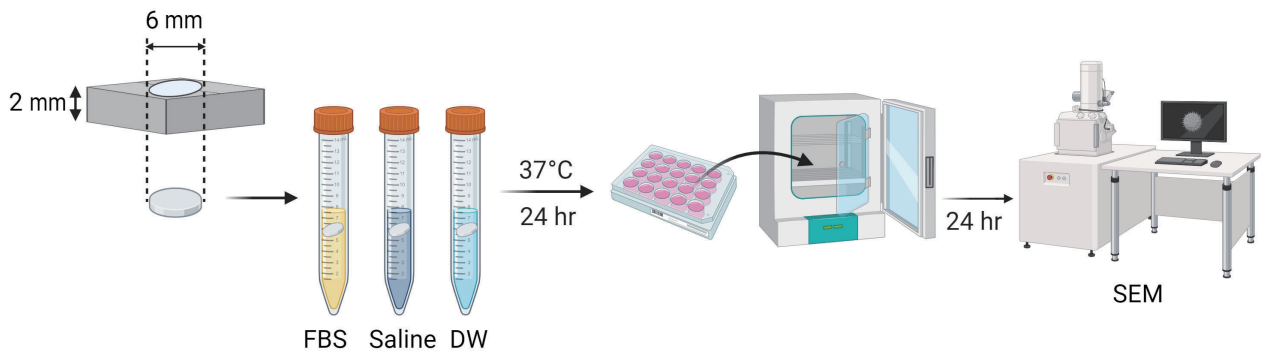
test procedure.

### 3. Surface morphology analysis through SEM

The material was placed into split molds of 4.0 mm diameter and 6.0 mm height, and stored for 1 hour in a 37°C cabinet with 95% relative humidity. 2 samples from each group were immersed in FBS, saline, or DW at 37°C for 24 hours. After immersion, the discs were coated with a 100 nm layer of platinum and examined under a scanning electron microscope (SEM, Hitachi S-4800, Tokyo, Japan) at ×2,000 magnification. Fig. 2 shows a schematic of the SEM analysis procedure.



**Fig. 1.** Schematic illustration of compressive strength test. The specimens were immersed in fetal bovine serum (FBS), saline, or deionized water (DW) after initial setting. The compressive strength was measured after 1 and 7 days.



**Fig. 2.** Schematic illustration of surface morphology analysis. The specimens were immersed in fetal bovine serum (FBS), saline, or deionized water (DW). The surface morphology was observed using scanning electron microscopy (SEM).

#### 4. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp, Armonk, NY, USA). One-way analysis of variance (ANOVA) was conducted to determine significant differences in compressive strength according to the type of materials, followed by Tukey's post hoc test. Independent t-test was conducted to determine the effect of storage duration on compressive strength. Statistical significance was set at a *P*-value of less than 0.05.

## Results

### 1. Compressive strength

The compressive strengths of the specimens are detailed in Table 2 and illustrated in Fig. 3. Compressive

strength generally increased across all groups with longer CSC storage durations; however, these differences were not statistically significant.

The compressive strength of EMPR samples significantly decreased after 1 day of immersion in FBS compared to those immersed in saline and DW ( $P < 0.05$ ). Moreover, EMPR specimens immersed in FBS for 7 days showed significantly lower compressive strengths compared to those in saline and DW ( $P < 0.05$ ).

For the PMTA group, immersion in FBS for 1 day resulted in the lowest average compressive strength, but it was not significantly different from that of the saline and DW groups ( $P > 0.05$ ). No significant difference was observed after 7 days of immersion in the PMTA group.

After 1 day of immersion, the compressive strength of the EMPR group exposed to FBS was significantly

**Table 2.** Mean and standard deviation of compressive strength of calcium silicate cements in contact with storage media for 1 and 7 days

Storage media	EMPR			PMTA		
	FBS	Saline	DW	FBS	Saline	DW
1 day	35.12 ± 9.69 <sup>b</sup>	57.32 ± 3.30 <sup>a</sup>	52.75 ± 5.97 <sup>a</sup>	44.88 ± 11.56 <sup>ab</sup>	54.34 ± 9.68 <sup>a</sup>	52.59 ± 6.14 <sup>a</sup>
7 days	39.04 ± 14.54 <sup>b</sup>	60.65 ± 15.58 <sup>ab</sup>	59.58 ± 11.54 <sup>a</sup>	50.10 ± 11.06 <sup>ab</sup>	59.19 ± 7.13 <sup>a</sup>	56.11 ± 11.72 <sup>a</sup>

Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

a – c : Different superscript letters denote significant differences between groups stored in the solution for the same period ( $P < 0.05$ ).

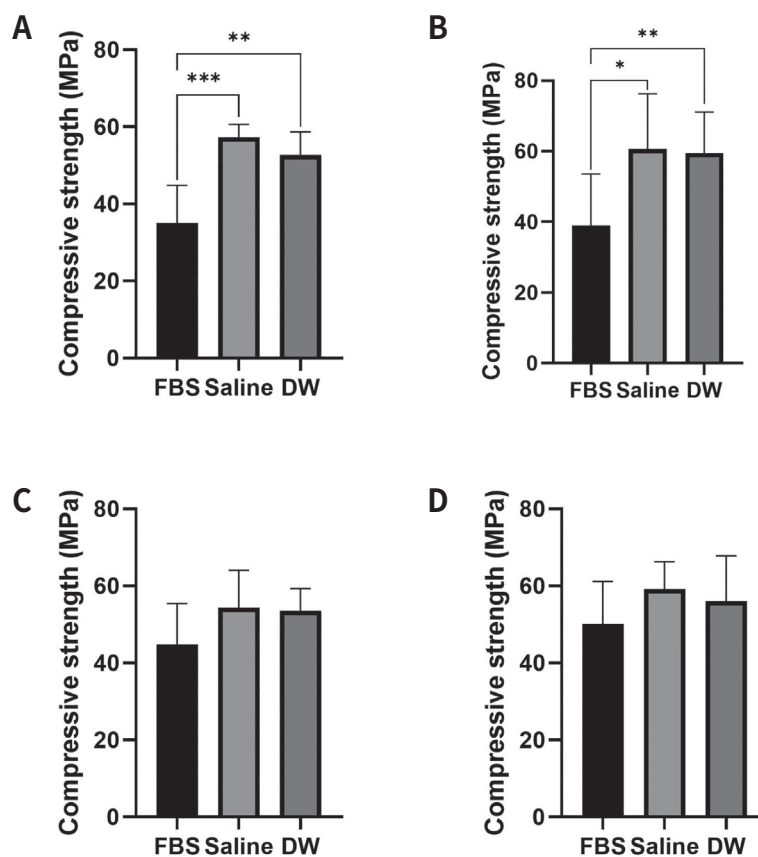
EMPR: Endocem MTA Premixed Regular; PMTA: ProRoot MTA; FBS: Fetal bovine serum; DW: Deionized water.

**Fig. 3.** Compressive strength of (A) EMPR after 1 day of immersion, (B) EMPR after 7 days of immersion, (C) PMTA after 1 day of immersion, (D) PMTA after 7 days of immersion.

Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

EMPR: Endocem MTA Premixed Regular; PMTA: ProRoot MTA; DW: deionized water; FBS: fetal bovine serum.



lower compared to that of EMPR and PMTA groups exposed to saline and DW. No significant difference was found between the EMPR and PMTA groups when both were exposed to FBS. Additionally, the compressive strength of the PMTA group in contact with FBS was not significantly different from those of the EMPR and PMTA groups exposed to saline and DW. The observed differences in compressive strength between the groups remained consistent after 7 days of immersion, with EMPR exposed to FBS showing significantly lower compressive strength compared to the other groups.

## 2. Surface morphology

Both EMPR and PMTA surfaces displayed distinct crystalline structures when exposed to either DW or saline solution, as shown in Fig. 4. The surfaces of

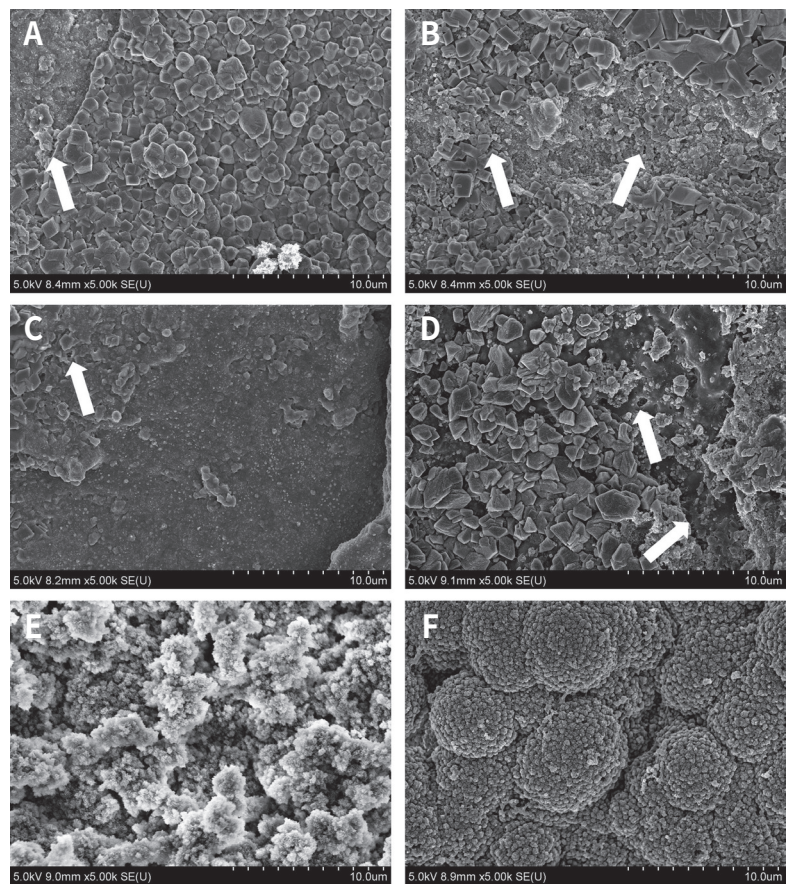
CSCs in contact with DW and saline exhibited angular crystals of various shapes embedded within an uneven matrix, with acicular projections also present (Fig. 4A - 4D). In contrast, CSC surfaces exposed to FBS showed a different morphology, characterized by the absence of angular and acicular crystals. Instead, the surfaces displayed smaller globular crystals. No noticeable differences were observed between the EMPR and PMTA surfaces.

## Discussion

We investigated the impact of blood exposure on the compressive strength and surface morphology of two CSCs. The compressive strength of the CSCs was measured, and surface morphology changes were analyzed after immersion in various solutions. The results

**Fig. 4.** SEM micrographs of surface of CSCs ( $\times 5000$ ). (A) EMPR surface set in the presence of DW (B) PMTA surface set in the presence of DW, (C) EMPR surface set in the presence of saline (D) PMTA surface set in the presence of saline, (E) EMPR surface set in the presence of FBS, (F) PMTA surface set in the presence of FBS. The arrows indicate acicular crystal formation, which is known to be related to setting process of CSCs.

SEM: scanning electron microscopy; CSC: calcium silicate cement; EMPR: Endocem MTA Premixed Regular; DW: deionized water; PMTA: ProRoot MTA; FBS: fetal bovine serum.



showed a reduction in compressive strength when EMPR and PMTA were exposed to FBS, with EMPR experiencing a more pronounced decline. Additionally, changes in surface crystallization patterns were observed following exposure to FBS.

Compressive strength reflects several material properties, including the hydration reaction, which is crucial for the setting process of hydraulic silicate cements<sup>26-28</sup>. It serves as a representative measure of both the setting and hydration processes<sup>27-29</sup>. The compressive strength test simulates the masticatory forces applied to the material when CSCs are used as base or restorative materials. Although there are no specific requirements for CSCs, ISO 9917-1 suggests that the standard compressive strength of dental hydraulic cements should exceed 50 MPa<sup>25</sup>. This study followed the standardized procedure delineated in ISO 9917-1, and the compressive strengths of both CSCs exceeded

50 MPa, except when in contact with FBS.

In this study, PMTA, the first commercially available MTA product, was selected due to its widespread recognition and extensive investigation across many countries. Premixed type CSC is a combination of calcium phosphate cement powders and nonaqueous liquid with several additives. For example, anti-wash-out agents, such as methylcellulose or glycerin and accelerators such as calcium chloride or zirconium oxide are contained. Premixed CSCs currently on the market include Well-Root PT (WRP, Vericom Co., Chuncheon, Korea), which is a putty type with additives such as glycol and zirconium oxide, and Endocem MTA premixed regular which is premixed type composed of dimethyl sulfoxide and zirconium oxide. EMPR was chosen because it is widely utilized and distributed in the Republic of Korea and has been the subject of numerous research studies.

The findings indicate that the compressive strength of EMPR is adversely affected by FBS exposure. Previous research has demonstrated that blood contamination reduces the compressive strength of MTA<sup>21</sup>. However, this effect was statistically significant primarily in the EMPR group. The compressive strength of EMPR in the FBS group was notably lower than that in other experimental groups after 1 and 7 days of immersion. Several studies suggest that modifications aimed at reducing the setting time can yield varying results in compressive strength. For instance, adding NaOCl gel, KY Jelly, and 5% CaCl<sub>2</sub> to MTA as hydration accelerators improves setting time but decreases the compressive strength<sup>30-32</sup>. Conversely, exposure to simulated body fluid (SBF), which has ion concentrations similar to those of human blood serum, did not significantly affect the compressive strength of EMPR compared to DW or saline groups<sup>33</sup>. The lack of proteins in SBF, unlike FBS, accounts for the differences observed in this study. FBS was selected for this study due to its availability, biosafety, and biochemical composition which closely resembles that of human blood serum<sup>23,24</sup>.

The compressive strength of PMTA in the FBS group was lower compared to the other groups; however, this difference was not statistically significant. No significant differences were found between the saline and DW groups. These findings suggest that blood contact does not have a lasting impact on the compressive strength of PMTA.

SEM was employed to examine the effect of blood contamination on the hydration of CSCs. Acicular crystals, also known as ettringite or hexacalcium aluminate trisulfate hydrate, were observed in both PMTA and EMPR groups exposed to saline or DW<sup>34</sup>. However, surface of FBS groups was morphologically homogenous, lacking acicular or angular crystals. Previous research has shown that blood contamination impairs the formation of acicular crystalline structures in MTA, which are characteristic of the ettringite phase<sup>34</sup>. Moreover, the ettringite phase formed during MTA hydration is particularly susceptible to blood

contamination<sup>21</sup>. The absence of acicular crystals, along with reduced compressive strength and surface microhardness, has been reported for MTA contaminated with blood<sup>24,34,35</sup>. The setting and strength of hydraulic cements have been attributed to the formation of calcium silicate hydrate and ettringite on nucleation sites of calcium hydroxide crystals<sup>34,36</sup>. The absence of acicular crystals in the FBS group can be attributed to the inhibition of the hydration process caused by the adhesion of proteins to crystal nucleation sites.

Numerous studies have demonstrated that proteins in the blood can hinder the setting process of MTA and Portland cement. For instance, mixing hemoglobin, which produces an air-entrainment admixture, with Portland cement has been shown to reduce compressive strength<sup>37</sup>. Similarly, incorporating red blood cell powder into Portland cement significantly extends the setting time and decreases compressive strength<sup>38</sup>. Given the similarities in composition and hydration mechanisms between CSCs and Portland cement, it is plausible that these effects could also occur in CSCs<sup>39</sup>. Additionally, the binding of blood proteins to crystal nucleation sites can impede the hydration process of CSCs.

Compressive strength is crucial in determining the suitability of CSCs for clinical applications. In scenarios where the material is exposed to additional forces, especially with blood presence, CSCs with higher compressive strengths are preferred. Conversely, although EMPR may have lower compressive strength, it could still be suitable for specific clinical applications where rapid setting is more critical than compressive strength.

This study had several limitations. Firstly, FBS was used instead of human blood, which may not fully replicate human blood contamination conditions. Only one newly developed premixed CSC product was evaluated. Additionally, chemical analyses of the crystals identified by SEM were not conducted. Further research should include a broader range of materials and more comprehensive analyses to better understand the effects of blood contamination on CSCs.

## Conclusion

Exposure to FBS resulted in reduced compressive strength and changes in surface morphology. EMPR, a premixed CSC, was significantly affected by FBS exposure. These findings suggest that minimizing blood contamination during VPT is important for ensuring the optimal setting of CSCs. In clinical scenarios where achieving complete hemostasis is challenging and substantial occlusal forces are anticipated, PMTA may be preferred than EMPR. Various factors should be considered when selecting materials, depending on the specific clinical context of VPT.

## Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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