





Evaluation of retrograde filling quality with a combination of calcium silicate cement and calcium silicate-based sealer

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정재헌



Table of Contents

List of figures and tableii
Abstract
I. Introduction
II. Materials & Methods5
III. Results
IV. Discussion
V. Conclusion
References ······29
Abstract in Korean

List of Figures

Fig. 1. Representative images of resected roots
Fig. 2. Images of the KiS-1D ultrasonic tip and root after retro-preparation
Fig. 3. Representative images of retrograde filling14
Fig. 4. Representative periapical X-ray images16
Fig. 5. Representative 3D images from micro-CT scans
Fig. 6. Representative micro-CT images of a specimen from each group18
Fig. 7. Representative SEM images

List of Table

Table 1. Volume percentage of gaps between the filling materials and the tooth structure $(%V_{out})$, internal voids $(%V_{in})$, and total defects $(%V_{total})$ 19



ABSTRACT

Evaluation of retrograde filling quality with a combination of calcium silicate cement and calcium silicate-based sealer

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In apical surgery, retrograde filling is a crucial procedure used to form a hermetic seal at the root-end area. However, it is very technique-sensitive because of limited access and visibility in clinical settings and the difficulty of handling retrograde filling materials. Therefore, the use of calcium silicate-based sealer, which has good flowability and



maneuverability, as a retrograde filling material could be expected to improve retrograde filling quality. The aim of this study was to assess the quality of retrograde filling with a combination of calcium silicate cement and calcium silicate–based sealer.

Twenty single-rooted, extracted human teeth were instrumented with nickel-titanium instruments and obturated with gutta-percha cones. Root resection at 3 mm from the apex and root-end preparation were performed. The root-end cavities were filled using two different retrograde filling methods (n=10). In group 1, the root-end cavities were filled with Endocem Zr (Maruchi, Wonju, Korea), while in group 2, the root-end cavities were filled with an approximately 1-mm-thick layer of Endoseal MTA (Maruchi) followed by Endocem Zr. Then, the samples were scanned using micro-computed tomography (micro-CT) and three-dimensional images of the samples were reconstructed. The volume of the yoids in the filling materials were measured, and the percentages of these volumes were calculated. Data were analyzed using the Mann-Whitney U test at a significance level of 95% to compare the difference in the volume percentage of voids between the two groups. Selected specimens were further observed using scanning electron microscopy (SEM).

No significant difference was found between the two groups in the volume percentage of the gaps and internal voids. The SEM examinations showed that the calcium silicate based-sealer applied in the retro-prepared cavity demonstrated good adaptation to the cavity wall and the calcium silicate cement.



Within the limitations of this study, the quality of retrograde filling with a combination of calcium silicate cement and calcium silicate–based sealer showed no statistically significant difference from that of retrograde filling with calcium silicate cement only.

Key words: gap, void, micro-computed tomography, calcium silicate-based sealer, calcium silicate cement, root-end cavity, retrograde filling, scanning electron microscope, surgical endodontics



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I. Introduction

In surgical endodontic treatment, the essential factors that affect treatment outcomes include not only surgical removal of the irritants and inflammatory tissues, but also the formation of a hermetic seal between the retrograde filling material and root canal wall at the root-end area (1, 2). When traditional materials such as amalgam, with clear



disadvantages of leakage and cytotoxicity, were used for retrograde fillings, the success rate for endodontic surgery was reported to be approximately 60% (3). Since its introduction in the 1990s, mineral trioxide aggregate (MTA) has emerged as the gold standard for retrograde filling materials in apical surgery by virtue of its superior sealing ability, biocompatibility, and capacity to induce hard tissue formation (5-7). Many studies using MTA as a retrograde filling material have reported a success rate of over 90% for endodontic surgery (5, 8, 9). Furthermore, various calcium silicate cements have been introduced and widely used as retrograde filling materials to supplement the commonly encountered drawbacks of MTA, including its long setting time and heavy metal content (10-12).

Another potential problem with the use of MTA is its handling difficulty. Despite the development of new calcium silicate cements and instruments, filling the cavity of the retro-prepared cavity during endodontic surgical procedures remains a potential challenge. Especially during apicoectomy, limited access and visibility can result in unexpected voids in the retro-filling, which can affect treatment outcomes. A previous study reported that inadequacy of the existing root-end filling was a common feature in cases of failure of apical surgery (4, 13). Furthermore, the presence of anatomic complexities, such as an isthmus, can reduce the success rate of apical surgery because of the resulting difficulties in adequate retro-preparation and retro-filling (14). In cases of intentional replantation, although access and vision limitations are less of an issue, clinicians should finish the procedure in approximately 15 minutes to obtain a favorable prognosis. Under these



conditions, clinicians are pressed for time, which it can result in low-quality retrograde filling and potential treatment failure (15).

Endosequence Root Repair Material (Brasseler, Savannah, GA, USA), a frequently used calcium silicate cement–based material, is available in two formulated consistencies (syringable paste or condensable putty) and can be used as a retrograde filling material and/or perforation repair material due to its biocompatibility and favorable handling properties (19). Likewise, the recently developed Endoseal MTA (Maruchi, Wonju, Korea) is an injectable premixed bioceramic endodontic sealer. Because of its good flowability and convenience in handling, it can be used as a sealer in the single-cone technique in non-surgical endodontic treatment and this showed similar filling quality to the continuous wave technique (16, 17). Furthermore, a three-dimensional cell culture model study demonstrated that Endoseal MTA is cytocompatible (18). In in vitro study using open apex model, Tran et al. demonstrated that the application of calcium silicate–based sealer to the canal walls before orthograde delivery of calcium silicate cement–based material enhanced its adaptation to the root dentin wall (33).

In apical surgery, because packing of mixed calcium silicate cement into the root-end cavity is a difficult and technique-sensitive procedure, if calcium silicate based–sealer with good flowability is adapted to the root-end cavity first, and the remainder is filled with conventional calcium silicate cement, it would be reasonable to expect that manipulation would become easier, the procedure time would be reduced, and the retrograde filling quality would improve.



Given this background, the aim of this study was to assess the quality of retrograde filling with a combination of calcium silicate cement and calcium silicate–based sealer using micro-computed tomography (micro-CT) and scanning electron microscopy (SEM). The null hypothesis was that the technique with a combination of calcium silicate cement and calcium silicate–based sealer would demonstrate a retrograde filling quality similar to the technique with calcium silicate cement only.



II. Materials & Methods

1. Sample preparation

Twenty single-rooted, extracted human maxillary premolars before orthodontic treatment were collected. The protocol of this study was approved by the institutional review board of our institution (IRB:3-2019-0022). Only teeth with two canals confirmed using periapical radiographs were included, and teeth with previous root canal treatment, caries, cracks, fractures, or perforations were excluded from the study. The working length was determined to be 0.5 mm short of the apical foramen, and all canals were instrumented with the ProTaper Gold nickel-titanium instrument system (Dentsply Maillefer, Ballaigues, Switzerland) to a size of #40 (F4) using a crown-down technique. Between instrumentations, each canal was irrigated with 2.5% sodium hypochlorite (NaOCl) solution. After a final irrigation with ethylenediaminetetraacetic acid and NaOCl, they were obturated with gutta-percha cones and AH plus sealer (Dentsply Maillefer) by using a heat plugger connected to System B (Kerr, Orange, CA, USA) and the Duo-beta system (B&L Biotech, Seoul, Korea). Root resection was then performed at 3 mm from the apex, at 90° to the longitudinal axis of the teeth with a tapered diamond bur in a high-speed handpiece under water cooling (Figure 1). Root-end preparation was performed 3 mm into the root canal space along the long axis of the root with a KiS-1D ultrasonic tip (Obtura Spartan, Algonquin, IL, USA) that had a 0.5-mm diameter and 3-



mm cutting surface (Figure 2-A). To standardize for uniformity of the root-end preparations, efforts were made to insert the tips approximately 3 mm into the canals and to ensure that the size of the preparation diameter was 1.0 mm (Figure 2-B). The teeth were then randomly assigned to two groups according to the retrograde filling method (n=10).

2. Retrograde filling methods

In group 1, the root-end cavities were filled with Endocem Zr (Maruchi). After mixing the material according to the manufacturer's instructions, delivery into the root-end cavity was performed using an MTA carrier block (B&L Biotech) and applicator (B&L Biotech), followed by incremental packing using a microplugger (B&L Biotech). The outermost surface was then cleaned with a cotton pellet (Figure 3-A).

In group 2, the root-end cavities were first filled with Endoseal MTA (Maruchi) to a thickness of approximately 1 mm. After a 24-gauge needle tip provided by the manufacturer was connected to the syringe containing the material, the tip was carefully placed into contact with the root-end cavity floor (gutta-percha filling) and the sealer was injected slowly. After confirming with a microscope that the floor of the root-end cavity was coated with sealer (Figure 3-B), the rest of the retro-prepared cavity was filled with Endocem Zr using the same method described for group 1 (Figure 3-C).



All apicoectomy procedures were performed under a dental microscope (OPMI PICO; Carl Zeiss, Gottingen, Germany) at ×10 magnification. After retrograde fillings were performed, periapical radiographs were taken of all samples to ensure the quality of the filling (Figure 4). All samples were allowed to set and were stored in a humidified chamber at 100% relative humidity and 37°C for 7 days until they were investigated using micro-CT. All specimens were prepared by a single operator (JH Jung) and a skilled dental hygienist who helped to mix the material during the procedure because of its fast setting time.

3. Micro-CT evaluation

To evaluate the amount of voids in the root-end area, specimens were scanned using a high-resolution micro-CT scanner (SkyScan1173; Bruker, Kontich, Belgium). The micro-CT scanner had an X-ray source voltage of 130 kV, a source current of 60 μ A, a pixel size of 12.04 μ m, a 1-mm-thick Al filter, an exposure time of 500 milliseconds, and a rotation step of 0.3°. After the micro-CT images were taken, a software program (Nrecon v1.7.0.4., Bruker) was used to reconstruct three-dimensional (3D) images of the samples (Figure 5).

After micro-CT scanning, the range of measurement was set to be 2 mm apical from the interface between the gutta-percha and root-end filling materials in order to measure the volume of the gap between the tooth surface and the root-end filling materials and the voids in the filling materials, following a similar method to that described in a previous



study (17). By analyzing successive micro-CT images of each tooth, specific gray-scale ranges were assigned to the volume of the filling materials (V_m), the gap between the filling materials and the root canal wall (V_{out}), and the voids inside the filling materials (V_{in}). The percentage of defects (V%) was calculated as follows:

$$\% V_{out} = V_{out}/(V_{out}+V_{in}+V_m) \times 100$$
$$\% V_{in} = V_{in}/(V_{out}+V_{in}+V_m) \times 100$$

4. Scanning electron microscopy evaluation

After micro-CT scanning, three teeth from each group were selected for SEM analysis. Roots were sectioned longitudinally in the buccolingual direction using a diamond saw. Dehydration of samples was performed in a graded series of aqueous ethanol (30%, 50%, 70%, 90%, and 100%). Sections were vacuum-dried, sputter-coated with gold, and then examined using SEM (Sigma 500, ZEISS Microscopy, Germany). The adaptation of each root-end filling material to the dentin and the interface of each root-end filling material were examined at ×20 and ×500 magnification, and finally, microphotographs were taken.



5. Statistical analysis

The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to verify whether the data were normally distributed. The data were not normally distributed, ; thus, the Mann-Whitney U test was used to determine assess the statistical significance of differences between the two groups using SAS version 9.3 (SAS institute, Cary, NC, USA) at with a significant significance level of 95%.



III. Results

1. Micro-CT evaluation

The volume percentages of the gaps between the root-end filling materials and the tooth structure and the internal voids are shown in Table 1. No significant differences were found between the two retrograde filling methods for the volume percentages of the gaps between the root-end filling materials and the tooth structure ($%V_{out}$) or the internal voids ($%V_{in}$) (Table 1). The volume percentage of total defects ($%V_{total}$) in both groups also demonstrated no statistically significant difference (Table 1).

In the micro-CT images, it was straightforward to distinguish the calcium silicatebased sealer from the calcium silicate cement, and micro-CT revealed that these two materials were well packed, without remarkable gaps or voids in the root-end area (Figure 6).

In the 3D images from micro-CT scans, it was likewise possible to differentiate the calcium silicate–based sealer from the calcium silicate cement, and the gaps between the tooth surface and the root-end filling materials and the voids in the filling materials could be visualized as specialized colored dots. These images showed that there were very few internal voids in the calcium silicate–based sealer; instead, most of the internal voids were found in the calcium silicate cement (Figure 5).



2. Scanning electron microscopy evaluation

There was no remarkable gap between the calcium silicate–based sealer and the calcium silicate cement. Furthermore, the calcium silicate–based sealer showed acceptable sealing at its interface with dentin. There were some visible internal voids in the calcium silicate cement in both groups (Figure 7).



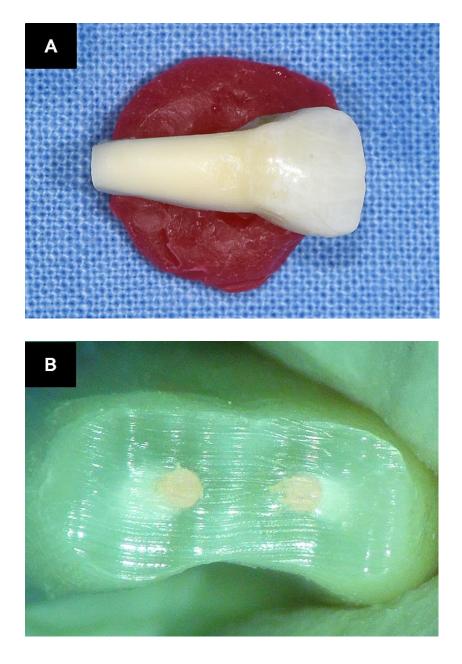


Figure 1. Representative images of resected roots

(A) Buccal view of a resected root. (B) Cross-sectional view of a resected root.



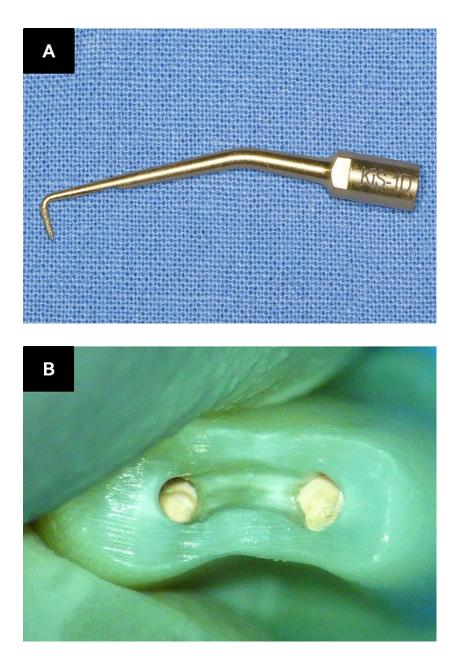
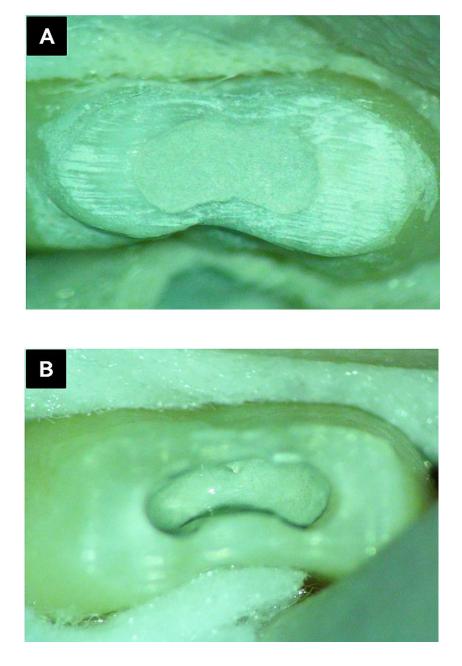


Figure 2. Images of the KiS-1D ultrasonic tip and root after retro-preparation

(A) KiS-1D ultrasonic tip for retro-preparation. (B) Cross-sectional view of a root after retro-preparation.







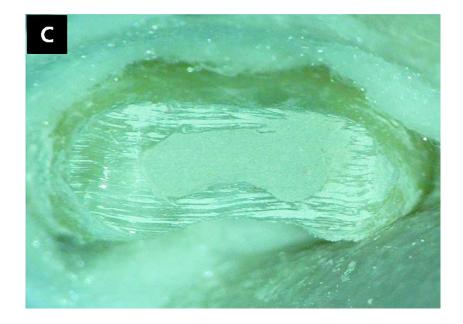


Figure 3. Representative images of retrograde filling

(A) Group 1 (cement), image after retrograde filling with Endocem Zr.

(B) Group 2 (sealer + cement), image after application of an approximately 1-mm-thick layer of Endoseal MTA.

(C) Group 2 (sealer + cement), image after retrograde filling with Endoseal MTA and Endocem Zr.



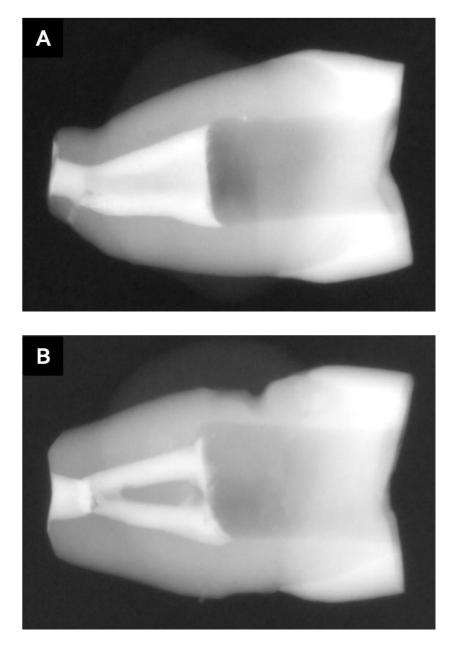


Figure 4. Representative periapical X-ray images

(A) Group 1 (cement). (B) Group 2 (sealer + cement).



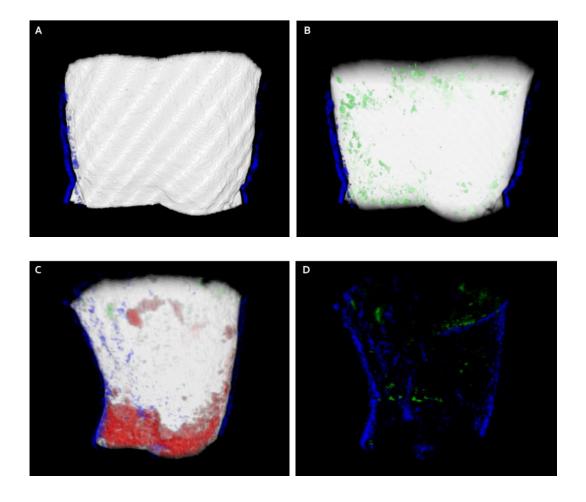


Figure 5. Representative 3D images from micro-CT scans

The white portion is the calcium silicate cement, the red portion is the calcium silicate– based sealer, the blue dots represent the gap between the root-end filling materials and the tooth structure, and the green dots represent the internal voids in the root-end filling materials.

(A) Group 1 (cement). (B) Group 1 (cement), image with the transparency of the filling material set to 50%.

(C) Group 2 (sealer + cement). (D) Group 2 (sealer + cement), image with the transparency of the filling materials set to 100%.



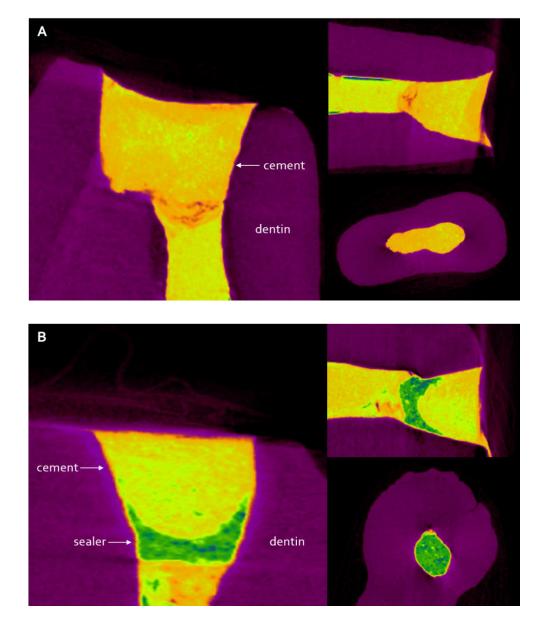


Figure 6. Representative micro-CT images of a specimen from each group

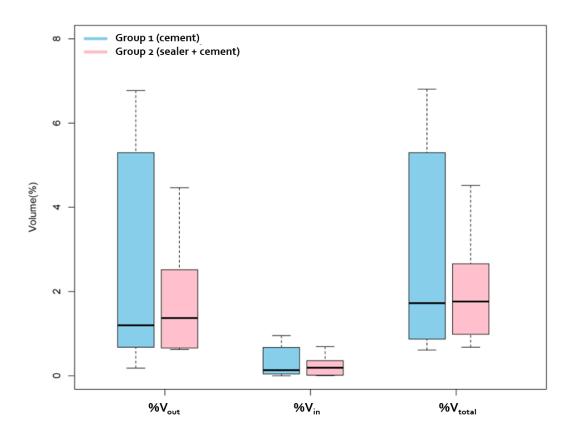
The yellow portion in the root-end cavity is the calcium silicate cement, and the green portion in the root-end cavity is the calcium silicate-based sealer.

(A) Group 1 (cement). (B) Group 2 (sealer + cement).

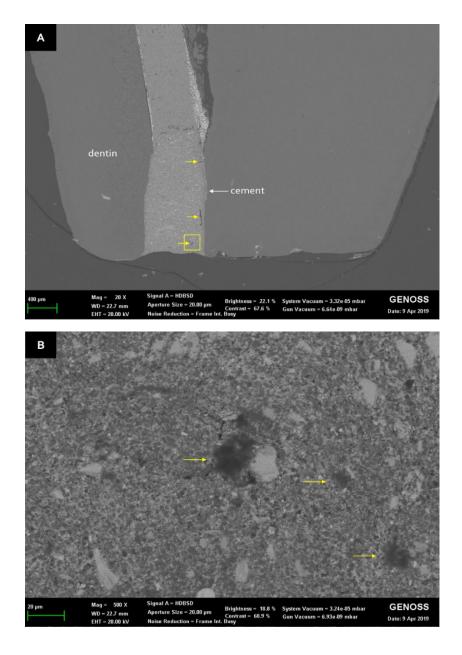


Table 1. Volume percentage of gaps between the filling materials and the tooth structure (% V_{out}), internal voids (% V_{in}), and total defects (% V_{total}) (n=10 for each group)

Variables	Group 1 (cement)	Group 2 (sealer + cement)	p-value
	median(Q1-Q3),(min-max)	median(Q1-Q3),(min-max)	
Gap (%V _{out})	1.20(0.68-5.30),(0.19-6.77)	1.37(0.66-2.52),(0.63-4.47)	0.9097
Internal voids (%V _{in})	0.13(0.04-0.67),(0.00-0.96)	0.19(0.02-0.36),(0.01-0.70)	0.7337
Total defects (%V _{total})	1.72(0.87-5.30),(0.61-6.80)	1.76(0.99-2.66),(0.68-4.52)	0.7913









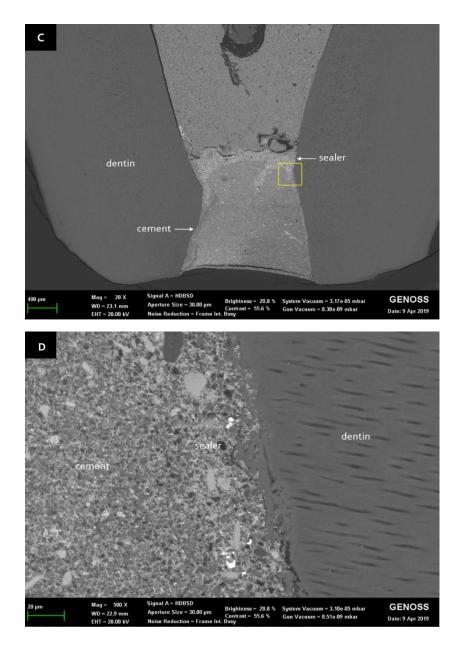


Figure 7. Representative SEM images

(A) Group 1 (cement) (×20). Calcium silicate cement was well packed into the root-end cavity, but some internal voids were visible (arrows).



(B) Group 1 (cement) (\times 500). A high-magnification image of the square area in (A) showing some internal voids (arrows).

(C) Group 2 (sealer + cement) (×20). Calcium silicate–based sealer and calcium silicate cement were well packed into the root-end cavity and easily distinguished.

(D) Group 2 (sealer + cement) (×500). A high-magnification image of the square area in (C) showing acceptable sealing at the interface between the calcium silicate–based sealer and dentin.



IV. Discussion

Many studies have evaluated the various physicochemical properties of recently introduced calcium silicate cements in the root-end filling (20, 23-25). These materials have been proven to have similar sealing ability, marginal adaptation (20, 23, 24), and cytotoxicity (25) to MTA, indicating that calcium silicate cements could be considered as alternative materials for root-end surgery procedures. In fact, endodontic microsurgery using calcium silicate cements as root-end filling materials has shown excellent clinical outcomes, comparable to those of apical surgery using MTA (10-12).

Furthermore, various *in vitro* studies of the use of calcium silicate–based sealer as a filling material have been published, but those studies have been limited to conventional root canal treatment (16, 17, 26). In those studies, using gutta-percha cones and calcium silicate–based sealer in orthograde root canal fillings showed good filling quality, sealing ability, and clinical outcomes. To best of our knowledge, the present study was the first to test the possibility of using a calcium silicate–based sealer as a retrograde filling material.

This study evaluated the quality of retrograde fillings made using two different methods through micro-CT and SEM. High-resolution micro-CT is a nondestructive, highly accurate method that is increasingly used for the assessment of 3D microstructures. In addition to measuring volume, micro-CT facilitates qualitative analyses of images and differentiates between filling materials, voids, and tooth structures, based on the gray-scale



values of the images (16, 17, 27, 28). As shown in Figures 5 and 6, the two different materials used in this study and two types of defects could be straightforwardly distinguished and detected. Using micro-CT, the volume of the gap and/or voids in the root-end area was measured. Non-obturated areas, such as a gap between the tooth structure and the root-end filling materials and/or voids inside the filling materials, may allow bacteria to penetrate from the root canal to the peri-radicular area, which can result in treatment failure. Therefore, these are useful measures for evaluating root canal filling quality (16, 28-30) or retrograde filling quality (20).

Unlike a previous study (20) that measured only the volume of gaps between the tooth structure and the root-end filling materials, this study additionally measured voids within the filling materials. It was expected that gap volume could directly affect the retrograde filling quality, whereas the volume of internal voids may have less effect on the quality. However, their actual relationship with apical leakage or treatment outcomes has yet to be determined. Furthermore, the percentage of gaps and internal voids was calculated in the range of 2 mm apical from the interface between the gutta-percha and root-end filling materials. The reason for this is that calcium silicate–based sealer was present in the upper part of root-end cavity, making only the upper 2-mm layer necessary for an adequate comparison with conventional methods using the micro-CT images.

As a result of the present study, group 2 showed generally lower values for the volume of the gaps and internal voids than group 1, but there was no statistically significant difference between the two groups. Furthermore, the values for the volume of the gaps and



internal voids in group 2 showed more narrow range than in group 1, which may be interpreted as implying that the retrograde filling method with a combination of calcium silicate cement and calcium silicate–based sealer was less technique-sensitive.

We used SEM to observe the interface between each root-end filling material and root dentin. The limitations of using SEM in this study were its two-dimensional nature and the potential detachment of the filling material from the root dentin when using the high vacuum setting. In addition, expansion or contraction or destruction of the tooth and/or filling material may have occurred. Despite its limitations, it should be noted that SEM examination is an effective method for assessment of marginal adaptation due to its good resolution and high magnification (21, 22).

The SEM observations of specimens in this study demonstrated that the combination of calcium silicate–based sealer and calcium silicate cement was acceptable, without remarkable gaps and/or voids at the interface. This may be attributed to the presence of similar components in two materials. Endocem Zr is derived from pozzolan cement and is composed of calcium oxide, silicon dioxide, aluminum oxide, zirconium oxide, and other metallic oxides (31). Endoseal MTA was developed by the same company and is also based on pozzolan cement, according to the manufacturer. Furthermore, Endoseal MTA showed excellent sealing morphology at the interface with tooth structure in high-magnification SEM images. Yoo et al. reported that when Endoseal MTA was used in root canal obturation, mineralized apatite structures throughout the dentinal tubules were detected, and its very fine texture (with a mean particle size of $1.5 \,\mu$ m) contributed to this phenomenon (32). This



biomineralization ability may have contributed to the superior sealing morphology at the interface between Endoseal MTA and tooth structures in this study.

There may be many advantages of using a calcium silicate-based sealer for root-end fillings. First of all, in previous techniques for apical surgery using MTA or calcium silicate cement, the procedures for delivering the filling material from the MTA carrier block to the root-end cavity by applicator and condensing the material in the root-end cavity are time-consuming, and dropping material in the operative field is a real concern. In contrast, the paste-like consistency of sealer means that it has better flowability than the previously used root-end filling material. Moreover, it can be delivered in an injectable premixed form via a thin needle tip. These factors can contribute to easy handling, decreased procedure time, and accessibility of narrow regions. In particular, when a tooth has an area of anatomic complexity, such as an isthmus, the root-end preparation should include the isthmus area, and the consequent reduction in dentin thickness could result in treatment failure due to vertical root fracture (14). Instead, if calcium silicate-based sealer is applied through a thin needle tip in such cases, only minimal preparation of the isthmus would be needed, increasing the likelihood of a successful outcome.

This study had some limitations. First, the teeth used in this study were human maxillary premolars with two canals, but it was difficult to collect and use teeth with a more obvious isthmus. Although efforts were made to standardize the retro-preparation and retro-filling procedures, anatomic variations were present in each tooth, which could affect the study results. In addition, this was *in vitro* study and all procedures were performed



under an environment similar to that of intentional replantation. If the same procedure had been performed under conditions mimicking apicoectomy, with the teeth fixed in dentiform and using a dummy, the results might have been somewhat different. We did not use an apicoectomy model, since doing so would have resulted in the inclusion of too many variables that could affect retrograde filling quality, such as root length, inclination, visibility, and the osteotomy and root resection technique.

When using this new method of retrograde filling, it would be expected that in actual apical surgery, the needle connected to the syringe of the sealer should be bent properly for an easy approach. If this is impossible, a specially designed needle or tip may be needed. Furthermore, in clinical procedures, the difficulty could vary according to the tooth type, tooth position, number of roots, and root form; therefore, consistent guidelines for this procedure would be needed. For example, it would be necessary to arrive at a consensus regarding the proper amount of calcium silicate–based sealer to apply in the root-end cavity. In the present study, we applied an approximately 1-mm-thick layer of Endoseal MTA, and there was no extrusion of sealer outside the root-end cavity when applying and condensing the calcium silicate cement. Most sealer materials were detected within 2 mm from the floor of the root-end cavity according to the micro-CT and SEM analysis. If a greater amount of sealer is used, extrusion of sealer out of the root-end cavity when the calcium silicate cement condenses may occur, leading to exposure of sealer at the outermost surface of the root-end filling. Further studies will be needed, as these factors may affect treatment outcomes.



V. Conclusion

Within the limitations of this study, from the analysis of micro-CT and SEM images, we can conclude that the retrograde filling quality with a combination of calcium silicate cement and calcium silicate–based sealer had no statistically significant difference from that with calcium silicate cement only.



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국문요약

칼슘 실리케이트 시멘트와 칼슘 실리케이트 계통 실러를

함께 사용 시 치근단 역충전의 질 평가

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정재 헌

치근단 수술 시, 치근단 역충전은 근관 내 세균이 치근단 조직으로 누출 되지 않도록 긴밀한 밀폐를 형성한다는 점에서 매우 중요한 과정이다. 하지만 실제 수술 시 제한된 시야와 접근의 어려움, 재료 및 기구 조작의 어려움으로 인해 치근단 역충전은 난이도가 높고, 이로 인해 결함이 발생할 수 있다. 만 약 흐름성이 좋고, 적용이 간편한 칼슘 실리케이트 계통 실러를 치근단 역충 전 재료로 사용한다면 보다 쉬운 적용 및 양질의 치근단 역충전을 기대할 수 있다. 이 연구의 목적은 칼슘 실리케이트 계통 실러와 칼슘 실리케이트 시멘 트를 순차적으로 치근단 와동에 역충전 재료로 적용했을 때 void의 비율을

34



micro-CT로 측정하여 충전의 질을 평가하는 것이며, 주사전자현미경(SEM)을 사용하여 치아와 재료 간, 서로 다른 재료 간 계면을 관찰하였다.

20개의 사람의 상악 소구치를 근관충전 후 치근단 3mm를 절제한 뒤 초음 파 기구를 사용하여 3mm 깊이로 치근단 와동 형성을 시행하였다. 무작위로 두 그룹으로 나누어 첫 번째 그룹은 칼슘 실리케이트 시멘트로 충전하고, 두 번 째 그룹은 칼슘 실리케이트 계통 실러를 근단부 와동에 1mm 적용하고, 남은 부위를 칼슘 실리케이트 시멘트로 충전하였다. 이후 micro-CT로 스캔 후 충전 재와 근관 벽 사이, 충전재 내부 void의 비율로 나누어 통계적 처리를 시행하 였다. 이후 시편은 SEM으로 관찰하였다.

충전재와 근관 벽 사이 void의 비율 (%V_{out}), 충전재 내 void의 비율 (%V_{in}) 모두 두 번째 그룹에서 상대적으로 적게 나타나지만 두 가지 충전 방 법 간 통계적 유의차는 없었다. SEM 이미지에서는 칼슘 실리케이트 계열 실러 가 근관 벽 및 칼슘 실리케이트 시멘트와 잘 적합 되는 것이 관찰되지만 칼슘 실리케이트 시멘트 재료 내의 void가 관찰되었다.

핵심이 되는 단어: Micro-CT, 칼슘 실리케이트 계통 실러, 칼슘 실리케이트 시멘트, 치근단 와동, 치근단 역충전