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Use of Micro-Computed Tomography and Computer-Aided Design in Comparative Volumetric Analyses of Endodontic Armamentarium

미세전산화단층촬영 및 의료용 CAD 를 이용한

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Abstract

Use of Micro-Computed Tomography and Computer-Aided Design in Comparative Volumetric Analyses of Endodontic Armamentarium

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1. Objectives

The purpose of root canal treatment (RCT) is to chemo-mechanically disinfect and shape the canal space, and thereafter create hermetic seal of the canal system using obturant and sealer.

Part one of the present study is a comparative analysis of the root canal morphological changes induced by the two different file systems, ProTaper Next (PN) (Dentsply Maillefer, Ballaigues, Switzerland), the conventional rotary full sequence system (RFSS), and the newly introduced WaveOne Gold (WG) (Dentsply Maillefer), which is a reciprocating file (RF) system. This is represented by the volumetric changes before and after the shaping of canals with varying degrees of curvature severity.

Part two of the present study is a comparative analysis of the volumetric changes of the three different sealers spanning 8 weeks post-obturation *ex vivo*. The sealers studied include the conventional epoxy resin-based AH Plus (AP) (Dentsply Maillefer), a calcium silicate-based EndoSeal MTA (ES) (Maruchi, Wonju, Republic of Korea), and a cold flowable gutta-percha (GP) named Guttaflow 2 (G2) (Coltène Whaledent, GmBH+Co KG, Langenau, Switzerland).

2. Material and Methods

The sample teeth were selected following the inclusion criteria: no root caries, un-treated root canals, and complete apexification. To compare the effect of the two different file systems, the mesiobuccal and distobuccal canals were randomly categorized into two groups (n = 32/group) – the rotary PN group and the reciprocating WG group. To compare the effect of the three different sealers, the palatal canals were randomly classified into three groups (n = 12/group) and were named the AP group, ES group, and G2 group respectively for AH Plus, EndoSeal MTA and Guttaflow 2.

In part one, the canal preparation was executed to the pre-determined working length up to the apical size of 25 (PN: X2 25/06; WG: Primary 25/07) following the respective manufacturer's protocols. In part two, the canals were prepared up to the apical size of ISO 40 with Protaper NEXT X4 for standardization.

In part two, each AP, ES, G2 sealer was prepared whilst adhering to the corresponding manufacturer's instructions, and single-cone technique with ISO 40 GP cone (Meta Biomed, Chungju, South Korea) was used. After the canal filling, the coronal cavity was sealed with Fuji II LC (GC, Tokyo, Japan). All root surfaces, except for the 2 mm radius encircling the apical foramen, were covered with varnish before being stored in a separate sealed container with 5 mL of phosphate-buffered saline solution at 37 °C.

The micro-computed tomography (microCT) images (SkyScan1172, SkyScan, Aartselaar, Belgium) were taken at 24 hours, 1 week, 2 weeks, 4 weeks and 8 weeks post-obturation. The scanned images were reconstructed using NRecon version 1.6.2.0 (SkyScan, Belgium). The reconstructed images were visualized into three-dimensional (3D) models for volumetric analyses using two medical computer-aided design (CAD) software programs (Mimics, Materialise N.V., Leuven, Belgium; 3-matic, Materialise N.V.).

All used PN and WG files, as well as the obturated specimen were prepared for viewing under field emission scanning electron microscopy and energy dispersive x-ray spectroscopy.

The statistical analyses was performed using SPSS version 24 (SPSS, Chicago, USA) with the statistical significance set at $P < 0.05$, whilst using

Levene's test, one-way analysis of variance, Tukey's HSD test, Dunnett's T3 test and independent samples t-test for different comparison types.

3. Results

In part one, WG group resulted in statistically significant canal deviation at coronal-third of straighter canals compared to that of curved canals, which may clinically translate to the risk of cervical canal transportation during relatively routine root canal treatments of straight canals. Its shaping ability, however, was not significantly different from that of ProTaper Next at a given canal-third at a given curvature severity.

In part two, ES group showed a statistically significant increase in the total volume at 8-weeks, compared to that of the AH group. There was an increase in volume of the G2 group, but without statistical significance compared to the ES group. The volumetric increase in the ES group, further supported by the FE-SEM observations, clinically translates to the potential for ES to provide continued filling of the canal wall interface as an endodontic sealer, via continued hydroxyapatite (HA) crystal growth in the presence of bodily fluid.

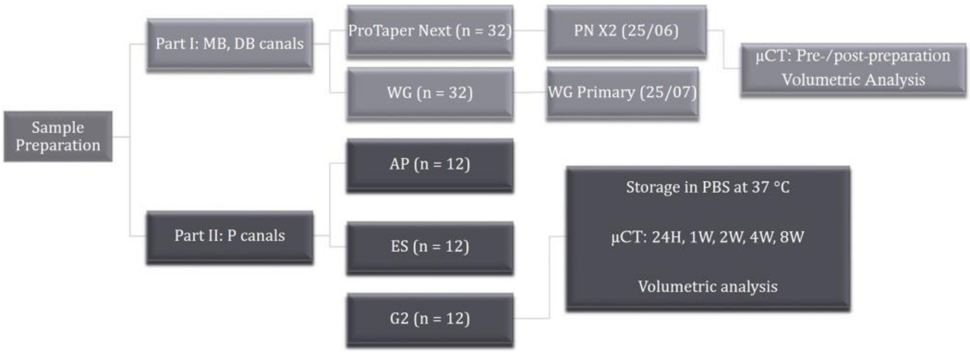

Figure 1. Flowchart of Methodology.

Keywords: Root canal treatment, endodontic file, endodontic sealer, micro-computed tomography, CAD, volumetric change

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I. Shaping Ability of ProTaper Next and WaveOne Gold NiTi File Systems: A Volumetric Evaluation

Introduction

One of the main challenges of root canal treatment (RCT) is safe preparation of canals with higher degree of curvature. Curved canals are associated with the incorrect adaptation of endodontic files, leading to canal aberration via perforation, ledge or zip formation (1). It is reported that ledge formation was twice as frequent in J- or C-curved canals (40% and 42.1% respectively) as the straight canals (19.3%) (2). This phenomenon can be attributed to the metallic memory of the nickel-titanium (NiTi) files, where the they exert straightening force in curved anatomies (3).

Harnessed with its mechanical advantages, including flexibility, reduced debris extrusion, and canal centering ability (4-6), different NiTi file designs have become available in order to address such deviation from the original canal anatomy. These designs involve various canal shaping movements. The conventional type of such is the rotary full sequence system (RFSS)(7) as seen in ProTaper Next (PN) (Dentsply Maillefer, Ballaigues, Switzerland). This file system, introduced into the market in 2007, involves a series of file sequence per procedure (SX, X1, X2 for final finishing size of ISO 25), and features an off-center rotation with rectangular cross-section.

The reciprocating file (RF) system, compared to the conventional counterparts, allows streamlined procedural steps and safer instrumentation process (8). One recently introduced system of such is WaveOne Gold (WG)

(Dentsply Maillefer). This system promotes single-file preparation of the curved root anatomies (9). Such simplification comes from the unequal clockwise/counterclockwise angles with variable taper of the parallelogram cross-sectional shape where all of the canal surface is theoretically touched after 3 cycles of partial reciprocation (10), thereby shaping the canal to full length using a single file. Its metallurgical property arises from the proprietary *Gold-wire* supermetal technology, which imbues golden color and promises resistance to cyclic fatigue via enhanced shape-memory and reduced file stiffness (8, 10).

Currently, no three-dimensional (3D) volumetric data is available regarding the canal shaping performance of WG. Furthermore, existing studies featuring micro-computed tomography (microCT) on the performance of RFs rely on the method of extrapolation of cross-sectional distance (11-18); thus lacking on true volumetric comparison. The present study employs medical computer-aided design (CAD) software programs for 3D reconstruction of μCT images to investigate the accuracy of PN and WG in their adaptation to curved canal anatomies during RCT.

Material and Methods

1. Sample Preparation

The mesiobuccal and distobuccal canals of extracted human maxillary molars were used with the following inclusion criteria: no caries beyond cemento-enamel junction; un-treated root canals; complete apexification; no visible sign of fracture. The lengths of the roots were unified to 12 ± 1 mm from the anatomical apex by cutting at the region of cemento-enamel junction with a diamond mill.

Each canal was randomly assigned with either continuous rotational (PN) or reciprocating (WG) file system, and the resultant two groups (n = 32/group) were named PN and WG respectively. The working length was established by pulling an ISO 10 K-file 0.5 mm short from the major apical foramen. After the glide-path formation with ISO 10 K-file, canal shaping was performed with X-Smart Plus motor to the pre-determined working length up to the apical size of 25 (PN: X2 25/06; WG: Primary 25/07) following the respective manufacturer's protocols with slow pecking motions; and irrigated throughout with 3.25% sodium hypochlorite (NaOCl) using 27-gauge side-vent needle. The entire preparation was performed in a single-session by an endodontist. All samples were stored at 37°C in distilled water.

One file from the WG group was excluded due to file separation during the canal preparation. A new specimen was added to the group to equalize the number. The separation file was included for the topographic analyses using field emission scanning electron microscopy (FE-SEM) and energy dispersive x-ray spectroscopy (EDS).

2. Volumetric Analyses

The high-resolution microCT images (SkyScan1172, SkyScan, Aartselaar, Belgium) were taken before (pre-RCT) and after the root canal preparation (post-RCT) at 100 kV source voltage and 100 μA source current with a 0.5 mm aluminum filter under an isotropic resolution of 10 μm. The images of each root were acquired via 180° rotation of the specimen around its mid-vertical axis using 0.4 single rotation step at 2096 x 4000 pixels.

The microCT images were reconstructed using NRecon version 1.6.2.0 (SkyScan, Belgium), and the data for each root extracted as approximately 1000 JPG images of axial slices.

The reconstructed images were converted into STL-files compatible with the two medical CAD software programs (Mimics, Materialise N.V., Leuven, Belgium; 3-matic, Materialise N.V.) used in the present study to allow for the volumetric analyses.

The total volumetric change between the pre-RCT and the post-RCT groups were determined via superimposition with *N-Point Registration* and *Global Registration,* and subsequent *Boolean subtraction* and *interaction* operations in the 3-matic software program.

The extent of canal transportation from the central axis of the canal at the coronal-, middle- and apical-thirds were then quantified by creating a *datum plane* at each canal-third, perpendicular to the 3-dimensional *centerline* of the canal.

The root curvature was measured at the point of the greatest convexity along the centerline of the sample's pre-RCT model via Schneider's technique (19). The degree of canal curvature were classified into four categories: straight (≤10°), mild (10-20°), moderate (20-35°) and severe (35-70°), modified from Schneider's classification of ≤5°, 10-20°, 25-70° respectively for straight, moderate and severe (19).

3. Topographic Analyses

All used NiTi files were prepared for examinatioin under FE-SEM (S-4700, Hitachi, Ltd., Tokyo, Japan). The debris on files were removed for accurate imaging via 5-minute cycles of ultrasonic bath in the order of distilled water, alcohol, and distilled water. A new file from each system was also observed as control. The files did not undergo any coating process to enable the EDS analysis. A new and a used file from each system were manually separated to analyze their cross-sectional surface composition via EDS (EMAX 7200-H, Horiba, Ltd., Kyoto, Japan) equipped on the FE-SEM.

4. Statistical Analyses

In order to account for the difference in the file taper of PN and WG, and to facilitate the comparison between the two groups, percentage change was used in the analyses of the data collected in the CAD section of the present study. The following basic mathematical formula was established for the purpose of this study:

$$Volumetric\ Deviation\ Ratio = \ c/(b + c)$$

where a is the portion of original canal space untouched by the file (not depicted in *volumetric deviation ratio*); b is the portion of original canal space touched by the file; and c is the new canal space created by the file (Fig. 3θ).

Upon verifying equality of variances via Levene's test, one-way analysis of variance (ANOVA) was performed for intra-group comparison of each curvature category at a fixed canal-third (*factor*: apical-third; *dependent variables*: means of WG in mild, moderate, and severe curvature categories). One-way ANOVA was also applied for the intra-group comparison of each canal-third in a fixed curvature category (*factor*: severe curvature category; *dependent variables*: means of WG at apical-, middle- and coronal-thirds). For all cases where the equality of variance was present, post hoc comparison was performed via Tukey's HSD test; while Dunnett's T3 test was used when the equality of variance assumption was invalid. Independent samples T-test was used for inter-group comparison of PN and WG in each curvature category at each canal-third (*grouping variable*: file system; *test variable*: means at each canal-third in each curvature category). All statistical analyses were performed using SPSS version 24 (SPSS, Chicago, USA) with the statistical significance level set at $P < 0.05$.

The study's protocol was evaluated by the Seoul National University School of Dentistry Institutional Review Board (IRB #S-D20160011).

Results

1. Volumetric Analyses

Differences in the volumetric deviation ratio between PN and WG groups are tabulated in Table 1. There was no statistical difference between the two groups at a given canal-third at a given curvature severity. When compared within the group, the volumetric deviation ratio of WG group at coronal-third showed significant difference between mild and severe curvature categories ($P = 0.042$). However, all other canal-thirds within WG group and all canal-thirds in PN group showed no significant difference between each curvature categories.

2. Topographic Analyses

Surface analyses using FE-SEM revealed higher incidence of file blunting in the PN group (81%) than WG group (61%), while an incidence each of file separation (6%) and elongation (6%) was observed in WG group. Spectroscopy using EDS revealed traces of calcium, phosphorus and oxide on both separated surface (Fig. 2*D*) of a WG file and on the debris embedded in the lateral edge of the same file (Fig. 2*D eyelet*). In contrast, only nickel and titanium were noted on both surfaces of unused PN and WG files.

Discussion

The majority of studies regarding the shaping abilities of endodontic files have focused on the distance measurement at a given point of the root canal (11-18); and studies have revealed better (20, 21) or similar (15, 22) centering ability of RFs compared to that of RFSSs. To achieve successful clinical translation of *in vitro* or *ex vivo* research findings, volumetric analysis becomes imperative when one considers the limitations of distance measurement at static points. The static distance measurement does not account for the dynamic interaction of files with the canal walls; which occurs in all directions, with the center of rotation constantly changing beyond the single axis for both PN and WG (10, 23). This dynamicity could be omitted when selective measurement occurs at the mesial and distal edges of the canal (11, 13, 15, 17, 18); a method which was first introduced in 1988 by Calhoun and Montgomery for use with a stereomicroscope (24). The present study addressed the limitations arising from two-dimensional extrapolation through the use of the specialized medical CAD software programs to analyze the 3D changes. Part of this analysis is the novel method of curvature calculation using the true centerline of the canal (Fig. 3*θ)*.

The primary aim of root canal treatment is the cleaning of the canals by chemical and mechanical means (7). Pertinent to this aim is the movement and the adaptation of the files to the canal curvature. The extent of canal transportation was measured via post-instrumentation dentinal volume loss – for which there was no significant difference between PN and WG groups. This finding concurs with that of the pre-existing two-dimensional investigations involving RFSSs and RFs (15, 22). Of note, more coronal-third deviation occurred in less curved canals for both PN and WG groups; and the difference between mild and severe curvatures were statistically significant for WG group (Table 1). One interpretation for this is the potentially larger coronal core-diameter of WG. While WG exhibits smaller maximum-fluted diameters than its predecessor due to its regressive taper (25), its 7% apical taper (10) is still greater than PN's variable taper starting with the apical 6% (26). This finding also suggests that NiTi files, regardless of the movement kinematics, perform with better centering ability in severely curved canals than in straighter canals – but further evaluation is required for this generalization. An additional speculation is that the straighter canals were inadvertently exerted with more mechanical force in the coronal-third during the experiment, as opposed to passive file penetration and withdrawal, due to the routineness of straight canals.

Both systems have variable flute forms and WG exhibited one less flute number than PN per unit length (Fig. 1*A,* 2*A*). It is reported that WG's smoother tip transition (Fig. 2*A*) influences the cutting efficiency (25), and the lower number of spiraling flutes causes less canal aberration due to reduced stiffness of the tip (27). Within the limitations of this study, said properties did not induce a significant difference in the overall performances of the two groups.

The author recommends high magnification (50–300 μm) SEM for standardized assessment of topographical features such as file blunting and elongation. Both elongation and separation occurred in WG group from the severe curvature category (Fig. 2*B, D*); while none occurred in PN group.

Although the sample size is relatively small to draw conclusions, this occurrence is in contrast to the main consensus that rotary NiTi files have higher separation rate than reciprocal NiTi files (6, 28). The reason may be the single file status of WG group, compared to the RFSS of PN group. The loading stress is distributed across the entire sequence of the PN files, whilst the same load is borne by a single WG file; although the benefit of WG includes the reciprocating kinematics of the disengaging clockwise movement and subsequent reduction of torsional stress (29-31).

It is reported that for other RFs including the predecessor to WG, a file could be reused for up to 5 canals (32). However, greater curvature severity heightens the risk of complications arising from the multiple use of a file – such as in a multi-rooted tooth. This is especially pertinent to WG, due to the repeated cycles of thermal treatment for flexibility. The thermal process makes WG file comparatively malleable; such mechanophysical property can be observed from the incorporation of debris along the file edge (Fig. 2*D eyelet*). The chemical constituents of the debris were Ca-P-O (Fig. 2 *Spectrum 1*), which may represent the displaced apatite from the cut dentin. Both PN and WG are specified as single use, or single patient use (10, 33). As curved canals trigger flexural fatigue in addition to the torsional fatigue, clinicians should exercise caution when treating multi-rooted teeth and adhere to the manufacturer's recommendations.

The author suggests further study on efficiency via time measurements. Given the comparable performances of PN and WG, clinicians may derive their selection for the instrument from total shaping time. Studies have shown the reduction of canal preparation time with RFs compared to

RFSSs (7, 22). Another consideration for studies involving μCT and CAD is the degree of inherent mechanical errors in the computerized systems – pixel to voxel extrapolation in reconstructing the two-dimensional slices to a continuous 3D object for μCT images (34); and the chord error filtering parameters for CAD software programs (35). The combination of the two methods, however, may be the current most reproducible and nondestructive characterization for dynamic volumetric analyses.

Within the limitations of the present methodology, WaveOne Gold showed statistically significant reduction in its shaping accuracy in coronal-third of less curved canals; which may clinically translate to the risk of cervical canal transportation during root canal treatments. There was no significant difference in the total dentinal volumetric loss between the two systems. The blunting of the file edges in SEM suggests that clinicians should employ both ProTaper Next and WaveOne Gold solely for single-use, in order to maintain their maximum shaping abilities.

Figures and Tables

Table 1. Volumetric deviation ratio (%) of PN and WG Groups at coronal-third

	Volumetric Deviation Ratio (%), mean ± SD	
Curvature	PN (n = 32)	WG (n = 32)
Mild (10-20°)	59.76 ± 22.30	74.75 ± 15.63[a]
Moderate (20-35°)	53.01 ± 20.41	60.29 ± 17.56
Severe (35-70°)	39.20 ± 22.44	48.18 ± 24.94[a]

Superscript within the same group denote a significant difference at $P < 0.05$.
SD: standard deviation.

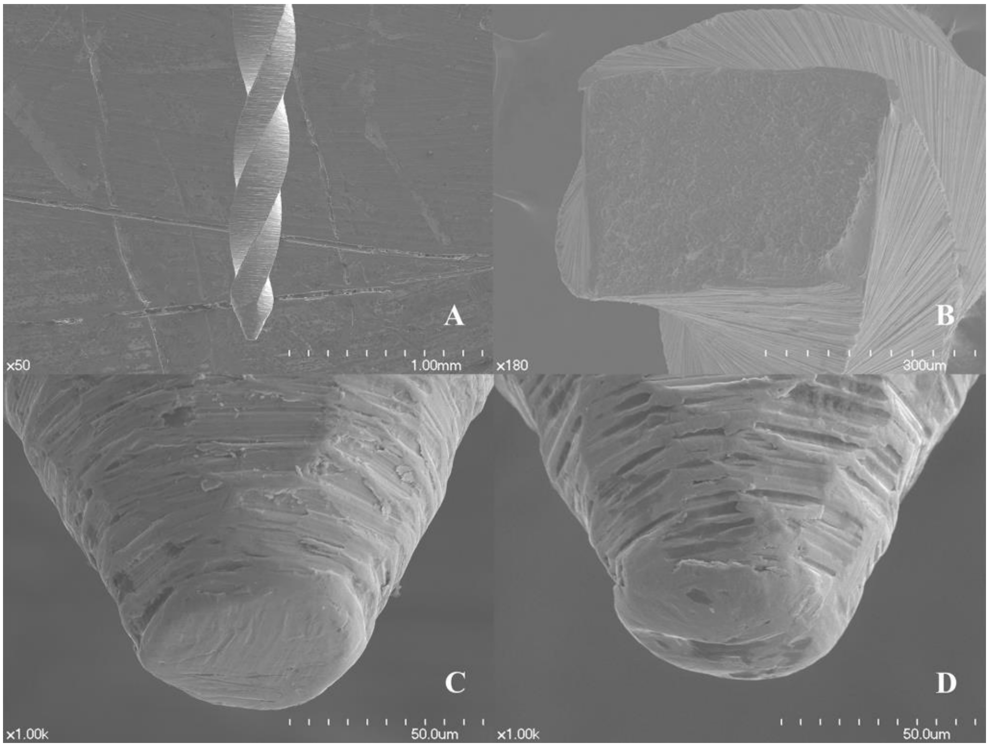

Figure 1. SEM of (*A, B*) control and (*C*) blunted PN.

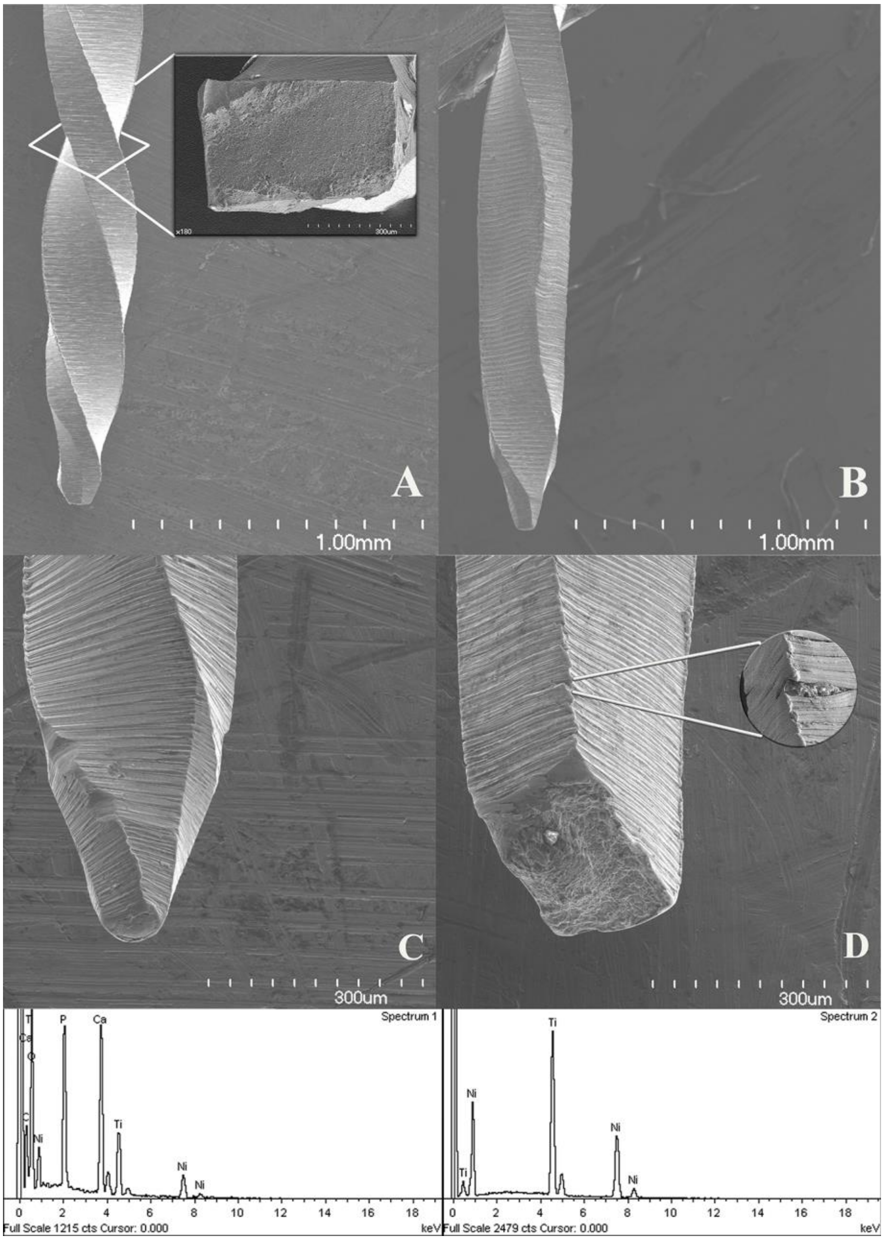

Figure 2. SEM of (*A*) control, (*B*) elongated, (*C*) blunted and (*D*) separated WG; surface chemical spectra of embedded debris in Fig. 2*D eyelet* (*Spectrum 1*) and control WG (*Spectrum 2*) as observed using EDS.

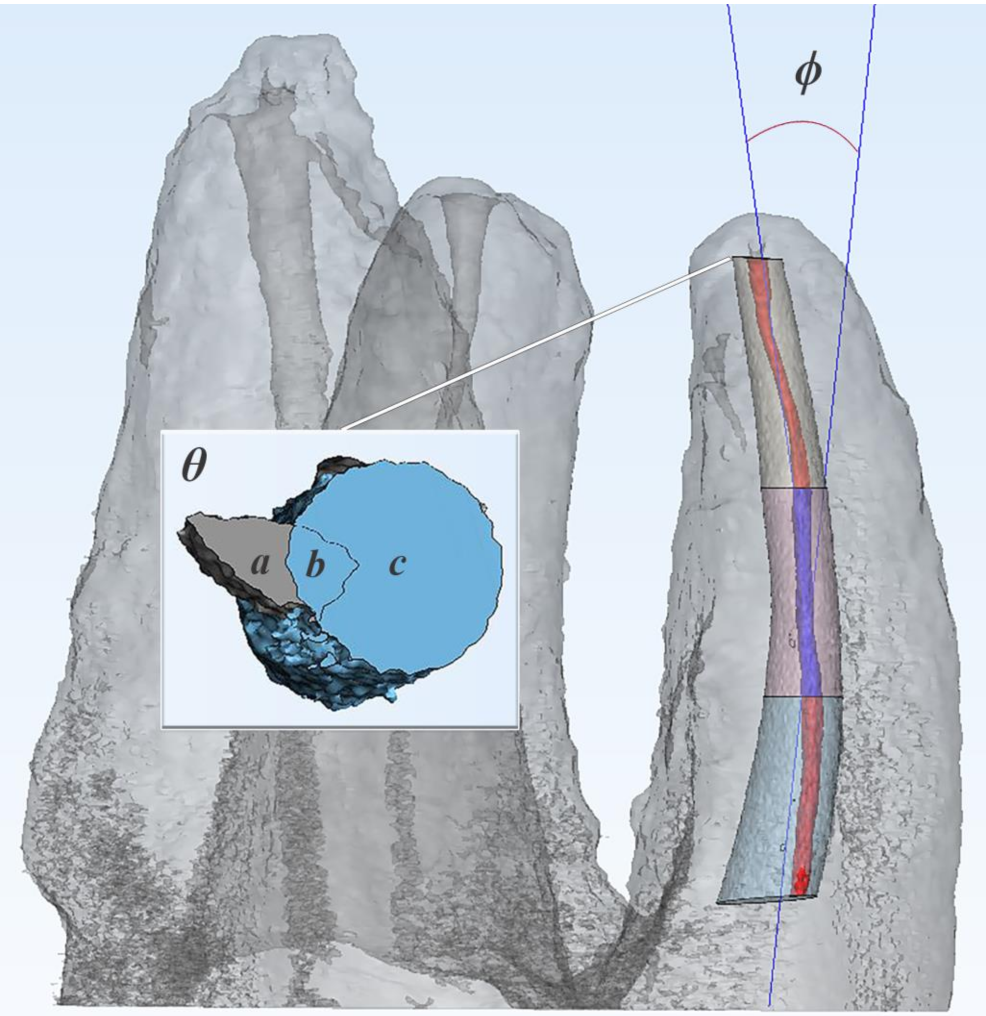

Figure 3. Representation of computer-aided design workflow including Schneider's method of curvature measurement (ϕ) along the canal centerline; with datum plane separating the apical- (*yellow*), middle- (*red*) and coronal- (*cyan*) thirds of superimposed pre- and post-instrumentation images. Predefined a, b, c depicted in the cross-sectional view (θ), where a is the portion of original canal space untouched by the file; b is the portion of original canal space touched by the file; and c is the new canal space created by the file.

II. Volumetric Study of Endodontic Sealers via Micro-Computed Tomography and Computer-Aided Design

Introduction

A successful endodontic treatment requires an amalgamation of multifactorial conditions which are carefully approached and satisfied. One of such is the hermetic sealing of the disinfected root canal space (36-41). The importance of the seal is emphasized by many (42-48), including Hoen et al. who reported the presence of microleakage in 13% of endodontic retreatment cases (36).

A conventional type of endodontic sealer, AH Plus (AP) (Dentsply Maillefer), is reported to exhibit high flowability (49) and high bond strength (50, 51); but it is also reported to show dimensional shrinkage in presence of moisture for a given period of time (52). This property is clinically less desirable in preserving the long-term endodontic seal.

Many other materials have been introduced for application as endodontic sealers. Two materials to be investigated in the present study are Guttaflow 2 (G2) (Coltène Whaledent, GmBH+Co KG, Langenau, Switzerland), a cold flowable gutta-percha (GP), and EndoSeal MTA (ES) (Maruchi, Wonju, Republic of Korea), a calcium silicate-based sealer. G2 is the most updated version from its predecessor Guttaflow and the original RoekoSeal Sealer, which contains GP filler particles (53).

The main constituent of ES is mineral trioxide aggregate (MTA). The substance was introduced in the 1990s as a reparative filling material for retrograde endodontic treatments (54-57), and its application as an orthograde endodontic sealer is a relatively more novel approach. The substance has been lauded for its biocompatibility, sealing ability and its tolerance to moisture contamination during setting reaction (54, 58, 59). In its approach to address the technique-sensitive handling issue of the conventional powdered MTA (60, 61), ES is manufactured as a ready-to-use paste form. In clinical condition, this pre-mixed form is likely to further diminish the issues present in the powdered forms.

The present study aims to investigate the volumetric changes of the three different sealers through 3D reconstruction of the obturated root canal systems using μCT and CAD via sequential scanning over a period of 8 weeks. The null hypothesis to be used in the present study is that there is no change in volume for the three different types of sealer over the 8 weeks.

Material and Methods

1. Sample Preparation

The sample teeth were selected following the inclusion criteria: no root caries, un-treated root canals, and complete apexification. These selection criteria, allowing for the standardization of the sample specimens in their root canal configuration, were devised following a literature review during the protocol design process (51, 62, 63).

A total of 36 palatal roots were selected for the specimen pool. The residual periodontal soft tissues were removed. The teeth were then rinsed and stored in distilled water to avoid dehydration. The crown of each specimen was trimmed with a diamond mill to produce the total root length of 12 ± 1 mm.

Each sample was randomly distributed into one of three groups (n = 12/group) with each group named AP, ES and G2 for obturation protocols involving AH Plus, EndoSeal MTA and Guttaflow 2 respectively.

The working length was determined by pulling the ISO 10 K-file (Mani, Tochigi, Japan) 0.5 mm short from the apical foramen. The canal preparation was executed using X-Smart Plus motor (Dentsply Maillefer). The glide path was formed by ProGlider (Dentsply Maillefer), and WaveOne Gold ISO 25 (Primary) was used for canal shaping. The canals were prepared to the apical size of ISO 40 with Protaper NEXT X4 for the purpose of standardization. The irrigant used throughout the preparation was 2 mL of 3.25% NaOCl. A 10 mL syringe with a 27-gauge side-vent needle irrigant delivery system was used.

The final irrigation was performed with 1 mL of NaOCl and the canals were dried with sterile paper points (DiaDent, Almere, the Netherlands) in corresponding canal sizes after the patency check with ISO 10 K-file. Each sealer was prepared under the following protocols adhering to the manufacturers' instructions.

Group AP

1. A freshly opened product was used. Equal volume units (1:1) of Paste A and Paste B were mixed on a mixing pad using a spatula to achieve a homogeneous consistency.
2. A light coating of AH Plus root canal sealer was applied along the canal wall to the working length with ISO 10 K-file using anticlockwise rotation. A light coating of sealer was then applied onto the surface of the master GP cone (Meta Biomed, Chungju, South Korea) and the cone was inserted into the canal with a single pumping motion to achieve a homogenous spread of the sealer. The resultant apical puff of extruded sealer was wiped with gauze.

Group G2

1. A freshly opened product was used. A light pressure was applied on the plunger to dispense the premixed sealer on a mixing pad.
2. A sufficient coating of Guttaflow 2 root canal sealer was applied along the canal wall to the working length with ISO 10 K-file using anticlockwise rotation. A light coating of sealer was then applied onto the surface of the master GP cone and the GP cone was inserted into the canal with a single pumping motion to achieve a homogenous

spread of the sealer. The resultant apical puff of extruded sealer was wiped with gauze.

Group ES

1. A freshly opened product was used. The needle tip was inserted into the canal to the level of coronal one-third. A light pressure was applied on the syringe to dispense the sealer directly into the canal space for coronal plug formation.
2. The master GP cone was inserted into the canal with a single pumping motion to achieve a homogenous spread of the sealer. An ultrasonic vibration (t = 15 seconds) was applied to the pincette in contact with the cone for sealer compaction and apical plug formation. The resultant apical puff of extruded sealer was wiped with gauze.

The canals were filled with the sealer via the single-cone technique using tapered ISO size 40 sterile GP cone, which were inserted into the canals up to the pre-determined working length and cut with a heated hand instrument at the canal orifice.

The coronal orifices were sealed with Fuji II LC (GC, Tokyo, Japan), and all root surfaces were covered with varnish, except for the 2-mm radius encircling the apical foramen. The samples were stored in a separate sealed container containing 5 mL of phosphate-buffered saline solution as a human salivary substitute and incubated at 37 °C in mimicry of bodily environment.

2. Volumetric Analyses

The μCT images were taken at 24 hours, 1 week, 2 weeks, 4 weeks and 8 weeks after the obturation. The scanning parameters are specified in part one of the present study. The scanned Images were reconstructed using NRecon version 1.6.2.0. The reconstructed images were visualized into 3D models for volumetric analyses using the two medical CAD software programs, Mimics and 3-matic.

The 3D models of the samples taken at each time interval were analyzed for the volumetric change over a set distance (4 mm) from the minor apical constriction. The point of minor apical constriction was measured by selecting the coronal slice with the smallest diameter at apical portion of the canal along its centerline. The model was trimmed perpendicular to the canal centerline to measure 4 mm from the pre-determined minor apical constriction, before obtaining the volume.

3. Topographic Analyses

After 8 weeks of storage, the specimens were cut into hemisections, silver coated, and viewed under FE-SEM to analyze the topographical details of the three different sealer groups especially along the tubular openings.

4. Statistical Analyses

The statistical analyses were all performed using SPSS version 24 and the statistical significance was set at $P < 0.05$. Levene's test was performed first to discern the equality of variances. The datasets from three groups at each time interval were compared via ANOVA. With equal variance, Tukey's HSD test was performed; while Dunnett's T3 test was performed when the equality was not present.

The present study's protocol was evaluated by the Seoul National University School of Dentistry Institutional Review Board (IRB #S-D20160011).

Results

1. Volumetric Analyses

The volumetric changes of the three groups over 8 weeks is represented in Figure 2. Both G2 and ES showed statistically significant increase compared to AP ($P < 0.05$), but nil statistical difference was noted between G2 and ES. However, ES showed 4% more volumetric increase than G2 and 10% more increase than AP at 8 weeks post-obturation.

2. Topographic Analyses

Topographic analyses using FE-SEM showed a planar crystalline growth in ES group at the openings of dentinal tubules (Fig. 3-5). In dentinal tubules without the crystalline-like growth, the openings appeared to be mechanically blocked by the smear layer debris, and those showing the crystalline growth showed unhindered communication with the main canal space (Fig 3-4). Signs of odontoblastic process were present in the tubules of all groups (Fig. 6).

Discussion

The aim of the root canal treatment is to remove the soft tissue of the root canal system by chemical and mechanical cleaning of the canals using irrigants and endodontic files, then effectively sealing the cleaned root canal system using obturants and sealers (64). One of the factors contributing to the endodontic retreatment is the reinfection of the root canal system via microleakage (36-39). For this reason, various new materials have been introduced to enhance the hermetic sealing ability of endodontic sealers.

The conventional sealer of choice, the epoxy resin-based AP (65), has had clinical acceptance used due to its inertness with cell metabolism (66), high flowability (49), and high bond strength (50, 51). It is important to note, however, that the magnitude of sealer bond strength does not necessarily translate to the clinical sealing property of the material (67, 68). It is of the author's opinion that the filling of voids within the canal microsystem, represented by the volumetric increase in the present *ex vivo* study, may be a more robust predictor for the clinical sealing efficiency than the *in vitro* test of the sealer's push-out bond strength.

The dimensional shrinkage of AP, as previously mentioned, has been reported by Zhou et al. when incubated in distilled water over a period of 30 days (52). Most other studies agree with the result of the present study in that dimensional expansion, albeit to varying degrees, is observed with AP (69-71).

MTA is considered a comparatively novel material in its application as an endodontic sealer in non-surgical orthograde root canal treatments. It is a calcium silicate-based sealer whose setting pH has a strong alkaline, thus antibacterial, nature (72-74). In presence of moisture, the main constituent di- and tri-calcium silicates super-saturate the environment with calcium and phosphate ions and promote hydroxyapatite (HA) crystal formation (51, 57, 72, 73, 75, 76). The antibacterial activity and the continuous void filling potential marks MTA as a promising candidate for endodontic applications. Its use as a root-end filling material is well accepted (54, 77, 78). However since its first introduction as an alternative material for conventional orthograde treatments (60), its performance has not been unanimously concluded, with inferior (79), equal or superior (80) results compared to that of GP. The author suggests that such variance is influenced by the technique-sensitivity of the powder type MTA, as it requires mixing into a slurry followed by the subsequent handling difficulties.

Many techniques are used for the application of the powdered MTA, including hand condensation (60, 81), ultrasonic activation (82), and lentulo spiral method (79). Due to the enhanced maneuverability afforded by the thickening agent, ES diminishes the source of potential handling error, and the premixed injectable paste form allows uniform introduction into the root canal system whilst maintaining a constant powder-liquid ratio during the application.

One of the assumptions of the present study is that the radiopacifier of ES uniformly expands with the bulk of the material to linearly reflect its volumetric changes as observed via μCT. In this method, the volumetric expansion of ES is visualized by the μCT, and the resultant image is dependent on the uniform expansion of the entirety of the material including its constituent radiopacifier, zirconium oxide (83, 84).

Another point of consideration for ES is the presence of the thickening agent. Currently, its chemical composition and biochemical interaction is unknown. The handling of the product is greatly enhanced by the addition of the thickening agent to the hydraulic cement, but whether the agent contributes negatively or positively to the overall performance of the sealer is unknown.

Statistical analyses using ANOVA and Tukey HSD post-hoc test showed a statistically significant ($p = 0.021$) increase in the total volume at 8 weeks post-obturation in the ES group, compared to the AH group (Fig. 2). There was an increase in volume of the G2 group, but without statistical significance compared to the ES group. Such volumetric increase noted in the ES group is further supported by the FE-SEM images, which suggest the presence of laminate crystalline growth (Fig. 3-4) and crystallite nucleation (Fig. 5).

Within the limitations of the present study, the volumetric increase observed in the ES group clinically translates to the potential for Endoseal MTA to provide antibacterial activity and continued filling of the canal wall interface as an endodontic sealer, by continued HA-like crystal growth in the presence of bodily fluid in vivo. Further comparative investigation on the antibacterial activity, leakage and bond strength test of the three materials will contribute to a more solid understanding of the products.

Figures and Tables

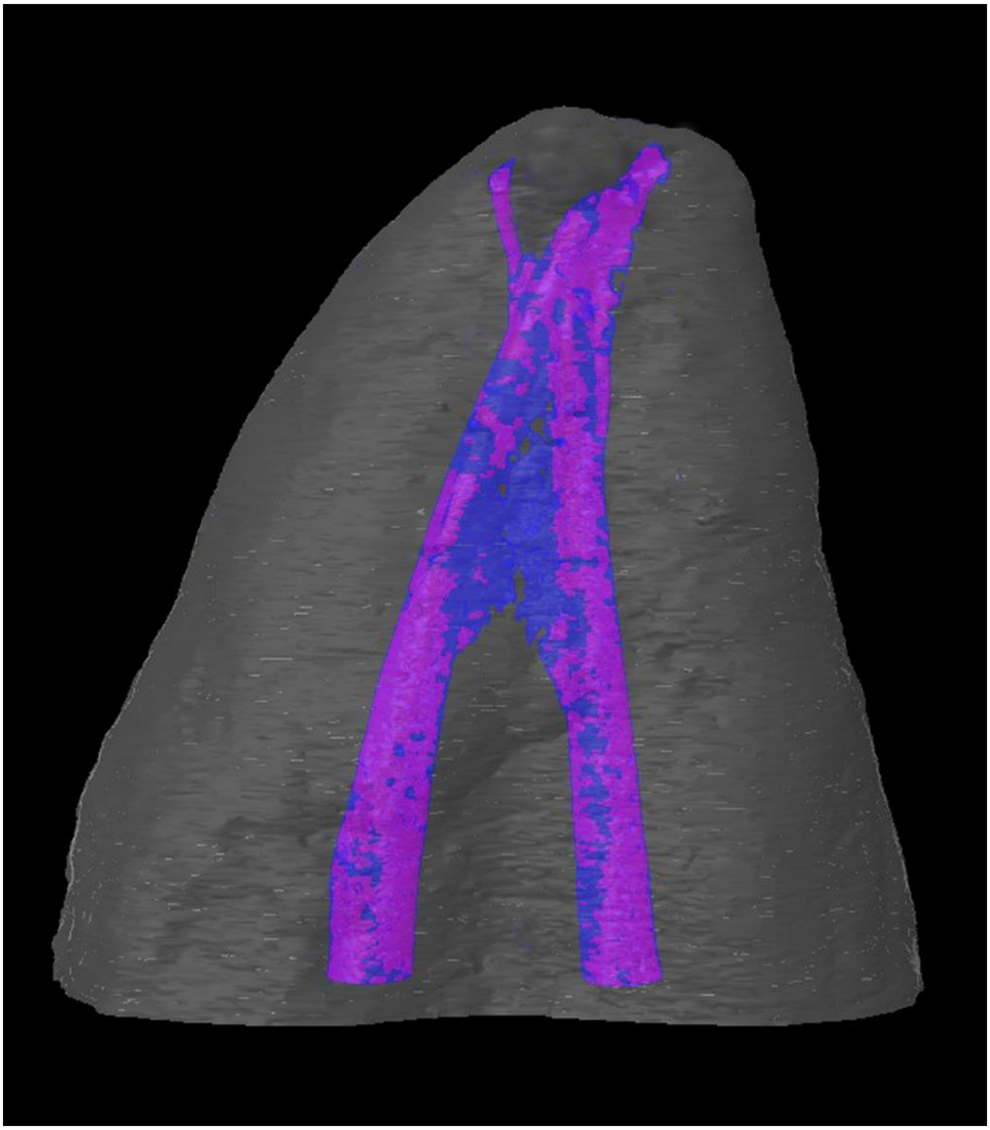

Figure 1. Representation of superimposed scanned images taken at 24 hours (blue) and 8 weeks (fuchsia) post-obturation, a 3D reconstructions of specimen from ES group.

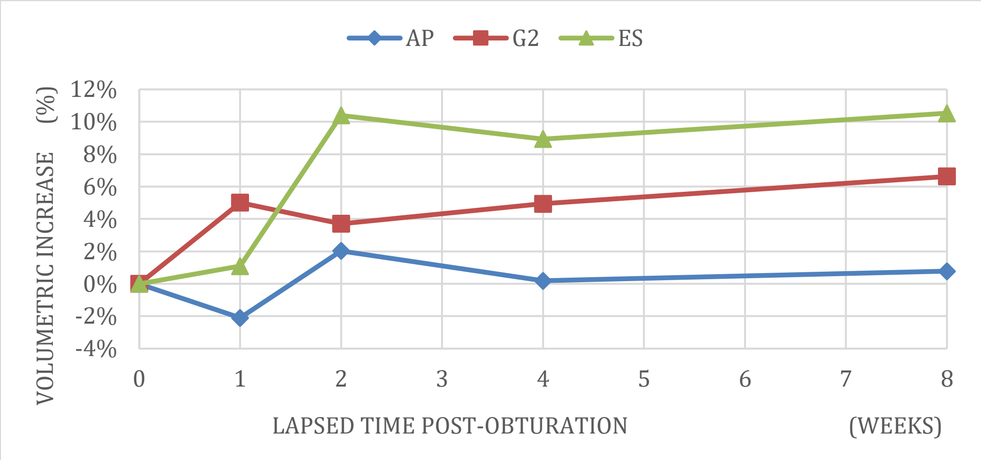

Figure 2. Volumetric changes of AP, G2 and ES over the course of 8 weeks.

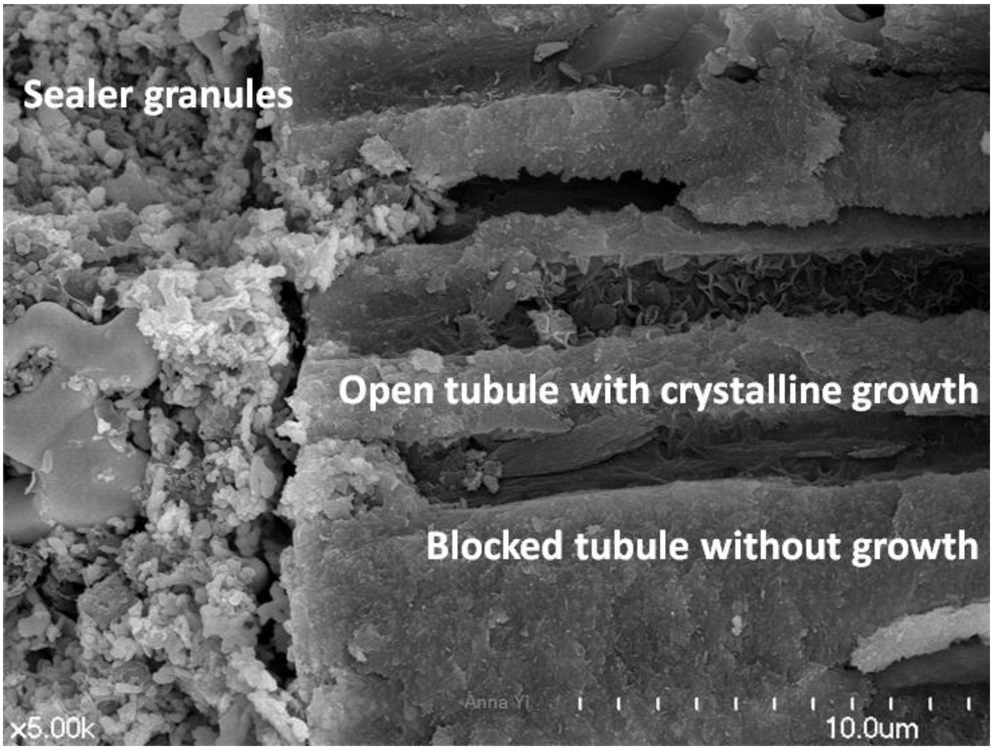

Figure 3. FE-SEM image of ES group showing cluster of sealer granules along the canal wall, crystalline growth within an open tubule and a lack of growth within a blocked tubule.

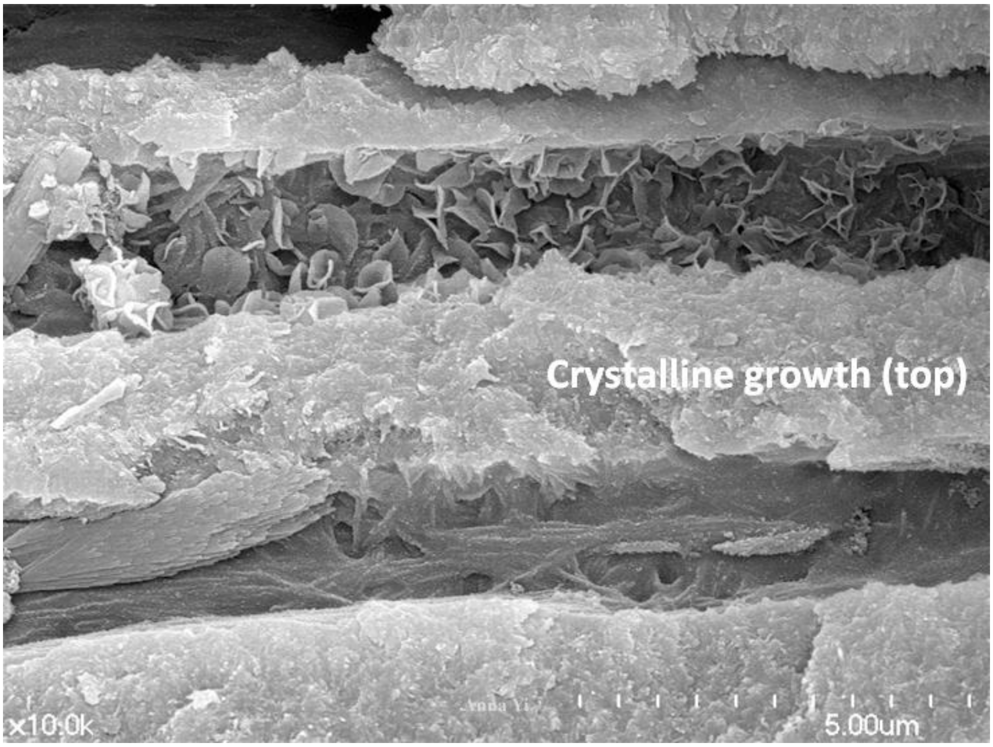

Figure 4. Magnified view of Fig. 3, showing planar crystalline growth (top) and dentinal microfibrils (bottom).

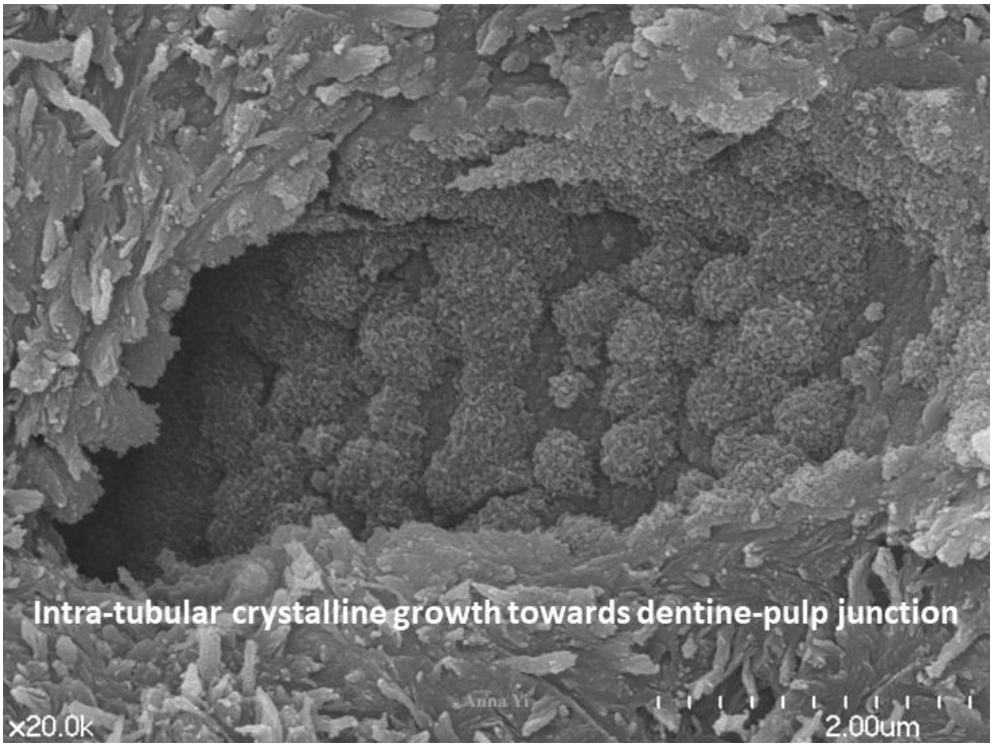

Figure 5. FE-SEM image of ES group showing clustered crystalline growth along the tubular wall towards the dentine-pulp junction, or the tubular opening.

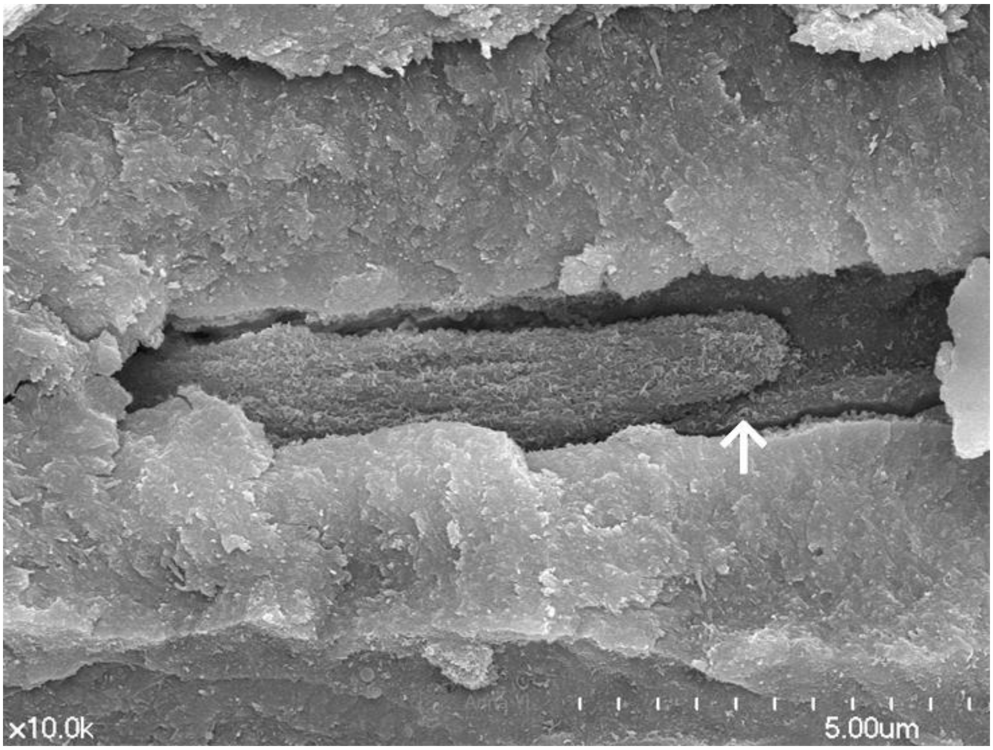

Figure 6. FE-SEM image of showing a remnant odontoblastic process within a dentinal tubule.

Table 1. Test Material Specifications

Product Name	Composition	Company	Lot Number
ProTaper Next	Nickel-titanium alloy	Dentsply Maillefer, Ballaigues, Switzerland	1365932
WaveOne Gold	Nickel titanium alloy	Dentsply Maillefer	1296392
AH Plus	Paste A Epoxy resins Calcium tungstate Zirconium oxide Silica Iron oxide pigments Paste B Amines Calcium tungstate Zirconium oxide Silica Silicone oil	Dentsply Maillefer	1603001007
EndoSeal MTA	Calcium silicates Calcium aluminates Calcium aluminoferrite Calcium sulfates Zirconium oxide Thickening agent	Maruchi, Wonju, Republic of Korea	SEE661123
Guttaflow 2	Gutta-percha powder Polydimethylsiloxane Silicone oil Paraffin oil Platinum catalyst Zirconium dioxide Microsilver (preservative) Coloring	Coltène Whaledent, GmBH+Co KG, Langenau, Switzerland	H09277

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국문초록

미세전산화단층촬영 및 의료용 CAD를 이용한 근관치료에 따른 체적 변화 분석

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1. 목 적

근관 치료는 화학적 또는 기계적 방법으로 근관을 세정하고 성형하여 근관내 연조직과 세균을 제거한 후, 근관 충전재료를 이용하여 완전하게 근관계를 밀폐하는 것이다. 본 논문에서는 근관 만곡도에 따른 재래식 회전형 시스템인 ProTaper Next (PN)와 최근에 출시된 왕복운동형 시스템인 WaveOne Gold (WG)의 근관 성형 전•후의 체적 변화를 측정하여 두 시스템간 근관 형성능을 비교하고자 하였고, 에폭시 레진계 근관 충전용 실러인 AH Plus (AP)와 칼슘 실리케이트계 실러인 EndoSeal MTA (ES) 및 거타퍼챠 계열인 Guttaflow 2 (G2)로 근관을 충전한 후 진행된 체적 변화를 비교하고자 하였다.

2. 방 법

근관용 파일 시스템이 성형된 근관의 체적에 주는 영향을 비교하기 위하여 발거된 건전한 상악 대구치의 만곡 근관을 무작위로 회전형인 PN 실험군과 왕복운동형인 WG 실험군으로 분류하였다 (n = 32/실험군). 근관은 치근첨 크기가 ISO 25 (PN: X2 25/06; WG: Primary 25/07)까지 제조자의 설명서에 따라 성형하였고, 근관 성형 전과 후는 미세전산화단층촬영하여 의료용 CAD 프로그램인 Mimics와 3-matic으로 분석하였다. 또한 주사전자현미경과 에너지 분산형 X선 분광기를 이용하여 사용한 파일의 표면을 분석하였다.

적용한 근관 충전용 실러에 따른 체적 변화를 비교하기 위하여 발거된 건전한 상악 대구치의 만곡 근관을 무작위로 근관 충전용 실러에 따라 AP 실험군, ES 실험군 및 G2 실험군으로 (n = 12/실험군) 분류하였다. 근관은 제조자의 설명서에 따라 Proper Next X4 (40/06)까지 성형하였고, 멸균된 비표준화된 거타퍼챠 콘을 이용해 single−cone 술식으로 근관 충전하였다. 근관 입구는 Fuji II LC로 밀폐하고, 치근단 공의 주변 2 mm를 제외한 표면은 바니쉬로 처리하였다. 시편은 5 mL 인산완충액과 함께 실험용 밀폐용기에 넣고 37°C에 보관하면서 24시간, 1주, 2주, 4주 및 8주 경과한 시점에서 미세전산화단층촬영하여 Mimics와 3−matic으로 분석하였다. 또한 근관 충전된 치아는 주사전자현미경으로 관찰하였다.

SPSS version 24 (SPSS, Chicago, USA)를 이용하여 통계 분석하였고, 통계적 유의수준을 $P < .05$ 으로 정의하였다. 본 연구에서 수집된 데이터는 Levene 의 등분산 검정 후, 그룹 내 비교를 위해 일원배치 분산분석을 하였고, 등분산이 성립되었을 때에는 Tukey 의 정직유의차를 이용한 사후검정을 적용하였으며, 등분산이 성립되지 않았을 때에는 Dunnett 의 T3 사후검정을 적용하였다. 그룹 간 비교를 위해 독립 표본 T 검정을 하였다.

3. 결 과

근관용 파일 시스템이 주는 영향을 비교한 결과 만곡도가 낮은 근관일수록 관측 1/3 부분에서 WG 의 근관 성형 정확도가 통계적으로 유의하게 감소되는 것이 관찰되었다. 이러한 현상은 임상적으로 근관 성형 시 관측 근관 형태변화가 야기될 가능성이 높을 것으로 해석된다. PN 실험군과 WG 실험군의 총 상아질 손실의 체적 분석 결과에서는 두 실험군간 통계적으로 유의한 차이가 없는 것이 관찰되었다. 주사전자현미경 관찰에서 사용한 파일 표면이 무더진 정도를 고려하면 두 시스템의 최대 성형력과 안전성을 위하여 1 회 사용 원칙을 따라야 할 것으로 보였다.

적용한 근관 충전용 실러에 따른 체적 변화를 분석한 결과 근관 충전 8 주 후 ES 실험군의 총 체적이 유의하게 증가된 것이 관찰되었다.

이러한 체적 증가는 EndoSeal MTA 가 체액과 반응하여 형성한 수산화인회석이 지속적인 근관내 결정질 성장을 토대로 근관 충전재료로서의 근관벽 계면의 우수한 밀폐 역할을 할 수 있을 것으로 보였다.

주요어 : 근관치료, 근관치료용파일, 근관충전실러, 미세전산화단층촬영, CAD, 체적변화

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