

Comparison of Bacterial Leakage Resistance of Various Root Canal Filling Materials and Methods: Confocal Laser-Scanning Microscope Study

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Summary: This study evaluated the bacterial leakage resistance and root canal lining efficacy of various root canal filling materials and methods by using confocal laser-scanning microscope (CLSM). Sixty extracted human premolars with mature apex and single root canal were randomly divided into 2 control groups and 4 experimental groups. Group CW was filled with continuous wave technique using gutta-percha and AH Plus sealer. Group GC was coated with AH-Plus sealer and then obturated with soften GuttaCore. Group GF was obturated using GuttaFlow and gutta-percha. Group EM was filled with EndoSeal MTA and gutta-percha using ultrasonic vibration. The AH-Plus, GuttaFlow, and EndoSeal were labeled with Hoechst 33342 to facilitate fluorescence. The obturated root tip was incubated with Carboxyfluorescein diacetate succinimidyl ester (CFSE)-stained *E. faecalis* for 14 days. CLSM was performed to evaluate the sealer distribution and bacterial leakage for the apical 1-, 2-, 3-mm specimens. Statistically significant differences were determined by 1-way ANOVA with Tukey's post-hoc test and Pearson's correlation analysis. Group EM showed the better sealer distribution score than the other groups ($p < 0.05$). Group CW and group GC exhibited the less bacterial leakage than the group GF, while group EM showed the similar bacterial leakage score with the groups CW and GC. There was no significant correlation between the sealer distribution and bacterial leakage

($p > 0.05$). Under the conditions of this study, different root canal filling materials and methods showed different efficacy for canal distribution and bacterial leakage resistance. SCANNING 37:422–428, 2015. © 2015 Wiley Periodicals, Inc.

Key words: bacterial leakage, confocal laser scanning microscope, GuttaCore, GuttaFlow, root canal sealer, sealer distribution

Introduction

Root canal fillings aim to seal the root canal system to prevent reinfection of the periapex. Obturation should eliminate all routes of leakage from the oral cavity and the periradicular tissues into the root canal system by creating a fluid-tight seal. Therefore, ideally, root canal filling materials, and endodontic sealers should seal the canal laterally and apically and have good adaptation to the root canal dentin (Özcan *et al.*, 2011; Chandra *et al.*, 2012). The materials and methods used for root canal obturation are one of the critical determinants for the success or failure of endodontic treatment (Bodrumlu and Tunga, 2007).

Obturation of the root canal space has been performed using various techniques. The most commonly used technique is cold lateral condensation using gutta-percha (GP) cones which allows good length control and is predictable (Gutmann *et al.*, '93). The "continuous wave of condensation" which was designed to simplify the vertical condensation method has been found superior or similar to that provided by the lateral condensation (Buchanan '98; DuLac *et al.*, '99; Smith *et al.*, 2000; Özcan *et al.*, 2013). It is claimed that the heat source allows sufficient heat for the apical GP to be softened and adapted to the irregularities of the intracanal anatomy (Kytridou *et al.*, '99; Gilbert *et al.*, 2001). Thermoplasticized GP techniques have

Conflict of interest: None.

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Received 16 March 2015; Accepted with revision 21 May 2015

DOI: 10.1002/sca.21231

Published online 26 June 2015 in Wiley Online Library
(wileyonlinelibrary.com).

been advocated for root canal obturation because they may provide a more homogeneous obturation and better adaptation to the canal walls (Kytridou *et al.*, '99) which might result in a lower leakage compared with lateral condensation. GuttaCore (DENTSPLY Tulsa Dental Specialties, Tulsa, OK) is one of the carrier-based GP obturation system. As an improvement over the previous Thermafil technique, GuttaCore obturator carriers are not made from plastic, but from a GP elastomer with intermolecular cross links (Li *et al.*, 2014). This makes the procedures not only rapid and high-quality three-dimensional root canal obturation, but also easy post space preparation and root filling removal in a case where retreatment is required. EndoSeal MTA (Maruchi, Wonju, Korea) was recently introduced in a pre-mixed paste type MTA-based sealer with the characteristics of hardening even at the complex and moist canal environment. The company also claims that the ultrasonic method using the EndoSeal MTA may reduce lateral and vertical forces applied to the root dentin during filling procedure. GuttaFlow (Coltene Whaledent, Alstatten, Switzerland), a new flowable root canal filling paste, was introduced as a non-heated flowable obturation system that combines both the sealer and the gutta-percha in 1 injectable system (Li *et al.*, 2014). The manufacturer claims that this material has good homogeneity and adaptation to the root canal walls owing to its better flow properties.

As above, new obturation biomaterials and methods have been introduced over the past decades to improve the seal of the root canal system. However, it is not clear whether they really produce a three-dimensional impervious seal that is important for reducing root canal reinfection. Therefore, the purpose of this study was to evaluate the bacterial leakage resistance and root canal lining of various root canal filling materials and methods by using confocal laser-scanning microscope (CLSM).

Materials and Methods

Preparation of Teeth and Canal Obturation

Sixty extracted human premolars with mature apex and single root canal were used in this study. After preparing a conventional access cavity, a size 10 K-file was inserted into the canal until it was just visible at the apical foramen. Working length was determined by subtracting 0.5 mm from this length. The root canal of each tooth was cleaned and shaped using sizes S1, S2, F1, and F2 ProTaper Universal nickel-titanium files (Dentsply Maillefer, Ballaigues, Switzerland) and finally with the size #35/0.04 taper nickel-titanium file (BLX; B&L Biotech, Ansan, Korea). The root canals were irrigated profoundly using 2.5% sodium hypochlorite between each instrument. Then canals were

rinsed with 17% EDTA solution (MD-cleanser; Meta Biomed, Chungju, Korea) for 1 min to remove smear layer, followed by flushing again with 2 ml of sodium hypochlorite. The root canals were dried with paper point. After biomechanical preparation, all the teeth were sterilized in an autoclave for 20 min at 121°C. The teeth were randomly divided into two control groups and four experiment groups using continuous wave of condensation technique, GuttaCore obturation system, GuttaFlow system, and EndoSeal MTA, respectively (n = 10). To facilitate fluorescence under confocal microscopy, AH Plus, GuttaFlow, and EndoSeal were labeled with Hoechst 33342 (Sigma, St. Louis, MO).

Group CW (Continuous wave of condensation technique with GP and AH Plus sealer): Each canal was filled with epoxy resin-based sealer (AH Plus; Dentsply Caulk, Milford, DE) and a size #35/0.04 taper GP master cone using Duo-Alpha and Duo-Beta system (B&L Biotech). The GP was down-packed with a Duo-Alpha heat source to the 4 mm from the working length and backfilled using a Duo-Beta.

Group GC (GuttaCore obturation system with AH Plus sealer): The canals were coated with a thin layer of AH Plus sealer using K-file. The size 30 GuttaCore obturator was softened in the dedicated GuttaCore oven and slowly inserted to the working length. The carrier was twisted off and the filled material was compacted at the orifice.

Group GF (GuttaFlow obturation system with GP): Teeth were obturated using the GuttaFlow and GP as the manufacturer's instructions. GuttaFlow provided in a special capsule was mixed in the triturator for 30 s. The capsule was then loaded on the dispenser with attached canal tip. GuttaFlow was layered slowly into the apical canal part. The master GP cone was coated with GuttaFlow and inserted in the canal. The master GP cone was cut at the orifice level.

Group EM (Ultrasonic condensation with EndoSeal MTA and GP): The EndoSeal MTA was prepared as the manufacturer's instruction and loaded in a metal tip (Centrix; Centirx, Shelton, CT). EndoSeal MTA was then injected into coronal and middle root canal. By using lentulo spiral, EndoSeal MTA was adjusted to middle and apical root canal. Then the master GP cone was inserted into the canal. By ultrasonic vibration to the pincette which hold the master cone, the GP cone could reach all the way to working length. The master GP cone was cut at the orifice level.

Group PC (Positive control; n = 10): The canals of this group were not filled.

Group NC (Negative control; n = 10): The canals of this group were filled with GP and AH plus sealer. The negative control group was sealed around entire root surface with colored nail varnish.

Except the Group NC, the roots of 50 teeth were sealed with colored nail varnish, except for the apical 1 mm around the apical foramen.

Bacterial Leakage Experiment

Enterococcus faecalis (*E. faecalis* strain ATCC 19433) were grown in Brain Heart Infusion (BHI) broth (DIFCO, BD science, San Jose, CA) under aerobic conditions at 37°C. The bacteria were resuspended in phosphate buffer saline (PBS) at 5×10^7 CFU/ml and stained with cellTrace Carboxyfluorescein diacetate succinimidyl ester (CFSE, Molecular probes, Life technology, Carlsbad, CA) following the manufacture's instruction. Briefly, *E. faecalis* were stained with 10 μ M of CFSE at 37°C for 10 min. After incubation, cold PBS was added to stop the staining and *E. faecalis* were thoroughly washed. The prepared CFSE-stained *E. faecalis* were inoculated in BHI broth. After cutting the tapered end of 2 ml Eppendorf plastic tube (Eppendorf-Elkay, Shrewsbury, MA), the obturated root tip was inserted into the tube containing BHI broth inoculated with CFSE-stained *E. faecalis* until the root tip protruded through the end. The entire assembly was then incubated at 37°C for 14 days.

Specimen Preparation and Confocal Laser-Scanning Microscope for Scoring

The samples were washed with PBS and specimens were sectioned at every 1 mm from 1 to 3 mm level of apex using a low speed diamond disk (Horico, Berlin, Germany). The procedure was done with minimum pressure under water cooling to minimize the smearing of the GP.

All samples were observed by CLSM (LSM 700; Carl Zeiss, Oberkochen, Germany) at each 1 mm specimen. Each 10 samples were evaluated for a blue fluorescent ring around the canal wall, indicating the sealer-dye distribution. The ratio of bacterial penetration was detected for a green spot around the canal wall, indicating the CFSE-stained *E. faecalis*. At each level, tooth surface was divided into eight parts of same arc and if there was fluorescence (Blue or Green) in a eighth, 0.125 point was given (Fig. 1). Because the score given in each level is not clinically available to evaluate the efficiency of the materials and methods, the final NET score for each specimen was given by summing up of the points from the levels of 1 mm to 3 mm of apical root.

Statistics

Quantitative data were tabulated and analyzed for 1-way analysis of variance (ANOVA) and Tukey's post-hoc tests using Graphpad Prism software (GraphPad Software, San Diego, CA). The correlation between sealer distribution and bacterial leakage was presented by Pearson's correlation analysis. The statistical significance was set at a confidence level of 95%.

Results

The representative CLSM results of sealer distribution and bacterial leakage of the tested groups are shown in Figure 2. The specimens of positive control group show a consistent green fluorescent ring around the canal wall, indicating the bacterial penetration into the canal (Fig. 2(A)). The blue fluorescent ring around the canal wall of negative control group represents the sealer distribution in canal wall (Fig. 2(B)).

The results of the quantitative evaluation of the penetration of AH Plus, GuttaFlow, and EndoSeal MTA stained with Hoechst 33342 are shown in Table I and Figure 3 (A and B). Group EM showed the better sealer distribution score than the other groups ($p < 0.05$). The results of bacterial leakage scores at 1, 2, and 3 mm levels from the apex and net score are presented in Table I and Figure 3 (C and D). Group CW and group GC exhibited the less bacterial leakage than the group GF, while group EM showed the similar bacterial leakage score with the groups CW and GC (Table I). As shown in Figure 3E, there was no significant correlation between the sealer penetration and bacterial leakage ($p > 0.05$).

Discussion

The elimination of bacteria from the root canals by cleaning and shaping procedures and the prevention of recontamination of the obturated root canal are fundamental for the successful treatment of apical periodontitis (Ray and Trope, '95). Healing of periapical disease involves a combination of bacterial eradication during treatment through chemomechanical means along with sealing of the root canal (Ray and Trope, '95). Recently, new materials for root canal filling and methods for these materials were introduced. This study evaluated the various root canal filling materials and methods by comparing the sealer distribution to canal wall and bacterial leakage resistance under the CLSM.

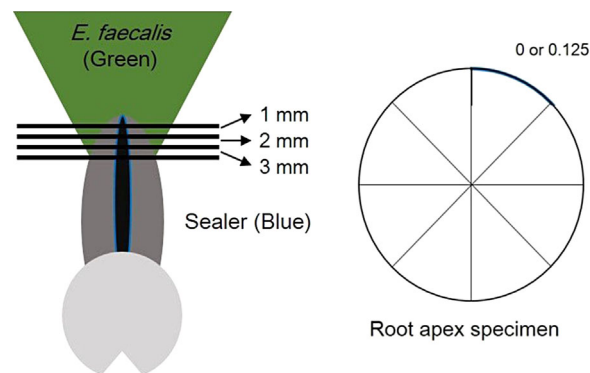


Fig. 1. The experimental designs for specimen preparation and scoring of fluorescences.

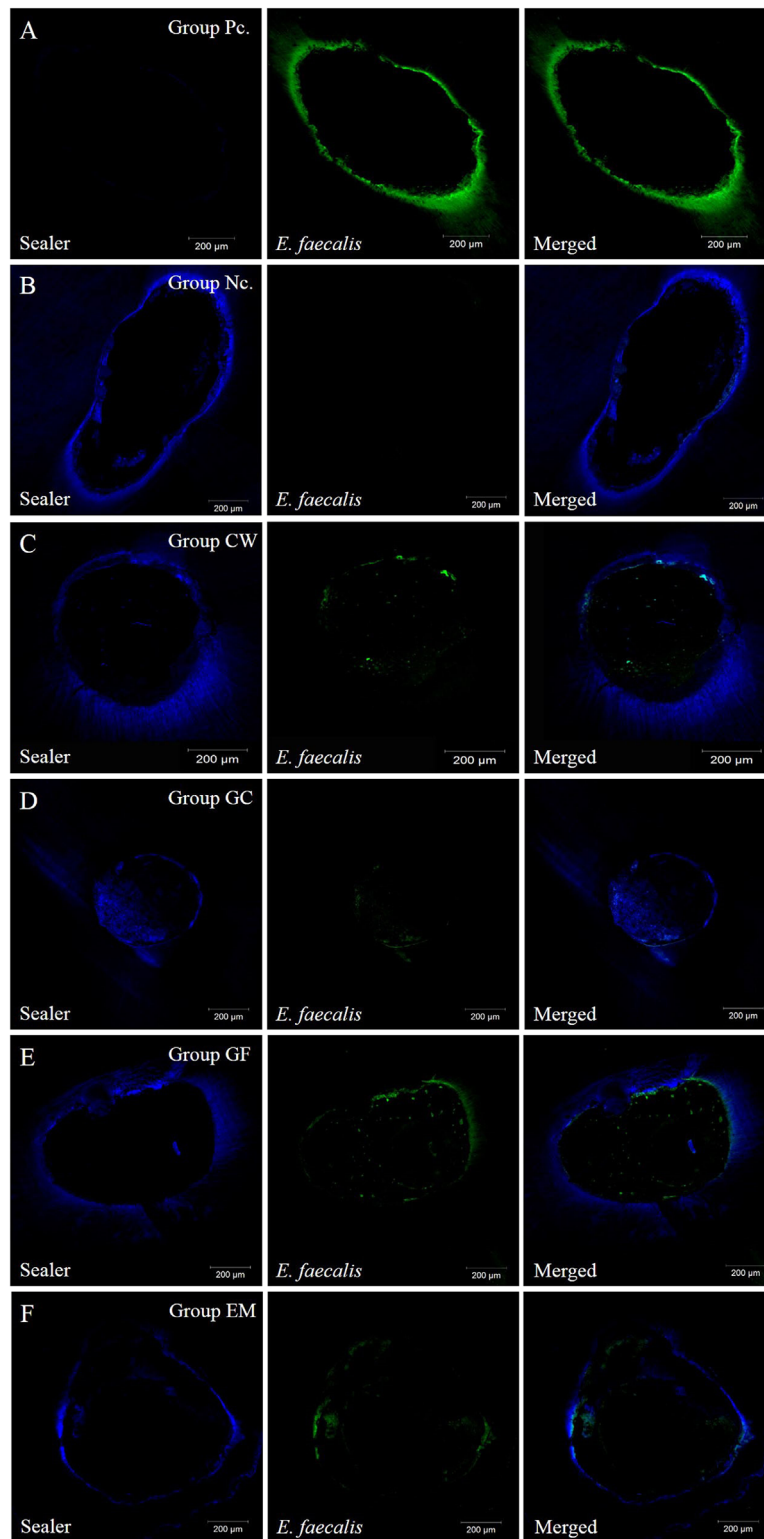


Fig. 2. The representative CLSM results of control groups and experimental groups: A. Group Pc (Positive control). B. Group Nc (Negative control). C. Group CW (continuous wave of condensation technique with GP and AH Plus sealer). D. Group GC (GuttaCore system with AH Plus sealer). E. Group GF (GuttaFlow obturation system with GP). F. Group EM (Ultrasonic condensation with EndoSeal MTA and GP).

TABLE I The sealer distribution score (%) and bacterial leakage score (mean \pm SD)

Group	Level Score	1 mm	2 mm	3 mm	Net
Group CW	sealer distribution	85 \pm 8	91 \pm 8	91 \pm 6	89 \pm 4A
	bacterial leakage	0.35 \pm 0.15	0.33 \pm 0.11	0.29 \pm 0.12	0.33 \pm 0.07a
Group GC	sealer distribution	86 \pm 9	86 \pm 9	90 \pm 5	88 \pm 3A
	bacterial leakage	0.46 \pm 0.15	0.27 \pm 0.05	0.29 \pm 0.10	0.32 \pm 0.09a
Group GF	sealer distribution	85 \pm 9	90 \pm 5	88 \pm 7	88 \pm 4A
	bacterial leakage	0.54 \pm 0.06	0.46 \pm 0.05	0.36 \pm 0.04	0.44 \pm 0.07 ^b
Group EM	sealer distribution	90 \pm 8	93 \pm 6	97 \pm 5	93 \pm 4 ^B
	bacterial leakage	0.38 \pm 0.12	0.39 \pm 0.12	0.32 \pm 0.06	0.37 \pm 0.08a ^b

CLSM technique may be useful as a complement to the established microbiological, histologic, standard electron microscopy, and PCR-based techniques for the identification of viable bacteria (Scivetti *et al.*, 2007). In the present study, CLSM offers advantages to evaluate clearly the distribution and interfacial adaptation of root canal sealers and the bacterial infiltration (Gharib *et al.*, 2007). Meanwhile, because *E. faecalis* is also one of the

most commonly isolated microbes from the root canal in secondary infections (Baumgartner and Falkler, '91; Timpawat *et al.*, 2001), this bacterial species was used in this study.

Ideally, a root canal sealer should be capable of producing a bond between the core material and the root dentin for preventing leakage effectively. A wide variety of root canal sealers are commercially available, however

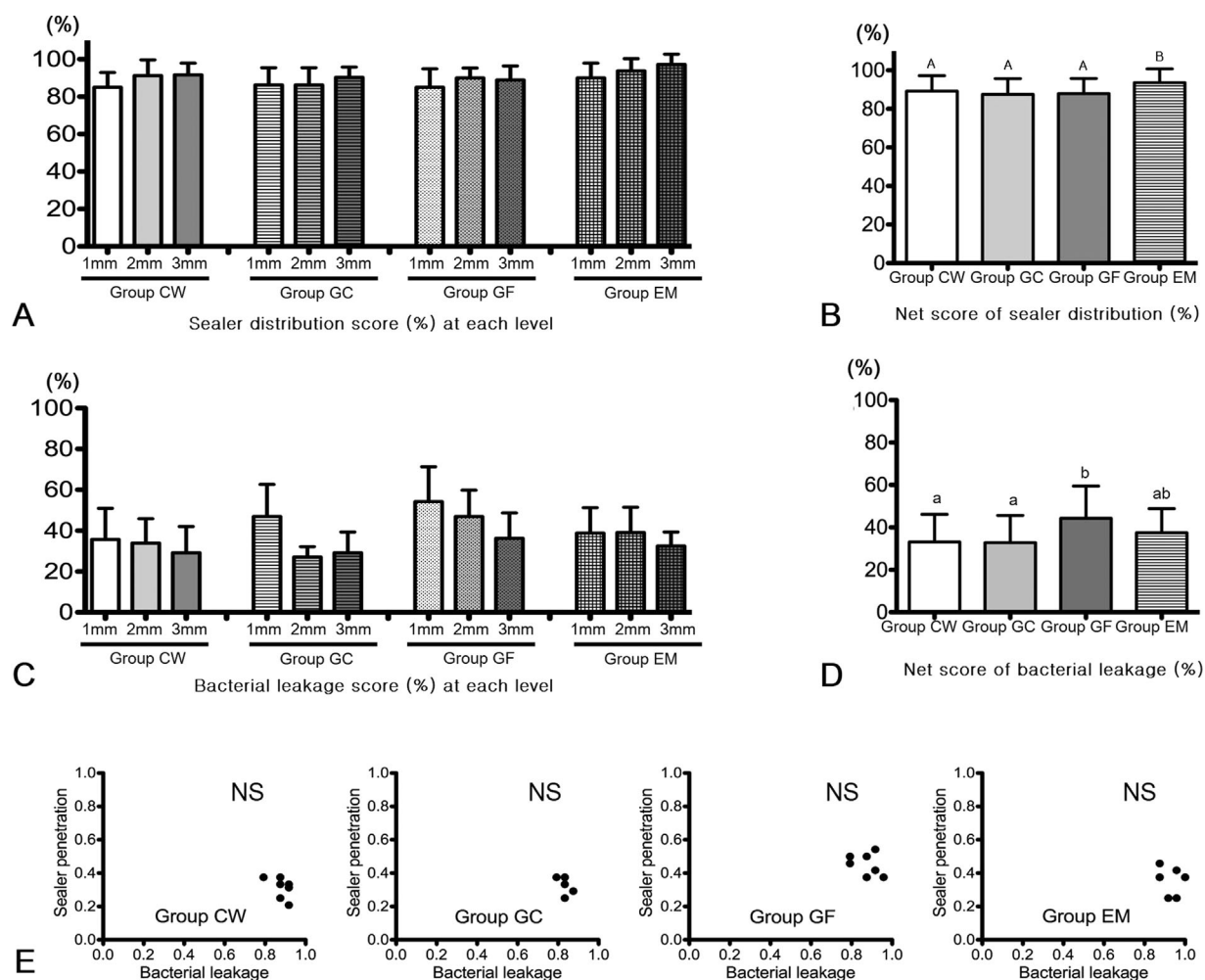


Fig. 3. A. The ratio of sealer distribution (%) in the 3 different levels. B. The net score of sealer distribution (%) of each group (groups with different superscripts have significant difference, $p < 0.05$). C. The score of bacterial leakage in the 3 different levels. D. The net score of bacterial leakage of each group (groups with different superscripts have significant difference, $p < 0.05$). E. The correlation between sealer penetration and bacterial leakage has no statistical significance (Pearson analysis, NS: not significant).

there is lack of consensus on which material seals the most effectively. AH Plus is an epoxy resin-based sealer. Some studies reported the apical seal of resin-based sealers are superior to the sealing ability of other sealers and shown to effectively seal the root canal (Oguntebi and Shen, '92; Cobankara *et al.*, 2002; Wu *et al.*, 2002; Gençoglu *et al.*, 2003). These were reported as to be related to the slight expansion during their setting reactions (Ørstavik *et al.*, 2001). In present study, it could be a same reason that the two groups of CW and GC had the less bacterial leakage than the groups which were AH Plus used. These results are coincidence with some previous reports regarding the efficacy of filling techniques (DuLac *et al.*, '99; Silver *et al.*, '99; Smith *et al.*, 2000; Wu *et al.*, 2001). The continuous wave of condensation technique has been found to be superior to some other canal filling techniques in terms of less apical leakage (DuLac *et al.*, '99; Smith *et al.*, 2000). However, some studies have found poor adaptation of GP to the canal wall by this technique (Silver *et al.*, '99; Wu *et al.*, 2001). Wu *et al.* (2001) reported that the apical GP ratio was low in some specimens and the sealer was too thick.

Meanwhile, the present results showed that group GF had significantly higher bacterial leakage than the continuous wave of condensation technique and GuttaCore system. However, on the contrary to the present results, Brackett *et al.* (2006) found no significant difference in sealing ability between GuttaFlow and vertically compacted GP used with AH Plus sealer. In a study by Hammad *et al.* (2008) GuttaFlow also showed good penetration into the dentinal tubules, and thus to help in preventing leakage. The good sealing ability exhibited by the GuttaFlow root-canal filling material could be attributed to its ability to flow into lateral grooves and depressions (Zielinski *et al.*, 2008).

On the other hand, considering the sealer distribution score, group GF had the similar efficacy with the groups CW and GC to line the canal wall but lower than the group EM which had the highest distribution score. The use of "ultrasonic" condensation method seemed to make the higher distribution score in the group EM. Ultrasonic vibration may have improved the flowability to ease in filling procedure or adjusting for varying working lengths and complex anatomy.

The EndoSeal MTA is similar to MTA Fillapex (Angelus, Londrina, PR, Brazil) which is a new calcium silicate-based sealer containing MTA and is known to possess favorable biocompatibility, antimicrobial activity, and good sealing ability (Torabinejad and Parirokh, 2010). EndoSeal MTA has a special concept of ultrasonic condensation (12). The ultrasonic vibration for sealer condensation may have brought positive effects to seal the canal and make higher sealer distribution scores and lining efficacy in this study. MTA Fillapex which has the similar composition mainly with MTA was also found to have greater flow values than AH Plus (Zhou *et al.*, 2013).

GuttaCore represents the latest generation of carrier-based root canal filling material that uses thermoplasti-cised GP as the core materials. In this present study, GuttaCore obturation system used with AH Plus sealer exhibited the less bacterial leakage than group GF and similar with the others. The reason for this finding might be similar with that found in group CW used with AH Plus sealer. The core-carrier may have enhanced the adaptation of GP to the canal wall and the flow of the molten GP material into irregular canal spaces. Previous studies reported that canals obturated with core-carrier techniques had the highest GP content within the filled canal space (Gençoglu, 2003; De-Deus *et al.*, 2006).

The flow of a sealer determines how effectively it fills accessory canals, irregularities on the dentinal wall, and spaces between the core filling materials (Zhou *et al.*, 2013). However, as found in Figure 3E in this study, the correlations of sealer distribution and bacterial leakage were not significant. It might be due to the technical sensitivity in filling procedures, although sufficient preliminary tests were done to minimize the procedural errors before the experimental tests. Probably the chemical and mechanical properties may have brought the different bacterial leakage resistances which were not correlated with the sealer distribution and penetration extents. This phenomenon should be studied further to see any potential correlations. Clinically, it is highly recommended to follow the manufacturers' instructions and do some sub-clinical practices using extracted teeth.

It is important to assess microbial leakage not only immediately after root canal filling but also with some time elapsed, because root canal sealing needs to be long lasting for clinical effectiveness. Thus, further studies are required to test the bacterial leakage with some time elapsed as well as thermos-mechanical loading considering clinical effects.

Conclusion

Within the limitations of present study, four different root canal filling materials and methods showed significant differences in their canal sealing efficiency and bacterial leakage resistance without correlations between the two variables.

Acknowledgment

Ji Hee Hwang and Jin Chung contributed equally to this work and have the first authorship shared.

References

- Baumgartner JC, Falkler WA, Jr. 1991. Bacteria in the apical 5mm of infected root canals. *J Endod* 17:380–383.

- Bodrumlu E, Tunga U. 2007. Coronal sealing ability of a new root canal filling material. *J Can Dent Assoc* 73:623.
- Brackett MG, Martin R, Sword J, Oxford C, Rueggeberg FA, Tay FR, Pashley DH. 2006. Comparison of seal after obturation techniques using a polydimethylsiloxane-based root canal sealer. *J Endod* 32:1188–1190.
- Buchanan LS 1998. Continuous wave of condensation technique. *Endod Prac* 1(7–10, 13-16):18.
- Chandra SS, Shankar P, Indira R. 2012. Depth of penetration of four resin sealers into radicular dentinal tubules: A confocal microscopic study. *J Endod* 38:1412–1416.
- Cobankara FK, Adanir N, Belli S, Pashley DH. 2002. A quantitative evaluation of apical leakage of four root-canal sealers. *Int Endod J* 35:979–984.
- De-Deus G, Gurgel-Filho ED, Magalhães KM, Coutinho-Filho T. 2006. A laboratory analysis of gutta-percha-filled area obtained using Thermafil, System B and lateral condensation. *Int Endod J* 39:378–383.
- DuLac KA, Nielsen CJ, Tomazic TJ, Ferrillo PJ, Jr, Hatton JF. 1999. Comparison of the obturation of lateral canals by six techniques. *J Endod* 25:376–380.
- Gençoglu N 2003. Comparison of 6 different gutta-percha techniques (part II): Thermafil, JS Quick-Fill, Soft Core, Microseal, System B, and lateral condensation. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 96:91–95.
- Gençoglu N, Türkmen C, Ahiskali R. 2003. A new silicon-based root canal sealer (Roekoseal-Automix). *J Oral Rehabil* 30:753–757.
- Gharib SR, Tordik PA, Imamura GM, Baginski TA, Goodell GG. 2007. A confocal laser scanning microscope investigation of the epiphany obturation system. *J Endod* 33:957–961.
- Gilbert SD, Witherspoon DE, Berry CW. 2001. Coronal leakage following three obturation techniques. *Int Endod J* 34:293–299.
- Gutmann JL, Saunders WP, Saunders EM, Nguyen L. 1993. A assessment of the plastic Thermafil obturation technique. Part I. Radiographic evaluation of adaptation and placement. *Int Endod J* 26:173–178.
- Hammad M, Qualtrough A, Silikas N. 2008. Extended setting shrinkage behavior of endodontic sealers. *J Endod* 34:90–93.
- Kytridou V, Gutmann JL, Nunn MH. 1999. Adaptation and sealability of two contemporary obturation techniques in the absence of the dentinal smear layer. *Int Endod J* 32:464–474.
- Li GH, Niu LN, Selem LC, Eid AA, Bergeron BE, Chen JH, Pashley DH, Tay FR. 2014. Quality of obturation achieved by an endodontic core-carrier system with crosslinked gutta-percha carrier in single-rooted canals. *J Dent* 42:1134.
- Oguntebi BR, Shen C. 1992. Effect of different sealers on thermoplasticized Gutta-percha root canal obturations. *J Endod* 18:363–366.
- Ørstavik D, Nordahl I, Tibballs JE. 2001. Dimensional change following setting of root canal sealer materials. *Dent Mater* 17:512–519.
- Özcan E, Eldeniz AU, Ari H. 2011. Bacterial killing by several root filling materials and methods in an ex vivo infected root canal model. *Int Endod J* 44:1102–1109.
- Özcan E, Eldeniz AÜ, Aydinbelge HA. 2013. Assessment of the sealing abilities of several root canal sealers and filling methods. *Acta Odontol Scand* 71:1362–1369.
- Ray HA, Trope M. 1995. Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration. *Int Endod J* 28:12–18.
- Scivetti M, Pilolli GP, Corsalini M, Lucchese A, Favia G. 2007. Confocal laser scanning microscopy of human cementocytes: Analysis of three-dimensional image reconstruction. *Ann Anat* 189:169–174.
- Silver GK, Love RM, Purton DG. 1999. Comparison of two vertical condensation obturation techniques: Touch 'n Heat modified and System B. *Int Endod J* 32:287–295.
- Smith RS, Weller RN, Loushine RJ, Kimbrough WF. 2000. Effect of varying the depth of heat application on the adaptability of gutta-percha during warm vertical compaction. *J Endod* 26:668–672.
- Timpawat S, Amornchat C, Trisuwan WR. 2001. Bacterial coronal leakage after obturation with three root canal sealers. *J Endod* 27:36–39.
- Torabinejad M, Parirokh M. 2010. Mineral trioxide aggregate: a comprehensive literature review-part II: Leakage and biocompatibility investigations. *J Endod* 36:190–202.
- Wu MK, Kast'áková A, Wesselink PR. 2001. Quality of cold and warm gutta-percha fillings in oval canals in mandibular premolars. *Int Endod J* 34:485–491.
- Wu MK, Tigos E, Wesselink PR. 2002. An 18-month longitudinal study on a new silicon-based sealer, RSA RoekoSeal: A leakage study in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 94:499–502.
- Zhou HM, Shen Y, Zheng W, Li L, Zheng YF, Haapasalo M. 2013. Physical properties of 5 root canal sealers. *J Endod* 39:1281–1286.
- Zielinski TM, Baumgartner JC, Marshall JG. 2008. An evaluation of Guttaflow and gutta-percha in the filling of lateral grooves and depressions. *J Endod* 34:295–298.