Acta Med. Okayama, 2022
Vol. 76, No. 6, pp. 715-721

Acta Medica Okayama

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Original Article

Graphene Oxide-based Endodontic Sealer: An *in Vitro* Study

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The failure of endodontic treatment is directly associated with microbial infection in the root canal or periapical areas. An endodontic sealer that is both bactericidal and biocompatible is essential for the success of root canal treatments. This is one of the vital issues yet to be solved in clinical dental practice. This *in vitro* study assessed the effectiveness of graphene oxide (GO) composites GO-CaF_2 and GO-Ag-CaF_2 as endodontic sealer materials. Dentin slices were coated with either the GO-based composites or commonly used root canal sealers (non-eugenol zinc oxide sealer). The coated slices were treated in 0.9% NaCl, phosphate-buffered saline (PBS), and simulated body fluid (SBF) at 37°C for 24 hours to compare their sealing effect on the dentin surface. In addition, the radiopacity of these composites was examined to assess whether they complied with the requirements of a sealer for good radiographic visualization. Scanning electron microscopy showed the significant sealing capability of the composites as coating materials. Radiographic images confirmed their radiopacity. Mineral deposition indicated their bioactivity, especially of GO-Ag-CaF_2, and thus it is potential for regenerative application. They were both previously shown to be bactericidal to oral microbes and cytocompatible with host cells. With such a unique assemblage of critical properties, these GO-based composites show promise as endodontic sealers for protection against reinfection in root canal treatment and enhanced success in endodontic treatment overall.

Key words: bioactive sealer, graphene oxide, mineral deposition, antimicrobial activity, radiopacity

The primary goal of endodontic treatment is to eradicate root canal infection and to protect against reinfection by filling and sealing the root canal system [1,2]. While mechanochemical root canal preparations radically reduce 40-60% of microorganisms, the chance of bacterial growth remains high [3,4]. Endodontic sealers play an important part by impeding the penetration of tissue fluid and killing residual bacteria to limit endodontic infections [5]. Sealers also act as a lubricant to facilitate the placement of the core filling material (*i.e.*, gutta-percha (GP), metal cone) [6]. Therefore, the sealers act synergistically with the core materials to create a hermetic seal [7]. The adhesive property of root canal sealers to dentin is important to seal the root canal thoroughly for preventing the penetration of fluids. In addition, sealers should have a clingy bond between the core material

Received March 31, 2022; accepted July 11, 2022.
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Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

and dentin for the long-term success of root canal treatment [8]. The sealer also must be soluble in a common solvent if it is to be removed from the root canal. Finally, the ideal sealer would be bacteriostatic, or at least should not encourage bacterial growth. Unfortunately, no available sealer has all these properties [7]. The selection of endodontic sealer, therefore, can affect treatment outcomes. Due to the relative biological and technical importance of sealers, their chemical and physical properties have been the subject of considerable attention to date [8].

The antimicrobial activity of root canal sealers increases the success rate of endodontic treatments by eliminating residual intra-radicular infections that might have survived in the root canal or later invaded the canal through microleakage [9]. Generally, the key antimicrobial properties of root canal sealers lie in their alkalinity and the release of calcium ions, which stimulate reparation and restoration via the deposition of minerals and mineralized tissues [10, 11]. Eugenol in the sealer is a potent antimicrobial agent that acts on microorganisms by degrading proteins. Similarly, formaldehyde in epoxy resin sealer exerts an antimicrobial effect by releasing hydroxyl ions and increasing pH. Some commercial sealers claim to be antimicrobial. However, a review of commonly used bioceramic sealers reported that they have various degrees of antimicrobial activity, but not to the extent that is clinically required [12].

At the same time, identical radiopacity between dentin and sealer is very important for sealer materials. Sealers should display an adequate radiopacity to distinguish adjacent anatomical structures from filler materials for the quality assessment of root fillings [13-16]. Extremely radiopaque sealers may lead to inadequacies in the filling when used with gutta-percha [17]. Thus, researchers and clinicians must establish a sealer that includes radiopacity among its properties.

The inclusion of graphene in materials to improve physical properties has been proposed for many materials including Portland cement, bioceramics, polymers, and metals. The addition of graphene oxide (GO) to bioactive materials was reported to improve their physio-mechanical properties, cell proliferation, and mineralization potential pertinent to their clinical use [18]. GO has large hydrophilic oxygen functional groups and a high surface area that enhances binding to dentin and seals against micropenetration [19]. The GO-dentin binding force may be enhanced by covalent bonding between epoxy groups of GO and dentin protein exposed to ethylenediaminetetraacetic acid (EDTA) treatment [20]. In this study, our previously developed antibacterial, biocompatible, and sealing-capable GO composites — graphene oxide calcium fluoride (GO-CaF_2) and graphene oxide silver calcium fluoride (GO-Ag-CaF_2) [21] — were assessed for their *in vitro* effectiveness as endodontic sealers. The aim of this study was to evaluate their properties of sealing ability, radiopacity, and potential bioactivity, all important for their use as endodontic sealers.

Materials and Methods

Synthesis of sample materials

1. GO synthesis. GO was synthesized according to Morimoto *et al* [22]. In brief, 3.0 g of graphite was added to 75 mL H_2SO_4. Then, 9.0 g of $KMnO_4$ was gradually added to the mixture, maintaining the temperature below 35°C and stirring at 200 rpm. Later, the mixture was stirred at 35°C for 2 hours prior to quenching. Then, H_2O (75 mL) was added under vigorous stirring and cooling to maintain the temperature below 50°C. Afterward, 7.5 mL H_2O_2 (30% (mass/mass) was slowly added with continuous stirring for 30 min at ambient temperature. The final product was purified by centrifugation.

2. GO-CaF_2 Synthesis. A one-pot synthesis method was used to synthesize 1-wt% GO-CaF_2 composite. A separate solution of 74.4 mg of KF, 70.5 mg of $CaCl_2$, and 50 mg of GO in 20 mL of MilliQ water was prepared. These ingredients were mixed gradually by magnetic stirring for 20 min. The final solution was purified by centrifugation [21].

3. GO-Ag-CaF_2 Synthesis. GO-Ag-CaF_2 was also synthesized as a 1-wt% composite following the one-pot synthesis method. A mixture of 74.4 mg of KF, 70.5 mg of $CaCl_2$, 78 mg of $AgNO_3$ solution, and 50 mg of GO dispersion was magnetically stirred for 20 min. Then 1% NaOH solution was gradually dropped into the mixture until the pH became 8.00. The final solution was purified by centrifugation [21].

Tooth selection and specimen preparation. Eighty freshly extracted bovine teeth were collected from local meat processing companies and preserved in −40˚C freezers. Sixty teeth were separated to prepare root dentin slices. The crowns were sectioned vertically with a diamond disc in a sectioning machine (BS-3000

EXAKT, Norderstedt, Germany) to create dentin discs from mid-crown-root canal dentin. Eighty-four root canal sectional specimens were prepared and polished with P1000 (P1000; Nihonkenshi Co., Ltd, Hiroshima, Japan) abrasive paper. All specimens used in this study presented the same range of root canal areas to reduce errors in the research as much as possible. To avoid error, five individual experiments were performed. Eighteen intact roots were used to evaluate the radiopacity of the different applied materials.

Digital radiograph procedure. On radiographs, root canal sealer should provide an outline that is distinct from root canal filler to confirm hermetic sealing and to identify the core materials. A digital intraoral sensor, HDI 1000 medical image processing unit (Rayence, Vatech Dental Manufacturing Ltd. UK) was exposed to a dental X-ray unit (Asahi, Japan) at 60 kV and 10 mA, for 0.12 s at a density setting of 2 using a standardized focus-to-sensor distance of 30 cm in perpendicular view. An optical bench was used to standardize the geometric projection. A total of seven exposures were made; six for different tooth samples (sealed and unsealed root canal specimens) and one for a metal ring sample (four different rings filled with different materials). The resulting images were transferred to a personal computer and analyzed by the EzDent Vet Administrator (Rayence & Myvet Inc. NJ, USA). The mean gray values of the images (region of interest (ROI)) were determined and analyzed using the Analyse/Measure tool of the ImageJ program [23-26]. For each image, three ROIs (upper third, middle third, and apical third area) were selected at the same rectangular format (100×30 pixels) for radiographs of gutta percha (GP) point-filled root canals and three different circular areas (60×60, 45×45 and 30×30 pixels) for material contrast radiographs.

Surface condition and sealing effect evaluation. Bovine root dentin was used. The dentin slices were washed with deionized water (d.H_2O) and submerged in EDTA solution for 2 min to remove all unwanted debris and for surface activation; then a final wash was done with d.H_2O. Clean dentin slices were dried at room temperature, and the exposed dentin was sealed with GO, GO-CaF_2, GO-Ag-CaF_2, or non-eugenol zinc oxide sealer (Nishika Canal Sealer N: NCSN), Nippon Shika Yakuhin Co., Ltd., Yamaguchi, Japan) using a dentin-bond brush on the surface of the dentin slice and kept for 5 min at 37°C. Later, it was dried and submerged in 0.9% NaCl, PBS, and SBF buffer and incubated at 37°C for 24 hours. For all solutions pH was maintained at 7.40. Samples were removed from the solution and cleaned by vigorous brushing to clean the dentin surface properly. The dried dentin slices were observed under scanning electron microscopy (SEM).

Results

Radiological findings. GP point-sealer contrast radiopacity and the sample-vs.-sealer contrast opacity was assessed. To obtain the maximum sealer in the root canal, condensation was avoided. The non-sealed canal showed a clear radiolucency in the radiograph (Fig. 1A-i). The GP-filled canal showed radiopacity of GP points and a radiolucent canal outline (Fig. 1A-ii). In contrast, the sealer-filled canal showed a maximum radiopacity that is analogous to GP points (Fig. 1A-iii). When associated with the GO-based materials, the GO-filled (Fig. 1A-iv) canal showed very little radiopacity whereas GO-CaF_2 (Fig. 1A-v) had increased radiopacity, and the GO-Ag-CaF_2-sealed canal (Fig. 1A-vi) had the greatest radiopacity among the GO-based materials. The radiopacity of the GO-Ag-CaF_2-sealed canal was comparable to that of a sealer-filled canal. However, in the mean gray value evaluation (Fig. 1B), it was noted to have GP point-dominated radiopacity which increased when different sealers were added with the GP point. In comparison to the control, the radiopacity of the experimented materials showed almost comparable and acceptable radiopacity. The mean gray value of non-sealed, GP point-, sealer-, GO-, GO-CaF_2-, and GO-Ag-CaF_2-filled canals were 118.031, 218.234, 229.291, 228.886, 226.15, and 229.756, respectively. On the other hand, in the prepared materials-vs.-sealer contrast radiographs (Fig. 2A), it was observed that sealer exhibited a noticeable radiopacity (Fig. 2A-i), whereas GO (Fig. 2A-ii) was radiolucent and GO-CaF_2 (Fig. 2A-iii) showed little radiopacity while the sealer (Fig. 2A-i) and GO-Ag-CaF_2 (Fig. 2A-iv) had almost similar radiopacity. However, in the mean gray value evaluation (Fig. 2B), in contrast to each material, it was noticed that sealer, GO, GO-CaF_2, and GO-Ag-CaF_2 showed mean gray values of 169.517, 37.744, 89.709, and 204.32, respectively.

Sealing effect on root dentin. Root dentin slices were treated in NaCl (0.9%), SBF (pH 7.40), and PBS (pH 7.40) buffer for 24 hours at 37°C, and the surface conditions of the dentin slices were observed under SEM. The one not treated with anything showed numerous open dentinal tubules approximately 2-μm in diameter (Fig. 3A-i, B-i, C-i). Once the dentin slice was soaked in 0.9% NaCl solution without any sealing agent, slight changes in the tubular dimensions were observed (Fig. 3A-ii). After treatment with sealer (Fig. 3A-iii), it was found to have partial sealing and opening of the orifices with tubular dimensional changes. GO-treated dentin (Fig. 3A-iv) showed complete sealing whereas GO-CaF_2 (Fig. 3A-v) showed some open tubules. On the other hand, GO-Ag-CaF_2 (Fig. 3A-vi)-treated dentin slices showed a sealed surface along with some additional deposits on the surface.

A similar condition was found with the number of open tubules in untreated dentin (Fig. 3B-ii) in PBS. However, sealer (Fig. 3B-iii)-treated dentin slices showed some dimensional changes and a sealing effect in PBS with some visible open tubule orifices. GO (Fig. 3B-iv) showed some sealing effect, but open tubules could nonetheless be seen. On the other hand, GO-CaF_2 (Fig. 3B-v) and GO-Ag-CaF_2 (Fig. 3B-vi) showed complete sealing, where GO-Ag-CaF_2 showed a huge additional deposition on its surface. In SBF, untreated dentin (Fig. 3C-ii) showed some sealing potential, but a sealer-treated (Fig. 3C-iii) dentin slice showed the same tendency as that shown in NaCl and PBS. GO (Fig. 3C-iv) has a potential sealing effect in SBF as well. GO-CaF_2 (Fig. 3C-v) also showed a sealing effect. GO-Ag-CaF_2 (Fig. 3C-vi) showed a sealing effect

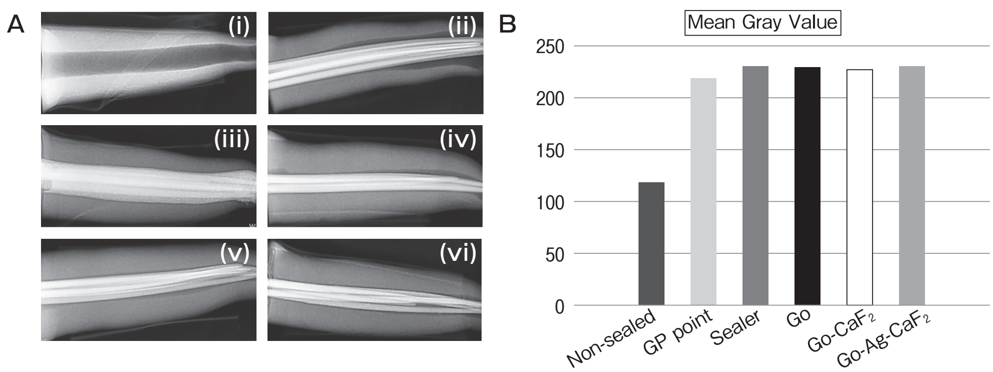

Fig. 1 A, X-ray of the root canal. Images at 60 kV and 10 mA, for 0.12 s at a density setting of 2 using a standardized focus-to-sensor distance of 30 cm in perpendicular view. **(i)** Intact (non-sealed); **(ii)** Filled with GP points only; **(iii)** Nishika Canal Sealer N (NCSN)-sealed with GP points; **(iv)** GO-sealed with GP points; **(v)** GO-CaF_2-sealed with GP filler; **(vi)** GO-Ag-CaF_2-sealed with GP filler. (An optical bench was used to standardize the geometric projection); B, The Mean Gray Value of ROIs.

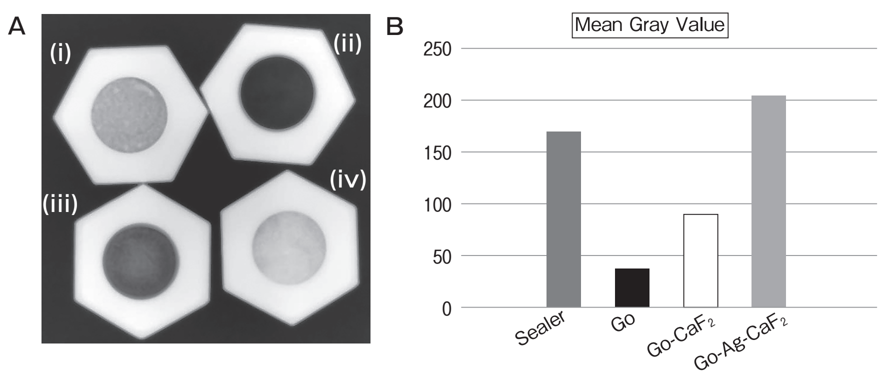

Fig. 2 A, X-ray of the specimens. Images at 60 kV and 10 mA, for 0.12 s at density 2 using a standardized focus-to-sensor distance of 30 cm in perpendicular view. **(i)** Nishika Canal Sealer N (NCSN)-treated specimen showing dense radiopacity and an identical outline; **(ii)** GO-treated specimen showing complete radiolucency **(iii)** CO-CaF_2-treated specimen showing slight radiopacity with marked haze radiolucency **(iv)** GO-Ag-CaF_2-treated specimen showing dense radiopacity with an identical outline. (An optical bench was used to standardize the geometric projection); B, Mean Gray Value of ROIs.

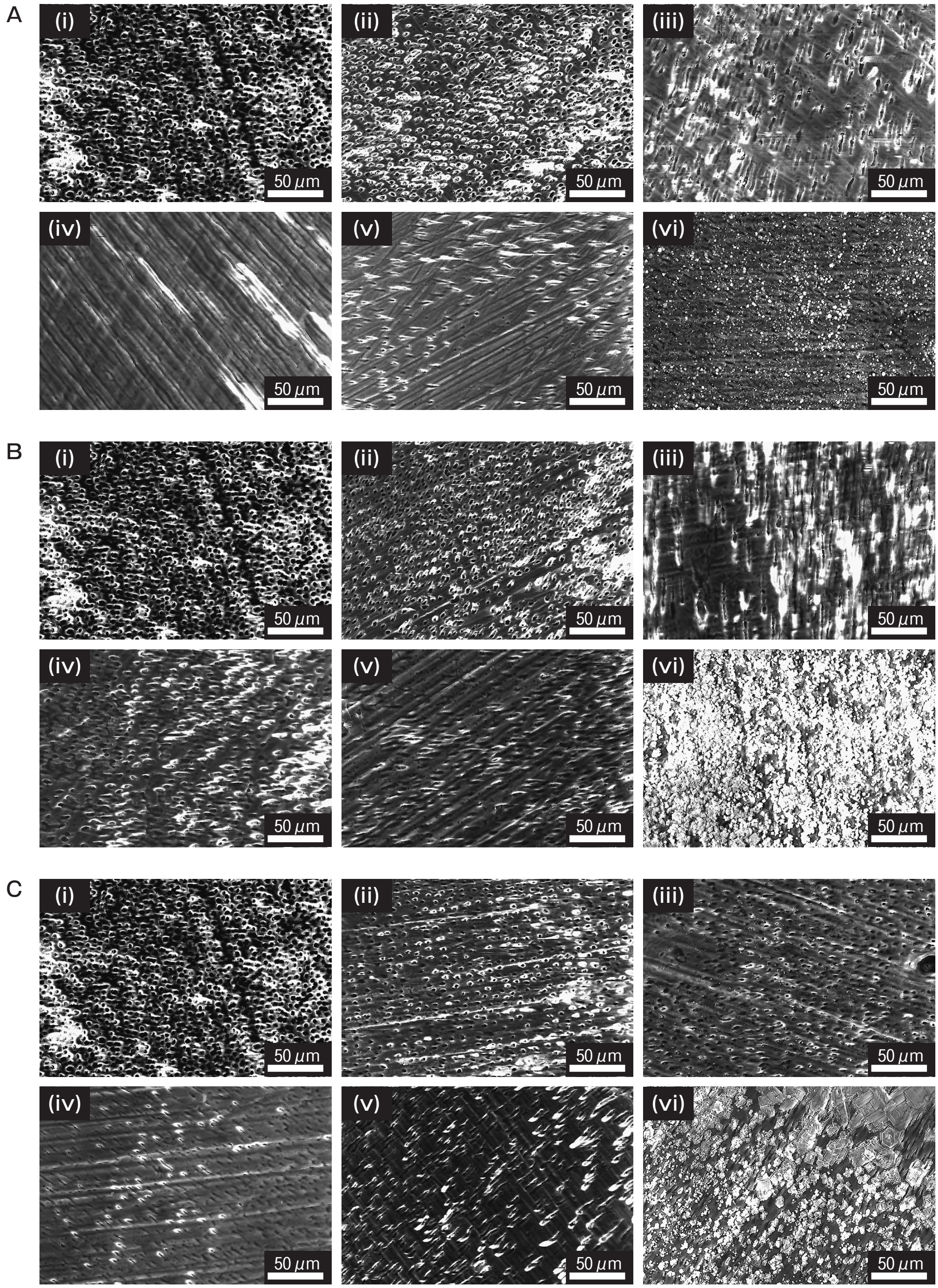

Fig. 3 A, SEM views of dentin slices soaked in NaCl (0.9%). **(i)** Intact dentin slice (non-treated); **(ii)** non-coated dentin slice; and **(iii)** NCSN-coated; **(iv)** GO-coated; **(v)** GO-CaF_2-coated; and **(vi)** GO-Ag-CaF_2-coated. dentin slices. The scale bar shows 50 μm; B, SEM views of dentin slices soaked in PBS (pH 7.40). **(i)** Intact dentin slice (non-treated); **(ii)** non-coated dentin slice; and **(iii)** NCSN-coated; **(iv)** GO-coated; **(v)** GO-CaF_2-coated; **(vi)** GO-Ag-CaF_2-coated dentin slices. The scale bar shows 50 μm; C, SEM views of dentin slices soaked in SBF (pH 7.40). **(i)** Intact dentin slice (non-treated); **(ii)** non-coated dentin slice; and **(iii)** NCSN-coated; **(iv)** GO-coated; **(v)** GO-CaF_2-coated; **(vi)** GO-Ag-CaF_2-coated dentin slices. The scale bar shows 50 μm.

as well as a crystal deposition, which is evidence of bioactivity.

Discussion

An ideal root canal sealer should be biocompatible and well tolerated by the peri-radicular tissues [8]. Commonly used sealers exhibit some degree of toxicity when freshly mixed; however, their toxicity is greatly reduced by setting [27]. Apart from its biocompatibility, a sealer's antimicrobial activity is the critical characteristic for the success of root canal treatment [28]. Each of the new-generation sealers claims a broader-spectrum antimicrobial efficacy [28]. It has been demonstrated that sealer material based on zinc oxide-eugenol releases potentially cytotoxic concentrations of eugenol. Calcium hydroxide-based sealers promote calcification but tend to dissolve over time and compromise the endodontic seal. Glass ionomer sealers may bond with tooth structure, but may also activate the release of prostaglandins in periapical tissues [29]. Among resin-based sealers, Epiphany™ root canal sealer has been the only material to present intraosseous biocompatibility [30,31]. The materials used in this study were previously reported to have excellent antimicrobial activity against cariogenic bacteria *Streptococcus mutans* and cytocompatibility with human epithelial cells (HeLa cells) [21].

The sealer used in endodontic treatment should have some radiopacity so that it can be seen on a radiograph and contrast with dentin and bone??? [13,32]. However, the sealer should not be more radiopaque than the core material (GP) and thereby mask any voids or imperfections of the obturation [33]. It is assumed that greater radiopacity might give the impression of a tight, compact filling, despite the existence of gross imperfections. On the other hand, a less radiopaque material might be assessed as absent where it is in fact present in small amounts [13,17]. In this study, GO-Ag-CaF_2 showed perfect radiopacity in contrast to dentin and radiopaque core materials (GP point), complying with the ideal radiopaque properties of sealer materials. However, the GO showed very negligible radiopacity. In the prepared materials-vs.-sealer contrast radiograph, it was clearly observed that the commercial sealers, GO-CaF_2, and GO-Ag-CaF_2, had similar radiopacity. Thus, comparing radiopacity in contrast to dentin, radiopaque core materials, and commercial sealer opacity, it can be stated that GO-CaF_2 and GO-Ag-CaF_2 have ideal characteristics of radiopacity for sealer materials. The mean gray values of all tested materials showed acceptable radiopacity in contrast to commercial sealers as well as a 2-mm aluminum stoppage mean gray value [34-37].

In general, root canal sealers are used in conjunction with a biologically acceptable semi-solid or solid obturating material to establish an adequate seal of the root canal system [38]. The commonly used core material is GP, which occupies the bulk of the canal space while the root canal sealer fills the interface between the core material and the dentin wall, the voids in the core materials, the accessory canals, and irregularities [6]. Root canal sealers usually overcome the limitations of GP points and obturation techniques by filling the space between the GP and the dentinal wall. Sealers that possess the excellent sealing capability and bactericidal activity would be clinically advantageous [39]. Adhesion of sealers to dentin and sealing of the orifices of the dentinal wall is important to seal the root canal system thoroughly and prevent incursion of endogenous and exogenous fluids. In our study, GO-based materials showed a coated layer on the dentin surface and the orifice of the dentinal tubules. GO-Ag-CaF_2 almost entirely covered the dentin surface as well as showing numerous mineral depositions on the dentin surface. Hence, it can be assumed that GO-Ag-CaF_2 is capable of sealing the orifice of the dentinal tubules and dentin surface as well as contributing to regeneration of apatite (dentin) like structures. The stability and bonding of these GO-based compounds to dentin were also confirmed in the early study under demineralization and acidic conditions [21]. Although this NCSN has been widely used as an endodontic sealer in clinical dental practice, its surface sealing ability has not yet been demonstrated. In contrast, GO-coated dentin was covered by a layer, resulting in the dentinal tubule seeming to be effectively sealed under 0.9% NaCl, SBF, and PBS conditions. Conversely, the dentin slices coated with GO-CaF_2 seemed to be almost covered, but a few unsealed dentinal tubules were observed. Meanwhile, GO-Ag-CaF_2 almost entirely covered the dentin surface in all conditions. In addition, there was mineral deposition on the dentin surface treated with GO-Ag-CaF2. These depositions correspond to apatite formation on the dentin surface confirming this material's bioactive characteristics as well.

In conclusion, GO-Ag-CaF_2 showed a combination of properties needed to be successfully utilized in a dental application. Namely, its dentin bond and sealing, antimicrobial activity, biocompatibility, radiopacity, and bioactivity all suggest its promise as a novel endodontic sealer for use in endodontic treatment and regeneration. However, this *in vitro* study had some limitations. Thus, future *in vivo* studies are expected to evaluate durability and the regenerative effects using normal and infected root canal models and to optimize the application conditions.

References

1. Gagliani MM, Gorni FGM and Strohmenger L: Periapical resurgery versus periapical surgery: a 5-year longitudinal comparison. Int Endod J (2005) 38: 320–327.
2. Hancock HH 3rd, Sigurdsson A, Trope M and Moiseiwitsch J: Bacteria isolated after unsuccessful endodontic treatment in a North American population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod (2001) 91: 579–586.
3. Bystrom A and Sundqvist G: The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J (1985) 18: 35–40.
4. Siqueira JF Jr, Magalhães KM and Rôças IN: Bacterial reduction in infected root canals treated with 25% NaOCl as an irrigant and calcium hydroxide/camphorated paramonochlorophenol paste as an intracanal dressing. J Endod (2007) 33: 667–672.
5. Kapralos V, Koutroulis, A, Ørstavik D, Sunde PTand Rukke HV: Antibacterial activity of endodontic sealers against planktonic bacteria and bacteria in biofilms. J Endod (2017) 44: 149–154.
6. Salz U, Poppe D, Sbicego S and Roulet JF: Sealing properties of a new root canal sealer. Int Endod J (2009) 42: 1084–1089.
7. Hargreaves K, Berman L and Rotstein I: Cohen's pathways of the pulp, 11th Ed, Elsevier, St Louis, Missouri (2015) pp 2–24.
8. Orstavik D: Materials used for root canal obturation: technical, biological and clinical testing. Endod Topics (2005) 12: 25–38.
9. Sjogren U, Figdor D, Persson S and Sundqvist G: Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J (1997) 30: 297–306.
10. Desaiand S and Chandler N: Calcium hydroxide-based root canal sealers: a review. J Endod (2009) 35: 475-480.
11. Okabe T, Sakamoto M, Takeuchi H and Matsushima K: Effects of pH on mineralization ability of human dental pulp cells. J Endod (2006) 32: 198–201.
12. Šimundić MM, Poklepović PT, Utrobičić A, Bago I and Puljak L: Antimicrobial efficacy of commercially available endodontic bioceramic root canal sealers: A systematic review. PLoS One (2019) 14: e0223575.
13. Beyer-Olsen EM and Orstavik D: Radiopacity of root canal sealers Oral Surg Oral Med Oral Pathol (1981) 51: 320–328.
14. Imai Y and Komabayashi T: Properties of a new injectable type of root canal filling resin with adhesiveness to dentin. J Endod (2003) 29: 20–23.
15. Katz A, Kaffe I, Littner M, Tagger M and Tamse A: Densitometric measurement of radiopacity of Gutta-percha cones and root dentin. J Endod (1990) 16: 211–213.
16. McComb D and Smith DC: Comparison of physical properties of polycarboxylate-based and conventional root canal sealers. J Endod (1976) 2: 228–235.
17. Gambarini G, Testarelli L, Pongione G, Gerosa R and Gagliani M: Radiographic and rheological properties of a new endodontic sealer. Aust Endod J (2006) 32: 31–34.
18. Nizami MZI, Takashiba S and Nishina Y: Graphene oxide: A new direction in dentistry. Appl Mater Today (2020) 19: 100576.
19. Pawar SS, Pujar MA and Makandar SD: Evaluation of the apical sealing ability of bioceramic sealer, AH plus & epiphany: An *in vitro* study. J Conserv Dent (2014) 17: 579–582.
20. Kim DS, Park SH, Choi GW, Choi KK and Kim SY: Effect of EDTA treatment on the hybrid layer durability in total-etch dentin adhesives. Dent Mater J (2011) 30: 717–722.
21. Nizami MZI, Nishina Y, Yamamoto T, Shinoda-Ito Y and Takashiba S: Functionalized graphene oxide shields tooth dentin from decalcification. J Dent Res (2020) 99: 182–188.
22. Morimoto N, Suzuki H, Takeuchi Y, Kawaguchi S, Kunisu M, Bielawski CW and Nishina Y: Real-time, in situ monitoring of the oxidation of graphite: Lessons learned. Chem Mater (2017) 29: 2150–2156.
23. Freitas P, Yaedú RYF, Rubira-Bullen IRF, Escarpinati M, Vieira MC, Schiabel H and Lauris JR: Reproducibility of pixel values for two photostimulable phosphor plates in consecutive standardized scannings. Braz Oral Res (2006) 20: 207–213.
24. Wenzel A: Sensor noise indirect digital imaging (the RadioVisioGraphy, Sens-a-Ray, and Visualix/Vixa systems) evaluated by subtraction radiography. Oral Surg Oral Med Oral Pathol (1994) 77: 70–74.
25. Rubira-Bullen IRF, Escarpinati MC, Schiabel H, Vieira MA da C, Rubira CMF and Lauris JRP: Evaluating noise in digitized radiographic images by means of histogram. J Appl Oral Sci (2006) 14: 410–414.
26. Nelvig P, Wing K and Welander U: Sens-A-Ray A new system for direct digital intraoral radiography. Oral Surg Oral Med Oral Pathol (1992) 74: 818–823.
27. Langeland K: Root canal sealants and pastes. Dent Clin North Am (1974) 18: 309–327.
28. Tanomaru JMG, Tanomaru-Filho M, Hotta J, Watanabe E and Ito IY: Antimicrobial activity of endodontic sealers based on calcium hydroxide and MTA. Acta Odontol Latinoam (2008) 21: 147–151.
29. Khashaba RM, Chutkan NB and Borke JL: Comparative study of biocompatibility of newly developed calcium phosphate-based root canal sealers on fibroblasts derived from primary human gingiva and a mouse L929 cell line. Int Endod J (2009), 42: 711–718.
30. Sousa CJA, Montes CRM, Pascon EA, Loyola AM and Versiani MA: Comparison of the intraosseous biocompatibility of AH Plus, EndoREZ, and Epiphany root canal sealers. J Endod (2006) 32, 656–662.
31. American Dental Association Specification no 57; Endodontic sealing materials; National Standards Institute, ANSI/ADA, Chicago (2000).
32. Baksi Akdeniz BG, Eyüboglu TF, Sen BH and Erdilek N: The effect of three different sealers on the radiopacity of root fillings in simulated canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod (2007) 103: 138–141.
33. Gorduysus M and Avcu N: Evaluation of the radiopacity of different root canal sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod (2009) 108: e135–e140.
34. Ay S, Gursoy UK, Erselcan T and Marakoglu I: Assessment of mandibular bone mineral density in patients with type 2 diabetes mellitus. Dentomaxillofac Radiol (2005) 34: 327–331.
35. Knezovic-Zlataric D and Celebic A: Mandibular bone mineral density changes incomplete and removable partial denture wearers: a 6-month follow-up study. Int J Prosthodont (2003) 16: 661–665.
36. McArthur DR and Taylor DF: A determination of the minimum radiopacification necessary for radiographic detection of an aspirated or swallowed object. Oral Surg Oral Med Oral Pathol (1975) 39, 329–338.
37. Nesrin D, Pelin G, Erinç O and Hayal, B: Construction of a copper stepwedge with aluminum equivalent mean gray values. J Appl Oral Sci (2010) 18: 390–396.
38. Carrotte P: Endodontics—a series overview. Br Dent J (2004) 197: 179.
39. Shin JH, Lee DY and Lee SH: Comparison of antimicrobial activity of traditional and new developed root sealers against pathogens related root canal. J Dent Sci (2018): 13: 54–59.