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Radiopacity quantification and spectroscopic characterization of OrthoMTA and RetroMTA

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Abstract

The aim of this article was to investigate the unknown radiopacity performances of OrthoMTA and RetroMTA via means of a contemporary image analyzing methods and energy dispersive X-ray spectroscopy. Three commercial hydraulic calcium silicate-based cements and a calcium hydroxide-based paste were used. Pure-grade zirconium oxide, bismuth oxide, zinc oxide, barium sulfate, and calcium hydroxide were as references. An energy-dispersive X-ray spectroscopy instrument was used for the elemental analysis. Radiographic image data was obtained according to the International Organization for Standardization 6876-2012 specifications. The region of interest was determined for each specimen. Mean (±SD) grey values of the X-ray image data was measured with an image analyzing software. The calibration curve was created by curve-plotting software and the mean grey-values were matched versus Al values (mm Al). Data were analyzed with one-way ANOVA followed by Tukey's multiple comparison test. Spectroscopic characterization of the commercial materials was shown with assigned Carbon, oxygen, aluminum, silicon, calcium, zinc, zirconium, barium, tungsten, bismuth, and sulfur elements. The major radiopacifier/s of OrthoMTA is Bi, of RetroMTA is Zr, of BioDentine are Ba and Zr, and of ProCal is Ba. The radiopacity values of all commercial materials are significantly different ($p < .05$). The rank of the radiopacity values: RetroMTA (3.36 ± 0.29mmAl) > OrthoMTA (2.56 ± 0.19mmAl) > BioDentine (2.02 ± 0.12mmAl) > ProCal (1.46 ± 0.60mmAl). The study reported that the radiopacity values and spectral characterization of RetroMTA and OrthoMTA cements. The pixel-based and reproducible method could be used universally to the quantification of the radiodensity of digitally collected X-ray data.

KEYWORDS
BioDentine, EDS, OrthoMTA, radiopacity, RetroMTA

1 | INTRODUCTION

Calcium hydroxide (CH) is widely used in Dentistry since 1920 (Hermann, 1920). CH has been included within varied dental materials for a century due to its beneficial properties both in soft and hard dental tissues. Currently, CH is still used for treatment modalities in such as pulp capping, pulpotomy, revitalization, pulpectomy, root resorptions, and dental trauma (Manfredi, Figini, Gagliani, & Lodi, 2016; Mohammadi & Dummer, 2011). Although CH is widely used in dentistry, CH formulations leave place to calcium silicate-based materials due to their superior properties than CH such as adhesion, low-solubility, and bioactivity.

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Dental bioceramic cements or hydraulic calcium silicate-based cements (CSCs) are bioactive filling and repair materials. CSCs have successfully used in dental operations over two decades thanks to its physico-chemical and biological properties (Wang, 2015). The first commercial representative of the Endodontic CSC is "Mineral trioxide aggregate" (MTA, Dentsply-Sirona, Tulsa, OK). MTA is known as the "gold-standard" material due to has mostly ideal reparative material properties (Camilleri & Pitt Ford, 2006; Parirokh & Torabinejad, 2010; Wang, 2015). Meanwhile, MTA successors or counterparts are mentioned as "MTA-like materials" (Gandolfi, Siboni, & Prati, 2012). Essentially, MTA-like cements or CSCs release Ca^{2+} ions, the structure of CSCs present Ca^{2+} conductivity, and biocompatible for stem-cells. Thus, calcified tissue formation adjacent to the CSCs occurs thanks to the bioactivity of CSCs (Gandolfi, Shah, Feng, Prati, & Akintoye, 2011).

Biodentine (Septodont, Saint-Maur-des-Fosses, France) is an MTA-like cement. Mainly, Biodentine acts as a "dentin substitute" in the coronal and radicular region of the dental tissues due to its advantages such as short setting time, controlled handling properties, improved adhesive and mechanical properties. According to its manufacturer, the powder consists of tricalcium silicate, calcium carbonate, and zirconium oxide. The manufacturer claims that ZrO_2 is a radiocontrast additive of the cement (BioDentine Product Brochure). Biodentine liquid contains calcium chloride suspension as an accelerator substrate of its hydration reaction (Grech, Mallia, & Camilleri, 2013; Wang, 2015). Appropriately to the International Organization for Standardization (ISO) 6876/2001, Biodentine demonstrates a radiopacity of more than 3 mm thickness of Al (Grech et al., 2013), which means it is a convenient material for root canal fillings and root repairing.

RetroMTA (BioMTA, Yuseong-gu, Daejon, Korea) is another MTA-like cement material. Fundamentally, RetroMTA possesses the fastest initial hardening reaction time after hydrated among its counterparts. Therefore, it is a suitable CSC for the vital pulp therapies and root repair. According to its manufacturer, RetroMTA consists of calcium carbonate, silicon oxide, aluminum oxide, hydraulic calcium zirconia complex, and water (Orhan, Irmak, & Mumcu, 2019). OrthoMTA (BioMTA, Yuseong-gu, Daejon, Korea) is also MTA-like cement material. On contrary to RetroMTA or BioDentine, OrthoMTA presents a long-hardening reaction time. Therefore, OrthoMTA is a suitable CSC for orthograde root canal filling or obturation instead of the vital pulp therapies and root repair. According to its manufacturer, the powder consists of tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetra calcium aluminoferrite, free calcium oxide, and bismuth oxide (Parirokh, Torabinejad, & Dummer, 2018). However, there is no available information about the radiodensity of neither RetroMTA nor OrthoMTA.

Radiocontrast agents reduce X-ray therefore, they are added into the compositions of dental materials for distinguishing dental tissues on radiographs. Thus, a practitioner could check the radiopacity featured materials via radiographic techniques in terms of proper or improper placement. If a dental material has a radiolucent character, therefore, radiocontrast or radiopacifier agents are added to detect by radiography. Therefore, the quantification of the radiodensity of MTA-like cements makes a clinical significance.

Mostly, barium sulfate is added to CH for this purpose (Uslu, Dikmen, & Orhan, 2020). However, different formulations of MTA-like cements use vary radiocontrast ingredients. The aim of this article was to investigate the unknown radiopacity performances of OrthoMTA and RetroMTA via means of a contemporary image analyzing methods and energy dispersive X-ray spectroscopy.

2 | MATERIALS AND METHODS

Three commercial CSCs and a radiopaque CH-based dental material were used for this study: The commercial CSC were OrthoMTA (Lot#OM1503D07, BioMTA, Yuseong-gu, Daejon, Korea), RetroMTA (Lot# RM1605D12, BioMTA, Yuseong-gu, Daejon, Korea), and Biodentine (Lot#B17299, Septodont, Saint-Maur-des-Fosses, Cedex, France). The commercial radiopaque CH-based material was ProCal (CE 1014, Lot#180216, Promida Co., Eskisehir, Turkey) . The powder was hand-mixed with its liquid (glycerin) and the mixture ratio of powder/vehicle was 1:1.25 (wt/wt) in accordance with the manufacturer's instructions (ProCal Product Brochure). The information on the commercial tested materials and instructions for handlings are listed in Table 1.

Pure-grade reference molecules in powder forms were used: ZrO_2 (Lot# HT0Z3YG4, Advanced Technology Industry Co., Eskişehir, Turkey), ZnO (CAS 1314-13-32, Hepşen Kimya, İstanbul, Turkey), Bi_2O_3 (Lot#1172065, ABCR GmbH &Co KG, Karlsruhe, Germany), $Ca(OH)_2$ (Lot# K45063410402, Merck KGaA, Darmstadt, Germany) and $BaSO_4$ (Lot# K48556550, Merck KGaA, Darmstadt, Germany) were as references.

2.1 | Radiographic data collection and image processing

Radiographic image data were obtained according to the ISO 6876:2012 specifications that specify the requisites and test methods for root canal filling materials. A pure-grade Al step-wedge was used as a reference. A photostimulable phosphor plate (Size #3, Planmeca Oy, Helsinki, Finland) was used as an image sensor. The dimensions of the sensor plate are 27×54 mm. The number of pixels is $771 \times 1{,}542$, and the pixel size is 35 μm in the default high-resolution-mode.

Custom radiolucent cylindrical molds (<0.1 mm Al) was prepared for the standardization of the groups. The dimension of each mold is 2 mm diameter and 1 mm thickness verified by a pachymeter (Mitutoyo Corp., Tokyo, Japan; Figure 1**)**. A Mylar strip was placed on the bottom of the mold to allow specimen filling (Supporting Information File 1). Specimens were mixed in accordance with the manufacturers' instructions and filled into the molds using a carrier (Biofilling kit, BioMTA). The filled molds were placed in a sealed container and were stored at 37°C for 24 hr in 100% humidity.

For each X-ray irradiation, a control specimen, a test specimen, and step-wedge were positioned on a plate sensor together (Figure 1). The specimens were irradiated using a dental X-ray unit (ProX,

TABLE 1 The information on the commercial tested materials and instructions for handlings are given

Group	Manufacturer	Powder compositions	Radiopacifier	Powder color	Liquid	Lot #	Setting time	Manufacturer's mixing instruction
OrthoMTA	BioMTA, Yuseong-gu, Daejon, Korea	Tricalcium silicate, Dicalcium silicate, Tricalcium aluminate, Tetracalcium aluminoferrite, Free calcium oxide, Bismuth oxide	Bismuth oxide	White	Distilled water	OM1503D07	Initial: 180 min Final: 360 min.	Sterile water is added into powder contained tube then the mixture is stirred by a clean applicator for 20 s. Excess water is removed with a cotton swab. Then apply it with the carrier of the "Biofilling kit" manufactured by BioMTA.
RetroMTA	BioMTA, Yuseong-gu, Daejon, Korea	Calcium carbonate, Silicon oxide, Hydraulic calcium zirconia complex, Aluminum oxide	Zirconium oxide	White	Distilled water	RM1605D12	Initial: 150–180 s. Final: 360 min	The 0.3 g powder pours onto the three drops of distilled water and wets it gently for 20 s. Waited until the shiny surface disappears, then the gel-form is applied with a carrier.
BioDentine	Septodont, Saint-Maur-des-Fosses, Cedex, France	Tricalcium silicate, dicalcium silicate, calcium carbonate, zirconium oxide, calcium oxide, iron oxide	Zirconium oxide	White	0.2 g water-based calcium chloride as the setting accelerator	B17299	Initial: 9-12 min Final: 45 min.	Five drop liquid is poured into the single-dose container of BioDentine, then the container is mixed for 30–60 s using a 4,000–4,200 rpm mixer.
ProCal	Promida Co., Eskişehir, Turkey	Calcium hydroxide, Barium sulfate	Barium sulfate	White	Glycerin	180,216	Non-set	The mixture ratio of powder/liquid is 1:1.25 (wt/wt). The paste-form is obtained by mixing the powder with liquid to a creamy consistency.

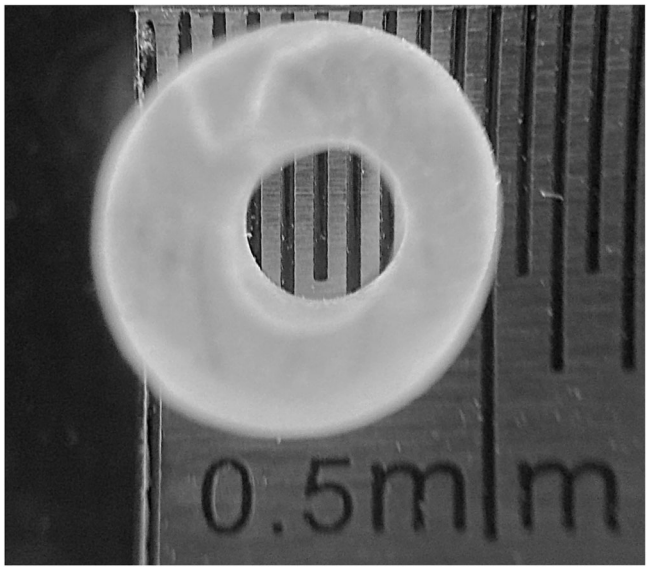


FIGURE 1 A representative image of the dimensions of mold at ×15 magnification (Peak 1962 loupe, peak optics, Tokyo, Japan)

Planmeca Oy) with 66 kV, 0.8 mA, 0.32 s exposure time parameters. The source-to-specimen distance was set at 30 cm. Radiographic image data were taken by a single operator. Total of 12 radiographic image data was collected for each test group.

Irradiated plate sensors were scanned with an imaging plate scanner (Proscanner, Planmeca Oy) during 7.2 s in high-resolution mode. The theoretical resolution is 14.3 lp/mm. Radiographic image data was exported to an 8-bit non-compressed file format (tagged image file format) from integrated viewer software (Romexis Viewer, Planmeca Oy). Image data was displayed at 13.3″ flat monitor and was analyzed on a macOS Catalina 10.15.5 computer (Apple Inc., Cupertino, CA) equipped with Intel Iris Plus Graphics 645 graphic card at 2,560 × 1,600 resolution (Figure 2).

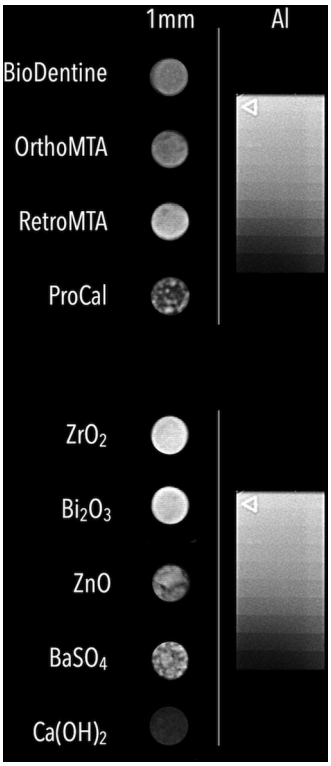


FIGURE 2 A representative X-ray image data shows of each test specimen and Al step-wedge

2.2 | Calculations and expression of results

Mean (±SD) grey values of the radiographic images were measured with an image analyzing software (Image J v1.5e, National Institute of Health, Bethesda, MD). First, a region of interest (ROI) was determined on the images. Care was taken to select the maximum surface area, provided that ROI was not too close to the edges, as X-ray absorption on the edges could be slightly different from that of the bulk of the object and could yield a more radiopaque image. For the circular specimens, a circular ROI with an area of 3,412 pixels was determined per specimen. For the step-wedge, a rectangular ROI (167,256 total pixels) was determined for each increment of the step-wedge.

After ROI determination, mean (±SD) grey value of ROI of each increment of the step-wedge and mean (±SD) grey ROIs of circular specimens were calculated with the software. The curve of the mean grey-values versus the thickness of each increment of the step-wedge was plotted using a curve fitting software (Curve Expert Pro, version 2.6.5, Hyams Development, curveexpert.net, Huntsville, AL). Finally, this calibration curve was used to interpolate the equivalent thickness Al values (mm Al) and their SDs for each circular specimen. The formula of quadratic function curve was below:

$$y = \rho\omega : a + bx + cx^2 + dx^3$$

2.3 | Spectroscopic characterization

The topographic features for each tested commercial material were examined using a high-resolution field emission scanning electron microscope (FE-SEM, Hitachi Regulus 8,230, Hitachi High-Tech Co., Tokyo, Japan). Visualization conditions were as follows: Accelerating voltage: 10.0 kV, magnification: x2.00k, and width: 11.6 mm at high-vacuum pressure. Prior to the FE-SEM examination, each sample was attached to metal stubs using carbon tape without coating application.

An energy-dispersive X-ray spectroscopy instrument (EDS) was used for the elemental analysis (Ultim Extreme, Oxford Instruments, High Wycombe, UK). All EDS data were collected at the central region of the image surface (50 μm × 50 μm area at ×2.00k magnification) of each material. Regarding the spectroscopic characterization of the commercial materials; Carbon, oxygen, aluminum, silicon, calcium,

zinc, zirconium, barium, tungsten, bismuth, and sulfur were assigned to their spectra using a software.

2.4 | Statistical analysis

Gray-values versus the thickness of each step of the step-wedge were analyzed with a curve fitting software (Curve Expert Pro, version 2.6.5, Hyams Development, curveexpert.net, Huntsville, AL). Mean (±SD) radiopacity values expressed as aluminum thickness (mm Al) of the CSC materials and powders were then interpolated using the calibration curve generated by the software. For each group, weighted mean and pooled SDs were calculated and data were analyzed with one-way ANOVA and Tukey's multiple comparison test (Prism 6.0, GraphPad Software, La Jolla, CA; $\alpha = .05$).

3 | RESULTS

3.1 | Radiopacity quantification

Data revealed normal distribution. The mean mm Al plot of the radiopacity values of CSCs and reference molecules are shown in Figure 3. All commercial materials were compared and the Mean ± SD of the radiopacity values of tested groups (mm Al) are given in Table 2. In multiple comparisons revealed that the radiopacity values of all groups are significantly different from each other ($p < .05$). RetroMTA showed the highest radiopacity values than other references ($p < .05$). The tested groups revealed the following rank in the radiopacity values: RetroMTA (3.36 ± 0.29 mm Al) > OrthoMTA(2.56 ± 0.19 mm Al) > BioDentine (2.02 ± 0.12 mm Al) > ProCal(1.46 ± 0.60 mm Al).

All reference molecules were compared and the Mean ± SD of the radiopacity values of reference agents (mm Al) are given in Table 3. In multiple comparisons revealed that the radiopacity values of all references are significantly different from each other ($p < .05$). ZrO_2 showed the highest radiopacity values than other references ($p < .05$). The references revealed the following rank in the radiopacity values: ZrO_2 (5.67 ± 0.04 mm Al) > Bi_2O_3 (4.78 ± 0.06 mm Al) > $BaSO_4$ (3.50 ± 0.12 mm Al) > ZnO (1.13 ± 0.08 mm Al) > $Ca(OH)_2$ (0.19 ± 0.02 mm Al).

3.2 | Spectroscopic characterization

In the topographic examination, it was observed that each hydrated CSC was homogeneous. The EDS results showed that the chemical composition of tested materials differed (Figure 4**)**. The quantitative results of EDS analysis (wt%) for the commercial materials are given in Table 4. The major radiopacifier/s of OrthoMTA is Bi, of RetroMTA is Zr, of BioDentine are Ba and Zr, and of ProCal is Ba. The amount of Zr and Ba elements in the OrthoMTA were lower than RetroMTA and BioDentine whereas, the amount of Bi in the OrthoMTA was higher than the rest of the groups. The Ca/radiocontrast media ratio of OrthoMTA, RetroMTA, BioDentine, and ProCal are 9.89, 6.22, 7.68, and 10.01, respectively.

4 | DISCUSSION

The present study investigated the first time radiopacity properties of "OrthoMTA" and "RetroMTA" using image analysis and energy-dispersive X-ray spectroscopy. American National Standard / American Dental Association specification #57 prescribes tests to measure the radiopacity of Endodontic sealing materials; these tests were adopted by the ISO 6876:2012 specifications (American National Standard/American Dental Association specifications No. 57 (ANSI/ADA no:57); ISO 6876:2012. Nevertheless, there is no specification or international standard for testing the radiopacity of CSCs. Therefore, ISO 6876 and ANSI/ADA#57 regulations cover the CSCs type of dental materials for analysis and set-up designs of studies.

Various test methods are designed to examine these clinically significant radiopacity properties of CSC materials. The above-mentioned international standards of root canal sealers are recommended to use an optic densitometer unit for measuring radiopacity of sealers on analog radiograms. However, contemporary dental radiography techniques are used digital X-ray images instead of analog

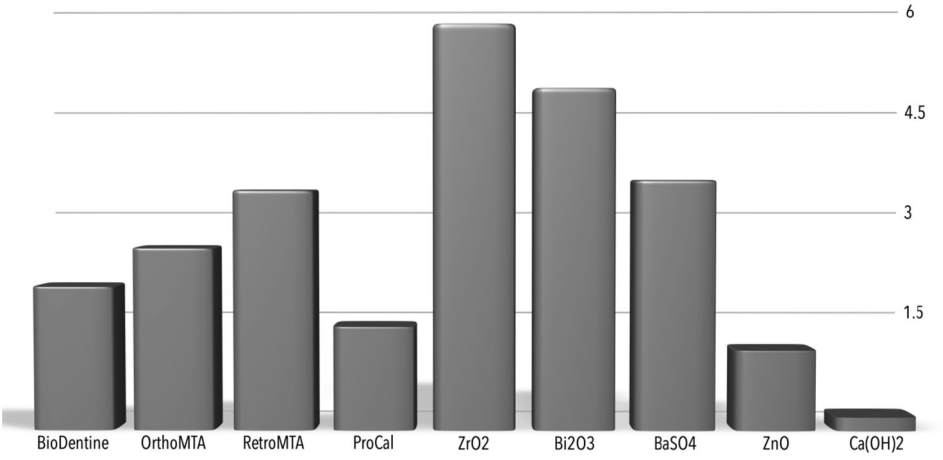


FIGURE 3 The mean mm Al plot of the radiopacity values of calcium silicate-based cements (CSCs) and reference molecules are shown

TABLE 2 Mean ± SD of the radiopacity values of tested groups (mm Al) are given

	BioDentine	OrthoMTA	RetroMTA	ProCal
Number of values	31,940	31,940	31,940	31,940
Mean	2.02[c]	2.56[b]	3.36[a]	1.46[d]
SD	0.12	0.19	0.29	0.60

Note: Means sharing a superscript letter are not significantly different ($p > .05$).

TABLE 3 Mean ± SD of the radiopacity values of reference agents (mm Al) are given

	ZrO_2	Bi_2O_3	$BaSO_4$	ZnO	$Ca(OH)_2$
Number of values	19,164	19,164	19,164	19,164	19,164
Mean	5.67[a]	4.78[b]	3.50[c]	1.13[d]	0.19[e]
SD	0.04	0.06	0.12	0.08	0.02

Note: Means sharing a superscript letter are not significantly different ($p > .05$).

radiograms and clinicians are making decisions with these digitalized X-ray image data.

In literature, image analyzing measurements have been utilized in numerous researches related to the medical and paramedical fields due to are capable to analyze high resolution images and huge data in a short time. (Dalen, Nootenboom, Vliet, Voortman, & Esveld, 2011; Flin, Brzoska, Lesaffre, Coleou, & Lamboley, 2011; Trong, Rozenbaum, Rouet, & Bruand, 2008). Digitalized X-ray image data was analyzed in the present study. First, the maximum surface area which is called ROI of the specimen was selected in this study. Then pixels in the selected ROI were dissolved to grey values (the pixels in interest were dissolved to grey values for each specimen). For the step-wedge, a rectangular ROI was determined for each increment of the step-wedge. Finally, the mean (±SD) grey value of specimens was matched with step-wedge. This pixel-based and reproducible method could be used universally to the quantification of the radiodensity of digitally collected X-ray data. By this way, quantification of the radiographic data will make it more clinically relevant.

Several devices and methods have been used to investigate in vitro radiopacity of these tested materials. In fact, variation in the specimen dimensions especially thickness might affect the radiopacity of medications and, consequently, the outcome of such these studies. All specimens were prepared exactly 1.00 mm thickness accordingly to the corresponding ANSI/ADA and ISO standards for Endodontic sealers. Furthermore, the irradiation parameters also complied with these standards. In the present study, multiple comparisons revealed that the radiopacity values of all tested materials are significantly different from each other. The results could be attributed to disclosed and non-disclosed radiocontrast agents with varied proportions of among the materials. There is no consensus about the type of the radiocontrast agent and their ratios in CSCs. Thus, the manufacturers of CSCs can use different radiocontrast media with varied proportions. However, the type of radiocontrast media can affect the physical properties of CSCs. Therefore, each material where is in the MTA-like cements pool should be considered separately due to fact that each CSC formulation is unique. Although there are no identified specific standards for CSCs or CH-based pastes, ISO 6876 or ANSI/ADA no:57 are usually followed for the associated researches. Therefore, it can be concluded that RetroMTA and OrthoMTA could be more distinguishable radiographically due to their higher radiopacity than those of BioDentine and ProCal.

In this study, the unknown spectral characteristic of radiocontrast contents of RetroMTA and OrthoMTA are shown. A dental material should have enough radiopacity that, in the radiograph, it could be distinguished from the surrounding dental tissues. For the CSCs, as they mostly are in contact with dentin, radiopacity values higher than dentin would be desirable (Cruz, Esteves, Poiate, Portero, & Almeida, 2014). According to ISO 4049, 1 mm-thick dentin has radiopacity value equivalent to 1 mmAl. In this study, all of the test materials exhibited higher radiopacity values than dentin, which conforms to ISO 4049, though this standard is for resin-based restorative materials. Since CSCs can also be used for vital pulp treatments, their radiopacity may be of importance when they are applied in coronal dentin (ISO 4049). ISO 6876 specifies that a root canal sealing material should have a minimum radiopacity value of 3 mmAl (ISO 6876). In this study, only RetroMTA (3.36 ± 0.29 mmAl) satisfied this requirement. This could be attributed to the Zr content of RetroMTA. In a material, elements with the higher atomic numbers bring higher radiopacity to that element (Cabasso, 2011). Atomic numbers for the radiopacifier elements detected in this experiment are; Al: 13, Zn: 30, Zr: 40, Ba: 56, Bi: 83. The Zn content of materials was very low; therefore, we speculate that it might not significantly contribute to the radiopacity of the materials. Bi, Ba, and Zr could have had a major impact on the radiopacity of the materials tested. In summary, RetroMTA (5.42% Zr, 0.38% Ba, 0.00%Bi) had the highest radiopacity value; followed by OrthoMTA (3.24% Bi, 0.01% Zr, 0.00% Ba) and BioDentine (2.80% Ba, 1.85 %Zr, 0.00% Bi). However, it is not quite possible to interpret the individual effects of the elements, since every material had varying amounts of the radiopacifying element. Therefore, we also measured the radiopacity of reference elements present in the CSC materials tested. According to our results radiopacity of the reference materials from the highest to the lowest were ZrO_2

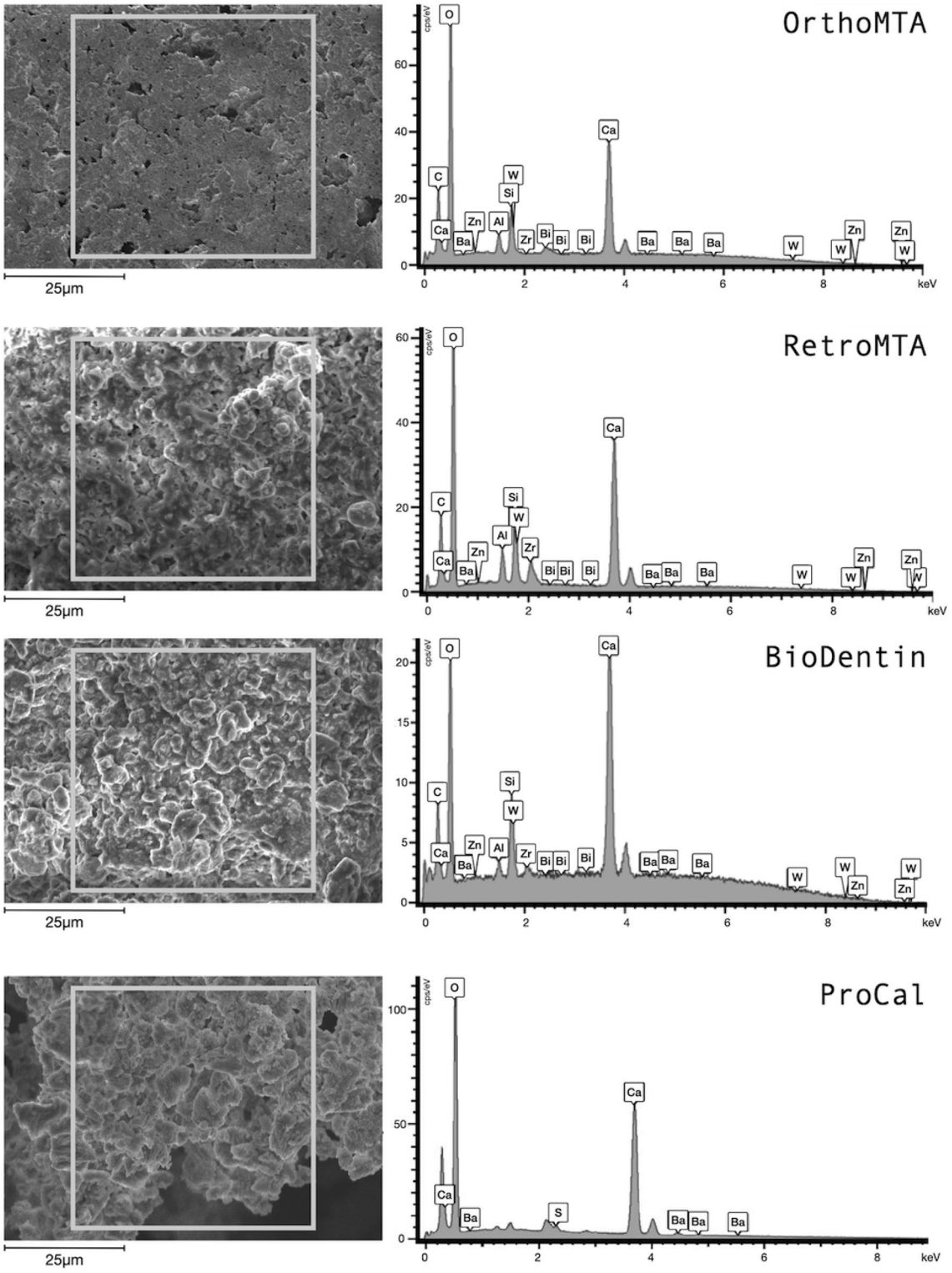


FIGURE 4 Representative EDX spectrum and FESEM images of each group at ×2,000 magnification

(5.67 ± 0.04), Bi_2O_3 (4.78 ± 0.06), $BaSO_4$ (3.50 ± 0.12; Table 4). This result could better correlate with the radiopacity values of the CSC materials tested. Since RetroMTA contained higher amounts of Zr, possibly in the form of ZrO_2, it had the highest radiopacity value. Although OrthMTA had only Bi as the main radiopacifier element, it was the second most radiopaque material tested. Its Bi content was possibly in the form of Bi_2O_3, which is the second most radiopaque compound. BioDentine had lower amounts of Zr compared to Retro MTA, although its Ba content was higher than that of the other CSCs tested. The Ba content of BioDentine did not result in higher

TABLE 4 The quantitative results of EDS analysis (wt% and wt% sigma) for the material groups are given

	Groups							
	OrthoMTA		RetroMTA		BioDentine		ProCal	
Elements	wt%	wt% sigma	wt%	wt% sigma	wt%	wt% sigma	wt%	wt% sigma
C	10.75	0.29	11.06	0.30	8.25	0.48	0.00	0.39
O	40.13	0.42	35.25	0.41	29.32	0.81	45.67	0.43
Al	1.61	0.08	2.26	0.08	0.80	0.12	0.00	0.11
Si	4.44	0.11	5.26	0.11	4.80	0.21	0.00	0.24
Ca	39.05	0.41	39.78	0.43	50.28	1.19	49.05	0.53
Zn	0.08	0.14	0.09	0.10	0.08	0.24	0.00	0.21
Zr	0.01	0.26	5.42	0.29	1.85	0.51	0.00	0.25
Ba	0.00	0.99	0.38	0.61	2.80	1.86	4.90	0.56
W	0.70	0.37	0.50	0.35	1.82	0.62	0.00	0.29
Bi	3.24	0.47	0.00	0.31	0.00	0.82	0.00	0.35
S	0.00	0.34	0.00	0.27	0.00	0.31	0.38	0.51
Total	**100.00**		**100.00**		**100.00**		**100.0**	

radiopacity due to the low radiopacity of $BaSO_4$ (Table 3). Interestingly, lowest radiopacity values were observed for ProCal, although it contained significant amounts of Ba, which were the highest amongst the tested materials (Table 4). This, again, could be attributed to the low radiopacity of BaSO4 than that of the other reference compounds. The amount of Ba in ProCal found in this study is in agreement with the information from its manufacturer. Interestingly, Ba content of BioDentine was not disclosed, neither by previous investigations nor by the manufacturer (Camilleri, Sorrentino, & Damidot, 2013; BioDentine product brochure: https://www.septodontusa.com/sites/default/files/2020-07/Biodentine-SDS.pdf). Although Ba is a radiocontrast agent, the reason for adding Ba to BioDentine cannot be elucidated by the results of this study as it was not the purpose of this study. The disclosed information about the radiocontrast media of OrthoMTA and RetroMTA was supported by the EDS results of this study. Trace elements were not considered in the results.

The case reports related to the toxicity of radiocontrast additions into CSCs are not available in the literature. However, micron-sized metal-oxides and metal complex additions into the dental materials can cause late-type hypersensitivity reactions (Gawkrodger, 2005; Stejskal & Stejskal, 1999; Syed, Chopra, & Sachdev, 2015). Bar suspensions have been used safely in clinical medicine since the 1950s (Hsu et al., 2020; Oliva et al., 2012). Similarly, the high atomic number of Bi brings about a high energy radiation attenuation larger than that of lead at almost negligible risk of toxicity (Bartoli, Jagdale, & Tagliaferro, 2020). Zirconia is the oxide form of zirconium. It has been reported that the zirconia presents no cytotoxic, mutagenic effects on the bone and fibroblasts after contact with muscles or bones (Sivaraman, Chopra, Narayan, & Balakrishnan, 2018).

Fundamentally, the CH-based medications are a type of a colloid (a solid diffused into a liquid) and this liquid (vehicle, medium, or matrix) may ease or limit the ionic dispersion from the medication (Fava & Saunders, 1999). The substrate additions or medium of CH medications might alter some of the physicochemical properties such as radiopacity, flow, and consistency (Athanassiadis, Abbott, & Walsh, 2007). It has been reported that is a potential correlation between the velocity of ionic dissociation of medication and the type of its vehicle and viscosity (Estrela & Pesce, 1996). Generally, the powder/liquid form of CH medications is used as a control group in the literature (Camões, Salles, Chevitarese, & Gomes, 2003). Therefore, ProCal as a conventional form of CH-based commercial material was added in this study. According to its manufacturer, ProCal is a radiopaque powder form of CH and it is mixed with its glycerin-based vehicle (ProCal product brochure: http://www.promida.com.tr/en/product-detail/111/procal-radiopaque-calcium-hydroxide). Glycerin is a viscous-type vehicle for intracanal medications (Steiner, Dow, & Cathey, 1968). Basically, it has transparent, non-toxic, hygroscopic (moistening substance) and can be mixed with water in any proportion (Fava & Saunders, 1999). Furthermore, A radio-contrast substance can be added in glycerin (Calişkan & Türkün, 1996; Salamat & Rezai, 1986).

To increase the radiopacity of the CH-based medications, it has been recommended to add barium sulfate to the CH powder before the preparation of the paste at a 1:8 (wt/wt) ratio; Webber, Schwiebert, & Cathey, 1981; Kleier & Barr, 1991). Similarly, barium sulfate to CH powder ratio (w/w) has been suggested as 1:4 in another study (Dumsha & Gutmann, 1985). Besides, it has been recommended that iodoform or bismuth carbonate could be added to increase the radiopacity of the CH (Holland, Maisto, Souza, Maresca, & Nery, 1981; Rezende, 1982).

The present study represents a pure radiopacity test, of which only one physical characteristic of the CSCs was measured. In the same way, as radiopacity of endodontic sealing materials is defined and measured by an international standard. Unfortunately, no universally accepted test exists for the quantification of the radiopacity of the MTA-like materials. Probably, this might be not easy to create a

standard for these bioceramics in endodontics. CSCs are widely used and the need for standardization of testing of their properties including radiopacity is required to ensure the uniformity of methodology and comparable results for a safer, efficient clinical use.

5 | CONCLUSION

The following conclusions can be drawn:

- The present study revealed that the radiopacity performance of OrthoMTA, RetroMTA, BioDentine are significantly different from each other. The results could be attributed to disclosed and non-disclosed radiocontrast agents with varied proportions of among the materials.
- The pixel-based and reproducible method could be used universally to the quantification of radiodensity of digitally collected X-ray data.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

Data are available figures in the article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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