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RESEARCH ARTICLE

A new *in vitro* method to evaluate radio-opacity of endodontic sealers

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Objectives: To evaluate a new method for assessing the radio-opacity of endodontic sealers and to compare radio-opacity values with a well-established standard method.
Methods: The sealers evaluated in this study were AH Plus® (Dentsply DeTrey GmbH, Konstanz, Germany), Endo CPM Sealer (EGEO SRL, Buenos Aires, Argentina) and MTA Fillapex® (Angelus Dental Products Industry S/A, Londrina, Parana, Brazil). Two methods were used to evaluate radio-opacity: (D) standard discs and (S) a tissue simulator. For (D), ten standard discs were prepared for each sealer and were radiographed using Digora® phosphor storage plates (Soredex; Orion Corporation, Helsinki, Finland), alongside an aluminium stepwedge. For (S), polyethylene tubes filled with sealer ($n = 10$ for each) were radiographed inside the simulator as described. The digital images were analysed using Adobe Photoshop® software v. 10.0 (Adobe Systems, San Jose, CA). To compare the radio-opacity among the sealers, the data were analysed by ANOVA and Tukey's test, and to compare methods, they were analysed by the Mann–Whitney U test. To compare the data obtained from dentin and sealers in method (S), Student's paired t-test was used (=0.05).
Results: In both methods, the sealers showed significant differences, according to the following decreasing order: AH Plus, MTA Fillapex and Endo CPM. In (D), MTA Fillapex and Endo CPM showed less radio-opacity than aluminium. For all of the materials, the radio-opacity was higher in (S) than in (D). Compared with dentin, all of the materials were more radio-opaque.
Conclusions: The comparison of the two assessment methods for sealer radio-opacity testing validated the use of a tissue simulator block.
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Introduction

Radio-opacity has been widely acknowledged as an important property of endodontic sealers. Among other physical/chemical properties, it has been stated that an ideal root canal filling material should have a certain degree of radio-opacity that allows for a clear distinction between the material and the surrounding anatomical structures, to facilitate the evaluation of the quality of root fillings.[1,2]

According to the American National Standards Institute/American Dental Association (ANSI/ADA), to determine the minimal requirement of radio-opacity for root canal filling materials, it has been established that they should have radio-opacity values of at least 3 mm of aluminium at a thickness of 1 mm.[3,4] The radiographic images must be obtained by chemical processing of the radiographic film,

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and radio-opacity must be evaluated by an optical densitometer.[3–6]

By contrast, the advantages of digital radiography systems have motivated researchers, and a number of studies have compared the radio-opacity of restorative materials measured from conventional and digital radiographic images.[7–20]

Furthermore, in the standard method, the samples are radiographed with no tissue association. The absence of tooth, bone and soft-tissue constitutes an important differential compared with clinical situations in which radio-opacity is investigated, and it could alter the perception of radio-opacity of dental materials. Aiming to simulate clinical conditions, Gegler and Fontanella[21] developed a "tissue simulator block". This experimental model has already been successfully used in studies on the diagnosis of external apical root resorption, but it has not been used to evaluate the radio-opacity of endodontic sealers.

Thus, the present study aimed to evaluate *in vitro* a new method for assessing the radio-opacity of three endodontic sealers [AH Plus® (Dentsply DeTrey GmbH, Konstanz, Germany); Endo CPM Sealer (EGEO SRL, Buenos Aires, Argentina) and MTA Fillapex® (Angelus Dental Products Industry S/A, Londrina, Parana, Brazil)] in digital images (phosphor storage plates) and to compare these radio-opacity values with a well-established standard method.

Methods and materials

To evaluate the radio-opacity of three root canal sealers—AH Plus, Endo CPM sealer and MTA Fillapex — the following methods were used:

(D) Materials in standard discs

Cylindrical samples were fabricated according to their manufacturers' instructions by pouring the manipulated sealers into plastic rings measuring 4 mm in diameter by 1.5 mm in thickness. Ten specimens were prepared from each sealer. The filled rings were stored at 37 °C (±1) in 95% (±5) humidity, until the material was completely set.

Each sample was then radiographed using Digora® phosphor storage plates (Soredex; Orion Corporation, Helsinki, Finland), alongside an aluminium stepwedge that was used as a reference. The radiographs were obtained using a radiographic unit (Dabi Atlante Spectro 70X, São Paulo, Brazil) operating at 70 kV and 10 mA, with a 0.3-s exposure time and a 30-cm focal distance set.

(S) Materials in a tissue simulator

The endodontic sealers were prepared according to their manufacturers' instructions. The freshly mixed sealer was introduced into polyethylene tubes (10 mm × 1.5 mm; Abbott Lab do Brasil, São Paulo, Brazil) with a syringe to avoid bubbles. The filled tubes were stored at 37 °C (±1) in 95% (±5) humidity, until the material was completely set.

The tubes with sealers ($n = 10$ for each) were individually placed in the root canals of teeth positioned in the tissue simulator, as described previously by Gegler and Fontanella.[21] Briefly, the maxillary anterior region of a human skull was used, divided by sagittal osteotomy into two segments fixed with wax (Wilson, São Paulo, Brazil) in a plastic container (length = 6 cm; width = 2.5 cm; depth = 3.5 cm). Distances of 1 cm were established between the external surfaces of the buccal and palatal segments and the container's walls, with this latter space filled with pored self-curing acrylic (Artigos Odontológicos Clássico, São Paulo, Brazil) that could simulate the soft tissues.

A distance of 0.5 cm was established between the internal surface of the buccal bone and the internal surface of the palatal bone. The space was filled with wax and was used to fix a human canine root with the root canal previously prepared. The root was inserted up to the point at which the cementum–enamel junction coincided with the level of the alveolar crest.

The set (tubes with sealers in the root canals of the teeth positioned in the tissue simulator) was radiographed as previously described.

A 24-inch liquid crystal display monitor at 1920 × 1980 resolution was used to display the images in a dimmed light room. One observer, a dental radiologist with several years experience in digital radiography, evaluated the images at a 50-cm distance from the monitor. The digital images were analysed in Adobe Photoshop® software v. 10.0 (Adobe Systems, San Jose, CA). For the materials in (D), a standard-size circle (400 pixels) was drawn in the centre of the standard disc, and another circle was drawn in the sixth step of the aluminium stepwedge, equivalent to 3 mm of aluminium. For the materials in (S), three standard-size circles (400 pixels) were drawn: one under the tube and another under the dentin, both in the cervical third, and the third in the sixth step of the aluminium stepwedge (Figure 1).

The average and standard deviation of the grey-scale pixel values of the area selected were measured using the histogram tool and were recorded. The pixel values obtained for the materials were subtracted from the pixel values obtained in the 3-mm aluminium stepwedge.

To compare the radio-opacity among the sealers, the data were subjected to statistical analysis using ANOVA and Tukey's test. To compare methods, the data were evaluated by the Mann–Whitney *U* test. To compare the data obtained from dentin and sealers in method (S), the Student's paired *t*-test was used. The significance level was set at 5%, and the data were processed using SPSS® software, v. 10.0 (SPSS Inc., Chicago, IL).

Results

When considering the difference in pixel density between the material and 3 mm of aluminium, it was observed that the materials in both methods showed significant differences (ANOVA and Tukey *post hoc* test; $p < 0.05$) according to the following order of decreasing radio-opacity: AH Plus, MTA Fillapex and Endo CPM. It is worth mentioning that in (D), MTA Fillapex and Endo CPM showed less radio-opacity than aluminium (negative values), which was not observed in (S) (Table 1, Figure 2).

When comparing both methods, regardless of the material used, the radio-opacity was higher in (S) than in (D) (Mann–Whitney U test; $p < 0.05$) (Table 1, Figure 2). Compared with dentin, in (S), all of the materials were significantly more radio-opaque (<0.05) (Table 2).

Discussion

The radio-opacity of root canal sealers has particular relevance for assessing the quality of endodontic treatment.[14,17] To evaluate this property, the ANSI/ADA standards have traditionally been employed. However, more recently, proposals to simplify this method have been presented, both to reduce the number of steps with the aluminium stepwedge[18] and to use digital radiographs and software to replace optical densitometry.[7–17,19,20] These changes have occurred not only for endodontic sealers but also for other categories of dental materials.[22]

Additionally, further progress towards the improvement of *in vitro* testing is expected to simulate clinical conditions more closely.[23] The inclusion of materials in the tooth structure was proposed in a radio-opacity study.[24]

In effect, our results showed differences in the relative radio-opacity of the sealers between methods, with increased radio-opacity of endodontic sealers when they were radiographed inside the simulator. The overlapping of soft tissues, bone and dental structures was intrinsic in the clinical situation and was an important issue when radio-opacity was investigated.[16,20] It is interesting to observe that the differences in radio-opacity among materials found in the standard method were reduced in the simulator method, owing to the overlapping of tissues, which resulted in a certain degree of radio-opacity that allowed for the distinctions between the materials and the surrounding anatomical structures.

With the standard method, certain sealers could present lower radio-opacity values than those recommended by ANSI/ADA, requiring the addition of radio-opaque substances to their compositions. These substances could negatively influence the other properties of the sealers.[25] The present study suggested that when radio-opacity was evaluated by the simulator method, the same sealer that was considered only slightly radio-opaque by the

Figure 1 Analysis of the images in Adobe Photoshop® software v. 10.0 (Adobe Systems, San Jose, CA). Std Dev, standard deviation.

Table 1 Comparison between sealers and methods, considering the difference in pixel density obtained from the materials and from 3 mm of aluminium

		Disc		*Tissue simulator*		
Groups	n	*Mean*	*SD*	*Mean*	*SD*	p-*value*
AH Plus®	10	7.6[aA]	28.2	50.4[aB]	5.2	0.0001
MTA Fillapex®	10	−18.6[bA]	19.7	34.0[bB]	5.2	
Endo CPM	10	−124.5[cA]	4.5	14.1[cB]	2.8	

SD, standard deviation.
Different lower case letters indicate significant difference among sealers in the same column (ANOVA and Tukey *post hoc* test).
Different capital letters indicate significant difference among methods in the same row (Mann–Whitney *U* test).
AH Plus was obtained from Dentsply DeTrey GmbH, Konstanz, Germany; Endo CPM was obtained from EGEO SRL, Buenos Aires, Argentina; MTA Fillapex was obtained from Angelus Dental Products Industry S/A, Londrina, Parana, Brazil.

Table 2 Comparison of pixel density between the materials and dentin with the simulator method

		Material		*Dentin*		
Groups	n	*Mean*	*SD*	*Mean*	*SD*	p-*value*
AH Plus®	10	205.8[a]	9.0	126.8[b]	9.8	0.0001[a]
MTA Fillapex®	10	201.5[a]	16.1	125.6[b]	15.5	
Endo CPM	10	187.7[a]	6.7	142.8[b]	9.8	

SD, standard deviation.
Different lower case letters on the same row indicate significant difference.
[a]Paired Student's *t*-test.
AH Plus was obtained from Dentsply DeTrey GmbH, Konstanz, Germany; Endo CPM was obtained from EGEO SRL, Buenos Aires, Argentina; MTA Fillapex was obtained from Angelus Dental Products Industry S/A, Londrina, Parana, Brazil.

standard method could be considered sufficiently radio-opaque to be used clinically with the original composition.

Furthermore, higher grey values were found in the sealers than in dentin in the simulator method, proving that the tested materials presented sufficient radio-opacity to be identified under clinical conditions. AH Plus, a two-component paste/paste sealer, has been continuously used in comparative studies of the physicochemical, biological and antimicrobial properties of root canal sealers.[26–28] This sealer contains zirconium and iron oxide, which contribute to its greater radio-opacity. Agreeing with the findings of this investigation, its adequate radio-opacity has been demonstrated in several studies that have used the standard method.[10–14,17,20]

By contrast, for the two MTA-based filling materials tested (MTA Fillapex and Endo CPM), which were introduced to the market with the promise of improving clinical performance, the findings demonstrated herein should be confirmed in future investigations.

According to the ANSI/ADA specifications, to evaluate the radio-opacity of endodontic filling materials, discs that are 1-mm thick should be imaged and compared with 3 mm of aluminium. Because the objective of this study was to compare methods, the material thickness of 1.5 mm was used to standardize this parameter in both methods. This fact did not allow for quantitative comparison of the data obtained regarding the radio-opacity of the endodontic sealers in the standard method with the data from other investigations that used the same method.

In addition, it is important to consider that the teeth used in the simulator were superior canines, owing to their large diameters in the cervical third of the root canal. This anatomy allowed the insertion of polyethylene tube within the canal. The interference of the tube in material radio-opacity was evaluated by Salles et al.[29] They compared the tooth radio-opacity with and without the tube within the root canal and found no significant differences. Based on these findings, it can be

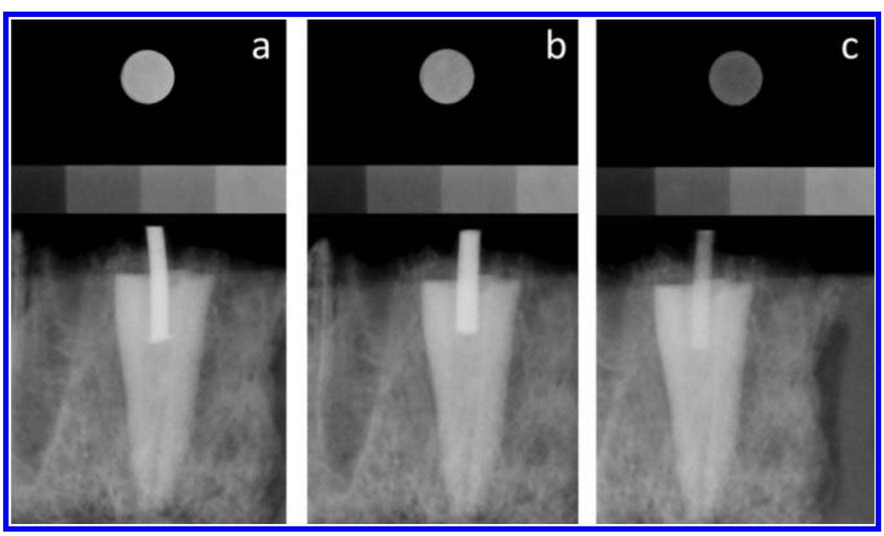

Figure 2 Image illustrating the differences between methods considering each material, showing higher radio-opacity in the tissue simulator than in standard discs: (a) AH Plus® (Dentsply DeTrey GmbH, Konstanz, Germany); (b) MTA Fillapex® (Angelus Dental Products Industry S/A, Londrina, Parana, Brazil); and (c) Endo CPM (EGEO SRL, Buenos Aires, Argentina).

inferred that, in this study, the tube did not affect the sealers' radio-opacity.

Future studies to develop methodologies that allow for the use of other dental groups, with different root diameters and bone cortical thicknesses, should be conducted to investigate whether these anatomical variations have any influence on the radio-opacity of the sealers inside the simulator.

In conclusion, the comparison of the two assessment methods for sealer radio-opacity testing, considering clinical reality, validated the use of a tissue simulator block.

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