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BIOCERAMIC ROOT REPAIR MATERIALS APPEAR MORE RADIOPAQUE IN A RADIOPACITY TEST SIMULATING CLINICAL REALITY THAN IN A TRADITIONAL CIRCULAR-DISKS TEST

Cimentos biocerâmicos aparecem mais radiopacos em um teste de radiopacidade que simula a realidade clínica do que em um teste tradicional com discos circulares

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ABSTRACT

Aim: This study assessed the radiopacity of three bioceramic root repair cements (NeoMTA Plus®, Biodentine®, and MTA Angelus®) with two radiopacity tests. **Materials and Methods:** Radiopacity tests were: a traditional radiopacity test using the circular-disk method with aluminium step wedges and digital radiography (we called "ANSI/ADA-based test"), and a tissue-simulator method using a cadaver model. Six circular specimens from each cement were fabricated to undertake the traditional ANSI/ADA-based radiopacity test; and six specimens from each cement were fabricated (into polyethylene tubes) to undertake the tissue simulator test. Radiopacity data was reported in greyscale pixels (from 0 to 255). Data were compared intra-group and between the two radiopacity tests. **Results:** The following radiopacity values were found for the traditional method and tissue simulator method, respectively: Biodentine® 120.6 ± 3.9 and 176.6 ± 2.8**;** NeoMTA Plus® 156.7 ± 6.5 and 191.7 ± 2.5; and MTA Angelus® 160.5 ± 6.7 and 192.7 ± 1.7. **Discussion:** The tissue simulator method was able to show that Biodentine®, although being less radiopaque than NeoMTA Plus® and MTA Angelus®, had higher radiopacity than 3mm-Al (fact that did not occur with the traditional method). This finding is critical because it refutes some previous beliefs that Biodentine® is not radiopaque enough to be used as a root repair material. **Conclusion:** The tissue simulator radiographic test showed all the cements more radiopaque in comparison to the radiopacity produced by the traditional circular-disks test (ANSI/ADA-based) ($p<0.05$). Biodentine® presented more radiopaque than 3 mm-Al, only in the tissue simulator method ($p<0.05$).

Keywords: Dental cements. Radiography, dental, digital. Endodontics. Cadaver.

RESUMO

Objetivo: Este estudo avaliou a radiopacidade de três cimentos biocerâmicos para reparo radicular (NeoMTA Plus®, Biodentine® e MTA Angelus®) com dois testes de radiopacidade. **Materiais e Métodos:** Os dois testes de radiopacidade foram: um método tradicional de disco circular, usando placas de alumínio com substituição de filmes por radiografia digital (denominado "teste baseado em ANSI/ADA") e um método simulador de tecido, usando um modelo de cadáver. Seis corpos de prova circulares de cada cimento foram fabricados para realizar o tradicional teste de radiopacidade baseado em ANSI/ADA; e seis corpos de prova de cada cimento foram confeccionados (em tubos de polietileno) para realização do teste do simulador de tecidos. Os dados de radiopacidade foram relatados em pixels em escala de cinza (de 0 a 255). Os dados foram comparados intragrupo e entre os dois testes de radiopacidade. **Resultados:** Os seguintes valores de radiopacidade encontrados para o método tradicional e para o método com o simulador foram, respectivamente: Biodentine® 120,6 ± 3,9 e 176,6 ± 2,8**;** NeoMTA Plus® 156,7 ± 6,5 e 191,7 ± 2,5; e MTA Angelus® 160,5 ± 6,7 e 192,7 ± 1,7.**Discussão:** O método simulador de tecidos foi capaz de demonstrar que o Biodentine®, apesar de ser menos radiopaco que o NeoMTA Plus® e o MTA Angelus®, apresentou radiopacidade superior ao 3mm-Al (fato que não ocorreu com o método tradicional). Este resultado e importante porque refuta algumas crenças anteriores de que o Biodentine® não é radiopaco o suficiente para ser usado como material de reparo radicular. **Conclusão:** O teste radiográfico do simulador de tecido mostrou todos os cimentos mais radiopacos em comparação à radiopacidade produzida pelo teste tradicional de discos circulares (baseado em ANSI/ADA) ($p<0,05$). Biodentine® apresentou mais radiopaco que 3 mm-Al, apenas no método simulador de tecido ($p<0,05$).

Palavras-chave: Cimentos dentários. Radiografia dentária digital. Endodontia. Cadáver.

INTRODUCTION

Bioceramic root repair materials (BRRM) are cements used in dentistry/endodontics for pulp capping, apexifications, apical microsurgeries, regenerative procedures, and for sealing undesirable communications between the root canal and the perirradicular tissues[1]. Some BRRM are powder-liquid systems requiring manual mixing, and some are premixed materials requiring moisture from surrounding tissues to set. Bioceramic materials have a setting process that promotes an appropriate sealing in the interface root canal-perirradicular tissues[2]. One of the expected properties for a root repair material is the sufficient radiopacity, which allows the material to be differentiated from anatomical structures in ental radiographs. A good radiopacity also allows the operator/dentist to evaluate the appropriability of the dental procedure[3-6].

The radiopacity of root repair materials is traditionally determined according to the American National Standard/American Dental Association (ANSI/ADA 2000, 2012) Specification No. 57 For Endodontic Sealing Materials, which establishes that materials must have a radiopacity not less than that equivalent to 3 mm of aluminium (Al). The ANSI/ADA protocol includes all endodontic cements and sealers, and it is not specific for root repair materials. The protocol requires that the radiographic images must be obtained by the chemical processing of radiographic film, using developing and fixation solutions, rinsing and drying; and that the radiopacity must be evaluated by an optical densitometer.

Overall, studies that evaluated the radiopacity of contemporary BRRM showed that cements, such as MTA Angelus®, are more radiopaque that a 3-mm Al step wedge. Nevertheless, radiopacity values are diverse between studies because of the different procedures adopted in the experiments, such as: the use of an aluminum step wedge with 2-mm increments in thickness (instead of 1-mm or 1.5-mm), or the use of digitalization of radiographic films[5-7].

Earlier studies reported the values of cements radiopacity in millimetres-Al. This unit of measure for radiopacity was based on measurement of radiodensity on occlusal films through an optical densitometer (8,9) or measurement of the pixel grey value using a specific software after digitization of conventional films (6, 10-13).One of the most studied BRRM, the MTA Angelus® (the previous version that was available in the market, containing bismuth oxide as the radiopacifier) has presented radiopacity from 3.0 mm-Al[6] to 6.45 mm-Al[5]. Nowadays, radiopacity values have been reported in grayscale pixels, because in the digital radiograph systems the output of the measurements is stored as absolute numbers of available grey shades (from 0 to 255) in contrast to the continuous density curve in the analogue film image[14]. MTA Angelus® (the new version currently in the market, containing calcium tungstate as the radiopacifier) has presented radiopacity of approximately 98.35 greyscale pixels[15].

Although the ANSI/ADA specification was useful for investigation of radiopacity in previous experiments, the guidelines are far from being in line with the contemporary advances in clinical dentistry – including the use of digital radiography and the creation of a scenario that better represents the clinical practice[7,11,15-18]. Evaluating the materials radiopacity in a test that could simulate the clinical scenario would help clinicians in the decision-making process to choose an adequate material for an individual procedure.

Therefore, this study assessed the radiopacity of three BRRM (NeoMTA Plus®, Biodentine®, and MTA Angelus®) with two radiopacity tests: 1) a traditional circular-disk method using aluminium step wedges with the substitution of films for digital radiography (we called “ANSI/ADA-based test”), and 2) a tissue-simulator method, using a cadaver model. The study expectation was to find higher radiopacity for the three bioceramic root repair cements when tested with the tissue-simulator method.

MATERIALS AND METHODS

This *in vitro* study evaluated radiopacity of three BRRM used in dentistry/endodontics: NeoMTA Plus® (Avalon Biomed Inc. Houston, TX, USA), Biodentine® (Saint-Maur-des-Fossés, France), and MTA Angelus® (Londrina, PR, Brazil) with two tests: 1) Traditional circular-disk method (ANSI/ADA-based), and 2) Tissue simulator method (cadaver model). This study was approved by the local Ethics Committee (approval number # 2.940.053), and it followed the PRILE 2021 guidelines for reporting laboratory studies in Endodontology (onlinelibrary.wiley.com/doi/abs/10.1111/iej.13542).

SAMPLES PREPARATION

This experiment used two types of molds to fabricate the cement samples: a circular mold (a stainless-steel ring with an opening side attachment, measuring 10 ±0.01 mm diameter x 1 ±0.01 mm height) was used for samples that undertook the traditional radiopacity test; and a cylindrical mold (a transparent polyethylene tube, measuring 1.5 ±0.01 mm diameter x 10 ±0.01 mm height) was used for samples that undertook the tissue simulator test. Sample size was based in previous studies reporting BRRM radiopacity[17-24].

The bioceramic cements were prepared according to manufacturer's instructions.

NeoMTA Plus® powder (one scoop, with a level powder of 0.1 gm) was deposited in a glass plate besides one drop of the provided gel (1:1 proportion to get a putty-like consistency). Small amounts of gel were incorporated into the powder until to get the putty. The material was then gently accommodated with a metal spatula into the stainless-steel rings (n=6), and with a small condenser into the polyethylene tubes (n=6). The radiopacifier into this cement was tantalum oxide.

Biodentine® one ampoule of liquid (calcium chloride-water-based) was opened and dispensed into a capsule of powder. The capsule was then closed and vibrated for 30 seconds in an amalgamator-like device. The capsule was opened to get the hydrated mixture, and the mixture was accommodated into the rings (n=6) and into the polyethylene tubes (n=6) as described above. The radiopacifier into this cement was zirconium oxide.

MTA Angelus® powder (one scoop) was deposited in a glass plate with a drop of distilled water (1:1 proportion) and mixed for 30 seconds until obtaining a sandy consistency. An MTA-applicator (Angelus, Londrina, PR, Brazil) carried the hydrated cement into the molds. The material was then accommodated into the ring (n=6) and into the polyethylene tubes (n=6) in the same way as described for the other two cements. The radiopacifier into this cement was calcium tungstate.

Each stainless-steel ring with material was individually covered with a small thin glass plate (1.2 mm thickness, 2x2 cm) fixed with tape over the mold. All samples (circular and cylindrical molds) remained in an incubator at 37°C and 95% humidity for 7 days to allow cements setting. The circular molds had side openings to remove the cement after setting and undertake the traditional circular disk radiopacity test. Immediately after removing samples from the rings, a digital caliper confirmed the sample diameter and height. Samples prepared for the tissue simulator test remained into the polyethylene tubes.

RADIOPACITY TESTS AND DATA COLLECTION

Traditional circular-disk method (ANSI/ADA-based)

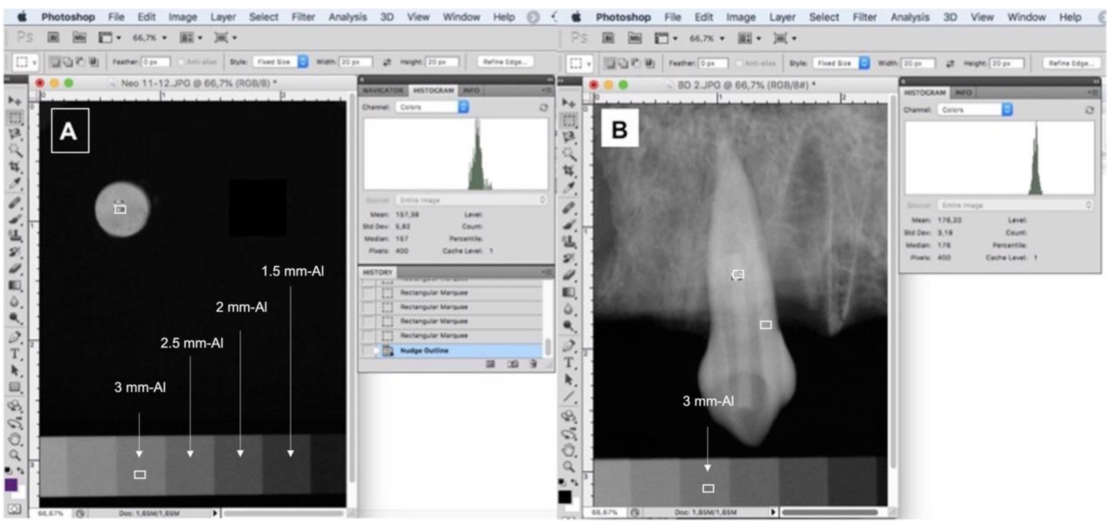
This method differed from the ANSI/ADA specification 57 (2000, 2012) because it used digital radiography, rather than conventional films. Samples were radiographed using a digital sensor (Fona CDR, Schick, Bratislava, Slovakia) alongside with an aluminum step wedge with incremental 0.5 (±0.01) mm steps. The x-ray unit operated at 70 kV, 8 mA, 0.2 s exposure time and 300 mm target distance[17]. The images were generated using the CDR Dicom software (Schick, Bratislava, Slovakia) and exported as JPEG files. Digital images were analyzed using the Adobe Photoshop software CS5 (Adobe Systems, San Jose, CA). A standard size square (400 pixels) was drawn in the center of the cement sample, and subsequently the same size square was drawn into the sixth Al step from right to left (representing 3 mm-Al in the used scale) (Figure 1A). Density values (mean and standard deviation) in grayscale pixels of the selected areas were measured using the histogram tool and converted into mm-Al[19].

TISSUE SIMULATOR METHOD

A tissue simulator was used to take radiographs of the samples. The tissue simulator was constructed using a cadaver maxilla according to a model previously developed[20]. The anterior portion of the maxilla was removed by one horizontal osteotomy (at the level of the floor of the nasal cavity) and two vertical osteotomies (at the canine region). This maxilla segment was divided into buccal and lingual halves which were then repositioned in a base of acrylic resin – creating a space in between the two halves. The space between the bony plates (5 mm) was filled with a thin layer of utility wax (Lysanda, Sao Paulo, SP, Brazil) to insert a tooth. A human extracted canine with previously enlarged root canal was then inserted in. The canine root was inserted up to the point at which the cementoenamel junction coincided with the level of the alveolar crest. Soft tissues were simulated by adding a thin layer of self-curing acrylic resin (powder-liquid)

(Jet Classico, Sao Paulo, SP, Brazil) in the buccal and lingual sides, external to the bony surfaces. The polyethylene tubes with cement were individually positioned inside the prepared root canal one at a time and radiographed using a digital sensor (Fona CDR, Schick, Bratislava, Slovakia) alongside with an aluminum step wedge with incremental 0.5 (±0.01) mm steps. X-ray parameters and digital storage were identical to those described in the traditional circular-disk, ANSI/ADA-based method. Digital images were imported to Adobe Photoshop and standard size squares were drawn to measure the density values (mean and standard deviation) in three locations: over the polyethylene tubes filled with cement, over the root canal dentin wall, and over the 3 mm-Al (Figure 1).

Figure 1 - Digital images opened in Adobe Photoshop.



Digital images opened in Adobe Photoshop with representative standard size squares that were drawn to measure the radiopacity density values (mean and standard deviation in greyscale pixels). The traditional circular-disks test (ANSI/ADA-based) is shown in ‘A’, the measurement is being taken in the center of the cement sample and in the 3 mm-Al stepwedge. The tissue simulator test is shown in ‘B’, the measurement is being taken in the cement sample [in an area with superimposition of cement, a thin layer of wax (to represent periodontal ligament), bone plates, and a thin layer of acrylic resin (to represent the soft tissues)], in the root canal dentin wall, and in the 3 mm-Al stepwedge.

DATA ANALYSIS

One single researcher worked as assessor for the radiopacity test – without knowing the type of cement that the specimen was originated from. Data in greyscale pixels of the cement radiopacity in relation to the 3 mm-Al radiopacity were used as reference and undertook an unpaired Student`s t test. Cements radiopacity values were compared using ANOVA and Tukey`s tests. Cement, dentin, and 3mm-Al radiopacity values for the samples that undertook the tissue simulator test were compared using a paired Student`s t test. The level of significance was set at 5%, and all data were processed using the SPSS® 10.0 (SPSS Inc., Chicago, IL).

RESULTS

No samples were missed following the incubation period (7 days at 37°C and 95% humidity), then, there was no need to fabricate additional samples. The results are showed in Table 1.

Table 1 – Sample results.

	Radiopacity Tests				
	Traditional circular-disks method (ANSI/ADA-based)		Tissue simulator method (cadaver model)		
	Cement	3mm-Al	Cement	3mm-Al	Root canal dentin wall
Biodentine®	120.6 ± 3.9 aA	119.8 ± 2.0	176.6 ± 2.8 aB*	112.5 ± 2.4	173.1±2.5
NeoMTA Plus®	156.7 ± 6.5 bA*	118.0 ± 1.7	191.7 ± 2.5 bB*	112.4 ± 2.4	173.9±2.1
MTA Angelus®	160.5 ± 6.7 bA*	119.3 ± 1.8	192.7 ± 1.7 bB*	116.2 ± 3.1	174.1±1.9

Mean and standard deviation (±) values in pixel density (grayscale pixels) of bioceramic root repair cements radiopacity and 3-mm Al's radiopacity after radiographs with the traditional circular-disks test (ANSI/ADA-based) and with the tissue simulator test. Root canal dentin wall's radiopacity (pixel density) is also showed for the simulator test.
Legend:
Different lowercase letters in the same column indicate significant difference between cements (P<0.05).
Different uppercase letters on the same line indicate significant difference between radiopacity tests/methods (P<0.05).
(*) Represent significant difference between cement and 3 mm-Al. In the Tissue Simulator test/method, it also represents significant difference between cement and root canal dentin wall (P<0.05).

The tissue simulator method showed cement samples (for all types of cement) more radiopaque than the ones evaluated by the traditional method ($p<0.05$).

Biodentine® was less radiopaque ($p<0.05$) than NeoMTA Plus® and MTA Angelus®, which had similar radiopacity between them ($p>0.05$); for both radiopacity tests.

Cements were more radiopaque that 3mm-Al standard required by ANSI/ADA (2000, 2012) in both radiopacity tests ($p<0.05$); except Biodentine® in the traditional method that had similar radiopacity to 3mm-Al ($p>0.05$).

Cements were more radiopaque than dentin ($p<0.05$) in the tissue simulator radiopacity test.

DISCUSSION

The radiopacity test using a tissue simulator in a cadaver model was considered more appropriate to represent the reality of bioceramic cements radiopacity in comparison with the traditional circular-disks method (ANSI/ADA-based test). The tissue simulator method was able to show that Biodentine®, although being less radiopaque than NeoMTA Plus® and MTA Angelus®, had higher radiopacity than 3mm-Al (fact that did not occur with the traditional method). This finding is critical because it refutes some previous beliefs that Biodentine® is not radiopaque enough to be used as a root repair material.

At first sight it could be assumed that the tissue simulator method should present the bioceramic cements with less radiopacity than in the ANSI/ADA-based test. However, it is known that the presence of layers (cadaver bone and materials simulating the soft tissues) makes everything more radiopaque (including the cement sample into the cylindrical polyethylene tube). Moreover, when digital sensors are used to radiograph several objects/materials that are interrelated to each other, in comparison with only a single object, they appear to become more sensitive to high-energy photons, producing higher variation in the grey levels[7,21]. In this current experiment, the digital radiographic system read the grayscale pixels in each one of the materials used in the tissue simulator – and the superimposition of those materials increased the number of available grey shades, which made the cement specimen highlight in the image. We therefore considered the tissue simulator method closer to the clinical reality since it read various 'tissue structures' (or materials) concomitantly. In addition, the simulator can measure the radiopacity of root canal dentin walls, which is essential to show the nuances between the BRRM and dentin - when the cement is clinically used to seal root perforations, for instance. In this study, the tissue simulator found that Biodentine®, NeoMTA Plus® and MTA Angelus® were more radiopaque than dentin ($P<0.05$), and more radiopaque than 3 mm-Al ($P<0.05$).

Similar tissue simulators to the one used in this current study were previously validated as radiopacity tests in investigations on root filling materials. Those investigations also evidenced the higher radiopacity for endodontic materials produced by the tissue simulator in comparison with the traditional ANSI/ADA method (with film)[22] and the ANSI/ADA-based test (with digital sensors)[18]. Up to date, only one study had tested radiopacity of BRRM using tissue simulators and, in a scale from 0 to 255 shades of grey, it found statistically smaller gray scale pixels for Biodentine® (191.1 ± 3.21) compared to the grey scale pixels of MTA Angelus® (194.3 ± 1.37)[15]. Our study also found that Biodentine® (176.6 ± 2.8) had less radiopacity that MTA Angelus® (192.7 ± 1.7) – but which matters was that the tissue simulator showed that Biodentine® is more radiopaque than 3mm-Al ($p<0.05$) and dentin ($p<0.05$). Earlier investigations that used the ANSI/ADA protocol considered some formulations of Biodentine® as having only suitable or even, insufficient radiopacity[23,24]. Some studies even tried to increase the amount of zirconium oxide or to substitute the radiopacifier to make Biodentine® more radiopaque[25]. Again, as stated before, this may be a problem of the traditional radiopacity tests that did not create a clinical scenario for dental/endodontic materials, and it used radiographic conventional films. Authors have already proved that digital radiography systems apply different methods of measuring X-ray radiation, showing that this was the reason because the radiopacity as recorded on traditional or digitized films was not indicative of the radiopacity as recorded on a digital sensor[7].

Interestingly, NeoMTA Plus® and MTA Angelus® had similar radiopacity values ($P>0.05$) in both radiopacity tests/methods – even being two bioceramic cements with different radiopacifiers. NeoMTA Plus® contains tantalum oxide (TaO, molecular weight of 196.947) while MTA Angelus® contains calcium tungstate (CaO_4W, molecular weight of 287.92). It is known that the radiopacity of a material depends only in part on the atomic numbers of its constituent atoms (more radiopaque if it contains atoms of high atomic number). Other material characteristics also need to be considered, such as: shape, other compounds of the powder, particle size, etc.[21]

This study had strengths and weaknesses. As strengths, it used a previous tested and validated tissue simulator constructed in a cadaver model. This simulator combined real bone tissue, wax, and acrylic resin to represent the soft tissues, and cement specimens in shape and size more appropriated to be inserted into the root canal, which made the scenario closer to the dental practice. Also, the use of the same digital radiography system in both radiopacity methods/tests. Because clinical use of film radiography is declining as digital systems are adopted, further studies on radiopacity should also focus on using digital systems[7]. A limitation of this study is the impossibility to say that identical radiopacity values would occur in clinical practice[26] (and the challenges of a clinical study in testing identical specimens in different clinical situations). However, our current results may sustain that Biodentine® is a bioceramic root repair cement with good radiopacity – and that radiopacity tests that mimic clinical situations are more efficient than the traditional method.

CONCLUSION

The tissue simulator radiopacity test showed all the BRRM (Biodentine®, NeoMTA Plus® and MTA Angelus®) more radiopaque in comparison to the radiopacity produced by the traditional circular-disks test (ANSI/ADA-based).

All tested BRRM presented adequate radiopacity in the tissue simulator test. Although Biodentine® had only 3 mm-Al radiopacity in the ANSI/ADA-based method, it was more radiopaque that 3 mm-Al when tested in the simulator.

Tissue simulator radiopacity test was also able to show that all BRRM were more radiopaque than root canal dentin – being appropriate to differentiate cements from the other structures.

This methodology is useful and reproducible to approximate the experiment to the clinical practice. Our current results may sustain that Biodentine® is a bioceramic root repair cement with appropriate radiopacity.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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