UNIVERSIDADE DE SÃO PAULO
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Analysis of the physicochemical properties of calcium silicate based cements used in retrograde and endodontic fillings

Análise das propriedades físico-químicas de cimentos à base de silicato de cálcio usados em obturações retrógradas e endodônticas

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Orientador: Prof. Dr. Marco Antonio Hungaro Duarte

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DADOS CURRICULARES

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*“Os que se encantam com a prática sem a ciência
são como os timoneiros que entram no navio sem timão
nem bússola, nunca tendo certeza do seu destino”.*

Leonardo Da Vinci

ABSTRACT

Objective 1: To evaluate volumetric change, pH, calcium release and radiopacity of calcium silicate repair cements: Endosequence RRM, Bio C Repair, MTA REPAIR HP, Ortho MTA, Ortho MTA plus 5% calcium oxide. **Objective 2:** Evaluate volumetric change, pH, calcium release, radiopacity and flow of three ready-to-use calcium silicate-based endodontic sealers: Endosequence BC Sealer, Bio C Sealer and Sealer Plus BC. **Methodology:** For repair cements, fifty acrylic teeth had their retro cavities filled with cements accordingly with the groups (n=10). The teeth were fixed in a plastic container and the apexes were kept in 10 mL of ultrapure water. Then, a Micro-CT 1174 scanning was performed to evaluate the volumetric change of the materials. The images were reconstructed, and the volume change of the materials was measured. The pH level and calcium ions release were evaluated using the ultrapure water of the plastic container in 3, 24, 72, and 168h. For radiopacity analysis, 3 specimens of each cements were placed on occlusal films together with an aluminum scale. The radiographs were digitalized and the radiographic density was assessed. Data were statistically analyzed by the ANOVA, Tukey, Kruskal-Wallis, and Dunn tests. For the sealers tests, root canals of thirty prototypes of upper incisor acrylic teeth were filled with sealer and gutta percha point, then immersed in containers with 13 ml of ultrapure water following the same methodology as the repair materials, including the flow test for this group of materials. **Results 1:** MTA Repair HP and Bio C Repair respectively presented the lowest and the highest volumetric change, ($P<0.05$). Endosequence RRM presented the highest radiopacity ($P<0.05$). All materials presented calcium release and alkalinization capacity. **Results 2:** Endosequence BC Sealer presented the lowest, and Bio C Sealer the highest volumetric change after 72 and 168h ($p<0.05$). Endosequence BC Sealer presented higher radiopacity ($p<0.05$). All materials showed alkalinization capacity. **Conclusions:** Among repair materials, Bio C Repair showed the highest volumetric change values, while MTA Repair HP the lowest values among them. All materials presented calcium release and alkalinization capacity, also their radiopacity values were above the minimum required by the ISO standard. Regarding the sealers. All materials presented alkalinization, calcium release capacity, radiopacity and flow values above the minimum required by the ISO standard. Bio C

Sealer showed the highest volumetric loss, while Endosequence BC Sealer was the one that lost the least volumetrically.

Keywords: Retrograde obturation. Root canal filling materials. MicroCT. Solubility.

RESUMO

Objetivo 1: Avaliar a alteração volumétrica, pH, liberação de cálcio e radiopacidade dos cimentos reparadores à base de silicato de cálcio: Endosequence RRM, MTA REPAIR HP, Bio C Repair e Ortho MTA Ortho MTA acrescido de 5% de óxido de cálcio. **Objetivo 2:** Avaliar alteração volumétrica, pH, liberação de cálcio, radiopacidade e escoamento de três cimentos obturadores endodônticos à base de silicato de cálcio prontos para uso: Endosequence BC Sealer, Bio C Sealer e Sealer Plus BC. **Metodologia:** Para os cimentos reparadores, cinquenta dentes acrílicos tiveram suas retro cavidades preenchidas com cimentos em conformidade com os grupos (n=10). Os dentes foram fixados em um recipiente plástico e os ápices foram mantidos em 10 mL de água ultrapura. Em seguida, foram realizadas leituras em micro tomografia 1174 para avaliar a alteração volumétrica dos materiais. As imagens foram reconstruídas, e a alteração de volume dos materiais foi mensurada. O nível de pH e a liberação de íons de cálcio foram avaliados utilizando-se a água ultrapura do recipiente plástico em 3, 24, 72 e 168h. Para análise de radiopacidade, três espécimes de cada cimento foram colocadas em filmes oclusais juntamente com uma escala de alumínio. As radiografias foram digitalizadas e a densidade radiográfica foi avaliada. Os dados foram analisados estatisticamente pelos testes ANOVA, Tukey, Kruskal-Wallis e Dunn. Para os testes dos cimentos obturadores, os canais radiculares de trinta protótipos de dentes acrílicos incisivos superiores foram preenchidos com cone de guta percha e cimento, depois imersos em recipientes com 13 ml de água ultrapura seguindo a mesma metodologia dos materiais reparadores, incluindo o teste de escoamento para este grupo de materiais. **Resultados 1:** O MTA Repair HP e o Bio C Repair apresentaram, respectivamente, a menor e a maior solubilidade, ($P<0,05$). O Endosequence RRM apresentou a maior radiopacidade ($P<0,05$). Todos os materiais reparadores apresentaram liberação de cálcio e capacidade de alcalinização. **Resultados 2:** O Endosequence BC Sealer apresentou a menor, e o Bio C Sealer a maior alteração volumétrica após 72 e 168h ($p<0,05$). O Endosequence BC Sealer apresentou maior radiopacidade ($p<0,05$). Todos os cimentos obturadores mostraram capacidade de alcalinização. **Conclusões:** Entre os materiais reparadores, o Bio C Repair apresentou os maiores valores de alteração volumétrica entre os materiais reparadores, ao passo que o MTA Repair HP obteve a menor

alteração volumétrica entre os materiais reparadores. Todos os materiais apresentaram liberação de cálcio e capacidade de alcalinização, seus valores de radiopacidade estavam acima do mínimo exigido pela norma ISO. Em relação aos cimentos obturadores, todos apresentaram capacidade de alcalinização, liberação de cálcio, radiopacidade e valores de escoamento acima dos valores mínimos recomendados. O Bio C Sealer apresentou a maior, enquanto o Endosequence BC Sealer apresentou a menor perda volumétrica.

Palavras-chave: Obturação retrógrada. Obturação do Canal Radicular. Microtomografia por Raio-X. Solubilidade.

LIST OF ABREVIATIONS AND ACRONYMS

%	percentage
<	less than
>	greater than
º	degree
µA	microampere
µm	micrometer
Al	aluminum
BC	bioceramic
C	Celsius
Ca	calcium
CaO	calcium oxide
cm	centimeter
g	gram
h	hour
HP	high plasticity
ISO	international organization for standardization
kV	kilovoltage
mA	milliampere
mg	milligram
Micro-CT	micro computed x-ray tomography
mL	milliliter
mm	millimeter
mm^3	cubic millimeter
MTA	mineral trioxide aggregate
n	number
P	statistical significance
pH	hydrogen potential
RRM	root repair material

SUMÁRIO

1 Introduction

1 INTRODUCTION

Calcium silicate-based cements, also known as bioceramic or hydraulic, are biomaterials widely used in restorative dentistry and especially in endodontics due to their biocompatibility, bioactivity (ALVES SILVA *et al.*, 2020) and excellent physical-chemical properties (BAEK; PLENK JR.; KIM, 2005). Basically composed of tricalcium silicate, these cements are radiopaque materials that form a hydrated silicate gel and become hard when manipulated with water (PARIROKH; TORABINEJAD, 2010). They are already established in the scientific literature for their skills such as: sealing capacity (TORABINEJAD; PARIROKH, 2010; PRATI; GANDOFFI, 2015); take setting in an environment with fluids; release of hydroxyl ions, which turns the environment alkaline and unsuitable for bacterial growth, and also release of calcium ions responsible for its bioactivity, which promotes the deposition of a layer of apatite, forming a mineralized barrier, when in contact with adjacent living tissues (GANDOLFI *et al.*, 2010).

A filling and repair endodontic material should ideally have the following characteristics: dimensional stability (CAMILLERI; MALLIA, 2011) color stability (MARCIANO *et al.*, 2014), adequate radiopacity (ISLAM; CHNG; YAP, 2006), be insoluble when in contact with tissue fluids (FRIDLAND; ROSADO, 2003), adequate flow and being easy to insert (HUNGARO DUARTE *et al.*, 2012) furthermore to presenting biological and chemical properties such as: alkaline pH, release of calcium ions (DUARTE *et al.*, 2003), bioactivity (GANDOLFI *et al.*, 2010), cell adhesion (BALTO, 2004) and biocompatibility (CAMILLERI *et al.*, 2004).

The MTA (Mineral Trioxide Aggregate) developed at Loma Linda University (TORABINEJAD; CHIVIAN, 1999) and introduced to the market in the mid-1990s, was the precursor of calcium silicate cements. Its original formulation was composed of gray Portland cement in addition of bismuth oxide as radiopacifier, being used in principle as a retro-filling material in endodontic surgeries (BAEK; PLENK JR; KIM, 2005) and later as root restorative material in the case of perforations (HASHEM; HASSANIEN, 2008), pulp capping (FARSI *et al.*, 2006), barrier in cases of pulp regeneration and apical plug (BANCHS; TROPE, 2004).

Although the original formulation has some excellent properties, others such as its sandy consistency, long setting time (PARIROKH; TORABINEJAD, 2010) and the potential to promote chromatic alteration in the dental structure (MOŻYŃSKA *et al.*, 2017), limited its use mainly in aesthetic areas and areas of difficult access.

In the last two decades, new generations of calcium silicate-based cements have been developed with the objective of overcoming the limitations of the original formulas and thus expanding their use within endodontics and restorative dentistry (DUARTE *et al.*, 2018). The new formulations introduced on the market aim to improve the plasticity and flow of the material, provide an appropriate setting time, ensure chromatic stability, as well as maintaining the properties already established as alkaline pH, release of calcium ions (PARIROKH; TORABINEJAD; DUMMER, 2018) and biocompatibility (ZOUFAN *et al.*, 2011). The inclusion of additives such as calcium chloride was incorporated into some formulations to accelerate setting time, zirconia oxide (CAMILLERI; CUTAJAR; MALLIA, 2011) and calcium tungstate replacing bismuth oxide (MARCIANO *et al.*, 2014) as radiopacifier in the new formulations which do not cause chromatic changes, are some examples of the evolution in the composition of these materials.

Another innovation within this group of materials was the forthcoming of ready-to-use cements, for which it is not necessary to manipulate to promote the mixture between two substances such as powder and liquid, whether a repair or root canal sealer, the material is already available ready for clinical application.

The setting reaction of these materials and the full usage of their physicochemical properties depend directly on their complex hydration reaction (CAMILLERI, 2007), which promotes the formation of portlandite (calcium hydroxide). In the case of ready-to-use materials, the amount of water becomes subjective, so we must count that the moisture of the dentin is sufficient to promote an adequate hydration reaction.

The objective of this research was to evaluate in vitro some physicochemical properties of silicate-based repair cements and root canal sealers, both in powder/liquid form and ready to use. For this purpose, the work was divided into two stages:

Article 1: To evaluate the volumetric change, pH level, calcium release and radiopacity of the following calcium silicate-based repair cements: Endosequence RRM, MTA Repair HP, BIO C Repair, Ortho MTA and Ortho MTA plus 5% CaO.

Article 2: To evaluate the volumetric change, pH level, calcium release, radiopacity and flow of the following calcium silicate-based endodontic sealers: Endosequence BC Sealer, BIO C Sealer and Sealer plus BC.

2 Articles

2 ARTICLES

2.1 ARTICLE 1 - ANALYSIS OF THE PHYSICOCHEMICAL PROPERTIES OF READY FOR USE AND POWDER/LIQUID REPAIR CALCIUM SILICATE-BASED CEMENTS

The article presented in this Thesis was written according to the **European Endodontic Journal** instructions and guidelines for article submission.

Analysis of the physicochemical properties of ready for use and powder/liquid repair calcium silicate-based cements

Physicochemical properties of five repair cements

ABSTRACT

Objective: To evaluate volumetric change, pH, calcium release and radiopacity of calcium silicate repair cement: Endosequence RRM, MTA REPAIR HP, Bio C Repair, Ortho MTA and Ortho MTA plus 5% calcium oxide.

Methodology: Fifty acrylic teeth had their retro cavities filled with cements accordingly with the groups (n=10). The teeth were fixed in a plastic container and the apexes were kept in 10 mL of ultrapure water. Then, a Micro-CT 1174 scanning was performed to evaluate the volumetric change of the materials. The images were reconstructed, and the volume change of the materials was measured. The pH level and calcium ions release were evaluated using the ultrapure water of the plastic container in 3, 24, 72, and 168h. For radiopacity analysis, 3 specimens of each cement were placed on occlusal films together with an aluminum scale. The radiographs were digitalized and the radiographic density was assessed. Data were statistically analyzed by the ANOVA, Tukey, Kruskal-Wallis, and Dunn tests.

Results: MTA Repair HP and Bio C Repair respectively presented the lowest and the highest volumetric change, ($P>0.05$). Endosequence RRM presented the highest radiopacity ($P<0.05$). All materials presented calcium release and alkalinization capacity.

Conclusions: Bio C Repair showed the highest volumetric change values, while MTA Repair HP the lowest volumetrically change. All materials presented calcium release and alkalinization capacity, also their radiopacity values were above the minimum required by the ISO standard.

Clinical relevance: Ready for use and powder/liquid calcium silicate-based cements have been widely used for conservative and surgical endodontics procedures because of their biological and physicochemical properties. However, it’s not elucidated if the formulation of these materials present identical physicochemical properties between them.

Keywords: Calcium silicate. Retrograde obturation. Solubility.

INTRODUCTION

During endodontic treatment, there may be accident and complications can affect the endodontic treatment success. Root canal perforation at the cervical or furcation portion is the most common, which will require the use of repair material (1). In addition, endodontic failure can be solved by apical surgery as adjuvant therapy (2). The apical root resection and a retrograde filling is the most used technique, employ repair materials to improve biological response and sealing ability (3).

An endodontic repair material should be radiopaque, presenting low solubility, not being irritating to periapical tissues, promoting adequate three-dimensional sealing (4), antimicrobial effect, mineralizing inducer and easy handling (5). The apical sealing of the root can prevent the egress of microorganisms and bacterial toxins from the inside of the root canal to the periradicular tissues, preventing fluid leakage into the root canal, besides stimulating tissue regeneration (6).

Mineral Trioxide Aggregate (MTA) is considered the gold standard material for various clinical applications due to its chemical-physical (6-8) and biological properties (9). Despite its excellent properties of MTA, its consistency and difficulty in handling imposed some limitations for clinical application (10). In this sense, improvements of this material were sought, such as insertion of calcium chloride to accelerate its setting time (11), alternative radiopacifiers (12) to avoid chromatic alteration of the tooth and alteration in the hydration vehicle to make it more plastic and facilitate its handling (13). In addition, new materials using the tricalcium and dicalcium silicate have been proposed.

Ortho MTA is a powder/liquid material and was developed by Bio MTA (Gwanak-gu, Seoul, South Korea), which basically presents the composition of the original MTA (calcium carbonate, silicon dioxide, aluminum oxide and bismuth oxide) (14).

MTA Repair HP is one of the liquid powder materials in which a polymer has been added to the liquid to improve handling and uses calcium tungstate instead of Bismuth Oxide as radiopacifier. Previous studies have demonstrated low cytotoxicity (15), good mineralization induction capacity (16), adequate radiopacity and low solubility (17).

Endosequence Root Repair Material is a pre-mixed putty consistency and ready for use. According to the manufacturer, its composition consists of: calcium silicates, monobasic calcium phosphate, zirconium oxide, tantalum oxide and thickening agents

(18). Previous studies have shown that it has good mineralization induction capacity and biocompatibility (19).

Bio C Repair is also a ready for use calcium silicate cement, which presents similar cytotoxicity, biocompatibility and ability to induce mineralization (20) similar to MTA based materials. However, its physicochemical properties have not yet been elucidated.

Tricalcium silicate-based cements depend of the hydration to achieve its setting (21). Pre-mixed materials depend on hydration from external environment (22), while powder/liquid materials already have liquid for their hydration and this may be favorable for their properties (23). The choice of suitable repair materials should be based on their biological and physicochemical properties. Thus, the aim of this study was to evaluate the volumetric change, radiopacity and release of calcium and pH level of premixed and liquid/powder repair materials. The null hypotheses tested were the following:

(1) There is no difference in the radiopacity among the cements;
(2) There is no difference in the volumetric change among the cements;
(3) There is no difference in the pH level and calcium release among the cements during the periods;

MATERIALS AND METHODS

In this study, five calcium silicate based cements were tested: Endosequence Root Repair Material, Bio C Repair, MTA Repair HP, Ortho MTA and Ortho MTA plus 5% calcium oxide. The methodology was based on the Guimarães et al. (24) study. MTA Repair HP (Angelus, Londrina, PR, Brazil) was manipulated using the total powder content of the capsule (0.085g) and mixed on a glass plate for 40 seconds with two drops of liquid (water and plasticizer). Ortho MTA (Bio MTA, Seoul, Korea) and Ortho MTA plus 5% calcium oxide (this addition aimed to observe whether the alkalinization capacity of this material could be increased) (25), were manipulated following the proportion of 1g of the powder to 0.3 mL of distilled water and 1g of the powder + 5% of CaO until a homogeneous mixture was formed. Regarding to Endosequence Root Repair Material and Bio C Repair, because they are ready for use, spatulation was not necessary.

Radiopacity

Radiopacity was analyzed in accordance with the ISO 6876:2012 standard. Three cylindrical samples (10 ± 0.1 mm diameter and 1.0 ± 0.1 mm height) were prepared.

The samples and the aluminum scale were positioned on occlusal films (F speed; Kodak Comp, Rochester, NY, USA), and then x-rayed using a radiographic unit (Gnatus XR 6010; Gnatus Ribeirão Preto, SP, Brazil) using 60 Kv and 10 mA for 0.3s. The focal distance to the film was 30 cm. After processing, the radiographs were scanned using a Canon t5i still camera and imported into a software (Adobe Photoshop CS5 Extended version12.0 x32).

The radiopacity value was determined according to radiographic density and converted into millimeters of Al. Following the conversion equation proposed in a previous study by Hungaro Duarte et al. (12). Six values of each material were recorded.

pH and calcium release

pH and calcium release level readings were performed after 3, 24, 72, and 168 hours. After each experimental period, the samples were transferred to a new vial with 10mL of ultrapure water. Measurement of pH was performed with a pH meter (Thermo model Orion 3 Star, MA, USA), previously calibrated with solutions with known pH (4,7 and 14). After removal of the specimens, the container was placed in a shaker (model 251; Farmen, São Paulo, SP, Brazil) for 5 seconds before pH measurement. The temperature of the room during the reading was 25°C.

The release of calcium ions was measured in atomic absorption spectrophotometer (AA6800; Schimadzu, Tokyo, Japan) equipped with a calcium-specific hollow cathode lamp. A standard solution of calcium was prepared, being: 20mg/L, 10mg/L, 5mg/L, 2,5mg/L, 1,25mg/L. The solution of nitric acid was used to bring the apparatus to zero absorbance. Calcium ion release calculations were performed using the standard curve straight equation.

Volumetric change analysis

Micro-CT scanning was used to evaluate volumetric change of the cements, as reported by Cavenago et al. (26). Fifty acrylic resin teeth (n=10) with standardized retro cavities were used. Before the scanning procedure, the cements were inserted in each specimen and immersed individually in acrylic containers containing 10 ml of ultrapure water and immediately scanned using a Microcomputed Tomography (SkyScan 1174v2; SkyScan, Kontich, Belgium) and at intervals of 24, 72 and 168 hours. The parameters used were 50 kV, 800 mA, 180º rotation with a step size of 0.6º, isotropic resolution of 14.1 µm and 1304 x 1024 pixels. Within the experimental period intervals, the samples were taken from the vials, dried with filter paper and scanned again using exactly the same parameters adopted for the first scan. Images of all specimens were reconstructed using dedicated software (NRecon v.1.6.3, Bruker-microCT). The reconstructed images of each period were geometrically coregistered with the preoperative data sets using DataViewer software v1.5.1 (Bruker-microCT, Kontich, Belgium), allowing quantitative comparison of the volume change among the periods. The volumetric change of the specimens was calculated using CTan software v. 1.12.4. Thus, volumetric change was determined by calculating the volume of cement samples lost during immersion, and the results found were converted into percentages to show the proportion of dissolved material.

Statistical Analysis

The tests values were submitted to the normality test using the Kolmogorov-Smirnov test. For radiopacity due to the presence of normality, the ANOVA and Tukey tests were used. In the analysis of pH, calcium release and volumetric change due to the absence of normality, the Kruskall-Wallis and Dunn tests were used. The degree of significance adopted was 5%.

Results

Volumetric change and radiopacity

The values of volumetric change (%) and radiopacity (mmAl) are summarized in Table 1. For radiopacity, all materials presented values above 3 mmAl, which is the minimum recommended by ISO 6876. Endosequence RRM presented highest radiopacity value (13.2 mmAl) ($p<0.05$). Ortho MTA and Ortho MTA + CaO presented similar radiopacity value between them (7.5 and 8.2 mmAl) ($p>0,05$). Bio C Repair presented (6.6 mmAl) radiopacity value, without statistically significant difference to Ortho MTA ($p>0,05$). MTA Repair HP (5.4 mmAl) and Bio C Repair (6.6 mmAl) presented the lowest radiopacity values ($p>0,05$).

TABLE 1. Values of the minimum and maximum median of the percentage of vol. change and mean and standard deviation of radiopacity in millimeters of aluminum. (- indicates volumetric reduction and + indicates volumetric increase)

	Volumetric change (%)			Radiopacity
	24h	**72h**	**168h**	
Brasseler RRM	-1,245 (-4,37 - +2,440) [a]	-2,140 (-6,730 - 0,02)[a]	-1,650 (-6,28 - 1,86)[a,b]	13.2 +/-0.4[a]
MTA Repair HP	-1,310 (-2,65 - -0,03) [a]	-0,2600 (-2,4 - +0,22)[a]	-0,0400 (-2,43 - 0,50)[a]	5.4 +/-0.8[b]
Bio C Repair	-4,59 (-9,21 - -0,81)[b]	-6,465 (-9,562 - -2,09)[b]	-5,370 (-13,22 - -1,78)[b]	6.6 +/-1.4[bc]
Ortho MTA	-1,605 (-5,539 - -0,8500)[a,b]	-2,405 (-7,13 -+1,12)[a,b]	-1,815 (-6,80 - 3,89)[a.b]	7.5 +/-0.5[cd]
Ortho MTA + CaO	-2,35 (-3,910 - -,0200)[a,b]	-2,040 (-3,61 - -0,18)[a]	-1,180[a] (3,61 - +0,6)[a]	8.2 +/-0.6[d]

Different letters show differences between the studied materials.

All studied materials showed volumetric alteration summing up during the experimental period, the percentage was higher within 72 hours, and decreased in the period after 168 hours. Endosequence RRM and MTA HP presented the lowest volumetric change in all periods with significant differences in relation the Bio C Repair (P<0.05). Bio C Repair and Ortho MTA presented similar values in all periods (P>0.05). Bio C Repair and Ortho MTA + CaO presented significant differences in 72 and 168 hours (P<0.05).

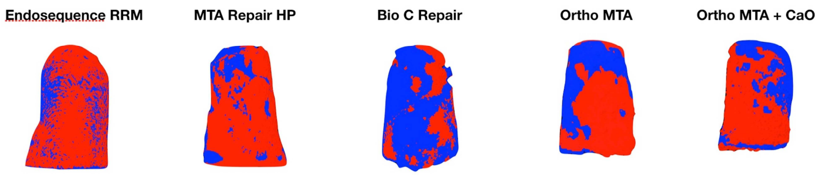

Figure 1. Images representative of the volumetric change of the materials after the final observation time showing the area where the material was solubilized (blue color) and the area where it did not suffer volumetric change (red color).

Alkalinization activity and calcium ion release

The values of pH are summarized in Table 2. The alkalizing activity was constant for all materials studied throughout the experimental period and after 168hs, there was no statistically significant difference (p>0.05). Ortho MTA + CaO presented the highest values in 3 hours (P<0.05). There was no significant difference among the cements in 24 hours and 168 hours (P>0.05). In the 72 hours the MTA HP presented the lowest values with no significant difference between the Ortho MTA and Ortho MTA + CaO (P<0.05).

TABLE 2. Median, minimum and maximum pH values of the materials studied in the different experimental periods

	pH level			
	3h	24h	72h	168h
Brasseler RRM	9.99(7.02 – 9.39)^{aA}	9.15(8.83 –10.02)^{aA}	9.02(8.51 – 9.23)^{aAB}	9.05(8.42 – 9.35)^{aA}
MTA HP	9.41(9.22 – 9.76)^{aAC}	9.37(9.10 – 9.61)^{aA}	8.82(8.29 – 9.29)^{bA}	9.41(8.65 – 9.68)^{aA}
BIO C REPAIR	9.12(8.70 – 9.60)^{aA}	9.39(8.93 – 9.61)^{aA}	9.17 (8.79 – 9.49)^{aAB}	9.25(8.16 – 9.82)^{aA}
ORTHO MTA	9.88(9.59 – 10.32)^{aBC}	9.18(8.46 – 9.84)^{bA}	9.53 (7.73– 10.14)^{abB}	9.34(8.16 – 10.04)^{abA}
ORTHO MTA+CaO	10.19(9.87 – 10.39)^{aB}	9.29(8.93 – 9.49)^{bA}	9.70 (8.35– 9.97)^{bB}	9.70(8.35 – 9.97)^{bA}

Different lowercase letters show significant differences between the periods analyzed within the same material (P<0.05).

The values of calcium ion release are summarized in Table 3. All materials presented potential release of calcium ions, especially in the first 24h, which was decreasing over the study period. Endosequence RRM presented significant difference in comparison with Orto MTA and Ortho MTA + CaO in the period of 3 hours (P<0.05). Ortho MTA + CaO presented lower values than the other cements in 24 hours (P<0.05). There was no significant difference among Endosequence RRM, Bio C Repair and MTA Repair HP (P>0.05). There was no significant difference among the cements in the period of 168 hours (P>0.05).

TABLE 3. Values of the median, minimum and maximum calcium (mg/L) released from the materials studied in the different experimental periods

	Calcium ions release			
	3h	24h	72h	168h
Brasseler RRM	18.20 (2.63 – 28.94)^{aA}	16.81(12.22 – 88.97)^{aA}	9.75(7.16 – 13.23)^{abA}	6.81(5.89 – 25.78)^{bA}
MTA HP	12.96 (8.56 – 26.68)^{aAB}	9.33(6.66 – 14.23)^{acAB}	4.56(2.98 – 7.81)^{bBC}	7.57(3.62 – 20.71)^{bcA}
BIO C HEPAIR	12.22 (7.15 – 23.69)^{aAB}	10.69(6.01 – 13.96)^{abAB}	7.44(5.87 – 11.20)^{bAB}	7.28(5.05 – 13.25)^{bA}
ORTHO MTA	9.66 (7.07 – 21.08)^{aB}	5.54 (1.49 – 14.06)^{bBC}	5.43(0.97- 14.92)^{abABC}	8.20(2.53 – 17.05)^{abA}
ORTHO MTA+CaO	11.54(3.73 – 18.79)^{aAB}	4.49 (2.23 – 8.00)^{bC}	3.70(0.0– 6.10)^{bBC}	7.42(6.58 – 10.24)^{aA}

Different lowercase letters show significant differences between the periods analyzed within the same material (P<0.05).

Discussion

Tricalcium Silicate-based cements are considered the gold standard material for root end filling materials and for root canal perforation (6,8,9) because of suitable physicochemical properties for good sealing ability and biological responses (8,9). The study evaluated the volumetric change, radiopacity, release of calcium and pH level of premixed and liquid/powder repair materials.

Radiopacity is an important feature in retro filling and repair materials which allows analyzing the quality of the fill of the material inserted into the cavity. All studied materials presented values higher than 3 mmAl, minimum recommended by the ISO 6876:2012 standard. Endosequence RRM presented the highest radiopacity values, while MTA Repair HP and Bio C Repair presented the lowest radiopacity. Therefore, the first null hypothesis was rejected. The radiopacifying agents of the cements differed among them. Endosequence RRM and Bio C Repair use the zirconium oxide as radiopacifying agents. In contrast, MTA Repair HP and Ortho MTA and Ortho MTA + CaO use calcium tungstate and bismuth oxide, respectively. These differences of composition and proportion can be the reason for the radiopacity differences.

Hungaro Duarte et al. (12) evaluated the radiopacity of Portland cement with different radiopacifying agents in the same proportions and observed more radiopaque values with the bismuth oxide and minor values with the barium sulfate and zinc oxide. The zirconium oxide and calcium tungstate presented intermediate values. Thus, it would be expected that Ortho MTA and Ortho MTA + Cao should present highest radiopacity values, while Endosequence RRM and Bio C Repair, similar values. It was previously reported that the amount and proportion of each radiopacifying agent can favor more or less radiopaque cement (27). Also, the difference in radiopacity between Endosequence RRM and BIO C Repair could be related to the presence of tantalum pentoxide in the composition of Endosequence RRM, a solid metal (18), which probably increases the radiopacity.

The volumetric change of the cements was measured using microcomputed tomography, as reported by Cavenago et al. (26). Micro-CT analysis allowed to evaluate the volumetric modifications of the materials (mm^3), which offers greater precision in the analysis and avoids limitations of conventional methodologies. Also, this methodology ensured standardized humidity conditions.

Low volumetric change is a desirable characteristic for retro-filling and repair materials (28), avoiding empty spaces and penetration of microorganisms (29). Ideally, a volumetric alteration should not be more than 3% in the first 24 hours (26). The results of this study showed significant difference among the cements. Therefore, the second null hypothesis was rejected. Bio C Repair did not present acceptable level of volumetric change within 24 hours (4.5%) and in the other periods. Previous studies reported that the silicate's particle size and amount of calcium aluminate could delay the hydration and setting time of the material, which may favor higher solubility (30). Also, Bio C Repair uses polyethylene glycol as a dispersing agent, which is considered as a water soluble material, and could corroborate with the volumetric degradation of Bio C Repair. The results of this study could be related to the different setting time and the silicate's particles size (31). The smaller particle size of the material favor a better hydration reaction and greater contact surface, improving its setting reaction and decreasing volumetric change, besides increasing its bioactivity (31). Finally, one should consider the difference between ready for use and liquid/powder silicate based materials. Ready for use materials can probably present alteration in its physicochemical properties because the hydration mechanism depends on the water from the root dentin, which could compromise its setting, justifying the results of this study.

The pH level and release of calcium ions is responsible for biomineralization process and antimicrobial action. During the hydration reaction of tri and dicalcium silicate occurs the formation of portlandite (calcium hydroxide), providing calcium and hydroxyl ions and an alkaline pH (21). All materials showed environment alkalinization capacity and release of calcium ions. The results demonstrated no significant difference among the cements only in the period of 168 hours. Thus, the third null hypothesis was rejected.

The calcium ion release factor and environment alkalinization do not seem to justify the high percentage of volumetric degradation presented by Bio C Repair in this study, because their pH and calcium levels were similar with Endosequence RRM, which presented lower percentage value of volumetric change. In addition, Endosequence RRM presented the highest calcium ion release value in the initial period and the lowest value in the final period. All materials had a decrease in calcium release reading over the experimental period. The addition of the 5% amount of CaO to the Ortho MTA powder did not increase its alkalizing capacity.

The repair process seems to be correlated with deposition of mineralized apatites, which depends on the pH level and the release capacity of calcium ions (32). The calcium ions released by the material react with the phosphate ions of the tissue precipitating hydroxyapatite crystals, allowing to create a mineral layer that present chemical adhesion to dentin and improve the sealing ability of the material (33). Therefore, it is desirable that the material promotes a high level of pH and calcium release during the setting reaction. It could be speculated that Endosequence RRM and MTA HP could present higher potential of chemically adhesion to dentin walls than the other cements.

In conclusion, with the limitation of this study, all repair cements presented radiopacity values above the minimum required by the ISO standard and presented alkalinization capacity and calcium ions release. BIO C Repair and MTA Repair HP presented the highest and lowest volumetric changes, respectively.

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2.2 ARTICLE 2 - EVALUATION OF EXPERIMENTAL MODEL IN VITRO FOR ANALYSIS OF BIOCERAMIC SEALERS

The article presented in this Thesis was written according to the **Clinical Oral Investigations** instructions and guidelines for article submission.

Evaluation of experimental model in vitro for analysis of bioceramic sealers

Abstract

Objective To evaluate physicochemical properties of three ready-to-use calcium silicate-based endodontic sealers: Endosequence BC Sealer, Bio C Sealer and Sealer Plus BC.

Methodology Radiopacity was evaluated according to ISO standard. For the flow test, 0.5 ml of each sealer was deposited between glass plates and the average of the diameters measurement were considered the sealer flow value. For pH and calcium release, root canals of thirty prototypes of upper incisor acrylic teeth were filled with sealer and gutta percha point, then immersed in containers with 13 ml of ultrapure water. The measurement of pH and release of calcium ions (atomic absorption spectrophotometer) were in 3, 24, 72 and 168h. Acrylic teeth were scanned by Micro-CT 1174 in the same periods. Obtained data were analyzed by the ANOVA, Tukey, Kruskal-Wallis and Dunn tests.

Results Endosequence BC Sealer presented the lowest, and Bio C Sealer the highest volumetric change after 72 and 168h ($p<0.05$). Endosequence BC Sealer presented higher radiopacity ($p<0.05$). All materials showed alkalinization capacity. All of them presented calcium ion release, with higher value for Sealer Plus BC.

Conclusions All materials presented alkalinization, calcium release capacity, radiopacity and flow values above the minimum required by the ISO standard. Bio C Sealer showed the highest volumetric loss, while Endosequence BC Sealer showed the lowest.

Clinical Relevance Ready-to-use silicate based sealers have been used for root canal filling; therefore, these materials should present suitable physicochemical properties for clinical application. Solubility and ions release were assessed, approach to clinical conditions using prototype teeth.

Keywords Calcium silicate. Root canal filling materials. MicroCT. Solubility.

Introduction

The filling and sealing of the root canal system after its proper modeling and cleaning are essential for long-term success [1,2] Endodontic sealers should fill areas of difficult access and promote sealing [3], presenting insolubility to the variable fluids, biocompatibility, and radiopacity greater than that of dentin [4].

Calcium silicate-based cements are known to have biocompatibility and bioactivity [5], their bioactive potential is associated with alkalinization and release of calcium ions, which occurs during their hydration reaction that produces calcium hydroxide that is the soluble component [6]. However, the solubility of cement may compromise the sealing quality of a root canal against recontamination by microorganisms or penetration of tissue fluids. [7].

Among the new endodontic calcium silicate-based sealers, premixed in syringes and ready to use, are: Endosequence BC Sealer (Brasseler, Savannah, GA, USA); Bio C Sealer (Angelus, Londrina, PR, Brazil); and Sealer Plus BC (MK Life, Porto Alegre, RS, Brazil). Endosequence BC Sealer is composed of tricalcium silicate, dicalcium silicate, colloidal silica, monobasic calcium phosphate, calcium hydroxide and zirconium oxide. As demonstrated in studies, it was biocompatible, especially 7 days after its application [8], presented lower inducing capacity of mineralized tissue formation than that of MTA, but higher than that of AH Plus [9], was effective against E. faecalis, its antimicrobial activity may be due to the combination of promoting a high level of pH, hydrophilia and active release of calcium hydroxide [10]. Bio C Sealer presents in its composition: tricalcium silicate, dicalcium silicate, tricalcium aluminate, silicon oxide, polyethylene glycol, iron oxide and zirconium oxide. This sealer showed greater biocompatibility and ability to induce mineralized tissue when compared to AH Plus [11], adequate flow, setting time and radiopacity, but high solubility [12]. Sealer Plus BC is composed of tricalcium silicate, dicalcium silicate, calcium hydroxide and zirconium oxide and presents biocompatibility when used in contact with periapical tissues, inducing a mild inflammatory reaction and favoring repair [13] and solubility above that recommended by ISO 6876 [14].

However, studies that analyzed solubility and volumetric alteration employed extensive specimens and tested the materials only after their setting. Testing the volume change, calcium release and pH immediately after filling the root canal, simulating the conditions that occur in the clinic, would provide a condition closer to reality. Standard solubility tests are based on the difference between weights before and after placing cement in ultrapure water [15,16]. These tests present methodological limitations, because material particles can detach from cements during the storage period, or cement can absorb water, which could influence weight and consequently results [17]. Micro-CT analysis has been used to evaluate the volumetric alteration of endodontic materials correlating solubility and dimensional alteration between different time intervals, thus allowing a more reliable observation of the dimensional behavior of the materials [18,14,19].

In order to simulate conditions closer to these sealers into clinical conditions, it is important that these filling materials have adequate physicochemical properties. Thus, the aim of this study was to evaluate the volumetric alteration, release of calcium and hydroxyl ions and flow and radiopacity of these sealers. The null hypothesis is that the materials have similar physical-chemical properties between them.

Materials and methods

The materials used in the study were pre-mixed calcium silicate-based endodontic sealers: Endosequence BC Sealer, Bio C Sealer and Sealer Plus BC. Because these are premixed and ready-to-use materials, no prior manipulation was required.

Radiopacity

Cylindrical samples were produced in accordance with ISO 6876:2012 standard using three rings measuring 10±0.1 mm in diameter and 1±0.1 mm thick. The molds were supported on a glass plate, and after filled with the injected sealer, they were covered by another glass plate. The samples and the aluminum scale were positioned on occlusal films (speed F; Kodak Comp, Rochester, NY, USA), and then x-rays were performed using a radiographic unit (Gnatus XR 6010; Gnatus Ribeirão Preto, SP, Brazil) using 60 Kv and 10 mA for 0.3s. The film focal distance was 30 cm. After processing, the radiographs were scanned using a Canon t5i still camera and imported into software (Adobe Photoshop CS5 Extended version12.0 x32), where a tool was used to identify the density areas in radiographic images. This procedure allowed the comparison between radiographic data, sealer density and radiopacity of the different thicknesses of the aluminum scale. The radiopacity value was determined according to radiographic density and converted into Al millimeters. Following the conversion equation proposed by Hungaro Duarte's previous study [20].

For solubility, pH and calcium release tests, 30 prototype specimens of superior central incisor teeth printed in transparent resin (IM from Brazil, São Paulo, SP, Brazil) were used, which were divided into 3 groups (n=10). The root canals of all specimens were prepared by automated asymmetric oscillatory motion using an Endodontic Reciproc VDW Silver engine (VDW, Munich, Germany) using R#25 instrument (VDW, Munich, Germany) up to the limit of the foramen and the preparation complemented with an R#50 instrument working at 1mm below the apical foramen limit. During mechanical preparation, the canals were irrigated with distilled water and final cleaning was done by shaking the volume of water present with an Easy Clean plastic instrument (Bassi Easy, Belo Horizonte, MG, Brazil) activated in rotational motion by pneumatic handpiece, for 20 seconds, in order to remove any remaining resin debris caused by mechanical preparation. Then the canals were irrigated once again and aspirated with Capillary tips (Ultradent do Brasil, Indaiatuba, SP, Brazil). The sealers were injected into the simulated canals through the syringe and its injection tip, which penetrated up to 4 mm below the actual length of the canal and was slowly removed as the sealer was deposited until an adequate refilling was observed. Then, gutta percha points Dia Pro R R50 (Diadent, Burnaby, BC, Canada) had their tips wrapped in sealer and inserted inside the canal to the work limit (1mm below foramen), the cervical excess was cut with heated instrument and the opening of the pulp chamber was sealed with temporary sealer Coltosol (Coltene Brasil, Rio de Janeiro, RJ, Brazil). Once filled, all specimens were included in vials containing 13 ml of ultrapure water. The resin teeth crowns were transfixed and glued to the lid of the containers, so that the root apexes were immersed in the ultrapure water throughout the experimental period, thus remaining in constant humidity. Then solubility, pH level and calcium release were tested following the methodology used by Guimarães et al. [21].

Flow

The flow test was performed according to ISO 6876:2001. A total volume of 0.5mL of sealer was deposited in the center of a glass plate. After 3 (three) minutes application on the plate with the syringe, another glass plate of 20 ± 2g of dough was installed on the plate containing the sealer and in both a weight corresponding to 100g. After 10 (ten) minutes of application, the weight was removed, and the largest and smallest diameter of cement was measured using a digital caliper (Mitutoyo MTI Corporation, Tokyo, Japan). The mean of the measurement of the two diameters was considered the sealer flow value. Three measurements were made for each sealer variable.

Volumetric change analysis

For this analysis, the methodology used by Cavenago et al. [22] was applied. Solubility was evaluated by volumetric measurements of sealers using Micro-CT images. Thirty prototypes of upper incisor acrylic resin teeth (n=10) were used, each sample was scanned four times after filling the root canals. They filled with injected sealer and percha gutta point, with proper care to avoid blisters during this step. After filling the canals, the samples were scanned using a Microfocus Computerized Tomographic Desktop by X-ray (SkyScan 1174v2; SkyScan, Kontich, Belgium). The scanning procedure was completed using 50 Kv X-ray strains, with 800 mA anode current. The image capture parameters used were a voxel size of 14.1 μm with rotation pitch of 0.7º in the rotation of 180º and zoom of 16.82°m. Each scan consisted of 266 tiff images with 1304 x 1024 pixels. The digital data were further elaborated by the reconstruction software (NReconv1.6.4.8, SkyScan) which was used for the volume of the measurements. In the CTan software, the samples were separated to be analyzed individually. This was limited to the (ROI) area of each sample and the new ROI data were saved in separate folders. Then, the sample data was opened, and the binary value were adjusted according to the raw images. This value was recorded to be used later in the second scan, with this, it was possible to obtain a quantitative analysis of the material volume through 3D plug-in analysis. This tool promotes an automated calculation of the total volume (mm^3) of the three-dimensional

(3D) image of selected binary objects (white color). These data were reported in a list of results after the '3D analysis' function was performed. Before the scanning procedure and the final setting time, the samples were individually immersed in acrylic bottles containing 13 ml of ultrapure water and scanned immediately and at the intervals of 24, 72 and 168 hours. Between the intervals of the experimental time, the samples were taken from the vials, dried with filter paper and scanned again using exactly the same parameters adopted for the first scan. The sample volume of the analysis after immersion in water was performed in the same way as the first. Thus, solubility was determined by calculating the volume of sealer samples lost during immersion, and the results found were converted into percentages to show the proportion of the dissolved material.

pH and calcium release

Thirty prototypes of upper incisor teeth printed in acrylic resin (10 per group) were used. The materials were injected into the root canals and the filling was completed with a gutta percha point wrapped in the same sealer and condensed vertically. After filling the canals, the specimens were placed individually in an acrylic bottle containing 13 ml of ultrapure water and stored at 37°C, where they remained throughout the experimental period. The acrylic teeth had their crowns fixed on the lid of the bottle, so that the apex of the roots was always immersed in the water. To avoid any interference in the results, all acrylic bottles were previously washed with ultrapure water. To obtain pH and calcium release level, readings were performed after 3, 24, 72 and 168 hours. After each experimental period, the teeth were transferred to a new bottle with the same volume of ultrapure water. The pH level was measured with a pH meter (Thermo model Orion 3 Star, MA, USA), previously calibrated using controls with pH at values of 4.7 and 14. After removal of the samples, the container was placed in an agitator (model 251; Farmem, São Paulo, SP, Brazil) for 5 seconds before measuring the temperature of the room during reading at 25°C. Deionized water was used as a control for the pH level measurements for all periods analyzed. Calcium ion release was performed using an atomic absorption spectrophotometer (AA6800; Schimadzu, Tokyo, Japan) equipped with a cathodic and specific lamp. For the wavelength and crack parameters, several tests were analyzed to determine the correct one. Standard calcium solutions were prepared at the following concentrations: 20 mg, 10 mg, 5 mg, 2.5 mg, 1.25 mg and 1 mg. Then, 2 ml of lanthanum nitrate solution were added to 6 ml of standard calcium or test solution. For the preparation of the blank solution, the same amount of lanthanum nitrate solution was added to 6 ml of ultrapure water. Calcium, white and test solutions were quantified in atomic absorption spectrophotometry. To place the device at zero absorbance, a nitric acid solution was used. The calcium ion release readings were compared with a standard curve obtained from standard solution readings.

Fig. 1 Root canal of prototype tooth filled with root apex in constant contact with moisture

Statistical analysis

The values obtained from the tests were then submitted to the normality test using the Kolmogorov-Smirnov test. Radiopacity and flow data showed normal distribution and ANOVA and Tukey tests were used for statistical comparison. The data of pH, calcium release and volumetric alteration did not present normal distribution and the Kruskal-Wallis and Dunn tests were used for the comparison among the groups. The degree of significance adopted was 5%. GraphPad Prism 6.0 Software was used.

Results

The results of flow and radiopacity tests are expressed in **Table 1**. All tested sealers presented flow and radiopacity values above the minimum recommended by ISO 6876:2001. The flow was similar for the three materials studied (P<0.05) The Endosequence BC Sealer presented the highest radiopacity value (9.0 mmAl) followed by Bio C Sealer (6.9 mmAl) and finally the Sealer Plus BC (4.8 mmAl) which presented the lowest radiopacity value. There were significant differences between the three materials regarding radiopacity (P<0.05)

Table 1. Median values and standard deviation of the flow (mm) and radiopacity (mmAl) tests

	Flow	**Radiopacity**
Endosequence BC	25.47 +/-0.3[a]	9.0 +/-1.6[a]
Bio C Sealer	25.46 +/-2.2[a]	6.9 +/-0.6[b]
Sealer Plus BC	27.84 +/-4.7[a]	4.8 +/-0.4[c]

Different lowercase letters demonstrate significant differences between the analyzed materials (P<0.05).

The median, minimum and maximum values of volumetric change percentage are expressed in **Table 2**. Regarding the volumetric change, all sealers presented volume loss above 3% in the fillings, with statistically significant difference between them. The fillings made by Bio C Sealer showed the highest values of volumetric loss, while the fillings performed with the Endosequence BC Sealer showed least volumetrically lost.

Table 2. Values of the minimum and maximum median of the percentage of volumetric change

	Volumetric change (%)		
	24	**72**	**168**
Endosequence	-5,000 (-8,460 - -2,780)[a]	-7,295 (-10,51 - -4,860)[a]	-7,050 (-10,49 - -4,440)[a]
Bio C Sealer	-11,43 (-15,17 - -9,780)[b]	-15,96 (-21,08 - -14,91)[b]	-16,92 (-21,08 - -15,42)[b]
Sealer Plus BC	-8,760 (-19,58 - -4,090)[b]	-13,36 (-21,96 - -8,170)[c]	-14,42 (-23,28 - -9,620)[c]

(-) identifies percentage of volumetric loss. Different lowercase letters demonstrate significant differences between the analyzed materials in each period (P<0.05).

The results of pH and release of calcium ions are shown in tables 3 and 4. The pH of the water which the materials were immersed in was 5.5. Within the methodology used in this study, all materials presented alkalinization capacity mainly in the first 3 hours of analysis, decreasing alkalinity to a level close to neutral after 24 hours and remaining so until the end of the experimental period (168 hours). The canal fillings performed with Sealer Plus BC showed the highest pH values, followed by Bio C Sealer, while the fillings performed with Endosequence BC Sealer presented lower alkalinization power. Regarding calcium release, the canal fillings performed with Sealer Plus BC released a greater amount of calcium ions throughout the experimental period, mainly in the initial periods of analysis (up to 24 hours), followed by fillings performed with Endosequence BC Sealer, which showed higher values of calcium ion release at the end of the 168 hours period. The canal fillings using Bio C Sealer presented the lowest value within the analyzed periods.

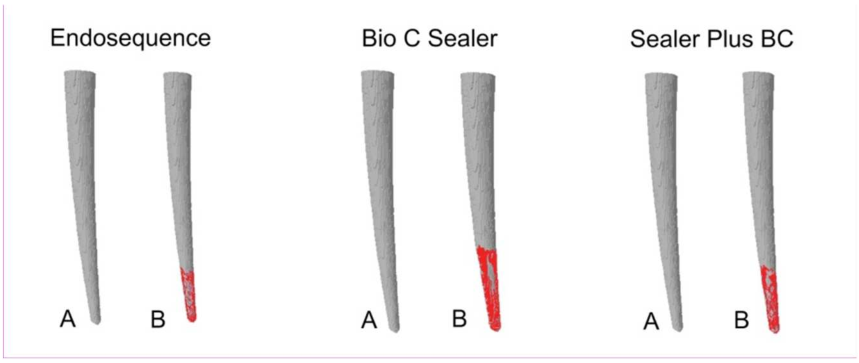

Fig 2. Representative images of the volumetric change of materials, where A is the material in the initial scan and B the volume change after 168 hours

Table 3. Median, minimum and maximum pH values of the materials studied in the different experimental periods

	pH level			
	3h	**24h**	**72h**	**168h**
Endosequence	6,775 (5,900-7,450)^{aA}	5,590 (5,340-5,650)^{bA}	5,660 (5,600-,810)^{cA}	5,945(5,790-6,550)^{dA}
Bio C Sealer	8,475 (6,360-9,500)^{aB}	7,145 (6,670-8,600)^{bB}	7,015 (6,650-,410)^{bB}	6,950 (6,210-7800)^{bB}
Sealer Plus BC	9,310 (6,840-9,980)^{aB}	7,545 (6,580-9,490)^{bC}	7,015 (6,470-,590)^{cB}	7,180 (6,430-8.770)^{bcB}

Different lowercase letters demonstrate significant differences between the periods analyzed within the same material and the uppercase letter indicate the differences between materials into each period analyzed (P<0.05).

Table 4. Median, minimum and maximum calcium (mg/L) values released from the materials studied in the different experimental periods

	Ca ions release			
	3h	**24h**	**72h**	**168h**
Endosequence	1,850 (0,9900-2,990)^{aAB}	1,025 (0,9600-1,500)^{bAB}	1,250 (1,010-1,520)^{abA}	1,770 (1,310-13,13)^{aA}
Bio C Sealer	0,9200 (0,3200-2,910)^{aA}	0,5850 (0,1700-1,360)^{aA}	0,5450 (0,2600-18,38)^{aB}	0,6200 (0,2200-3,770)^{aB}
Sealer Plus BC	2,745 (0,3900-14,28)^{aB}	1,600 (0,4100-2,920)^{abB}	0,8550 (0,6900-,240)^{bAB}	1,095 (0,750-3,770)^{abAB}

Different lowercase letters demonstrate significant differences between the periods analyzed within the same material and the uppercase letter indicate the differences between materials into each period analyzed (P<0.05).

Discussion

Calcium silicate-based sealers have been introduced in the recent history of endodontics, with the prospect of expanding the biological properties of endodontic fillings. These properties are dependent on the sealer's ability to alkalize the environment and promote the release of calcium ions, conditions that may be related to the solubility of the material. Another property that the sealer must present is the flow, which is important for these materials to penetrate and fill more critical anatomical areas of the root canal system such as isthmus, lateral canals and dentinal tubules, thus promoting a better sealing. However, it is important that flow is not highly elevated to avoid extravasation to the periapical tissues, which could interfere in the repair process, depending on the level of the material's cytotoxicity [23]. The three studied sealers presented flow values above the minimum required by ISO 6876:2012, and with similar behavior among them. The results for Sealer Plus BC and Endosequence BC Sealer corroborate some studies [24,25].

Radiopacity values above the dentin favors a visual analysis of the filling quality of the root canal system by the sealers. All studied materials presented radiopacity value above the ideal established by the ISO 6876/2001 standard. Endosequence BC Sealer presented the highest radiopacity value (9.0 mmAl) with statistically significant difference (p<0.05) compared to the other sealers, Bio C Sealer presented a value of 6.9 mmAl and the Sealer Plus BC, 4.8 mmAl. These results corroborate previous studies [26,24,12]. All the studied sealers have the same radiopacifying agent (zirconium oxide) in their compositions. The choice of zirconium oxide as a radiopacifier in the new calcium silicate-based sealers takes into account the aesthetic factor, for the bismuth oxide that has been used and is still in the composition of some cements, despite presenting higher values of radiopacity [20], can cause staining and discoloration of the dental structure [27]. In addition to this aspect, zirconium oxide does not affect the other physical-chemical and biological properties of calcium silicates [16], it can still be effective in inhibiting fungal infection by C. Albicans [28]. The different radiopacity values presented by the materials can be justified by the variability in the proportion of the radiopacifying agent used in each sealer, which can interfere in the values of the radiopacity of the material [29]. The bioactive properties of calcium silicate-based sealers highlight this material in relation to other classes of cements. The ability to induce repair and mineralized tissue formation, are dependent on the ability of these materials to alkalize the environment and promote the release of calcium ions. The calcium hydroxide present in the sealer formulation, or formed by the hydration of silicates and calcium oxide (Bio C Sealer) dissociates into calcium and hydroxyl ions, alkalizing the environment and thus making the environment inappropriate for bacterial development, besides participating in the formation of hydroxyapatite crystals. In clinical application, moisture is paramount for a premixed sealer to develop its hydration reactions and consequently the setting process; regarding this type of material, this can be a bias for its performance, because it is not known if the natural moisture of dentin is sufficient to promote the necessary reactions. So, this could lead to compromising some physicochemical properties of premixed calcium silicate-based sealers.

Manufacturers claim that the pH value of these sealers is near 12 in the first few hours. However, the present study analyzed the precise value of hydroxyl ions the sealer would release through the foramen, simulating the clinical condition and it was verified that the pH released to the apical region due to hydration was close to 10 in the first 3 hours of application, decreasing alkalinity to a level close to neutral after 24 hours, and thus remaining until the end of the experimental period. Sealer Plus BC and Bio C Sealer showed higher hydroxyl ions release capacity, differentiating from Endosequence BC Sealer. On the other hand, this was the one with the lowest volumetric loss. Regarding calcium release, Sealer Plus BC presented the highest calcium ion release value, while the lowest value occurred for Bio C Sealer. Possibly, the absence of calcium hydroxide in the composition of this sealer may have contributed to this result. It is important to highlight that the alkalinization capacity and the release of calcium ions may have been limited by the methodology used in this study, where the contact of the material with the external environment (ultrapure water) was restricted to the foramen diameter of acrylic tooth canals (about 0.25mm) which would be closer to clinical conditions.

The solubility of filling materials is related to the dissociation of the components of these materials or the degradation of particles when in contact with tissue fluids, which can undesirably cause gaps that can allow microbial colonization and lead to reinfection [15]. Under the methodology applied in this study, all sealers presented volumetric losses above 3% in the first 24 hours. Moisture can alter the properties of hydrophilic premixed calcium silicate-based sealers [30]. Until they complete their total setting time, the influence of the tissue fluids and blood on these materials can contribute to their solubilization. [15]. Simulating tissue fluids may provide greater similarity with clinical application [31]. Thus, the methodology of the present study was directed to simulate the clinical reality, where the premixed sealer inserted inside the root canal would immediately get in contact with moisture through the apical foramen. Endosequence BC Sealer presented the lowest percentage of volumetric change throughout the experimental period. In this study, the total volumetric loss percentage of this material was similar to that observed in the study by Zhou et al. [32]. Some factors may justify the lower volumetric loss of this sealer in relation to the other two studied sealers. Endosequence BC Sealer has calcium phosphate in its composition, which improves its setting time resulting in chemical composition and crystalline structure similar to apatites [33]. Another reason could be particle size; both EndoSequence and Sealer Plus BC have nanoparticles, which tend to have more efficient hydration reactions, since their surface area is more susceptible to the reception of water molecules, further optimizing their setting process, and consequently minimizing their solubility. On the other hand, Bio C Sealer presented the highest percentage of volumetric loss among the three investigated sealers.

In addition to not having calcium phosphate in its composition, it can be composed of particles larger than those of Endosequence BC and Sealer Plus BC. The larger the particle, the greater the volume loss of the material as it is solubilized. The percentage of volumetric loss observed in the present study was even higher than that presented by the previous research of Zordan-Bronzel et al. [12], which used a different methodology, waiting for the complete setting time of sealers before putting them in contact with moisture, unlike the present work, where the specimens were immersed in water immediately after the insertion of the filling materials, which may justify the higher solubility value obtained.

Conclusions

The null hypotheses tested were denied, except in flow test, in which there were no significant differences among the materials.

All materials presented alkalinization and calcium release capacity. The radiopacity and flow values were above the minimum required by the ISO standard. Bio C Sealer showed the highest values of volumetric loss, while Endosequence BC Sealer presented the lowest values. The pH and calcium release were higher for the Bio C sealer and the Sealer Plus BC.

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3 Discussion

3 DISCUSSION

An ideal endodontic material should have the following characteristics: promote good adherence to the canal walls after its setting; not to suffer contraction after its setting; promote a hermetic sealing; be insoluble to the tissue fluids; be biocompatible; have good flow; be radiopaque among others (GROSSMAN, 1974). Calcium silicate-based cements occupy an important place in modern endodontics, mainly from the perspective of expanding the biological properties of this group of materials. This study aimed to evaluate some chemical physical properties of five types of powder/liquid and ready-to-use repair cements, and three ready-to-use calcium silicate based root canal sealers.

The samples used for the repair material were 50 identical acrylic teeth, with retro cavity prepared, which provided a uniformity in the size of the retro cavities and therefore a standardization of the amount of repair cement used for analysis. For the root canal sealers study, 30 prototyped acrylic teeth were used, that had their canals prepared and filled with the respective sealers in addition to a cone of gutta percha. Both the prototypes used for the repair material and for the root canal sealers had their apexes kept in contact with moisture throughout the analysis process, so as to approach a more reliable clinical condition.

Radiopacity values above the dentin favors a visual analysis of the filling by the material. The radiopacity values presented by repair materials and root canal sealers were above 3mm Al, which is the minimum recommended by ISO 6876. Endosequence RRM presented the highest radiopacity value (13.2 mmAl) ($p<0.05$). Ortho MTA and Ortho MTA + CaO showed similar values of radiopacity among them (7.5 and 8.2 mmAl) ($p>0.05$). Bio C Repair presented radiopacity value (6.6 mmAl) without statistically significant difference for Ortho MTA ($p>0.05$). MTA Repair HP (5.4 mmAl) and Bio C Repair (6.6 mmAl) presented the lowest radiopacity values ($p>0.05$). Regarding the sealers, Endosequence BC Sealer presented the highest radiopacity value (9.0 mmAl) followed by Bio C Sealer (6.9 mmAl) and finally Sealer Plus BC (4.8 mmAl) which presented the lowest radiopacity value. There was a statistically significant difference among the three studied root canal sealers ($P<0.05$). Among all studied types of cements, those that use zirconium oxide as radiopacifier, presented the highest radiopacity values.

The biological, antimicrobial and bioactive properties of the cements are related to the ability to alkalinize the environment and promote the release of calcium ions, that are also related to the materials solubility. The materials solubility was analyzed by their volumetric change, which were measured using micro computed tomography (micro-CT), as described by a previous study (CAVENAGO *et al.*, 2014). This form of analysis allows evaluating modifications of materials in mm^3 measurements, which offers great accuracy and exceeds the limitations of conventional methodologies. Presenting a low volumetric change value is an interesting characteristic for a repair, filler or retro-filler material (TORABINEJAD *et al.*, 1995), this would avoid the formation of empty spaces and the penetration of microorganisms (SILVA *et al.*, 2016). According to the standard established by ISO, the volumetric change should not exceed more than 3% in the first 24 hours (CAVENAGO *et al.*, 2014).

All studied repair material showed volumetric change over the experimental period, the percentage was higher within 72 hours and decreased until the period of 168 hours. Endosequence RRM and MTA HP presented the lowest values in all periods without significant difference (P>0.05). Bio C Repair and Ortho MTA showed similar values in all periods (P>0.05). Between Bio C Repair and Ortho MTA + CaO there was no significant difference within the 24 hours period (P>0.05). Bio C Repair showed volumetric change levels lower than acceptable within 24 hours (4.5%) and also in subsequent periods. Previous studies have shown that the size of silicate particles and the amount of calcium aluminate can delay the hydration reaction and, consequently, the setting time of the material, which could favor greater solubility (SAGHIRI *et al.*, 2017). The results of this study may be related to different sizes of silicate particles (GANDOLFI *et al.*, 2014). Smaller particle size provides a larger contact surface and favors a better hydration reaction, improving their setting reaction, thus reducing volumetric change and increasing their bioactivity (GANDOLFI *et al.*, 2014). The difference between powder/liquid and ready for use materials should also be considered. Ready-to-use cements can probably present alterations in their physical-chemical properties because hydration mechanisms depend on water from root dentin, which could compromise their setting time, justifying the results obtained in this study.

Regarding the root canal sealers, all of them also showed volume loss (above 3%) in fillings with statistically significant differences between Endosequence BC Sealer and the others (Sealer Plus BC and Bio C Sealer). The fillings performed with Bio C Sealer showed the highest values of volumetric loss, while the fillings performed with the Endosequence BC Sealer showed the lowest values of volumetric change, corroborating results obtained by previous study (ZHOU *et al.*, 2013). Something what could explain the smaller volumetric change of Endosequence BC and Sealer Plus BC comparing to Bio C Sealer, is that they have nano calcium silicate particles in their compositions, which tends to promote a more efficient hydration reaction. Unlike the nano particles of the other two assessed sealers, Bio C Sealer has micro calcium silicate particles, so the larger the particle size, the greater the solubilization and the volume of material loss. Furthermore, it does not have calcium phosphate in its formulation, which may have contributed to a delay in setting time promoting greater solubility. The percentage of volumetric loss of Bio C Sealer found in this study exceeded the values found by another study (ZORDAN-BRONZEL *et al.*, 2019), which used a different methodology in which the complete setting time of the sealers was awaited before putting them in contact with moisture.

The alkalizing activity of all the repair cements was constant throughout the experimental period and, after 168 hours, there was no statistically significant difference ($P>0.05$). Ortho MTA + CaO presented the highest pH values in 3 hours ($P<0.05$). There was no difference among cements in 24 hours and 168 hours ($P>0.05$). In 72 hours, the HP MTA presented the lowest values without statistically significant difference from Ortho MTA and Ortho MTA + CaO ($P>0.05$).

Also, all the repair materials presented potential release of calcium ions, especially in the first 24h, which has decreased over the study period. Endosequence RRM presented significant difference in comparison to Ortho MTA and Ortho MTA + CaO in the period of 3 hours ($P<0.05$). Ortho MTA + CaO presented the lowest values compared to the other cements in 24 hours ($P<0.05$). There was no significant difference among Endosequence RRM, Bio C Repair and MTA Repair HP ($P>0.05$). There was no significant difference among the cements in 168 hours ($P>0.05$). The calcium ion release factor and environment alkalinization do not seem to justify the high percentage of volumetric degradation presented by Bio C Repair in this study, for their pH and calcium levels were similar to Endosequence RRM, which presented lower percentage value of volumetric change. Moreover, Endosequence RRM presented the highest calcium ion release value in the initial period and the lowest value in the final period. All materials had a decrease in calcium release reading over the experimental period. The addition of the 5% amount of CaO to the Ortho MTA powder did not increase its alkalizing capacity.

About the endodontic sealers, all materials presented alkalinization capacity mainly in the first 3 hours of analysis, decreasing alkalinity to a level close to neutral after 24 hours and remaining so until the end of the experimental period (168 hours). Manufacturers claim that the pH value of these sealers is near 12 in the first few hours. The canal fillings performed with Sealer Plus BC showed the highest pH values, followed by Bio C Sealer, while the fillings performed with Endosequence BC Sealer presented lower alkalinization power; on the other hand, this was the one with the lowest volumetric loss.

The canal fillings performed with Sealer Plus BC released a greater amount of calcium ions throughout the experimental period, mainly in the initial periods of analysis (up to 24 hours), followed by fillings performed with Endosequence BC Sealer, which showed higher values of calcium ions release at the end of the 168 hours period. The canal fillings using Bio C Sealer presented the lowest values within the analyzed periods. The absence of calcium hydroxide in the of Bio C Sealer formula may have contributed to this result. It is important to highlight that the alkalinization capacity and the release of calcium ions may have been limited by the methodology used in this study, where the contact of sealers with the external environment (ultrapure water) was restricted to the foramen diameter of acrylic tooth canals (about 0.25mm) which would be closer to clinical conditions.

Flow, which is an important property for these materials to penetrate and fill more critical anatomical areas of the root canal, was assessed for this group of sealers. Endosequence BC, Bio C Sealer and Sealer Plus BC presented flow values above the minimum required by ISO 6876:2012, and with similar behavior among them. The results for Sealer Plus BC and Endosequence BC Sealer corroborate some studies (MENDES *et al.*, 2018; CHEN *et al.*, 2020).

4 Conclusions

4 CONCLUSIONS

1- Root repair material group

a) All root repair material presented radiopacity values above the minimum required by the ISO standards;
b) All root repair material presented alkalinization capacity and calcium ions release.
c) The BIO C Repair and MTA Repair HP presented the highest and lowest volumetric changes, respectively.

2- Endodontic sealers group

a) All silicate based filling materials presented alkalinization and calcium release capacity;
b) The radiopacity and flow values of the silicate based filling materials tested were above the minimum required by the ISO standard.
c) The Bio C Sealer showed the highest values of volumetric loss, while Endosequence BC Sealer presented the smallest volumetrically loss.

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